Abstract

SY01

Carbon nanotubes and other possible causes of mesothelioma

SY01-1 Keynote Speaker

Responses of pulmonary cells to carbon nanotubes

Seishiro Hirano, Akiko Furuyama

Environmental Nanotoxicology Section, RCER, Natl. Inst. Environ. Studies, Japan

Health effects of carbon nanotubes (CNT) have been gaining attentions of toxicologists, because the shape of CNT is similar to that of asbestos and the potency of CNT to cause mesothelioma has been reported recently. We exposed macrophages (J774.1) and lung epithelial cells (BEAS-2B) to multi-walled CNT (MWCNT) in vitro and studied how the cells reacted with MWCNT and what signals were transduced inside the cells. We also injected MWCNT into the thoracic cavity of mice and investigated changes in the lung tissues. MWCNT were highly cytotoxic to both macrophages and epithelial cells compared to crocidolite. Electron microscopic studies suggested that plasma membranes of the cells were directly injured by MWCNT resulting in necrotic cell death rather than apoptosis. Studies with primer array, antibody array, and reporter gene assay suggested that exposure to MWCNT activated NF-κB, MAP kinases and finally increased pro-inflammatory cytokine production, suggesting that MWCNT caused oxidative stress to the cells. Macrophages took up MWCNT fast through a membrane receptor MARCO (macrophage receptor with collagenous structure) which is one of scavenger-type receptors and is expressed specifically in phagocytes. We transfected murine MARCO gene and revealed that MARCO-expressing cells took up MWCNT more rapidly than empty-vector transfected cells. We also expressed GFP-tagged MARCO in CHO-K1 cells. MWCNT adhered to MARCO first and were phagocytosed by the cells. Although BEAS-2B cells are not phagocytic cells, the cells were associated well with MWCNT. Those results indicate that MARCO is probably important for the fast-phase uptake of MWCNT and there are other mechanisms (non-MARCO mediated) in the slow-phase cellular uptake of MWCNT. There may be a chance that MWCNT migrate from the alveolar space to thoracic cavity. Our preliminary 1.5-year study suggested that intra-thoracic injection of MWCNT caused pleural thickening and lung tumors, suggesting that exposure to MWCNT should be avoided.

SY01-2

Diameter in nano-dimension of multi-walled carbon nanotubes and nanofibers is a critical factor in mesothelial injury and subsequent inflammation

Hirotaka Nagai[1,2], Nobuaki Misawa[1], Yoriko Yamashita[1], Midori Tanaka[3], Toshikazu Ishihara[1], Hiroyuki Yasui[4], Yutaka Yoshikawa[4], Yoriko Tanaka[1], Gaku Ichihara[5], Yasumitsu Miyata[6], Hisanori Shinohara[6], Shinya Toyokuni[1]

[1]Department of Pathology and Biological Responses, Nagoya University, Japan, [2]Department of Pathology and Biology of Diseases, Kyoto University, Kyoto, Japan, [3]Department of Pharmaceutical Sciences, Kyoto University, Kyoto, Japan, [4]Division of Analytical and Physical Chemistry, Kyoto Pharmaceutical University, Kyoto, Japan, [5]Department of Occupational and Environmental Health, Nagoya University, Nagoya, Japan, [6]Department of Chemistry and Institute for Advanced Research, Nagoya University, Nagoya, Japan

Multi-walled carbon nanotubes (MWCNTs) have potential applications in various fields. However, due to their needle-like shape and high durability, concerns have been raised that MWCNTs may induce mesothelioma. Previous studies have demonstrated the potential cytotoxicity of MWCNTs, but the features of MWCNTs that determine mesothelial cell cytotoxicity remains unclear. Here we show that the deleterious effects of MWCNTs on human mesothelial cells stem not from active phagocytosis, but from passive penetration. Thin dispersed MWCNTs with high crystallinity ($\phi \sim 50$ nm) showed mesothelial cell penetration and cytotoxicity in vitro and induced severe fibrotic inflammation in vivo, whereas thick ($\phi \sim 150$ nm) or aggregative MWCNTs ($\phi \sim$ 2-20 nm) did not. Notwithstanding this, every MWCNT studied contributed to macrophage-dependent local granuloma formation. Thus, the diameter of MWCNTs is a critical factor for cytotoxicity in mesothelial cells and subsequent diffuse inflammation. This work suggests that modulating the diameter of MWCNTs in production could reduce the threats to human health.

SY01-3

Nonasbestos fibers in the lung tissue of patients with mesothelioma give a hint for mesotheliomagenicity of nanofibers

Naomi Hisanaga[1], Kiyoshi Sakai[2], Eiji Shibata[3], Yoko Sakakibara[1], Michihiro Kamijima[4], Gaku Ichihara[5]

[1]Center for Campus Health and Environment, Aichi University of Education, Japan, [2]Environmental Health Department, Nagoya City Public Health Research Institute, [3]Department of Health and Psychosocial Medicine, Aichi Medical University School of Medicine, [4]Department of Occupational and Environmental Health, Nagoya City University Graduate School of Medical Science, [5]Department of Occupational and Environmental Health, Nagoya University Graduate School of Medicine

Objective: High concentrations of nonasbestos inorganic fibers having nano-level diameter are detectable in the human lung tissue. We aimed to clarify their role in the development of mesothelioma. Methods: The concentration and size of asbestos and nonasbestos fibers were determined in the lung tissues of 46 mesothelioma patients with an analytical transmission electron microscope.Results: The geometric mean (geometric standard deviation) concentrations (million fibers/g dry lung) of total asbestos, chrysotile, amphibole asbestos, total nonasbestos fibers, and aluminum silicate fiber that was the major type of nonasbestos fibers, were 4.8 (9.5), 1.4 (7.6), 2.3 (10.5), 41.0 (3.7), and 21.7 (4.9), respectively. Those of total asbestos, chrysotile, amphibole asbestos, total nonasbestos fibers, and aluminum silicate fiber having a diameter under 100 nm were 3.2 (9.7), 1.4 (7.4), 1.3 (9.9), 23.4 (4.1), and 13.4 (5.2), respectively. It was remarkable that the concentration of aluminum silicate fiber was significantly higher than that of asbestos. Discussion and conclusion: Because aluminum silicate fiber belongs to a group of clay mineral distributing widely in nature, a number of people would have been exposed heavily to it in dusty workplaces since before the era of massive industrial use of asbestos. If aluminum silicate has similar mesotheliomagenicity to asbestos, there would be a large number of mesothelioma patients since before the wide use of asbestos. Considering that the mass outbreak of mesothelioma clearly correlated to the use of asbestos, we speculate that mesotheliomagenicity of aluminum silicate fiber with nano-level diameter detected in the lung tissue would be less than that of asbestos.

SY01-4

Rat ERC/mesothelin and early lesions of tumorigenic and non-tumorigenic fibrous materials including multi-wall carbon nanotube

Shuichi Adachi[1], Miho Mizoi[1], Sei-ichi Omura[2], Takahiro Kobayashi[2], Okio Hino[3]

[1]Dept. Pub. Health, Grad.Sch.Nutr.Sci., Sagami Women's Univ., Japan, [2]Integrated Research Inst., Tokyo Inst. Tech., Kanagawa, Japan, [3]Dept. Mol. Pathogenesis, Grad.Sch.Med., Juntendo Univ., Tokyo, Japan

Nanomaterials are the most important new materials in various field of usage. Regarding the necessity of hazard identifications for nanomaterials, we have examined the lesions as an early sign of carcinogenic process in experimental animal after i.p. administration of nanomaterials. Multi-wall carbon nanotube (MWCNT), three different dimensional features of TiO2 (P:spheric particle, F100:short fiber, F400:long fiber), chrysotile asbestos (Chr), two types of crystalline whiskers (potassium titanate and silicon carbide) and vehicle (1% Tween 80 in saline) as control (V). At 1, 3, 5 days, 1, 2, 4, 10, 20 weeks after i.p. administration, histological lesions and plasma N-ERC level were examined. No significant change was observed in P and V groups without the coagulations of TiO2 particle on the surface of liver tissue. In contrast, 3 fibrous materials (F100, F400 and Chr) induced obvious inflammatory lesions between liver and diaphragm at 1 day and progressed to adhesion at 5days point. These lesions were the severest in Chr group and comparatively moderate in F400 and F100 groups. Plasma N-ERC levels in F400 were continuously high in Chr and whiskers, however, it gradually decreased to control level in fibrous TiO2 and MWCNT. These results suggest that plasma N-ERC is a possible indicator to evaluate the potency of mesothelioma inducible activity for fibrous nanomaterials.

PD01

Asbestos in the world

PD01-1

The magic stone: Asbestos between history and myth

Claudio Bianchi, Tommaso Bianchi

Center for the Study of Environmental Cancer - Italian League against Cancer, Italy

Asbestos was used in pottery in Eastern Finland from about 4000 B. C.. In the ancient era and in Middle Ages, magic properties were frequently attributed to this mineral. In the first century the Latin encyclopaedist Pliny the Elder reports in his Historia Naturalis that asbestos protects against all poisonings, particularly that of the magicians. Asbestos is often found in places of worship. Pausanias, a Greek geographer of the second century, reports the presence of the mineral in the lamp placed in a temple on the Acropolis of Athens. After the Liber Pontificalis, a book in which the biographies of the first Popes are collected, an asbestos wick was placed on the candle in the middle of the Lateran Baptistery in Rome. An apocryphal gospel, the Protoevangelium of James, reports that different materials, including asbestos, were used in making the curtain of the Jewish temple in Jerusalem. The Christian Fathers of the Church, Basil the Great (339-379) and Augustin of Hyppo (354-430), quoted asbestos in their theological works. The Italian traveler Marco Polo (1254-1324) in the Milione (Travels of Marco Polo) describes in a region of Central Asia towels that were placed in the fire without burning. He also reports that the Great Khan, the emperor of China, sent one of such towels as a gift to Rome; the tissue had to be used to wind the sudarium of our Lord. In the most ancient tale of the Japanese literature, Taketori Monogatari (The Tale of the Bamboo Cutter), 10th century, the legend is reported of the fire-rat fleece. Such fleece does not burn, even if placed in the fire. It may be found in China. In the 18th century, thanks to the Japanese scientist Hiraga Gennai, asbestos becomes a component of the samurai armour.

PD01-2

Impact of malignant mesothelioma in Taiwan: A 27-year review of population-based cancer registry data

Lukas Lee[1,2], Yu-Yin Chang[3], Jung-Der Wang[2,3,4]

[1]Division of Environmental Health and Occupational Medicine, National Health Research Institutes, Taiwan, [2]Department of Environmental and Occupational Medicine, National Taiwan University Hospital, Taipei, Taiwan, [3]Institute of Occupational Medicine and Industrial Hygiene, College of Public Health, National Taiwan University, Taipei, Taiwan, [4]Department of Internal Medicine, National Taiwan University Hospital, Taipei, Taiwan

Background and Objective: Malignant mesothelioma (MM) is primarily caused by exposure to asbestos. We conducted the study to describe the epidemiologic characteristics of MM in Taiwan and evaluate the impact of the condition. Methods: The Taiwan Cancer Registry Database was used to retrieve the cases of MM registered from 1979 to 2005. Only cases of histopathologically verified MM were included. For evaluating the impact of MM, the cancer sites of the pleura and peritoneum (ICD-O codes 163 and 158, respectively) were used for statistical analysis and estimation of the expected years of life lost (EYLL). Their survival was calculated by Kaplan-Meier analysis and extrapolated to obtain the EYLL using the Monte Carlo simulation by borrowing information from gender- and age-matched populations in Taiwan. Results: A total of 423 cases of MM were included; MM of the pleura and peritoneum accounted for 91% of all cases (387/423). The median survival of pleural and peritoneal MM was 7.6 and 13.5 months for males and females, respectively. The incidence of MM increased during the observation period. A total of 232 males and 155 females diagnosed with MM were used for estimation of EYLL: 14.8 [95% Confidence Interval: 13.1-16.6] life-years for males and 13.7 [11.2-16.2] life-years for females.Conclusion: The increasing incidence and significant EYLL for MM were observed for both males and females during 1979-2005 in Taiwan, although under-diagnosis and under-estimation were likely.

PD01-3

Study on the relationship between exposure to asbestos and asbestos-related diseases in Vietnam

Tran Thi Ngoc Lan

Environment Health Management Agency, Vietnam Ministry of Health, Vietnam

In Vietnam, asbestos has been used since 1960s, average comsumption is 60,000 to 70,000 tones of asbestos imported per year. 90% of asbestos is used in production of construction materials such as slate, pipe, brake or thermo-resistant products. There are 42 asbestos-cement roofing tile factories operating throughout 24 provinces and cities, with a total capacity of more than 100 million m^2/year.

According annual death record, there are around 150 death cases cause by membral cancers every year, there is including Mesothelioma. In order to clairify a relationship between asbestos exposure and its impact on the human health, in 2009-2010 a case control study on relationship between exposure to asbestos and asbestos-related diseases (ARD) in Vietnam has been established.

Objectives: i) to investigate the relationship between exposure to asbestos and pleural cancer in communities and ii) to Investigate the relationship between exposure to asbestos and ARD in patients admitted hospitals in 2009-2010.

Study Sites: 5 hospitals (Tumor hospital and Cho Ray hospital in Ho Chi Minh city; National Cancer Hospital, National Hospital of Respiratory Diseases and Hospital 103 in Hanoi)

Study methodology and sample size:

- Cross-sectional study: describe a relationship between exposure to asbestos and ARD among 150 reported death cases of membral cancers in community mortality registration in two years (2007-2008) .
- Case-control study:
  + Cases: 340 patients of ARD in 3 hospitals (including 40 cases of mesothelioma patients).
  + Controls : 680 patients (match compatible with cases) with the others diseases (non ARD) in 5 hospitals, (340 cases in the same hospital, 340 cases in others).

Expected outcomes:

•a report on asbestos consumtion in Vietnam and the relationship between asbestos exposure and mortality cases caused by mesothelioma in communities during a period 2007-2008.

•report on the relationship between asbestos exposure and asbestos-related diseases.

PD01-4

Pleural mesothelioma rates in South Africa: trends 1995-2006

Spoponki Kgalamono[1,2], Danuta Kielkowski[1,2], Braimoh Bello[1,3], Gill Nelson[1,2], Olojimi Fadahun[4], James Phillips[1,5]

[1]The National Institute for Occupational Health, Johannesburg, South Africa, [2]School of Public Health, University of the Witwatersrand, South Africa, [3]Reproductive Health Research Unit, Johannesburg, South Africa, [4]Demography Agincourt, South Africa, [5]School of Pathology, University of the Witwatersrand, South Africa

Introduction
South Africa has mined and refined chrysotile, amosite and crocidolite since the 19th century with peak production in 1977. At present, the mining and use of asbestos is banned in South Africa. In the 1970s and 1980s mesothelioma rates in South Africa were among the highest in the world. Trends in mesothelioma rates from 1995 to2006, were assessed to determine the burden of mesothelioma by year and gender.
Methods
Mesothelioma deaths were identified from death certificates. For each year, age and gender distributions were obtained from national statistics. Age-standardised mesothelioma rates and 95% confidence intervals were calculated annually by gender. Tests for trend were conducted using linear regression models.
Results
There were 2325 deaths due to mesothelioma: 1788 were men and 537 were women, a 3:1 ratio. The age standardized mortality rate was 11-15 per million and 3-5 per million in men and women respectively. The trends were stable over time for both genders.
Discussion
Mesothelioma rates were lower than expected, given the high rates in previous decades, and that asbestos production peaked 33 years ago. It is likely that the mesothelioma rate is an underestimate due to both under- ascertainment and shortened longevity due to HIV/AIDS.

PD01-5

Future burden of malignant mesothelioma in Western Australia

Nicholas de Klerk[1,3], William Musk[2,3]

[1]Centre for Child Health Research, University of Western Australia, Australia, [2]Dept of Respiratory Medicine, Sir Charles Gairdner Hospital, [3]School of Population Health, University of Western Australia

The main worldwide hazard for malignant mesothelioma (MM) has been occupational exposure to asbestos. The burden of disease from this exposure will continue for many years. More recent concerns about additional future incidence of MM arise out of exposure to fibres from: (i) the large amounts of existing asbestos-based products in the general environment; (ii) continued production of chrysotile and chrysotile-based goods particularly within developing countries; and (iii) general environmental exposure from naturally occurring asbestos or asbestiform minerals. While individual risks from these sources might be low when based on extrapolation from occupational risk estimates, these exposures could affect much larger populations and lead to more overall disease.Estimation of future risks and burden of disease can be made in at least three ways:(i) using extrapolation from exposure-response relationships derived from epidemiological studies of occupationally exposed cohorts; (ii) extending current age-period-cohort models into the future; (iii) applying current survey estimates of different types of exposure to current measures of disease incidence and extending these relationships into the future. These methods require various assumptions about the relationship between level and duration of exposure and disease occurrence.Applying these methods to Western Australian data produced consistent and similar results: the epidemic is likely to continue at around its present level for at least another 15-20 years before eventually declining.Additional contributions to MM incidence from non-asbestiform sources have been shown to be comparatively minimal and the genetic contribution to risk of MM appears to be similar to other more common cancers. So it is crucial to keep emphasising that prevention of exposure to asbestos still remains the urgent priority for prevention of MM.

PD01-6

Mesothelioma in England - results from the National Lung Cancer Audit 2006-2009

Mick Peake[1], Paul Beckett[1], Ian Woolhouse[1], Roz Stanley[2]

[1]Royal College of Physicians, UK, [2]The Information Centre for health and social care, Leeds, UK

The National Lung Cancer Audit is an audit of lung cancer run jointly by the Royal College of Physicians and The Information Centre for health and social care. The aim of the audit is to facilitate service improvement by recording outcomes in lung cancer on a large scale and through case-mix adjustment, start to explain the wide variations noted. Although Wales and Scotland also contribute to the audit, this abstract presents results for mesothelioma in England only (2006-2009).There were 4038 patients with mean age 71.5 yrs. Of these 3314 (82%) were male and 724 (18%) were female. 41% have right-sided disease, 25.5% are left-sided, 1% are bilateral and 0.1% are midline with the location recorded as unknown in 32%.46.5% are referred by their GP, 14.7% are emergency admissions, 16.9% are referred by another consultant, 5.6% are referred following an A&E episode, 8.5% come through other routes and in 7.7% the referral route is recorded as unknown.Overall the median survival is 267 days with a 1YS of 38.6%. Further analysis by histological subtype and by treatment will be provided. Further analysis of geographical variations in treatment will be included in a final presentation.This is the largest published series of mesothelioma cases in the UK and gives a fascinating insight into the presentation, diagnosis, treatment and outcome for the disease. There is a striking tendency for mesothelioma to be right-sided.as noted in earlier series. 25% of cases have no histological confirmation recorded, but where this is obtained, the epithelioid subtype has a much better prognosis. Patients receiving chemotherapy have the best survival but this finding is likely to be heavily influenced by patient selection.

PD02

Legal aspects

PD02-1 **Keynote Speaker**

Mesothelioma at the intersection of law and medicine

Steven Kazan

Kazan McClain Lyons Greenwood & Harley, USA

Modest use of asbestos in the late 19th Century led to the recognition that asbestos caused disease and death 100 years ago and produced evidence that asbestos caused lung cancer and mesothelioma by the middle of the last century. Subsequent increased use of asbestos has grave implications for increasing death rates now and for decades to come, particularly in Asia. This epidemic resulted in litigation in the United States from the late 1960s to today, with the past few decades demonstrating an increasing trend to worldwide litigation. Approximately 45 U.S. asbestos companies have undergone bankruptcy reorganization and have established trust funds with approximately $35 billion in current assets, after paying $10 billion to claimants. These funds are available to compensate anyone in the world who was exposed to products from these companies so long as they have claims valid under the law of their country of residence.

Doctors have moral and ethical obligations to advise patients of the causal connection between diagnosis and asbestos, and of the consequent right to compensation. These obligations have been recognized in the United States by state law and by the medical community. Patients today look to the Internet for information about disease, and physicians should know what their patients find. We will discuss search engine results in several languages and assess the potential reliability of that information which often comes from lawyers either acting on their own behalf or disguised as nonprofit public interest organizations. Patients often need additional funds for medical treatment and to replace earnings for the benefit of their families. Litigation can provide such funds and can also have a significant impact on patient survival. We will compare survival rates of patients involved in litigation with those undergoing various forms of treatment.

PD02-2

National tort claims by victims of asbestos textile industry in Japan

Kuniko Kobayashi

Attorney at Law, Member of Osaka Lawyers Advocacy for the Victims of Pneumoconiosis & Asbestos disease, Japan

There had been many small asbestos textile factories in the south area of Osaka since 1907 to 2005. They manufactured asbestos yarn, cloth, and other insulation materials that were used for ships, cars, and construction, etc. Many asbestos related disease cases in this area were discovered after 'Kubota shock' in June 2005.

But amazingly, the Japanese government had carried out a detailed investigation on asbestos hazards in this area between 1938~1940. Despite this, they did nothing to control the asbestos dust in those industries until 1970s. It's obvious that this government's negligence caused the spread of the damage.

In May 26, 2006, 8 asbestos victims brought the Japanese Government into court suing for compensatory damages for mental suffering. Eventually, the plaintiffs have increased to 29.

On 19th June 2010, the Court held the government responsible for failing to take measures against asbestos exposure, ordering it to pay a total of 435 Million yen in damages to 26 people. This is the first case that the court ruled the government's failure to regulate asbestos industry.

PD02-3

Brief history of Japanese asbestos litigations

Naoki Ikeda

Kwansei Gakuin University Law School, Japan

Until 2005 when Kubota Corporation publicly disclosed that its 79 former-employees and 2 nearby residents died from mesothelioma (the news is well known as "Kubota Shock"), we can find only less than 20 lawsuits filed by asbestos victims in public record.

After the Kobota shock, asbestos related applications for Workers Compensation dramatically increased. For example, mesothelioma applications jumped up from 149 in 2004 to 1,082 in 2005.

We are gradually having more court judgments or settlements related to asbestos exposure as well. In addition to traditional pattern of asbestos lawsuits filed by factory, mining, or shipyard workers, we have new collective lawsuits filed by construction workers, a tenant employee, nearby residents, family members or other category of victims.

Typical issues and remaining problems in such court cases will be shown and explained so that audience may roughly understand the social structure of asbestos exposures in Japan at the presentation.

PD02-4

Current situation on asbestos compensation in Japan

Sugio Furuya

Ban Asbestos Network Japan (BANJAN), Japan

Although Japan was the first country to impose a national asbestos ban in Asia, amongst the industrialized countries it was one of the last to act. Japan's "invisible asbestos epidemic," became public knowledge in the summer 2005 when news of occupational and environmental asbestos-related deaths amongst workers and the public in disease hotspots was widely broadcast. The scandal which developed over these deaths is widely referred to as the "Kubota Shock" .
Since then the numbers of cases awarded by workers' compensation scheme has been dramatically increasing. Also Japanese government introduced a new relief scheme for non-employees' asbestos victims since March 2006. This new relief scheme is now being under review. Also more victims and their families are bringing their cases into the courts. I will discuss the current situation of asbestos compensation in Japan.
So far six countries have introduced compensation scheme for non-employees' asbestos victims; France, Japan, Belgium, the Netherlands, the UK and Korea. However approaches are different country by country.

PD02-5

Summary of the situation in Europe

Jacobus Burgers

Department of Thoracic Oncology, The Netherlands Cancer Institute, The Netherlands

The actual burden of asbestos-related diseases in the Netherlands seems to remain stable the last few years. The estimations done in 2000 – 2005 predicted a rise in mesothelioma cases from 450/year to 800/year with the maximum to be reached around the year 2020. Whether the top incidence has been reached at 450-500 cases/year, or whether the incidence will follow the predictions needs to be seen at the next iMig conferences.
It remains a fact that asbestos mining has been banned in Western Europe. The Russian Republic however remains a large producer of asbestos. Despite the fact that mining and manufacturing of asbestos has been banned in Western Europe, asbestos industries like Eternit still hold office in most European countries. (see Google maps and look for Eternit)
In Europe, most countries have a financial support system for patients with mesothelioma. The systems differ largely between different countries e.g. some reimburse only patients with a occupational asbestos exposure, other countries reimburse all mesothelioma patients. The term of limitation ranges from 30 years after the last exposure to asbestos to no limitation at all, and the height of the reimbursements differs to a large extent. Therefore it is more attractive in some countries to apply for the national reimbursement, whereas in other countries filing a lawsuit is to be preferred.
All different systems have their drawbacks, but in the era of worldwide recession it is good that governments still hold on to their responsibilities towards asbestos victims. Whether an uniform reimbursement system throughout the whole European Union will be an improvement of the current situation is hard to say. Since such an effort will require large investments, my personal opinion is that this money might better be spend to compensate the victims or support mesothelioma research.

PD02-6

Experience in Egypt and Africa

Rabab Gaafar, Ibrahim Abdelsalam, Ola Khorshid, Saad Elguindy, Inas Elattar, Abeer Ashmawy, Hatem Abdelazim

Department of Medical Oncology, National Cancer Institute, Cairo University, Egypt

Background and purpose: Building-up evidences suggests that SMRP carries a diagnostic and a prognostic value in MPM. Egypt suffers endemic asbestosis and thus this study was conducted to evaluate the value of using SMRP in diagnosing patients with MPM and to correlate this marker with known clinico-pathological prognostic factors.Material and Methods: In the period from January 2006 till March 2008, serum samples were obtained from MPM presenting to the Egyptian National Cancer Institute, Cairo University. Serum samples were provided from patients with breast cancer and from healthy individuals to function as controls.The SMRP was assayed by ELISA using MESOMARK and correlations were made with different clinico-pathological prognostic parametersResults: 83 patients (50 MPM, 33 breast cancer) and 22 healthy individuals were examined in this study. Serum SMRP levels were not different between patients with breast cancer and healthy controls ($p>0.05$). However, there was a significant difference between MPM patients and the other two groups ($p<0.0001$). ROC analysis showed an AUC = 0.765 for differentiating between the controls and MPM with a best statistical cut-off of 7.22nM/L (sensitivity=66%, specificity=70.9%).The mean SMRP concentrations were significantly higher in patients with advanced disease ($p=0.038$), poor performance status ($p=0.017$) and high alkaline phosphatase ($p=0.015$). Mean SMRP concentrations were also higher in males, elderly patients, asbestos-exposed patients, epithelioid subtypes and patients with high platelet and leucocytic counts. However, these differences did not reach statistical significance.Conclusion: This study confirms that SMRP is of considerable sensitivity and specificity in MPM patients. Higher levels are frequently seen in patients with high tumor burden, which could be helpful in monitoring response to therapy.

PD02-7

Moving beyond mesothelioma diagnosis - empowering patients to improve efficacy of treatment

Linda Reinstein

Asbestos Disease Awareness Organization, USA

Although Linda Reinstein is neither a clinician nor scientist, she possesses an unusual breadth and depth of knowledge about asbestos and asbestos-related diseases. Since 2004, Reinstein, a mesothelioma widow and co-founder of the Asbestos Disease Awareness Organization (ADAO), has dedicated herself as a public health advocate focused on education, patient support, and advocacy for increased funding for research and a global asbestos ban. ADAO's collaborative efforts with the White House, Congress, and Agencies have shaped policies to eliminate all asbestos-related disease and unify the global voice of asbestos victims and their families. It is from this unique perspective that Reinstein can address 2010 IMIG conference attendees. Using both scientific data and anecdotal information, Reinstein will focus on three critical areas in need of attention regarding mesothelioma: improving health literacy, influencing social support network participation, and creating a patient/family Legacy Research Network (LRN). The very nature and extreme complexity of a mesothelioma diagnosis coupled with low health literacy can leave even the most educated patient confused and overwhelmed. Reinstein will address specific tactics to improve health literacy including: building pathways from mesothelioma experts to patients, strengthening patient support networks, and improving public awareness about treatment and research. Additionally, studies show that social support networks can play an influential role in cancer treatment success. Reinstein will explore how today's vast socially-focused networks can give critically-ill patients newfound hope, community-oriented support, the ability to more easily uncover and access key resources, and a more direct path to specific treatment options. Finally, Reinstein will address strategies for mesothelioma physicians, researchers, patients, and their families to create a Legacy Research Network (LRN). This network will build mesothelioma patient registries and increase research funding.

RD01

Pathology- Ⅰ : new insights

RD01-1

Prognostic significance of promoter methylation of *p16* in pleural mesothelioma

Kenzo Hiroshima[1], Toshikazu Yusa[2], Hiroshi Niwa[3], Hiroshi Ogawa[3], Michio Fujino[4], Toru Kameya[5], Ichiro Ito[5], Makiko Itami[6], Makoto Suzuki[7], Ichiro Yoshino[8], Toshiaki Kawai[9], Yuji Tada[8], Hideaki Shimada[10], Masatoshi Tagawa[6], Yukio Nakatani[8]

[1]Department of Pathology, Tokyo Women's Medical University Yachiyo Medical Center, Japan, [2]Chiba Rosai Hospital, Chiba, [3]Seirei Mikatahara General Hospital, Shizuoka, [4]Chiba East Hospital, Chiba, [5]Shizuoka Cancer Center, Shizuoka, [6]Chiba Cancer Center, Chiba, [7]Kumamoto University, Kumamoto, [8]Chiba University, Chiba, [9]National Defense Medical College, Saitama, [10]Toho University, Tokyo

Background: We encountered seven cases in whom the survival after extrapleural pneumonectomy (EPP) was surprisingly prolonged. The aim of this study was to evaluate the histological features shared by these cases.
Methods: We have analyzed 22 MPM cases. Sixteen were epithelioid, four were sarcomatoid, and two were biphasic mesotheliomas. Ten cases were treated with EPP and 12 were treated with therapy other than surgery. Patients with MPM were divided into two groups: those who survived for < 5 years in EPP cases or < 2 years in non-EPP cases (short-time survivor, SS), and those who survived for > 5 years in EPP cases or > 2 years in non-EPP cases (long-time survivor, LS). DNA was extracted from paraffin-embedded sections and modified by sodium bisulfite. We have analyzed the methylation status of p16, p14, p15, RASSF1A, and IGFBP-3 with methylation specific PCR.
Results: There were 15 SSs and 7 LSs. There was no visible tumor except one case in LS, and there was focal adhesion between parietal and visceral pleura. The thickness of the pleura was < 5mm in LS. Histologicaly, they showed papillary, tubular, or micorocystic patterns. Myxoid stroma was observed but desmoplastic change was not. Necrosis was not observed. Nuclear atypia was mild in two cases in LSs. Ki-67 labeling indices were 2-10% (mean 5%) in LS and 2-58% (mean 28%) in SS. The methylation frequency for individual genes was 20% for p16, 10% for p14, 53% for p15, 10% for RASSFA1, and 26% for IGFBP-3. Patients with p16 methylation had shorter survival than patients without p16 methylation (p = 0.0243).
Conclusions: There is a group of malignant mesothelioma with much less aggressive behavior than the usual MPM. These patients are diagnosed as mesothelioma at early stage. EPP with adjuvant therapy in these patients would prolong their survival.

RD01-2

Integrated profiling reveals a global correlation between epigenetic and genetic alterations in mesothelioma

Brock Christensen[1,2], E. Andres Houseman[2], Graham Poage[1], John Godleski[3], Raphael Bueno[4], David Sugarbaker[4], John Wiencke[5], Heather Nelson[6], Carmen Marsit[1], Karl Kelsey[1,2]

[1]Department of Pathology and Laboratory Medicine, Brown University, USA, [2]Department of Community Health, Brown University, [3]Department of Environmental Health, Harvard School of Public Health, [4]Division of Thoracic Surgery, Brigham and Women's Hospital, [5]Department of Neurological Surgery, University of California San Francisco, [6]Division of Epidemiology and Community Health, University of Minnesota

Integrated profiling of somatic molecular alterations present in tumors is necessary to further our understanding of the tumorigenic process. We investigated the potential relationships between gene copy number alterations and DNA methylation profiles in a case series of pleural mesotheliomas (n=23). Gene copy number (CN) alterations profiled with 500K SNP arrays and DNA methylation measured at over 750 cancer-related genes with methylation bead-arrays were examined concomitantly. Consistent with previous reports we observed prevalent allele loss at 1p36, 1p21.3, 4q22, 4q31-32, 3p21.3, 6q25, 9p21, and 22q. In addition, we observed prevalent gains at1q23, 5p, 7p, and 8q24. Considering each probed locus, there were no instances of significantly correlated CN alteration and methylation (no loci with $Q<0.05$) and averaging loci over their associated genes revealed only two genes with significantly correlated CN and methylation alterations (each $Q<0.04$). In contrast to the lack of discrete correlations, the overall extent of tumor CN alteration was significantly associated with DNA methylation profile when comparing CN alteration extent among methylation profile classes ($P<0.02$), and there was evidence that this association was partially attributable to prevalent allele loss observed at the maintenance DNA methyltransferase *DNMT1*. We observed a significant trend for increased methylation among tumors with no allele loss at *DNMT1* compared to tumors with allele loss ($P=0.05$). Further, *DNMT1* allele loss was associated with significantly reduced patient survival in a Cox proportional hazards model controlling for age, gender and tumor histology (HR, 5.07; 95% C.I. 1.23-20.9). Taken together, this work indicates a strong association between global genetic and global epigenetic dysregulation in mesothelioma rather than a discrete, local coordination of gene inactivation, and further highlights both the utility and necessity of integrative genomics approaches in cancer biology.

RD01-3

Stromal chronic inflammatory response is a significant predictor of survival in epithelioid diffuse malignant pleural mesothelioma (DMPM)

Kei Suzuki[1], Kyuichi Kadota[2], Camelia Sima[3], Maureen Zakowski[2], Valerie Rusch[1], William Travis[2], Prasad Adusumilli[1]

[1]Memorial Sloan-Kettering Cancer Center, USA, [2]Department of Pathology, Memorial Sloan-Kettering Cancer Center, [3]Department of Epidemiology and Biostatistics, Memorial Sloan-Kettering Cancer Center

Introduction: This study aims to determine whether a semiquantitative assessment of inflammatory response on routine H&E stain can be a predictor of survival in patients with epithelioid DMPM.
Methods:H&E sections of 232 epithelioid DMPM patient specimens from a single institution (1989-2009), with 14 stage I, 54 stage II, 123 stage III, and 34 stage IV, were reviewed. Each tumor was histologically assessed for acute and chronic inflammatory response both within the tumor and the stromal component. Inflammatory response was graded: low (none to mild infiltrate) or high (moderate to severe infiltrate). Log-rank test and Cox proportional hazards regression were used to investigate the association between the degree of inflammation (acute/tumor, acute/stroma, chronic/tumor, and chronic/stroma) and overall survival (OS).
Results: Among epithelioid DMPM, patients with high chronic inflammatory infiltration in stroma (n=82) had improved survival compared to low infiltration (n=152) (median OS=17.4 months vs 15 months, p=0.04). This prognostic stratification remained significant in a cohort of 187 patients who did not receive any neoadjuvant chemotherapy (p=0.023) and also in multivariate analysis (HR=0.69, 95% CI 0.51-0.94, p=0.018) that included laterality, staging, lymphatic invasion, and tumor histologic subtyping. Degree of acute/tumor, acute/stroma, or chronic/tumor inflammatory response did not show significant correlation with OS. In stage III patients, while T (p=0.96) or N (p=0.2) stage were not associated with OS, the predictive ability of chronic stromal infiltration remained significant in both univariate (median OS=17.4 months for high vs 9.4 months for low infiltration, p=0.046), and multivariate analysis (HR=0.65, 95% CI 0.44-0.96, p=0.029).
Conclusions: High degree of chronic inflammatory cell infiltration in the stromal component of epithelioid DMPM is associated with improved overall survival. Our result not only suggests the prognostic value of inflammatory response in epithelioid DMPM, but it also provides rationale for investigation of immunotherapy to benefit epithelioid DMPM patients.

RD01-4

Significance of pleomorphic, solid, and micropapillary subtypes in epithelioid diffuse malignant pleural mesothelioma (DMPM)

Kyuichi Kadota[1,2], Kei Suzuki[1,2], Camelia Sima[3], Maureen Zakowski[1], Valerie Rusch[2], Prasad Adusumilli[2], William Travis[1]

[1]Department of Pathology, Memorial Sloan Kettering Cancer Center, USA, [2]Division of Thoracic Surgery, Memorial Sloan-Kettering Cancer Center, New York, New York, USA, [3]Department of Biostatistics, Memorial Sloan Kettering Cancer Center, New York, New York, USA

Background:
In patients with epithelioid DMPM, T and N stage are current prognostic markers. We sought to investigate whether histologic subtyping can predict aggressive behavior among epithelioid DMPM patients.
Methods:
H&E slides of 232 epithelioid DMPM patients (14 stage I, 54 stage II, 123 stage III, 34 stage IV) from a single institution (1989 to 2009) were reviewed. We classified the tumors into 5 subtypes: pleomorphic (pleomorphism >10% of tumor) and predominantly trabecular, tubulopapillary, micropapillary, and solid subtypes. Lymphatic/vascular invasion was evaluated. Log-rank tests and Cox proportional hazard models were used to analyze the association between histological variables and overall survival.
Results:
Overall median survival of epithelioid DMPM was 1.4 years. Patients with pleomorphic subtype (n=34, 14.7%) had the worst median survival (0.67 years), followed by solid (n=89, 38.4%, 1.14 years), micropapillary (n=20, 8.6%, 1.32 years), tubulopapillary (n=51, 22.0%, 1.49 years), and trabecular (n=38, 16.4%, 2.08 years). On univariate analyses, pleomorphic subtype ($p<0.001$), lymphatic invasion ($p<0.001$), and vascular invasion ($p=0.005$) were significantly associated with poor survival, both in the entire cohort and stage III patients. Pleomorphic subtype showed significant association with lymphatic and vascular invasion ($p=0.003$ and $p<0.001$, respectively). Micropapillary subtype showed significant association with lymph node metastasis and lymphatic invasion ($p=0.022$ and $p<0.001$, respectively). Moreover, lymphatic invasion correlated with lymph node metastasis ($p<0.001$). In a multivariate analysis, pleomorphic subtype (HR=2.05, 95% CI 1.35-3.11, $p=0.0008$) and lymphatic invasion (HR=1.40, 95% CI 1.02-1.91, $p=0.033$) were independent predictors of poor survival. In stage III patients who underwent R1 resection, pleomorphic and solid subtype was a predictor of higher recurrence ($p=0.005$).
Conclusion:
Pleomorphic and solid subtypes are predictors of aggressive behavior in epithelioid DMPM. In patients with epithelioid DMPM, our data supports classification by histologic subtypes to better stratify survival.

RD01-5

Overexpression of CD26/DPPIV in mesothelioma tissue and mesothelioma cell lines

Vishwa Jeet Amatya[1], Yukio Takeshima[1], Taketo Yamada[2], Kei Kushitani[1], Chikao Morimoto[3], Kouki Inai[1]

[1]Department of Pathology, Hiroshima University Graduate School of Biomedical Sciences, JAPAN, [2]Department of Pathology, Keio University, Tokyo, Japan, [3]Division of Clinical Immunology, Advanced Clinical Research Center, Institute of Medical Sciences, University of Tokyo, Tokyo, Japan

Mesothelioma, a highly aggressive cancer with poor prognosis and refractory to currently available therapies show the increasing trends of its incidence in Japan and other developing countries. Although surgery is a gold standard for patients with early mesothelioma, most patients with advanced disease are not suitable for surgical resection and have option of palliative chemotherapy alone. One of the new treatment strategies for mesothelioma, the humanized anti-CD26 monoclonal antibody therapy is under development. CD26, a 110-kDa-transmembrane glycoprotein with known dipeptidyl peptidase IV activity, plays a role in tumor development and its expression was reported in various human malignancies. This study is to determine the preliminary selection criteria by immunohistochemistry for humanized monoclonal anti-CD26 antibody therapy.
Eighty-one epithelioid (49 differentiated and 32 less differentiated), 34 sarcomatoid, 19 biphasic mesothelioma and 8 mesothelioma cell lines were immunohistochemically examined using 8 different commercially available anti-CD26 antibodies for membranous and cytoplasmic expression. The cytoplasmic expression of CD26 was observed in all histological types of mesothelioma, while the membranous expression of CD26 was found in 88% of differentiated and 69% of less differentiated epithelioid mesothelioma, and none of sarcomatoid mesothelioma with anti-CD26 antibodies with rabbit polyclonal anti-DPP4 antibody and similar results were also obtained with goat polyclonal anti-DPP4/CD26 antibody. These antibodies absorbed with soluble human CD26 proteins do not show CD26 expression in mesothelioma tissue, suggesting these two antibodies localizes true CD26 protein. Seven mesothelioma cell lines, including sarcomatoid types, also showed membranous expression of CD26 in cellblock preparation. After CD26 vector transfection to CD26-negative MSTO-211H cells showed membranous expression of CD26 by flow cytometry, but not in tumor developed in NOD/SCID mice with inoculation of CD26 vector transfected MSTO-211H cells.
We found either rabbit or goat polyclonal antibodies are suitable for immunohistochemical evaluation of membranous expression of CD26 in mesothelioma.

RD01-6

High prevalence of atypical mesothelial proliferation in extrapleural pneumonectomy specimens; further evidence of a potential precursor lesion to invasive mesothelioma

Leona Doyle[1], Joseph Corson[1], David Sugarbaker[2], Lucian Chirieac[1]

[1]Department of Pathology, Brigham and Women's Hospital, USA, [2]Department of Thoracic Surgery, Brigham and Women's Hospital, Boston, MA, USA

Background: Atypical mesothelial proliferation (AMP) is thought to represent a potential precursor lesion to invasive pleural mesothelioma. To our knowledge there is no published literature describing the clinicopathologic characteristics of AMP. The aim of this study was to evaluate the prevalence of AMP in extrapleural pneumonectomy (EPP) specimens for invasive mesothelioma and to correlate AMP with clinicopathologic features.Design: We studied 46 consecutive EPPs with available surgical material (mean 22 slides per case, range 12-30), performed for invasive mesothelioma (IM) over 16 months. Each case was assessed independently by two pathologists for AMP according to currently established morphologic criteria. We evaluated architectural and cytologic features, the prevalence and extent of AMP and correlated clinicopathologic features between mesotheliomas with and without AMP. Results: All 46 EPPs (40M/6F, mean age 62.9 years; range 38-79) showed invasive mesothelioma (n=30 epithelioid, n=15 mixed and n=1 sarcomatoid). AMP was identified in 10 (22%) EPP specimens, in a mean of 3.5 slides (range 1-6). Nine cases (90%) were associated with epithelioid mesothelioma and 1 case (10%) with mixed mesothelioma. Common architectural patterns of AMP were a single cell layer proliferation (n=8), stratified proliferations (n=5) and papillary proliferations (n=5). Six cases (60%) had mixed AMP growth patterns. In AMP with a single cell layer proliferation, prominent nucleoli were present in at least 50% of lesional cells. AMP was present in EPPs weighing less (median 747g vs. 1110g, $p=0.03$) and in older patients (68 vs. 63 years, $p=0.02$).Conclusions: In our study the prevalence of AMP (22%) in EPPs is higher than anticipated, is more frequent in older patients and in specimens with lower weights. Further studies are needed to investigate the clinical significance of AMP and the role of AMP in the pathogenesis of mesothelioma.

RD01-7

Napsin A is helpful in separating adenocarcinoma of the lung from malignant mesothelioma

Andras Khoor[1], Pamela Kreinest[1], Philip Cagle[2]

[1]Department of Laboratory Medicine and Pathology, Mayo Clinic, USA, [2]Department of Pathology, The Methodist Hospital, Houston, Texas, USA

Background: Differentiating malignant mesothelioma from adenocarcinoma, particularly adenocarcinoma of lung origin, remains a difficult histopathologic problem. Consequently, it is important to identify markers that may facilitate this distinction. Napsin A is an aspartic proteinase involved in the maturation of surfactant protein B. It has been detected in the cytoplasm of type 2 pneumocytes and alveolar macrophages and is considered as a prospective marker for adenocarcinoma of the lung.
Design: The objective of this study was to investigate the expression of napsin A in adenocarcinoma of the lung versus malignant mesothelioma. Immunohistochemistry was performed on sections of tissue microarrays (114 cases of pulmonary adenocarcinoma and 46 cases of malignant mesothelioma) using a mouse monoclonal napsin A antibody (Novocastra, Newcastle, UK) and Dako´s EnVision™ FLEX+ detection system (Dako, Carpinteria, CA).
Results: Granular cytoplasmic immunoreactivity was detected in 92 pulmonary adenocarcinomas. All malignant mesotheliomas were negative.
Conclusions: These data indicate that immunohistochemistry for napsin A is a useful aid in separating adenocarcinoma of the lung from malignant mesothelioma with a sensitivity of 80% and a specificity of 100%.

RD02

Surgery-I

RD02-1

A standardized technique of radical pleurectomy for mesothelioma that achieves a macroscopic complete resection, regardless of tumor bulk

Joseph Friedberg, Keith Cengel, Melissa Culligan, James Stevenson, Stephen Hahn, Rosemary Mick, Daniel Sterman

University of Pennsylvania, USA

Background: Surgical debulking remains the cornerstone for aggressive multimodal treatment for mesothelioma. Achieving macroscopic complete resection (MCR) is the goal of all surgical debulking procedures. Two techniques, extrapleural pneumonectomy (EPP) and radical pleurectomy (RP) are two procedures employed for debulking. EPP, a more accepted technique to accomplish MCR, has evolved into a well-described and standardized procedure. Despite some recent enthusiasm for lung-sparing procedures to achieve MCR, there remains confusion surrounding technique, and even terminology, for RP.Purpose: The purpose of this work was to develop a standardized technique for RP that would predictably result in MCR, even in the setting of heavy bulk disease.Methods: 24 patients (ages 58 to 81, mean 66, and epithelial/nonepithelial subtypes 17/7) underwent RP as part of a multimodal treatment plan. The pleural space was divided into the following regions: lung surface, lung fissures, lung hilum, pericardium, chest wall, mediastinum, diaphragm and phrenic nerve. A systematic technique for debulking each area to no visible or palpable disease was developed. The primary goal was MCR and the secondary goal to preserve as much normal tissue as possible. These procedures were combined with an intraoperative adjuvant treatment (photodynamic therapy) and postoperative systemic therapy.Results: MCR was achieved in every case. The lung was spared in 100% of the cases. Required prosthetic reconstructions included, two diaphragm alone, three pericardium alone, one both. There was one postoperative mortality (stroke) and median OS not reached at 27.4 months.Conclusion: By reducing RP to a systematic technical approach, dictated by geographic region, it was possible to achieve MCR in every case even in the setting of bulk disease typically thought to preclude RP. This technique is readily described and performed and could serve as a step toward standardizing RP in much the same way the technique for EPP has become standardized.

RD02-2

Surgical approach to MPM: The Brigham and Women's Hospital experience

David Sugarbaker

Division of Theracic Surgery, Department of Surgery, Brigham and Women's Hospital, USA

Multimodality therapy based on surgical cytoreduction has yielded increased survival relative to single modality treatments or palliation. The goal of surgery is to provide a macroscopic complete resection (MCR), defined as the complete removal of all grossly visible tumor, while adjunctive therapies facilitate the cytoreductive process by eliminating micrometastatic disease systemically or at local surgical margins. Two operations have evolved: extrapleural pneumonectomy (EPP) and pleurectomy/decortication (P/D). The former is indicated for patients with advanced locally invasive disease; the latter for patients with more superficial spread of tumor that spares the lung, fissures, and mediastinal structures. The choice of therapy is influenced by the knowledge that the therapeutic impact of any modality is offset by the risk and injury imparted by the treatment. For appropriately selected patients, cytoreductive surgery followed by adjuvant therapeutic modalities achieves maximal collective cytoreduction. Of the two operations, EPP provides a more complete cytoreduction: MCR can be achieved in most cases. It forms the basis of a trimodality treatment regimen with adjuvant chemotherapy and radiotherapy and can be performed in appropriately selected patients with acceptable mortality (3.4% in our institution) and manageable morbidity. By contrast, P/D is associated with lower morbidity and mortality (1.8%); however, MCR is less frequently achieved, although with favorable tumor distribution, meticulous pleurectomy can result in MCR, and in less favorable cases combining P/D with adjunctive intraoperative therapy (i.e., laser, cautery, intracavitary chemotherapy) may also permit MCR. The influence of surgery goes beyond diagnosis and resection. Much of what we know about the biology of mesothelioma has come from studying the surgical pathophysiology, including delineation of histopathologic subtypes, disease stage stratification with survival, the propensity for local (in contrast to systemic) recurrence, as well as the prognostic effect of epithelial versus nonepithelial cell type, extrapleural nodal involvement, tumor bulk, and surgical margins.

RD02-3

Long-term outcome after extra-pleural pneumonectomy for patients with malignant pleural mesothelioma: A 10-year single center experience

Masaki Anraku[1], B. C. John Cho[2], Ronald Feld[3], Ming-Sound Tsao[4], Natasha Leighl[3], Michael R. Johnston[5], Shaf Keshavjee[1], Marc de Perrot[1]

[1]Division of Thoracic Surgery, Toronto General Hospital, University of Toronto, Canada, [2]Department of Radiation Oncology, Princess Margaret Hospital, University of Toronto, [3]Department of Medical Oncology, Princess Margaret Hospital, University of Toronto, [4]Division of Applied Molecular Oncology, Ontario Cancer Institute, University of Toronto, [5]Queen Elizabeth II Health Sciences Centre, Dalhousie University

Introduction: We report our 10-year experience of malignant pleural mesothelioma (MPM) treated with extra-pleural pneumonectomy (EPP), including a trimodality approach: induction chemotherapy (CTx), EPP, and adjuvant hemithoracic radiotherapy (RT). **Materials and Methods:** Retrospective analysis of 70 MPM patients undergoing EPP between 2001 and 2010 was conducted. Survival was estimated by Kaplan-Meier method. The log-rank and Wilcoxon comparison tests were used to compare groups. Multivariate analysis was performed to determine prognostic factors. All patients were followed prospectively with clinical and radiological evaluation by CT scan. **Results:** Of the 55 men and 15 women, the median age was 60 years (range: 26-78). Induction CTx was used in 59 patients (84%) and post-operative RT in 53 patients (76%). Tri-modality approach, as per protocol, was completed in 40 patients (59%). An additional 4 patients underwent induction RT over the past 15 months as part of our new protocol. A total of 3 patients (4%) died postoperatively. Morbidity rate after EPP was 37% (26/70), and 12 required re-operation. Indications for reoperation included: empyema/bronchopleural fistula (5), diaphragmatic disruption (3), and others (4). The overall 1-, 3-, and 5-year survival for all EPP (n=70) were 82, 31, and 16%. Tumor recurrence was seen in 36 patients (51%) clinically or radiographically. The sites of recurrence included: locoregional defined as chest wall, pericardium or mediastinum (15), abdomen (17), contralateral chest (15), brain (1), and bone (1). In multivariate analysis, female gender (p=0.0005), epithelioid histology (p=0.005), and no mediastinal nodal disease (p=0.005) remained significant favorable prognostic factors. **Conclusion:** Perioperative mortality rate of EPP was acceptable. The common sites of distant failure after EPP were abdomen and contralateral chest. A possible explanation is intraoperative tumor seeding. Therefore, preventing potential tumor seeding during surgery may help reduce the risk of distant failure and warrants further investigation.

RD02-4

Lung volume improvement in malignant pleural mesothelioma patients undergoing pleurectomy/ decortication

William Sensakovic[1], Samuel G. Armato III[1], Adam Starkey[1], Hedy L. Kindler[2], Wickii T. Vigneswaran[3]

[1]Committee on Medical Physics, The University of Chicago, USA, [2]Section of Hematology/Oncology, The University of Chicago, Chicago, IL, USA, [3] Dept. of Surgery, The University of Chicago, Chicago, IL, USA

Background: Malignant pleural mesothelioma (MPM) is a neoplasm that grows circumferentially along the pleura. The tumor and concurrent pleural effusion may reduce lung function by restricting or preventing lung expansion (i.e., trapped/encased lung or restrictive pleurisy). Pleurectomy/decortication (P/D) is a surgical tumor debulking procedure that attempts to relieve mechanical restriction of the lung by removing the pleura while leaving the lung intact. The purpose of this study was to provide objective evidence that P/D allows trapped lung to re-expand and to quantify the re-expansion based on computed tomography (CT) scans.**Methods:** A database of 12 patients demonstrating unilateral MPM was collected. Patients demonstrated substantially decreased pre-surgical ipsilateral lung volume with a median ipsilateral-to-contralateral lung volume of 0.43 (range: 0.08-0.75). Each patient underwent a pre-surgical CT scan, surgical debulking by P/D, and a post-surgical CT scan (at one month). The lung volume was measured in each scan using an automated algorithm and compared for each patient across time. **Results:** An increase in the ipsilateral post-surgical lung volume was observed for 10 of 12 patients (83%). The median ipsilateral lung volume increase was 44% relative to the pre-surgical ipsilateral lung volume and 21% relative to contralateral lung volume ($p=0.027$).**Conclusions:** Debulking of MPM with P/D substantially increased the ipsilateral lung volume relative to both the pre-surgical, ipsilateral volume and the contralateral lung volume. The substantial and significant increase one month after surgery provides strong evidence justifying the application of P/D as a palliative treatment option to free trapped lung.

RD02-5

Extrapleural pneumonectomy and pleurectomy/ decortication in patients with malignant pleural mesothelioma: Is diaphragm resection really needed?

Enrico Ruffini[1], Pier Luigi Filosso[1], Enrica Paze[2], Maria Cristina Bruna[1], Claudio Mossetti[1], Riccardo Carlo Cristofori[1], Umberto Ricardi[3], Dario Mirabelli[4], Alberto Oliaro[1]

[1]Thoracic Surgery, University of Torino, Italy, [2]Medical Oncology, S. Giovanni Battista Hospital, Torino, Italy, [3]Radiation Oncology, University of Torino, Torino, Italy, [4]Epidemiology, S. Giovanni Battista Hospital, Torino, Italy

OBJECTIVE: Extrapleural pneumonectomy (EPP) and pleurectomy/ decortication (P/D) have been proposed in selected patients with Malignant Pleural Mesothelioma (MPM). The techniques involve resection of parietal pleura along with the pericardium and the diaphragm. Concern has been raised about a possible peritoneal spread after diaphragm resection which may adversely affect the outcome. We retrospectively reviewed our series of EPP and P/D to evaluate whether diaphragm resection had an effect on the patient outcome and patterns of failure. METHODS: From 10/97 to 4/10 110 patients received EPP(73 patients) or P/D(37 patients) for MPM. The diaphragm was either resected and replaced with prosthetic material (Group1, 81 patients, 69EPP and 12P/D) or not resected leaving a residual (Group2, 29 patients, 4EPP and 25P/D), according to the surgeon preference. Postoperative chemotherapy and radiotherapy were used in most cases. We compared overall survival, median survival and patterns of failure in the 2 groups of patients. RESULTS: IMIG stage distribution and histology were similar among the 2 groups. Information about patterns of failure were available in 84 patients. Patterns of failure after surgery were local relapse (37 pts, 24EPP and 13P/D), distant relapse (19 pts, 15EPP and 4P/D) and abdominal relapse (28 pts, 20EPP and 8P/D). Local and abdominal relapses occurred in 26 and 25 patients in Group1 and in 11 and 3 patients in Group2 ($p=0.02$ and $p=0.14$). Two-year survival in group1 and 2 were 37% and 24% ($p=0.84$). Median survivals were 1.57 and 1.39 (years) respectively. In a multivariate regression analysis diaphragmatic resection was not an independent prognostic factor (HR 0.91, 95%CI 0.45-1.84, $p=0.82$).CONCLUSIONS: In our experience, preservation of the diaphragm after EPP and P/D had no adverse effect on the outcome of the patients. It was associated with a significant increased rate of local recurrence and a non-significant decreased rate of abdominal recurrence

RD02-6

MPM: Should we stop operating on presumed (clinical) stage III patients?

Hans Hoffmann

Dept. of Surgery, Thoraxklinik, University of Heidelberg, Germany

We retrospectively analyzed all available information on patients with biopsy-proven MPM who were admitted for treatment to the Thoraxklinik Heidelberg between January 2001 and December 2009. Treatment selection was based primarily on the tumor stage, the patient' s overall medical condition, and requirements of clinical trials conducted during this time period, but - in the majority of cases was highly individualized.

For the purpose of this analyses the patients were grouped into three distinct treatment groups:

A) BSC: Pts. who received no specific tumor-directed therapy, but were given all available palliative measures including VATS-pleurodesis/ decortication and/or palliative radiotherapy
B) CHT: Pts. who received Chemotherapy as their main treatment (up to 5 lines) with additional palliative VATS-pleurodesis/decortication and/ or palliative radiotherapy
C) EPP: Pts. who underwent EPP as their main treatment (either EPP alone, or CHT + EPP, or EPP + radiotherapy, or trimodal therapy)

A total of 482 consecutive patients with MPM were analyzed (BSC: n=116, CHT: n=266; EPP: n=100). Of the EPP group 6 had EPP alone; 4 had EPP+RT, 22 had CHT+EPP, and 68 underwent complete trimodal therapy.

Clinical Characteristics and survival:

	BSC (n=116)	CHT (n=266)	EPP (n=100)
Age (mean, range)	72 y (49-87)	65 y (28-86)	58 y (36-69)
ECOG 0-1	90 (78%)	248 (93%)	99 (99%)
ECOG >1	26 (22%)	18 (7%)	1 (1%)
Epitheloid Hist.	84 (72%)	195 (73%)	81 (81%)
Clinical stage I+II	75 (65%)	145 (55%)	69 (69%)
Clinical stage III	25 (22%)	76 (29%)	27 (27%)
Clinical stage IV	16 (14%)	45 (17%)	4 (4%)
Median survival stage I+II	8.2 m	15.2 m	24.4 m
Median survival stage III	10.9 m	13.5 m	13.9 m

Our results reflect the typical clinical characteristics and outcomes with acceptable survival for patients with stage I/II disease undergoing EPP but poor survival for resected patients with stage III tumors. In stage III there was no survival benefit of EPP relative to nonsurgical therapy (Chemotherapy).

WS01

Cytopathology -How reliable is it?-

WS01-1 **Keynote Speaker**

Cytopathology-How reliable is it ?

Françoise Galateau-Sallé

MESOPATH- Center-MESONAT Registry-INSERM ERB, France

As pleural effusion is usually the first clinical sign of malignant mesothelioma, cytology is the first diagnostic examination to be carried out. A definitive diagnosis based on cytologic samples is still contoversial , due to the fact that when mesothelioma cells are sufficiently well differentiated to recognize their mesothelial origin, they are difficult to separate from reactive mesothelium. The International Mesothelioma Interest Group panel in the last consensus statement published in august 2009, has recommended that a cytologic suspicion of malignant mesothelioma must be followed by tissue confirmation and be supported by both clinical and radiologic data. Additionnally the ERS TASK FORCE guidelines published in march 2010, are the following : [1] 1) It is not recommended to make a diagnosis of mesothelioma based on cytology alone because of the high risk of diagnostic error (grade 1B). 2) It is recommended that a cytological suspicion of mesothelioma is followed by tissue confirmation (grade 1B). 3)Disease recurrence and metastases can be ascertained on cytology alone. This recommendation is in agreement with that proposed by the International Mesothelioma Panel (grade 1B) [2] . Most authors agree that pleural fluid cytology can provide confirmation of a malignant pleural effusion but has a diagnostic yield of only 65%. For mesothelioma cases the sensitivity is lower of ~30%. However, additionnal ancillary techniques could complement standard cytology, when sufficient numbers of cells are present in cell block to permit immunohistochemical, ultrastructural studies or a combination of these tests. Groups of immunohistochemical antibodies on paraffin embedded cell blocks, could lead to a diagnosis in approximately 80% o patients with malignant mesothelioma. Among the new markers GLUT-1 appears promising together with EMA, desmin, p53 in distinguishing reactive mesothelial cells from malignant mesothelioma in cytologic effusions. In 2010, Savic S, reported that Fluorescence in situ hybridizaton is a sensitive and highly specific method for the definitive diagnosis of malignant mesothelioma. They also showed that in the subset of FISH-negative malignant mesothelioma, tumor suppressor genes on the chromosomal region9p21 are often inactivated by promoter methylation. Both genetic and epigenetic must be in the future useful and reliable tools in the identification of malignancies.

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WS01-2

How to make a definitive diagnosis of mesothelioma by effusion cytology?

Shotaro Maeda[1,2], Hosone Masaru[2], Katayama Hironori[2], Naito Zenya[3]

[1]Department of Pathology, Tama-Nagayama Hospital, Nippon Medical School, Japan, [2]Department of Pathology, Tama-Nagayama Hospital, Nippon Medical School, [3]Department of Pathology, Nippon Medical School

Objective: Malignant pleural mesothelioma(MPM) has poor prognosis in general. However, long-term survival was enabled by an extrapleural pneumonectomy of MPM at the early stage. Early detection of MPM by cytology is very important for the radical cure of MPM. The purpose of this study is to clarify whether MPM can be diagnosed definitively by effusion cytology using the cell transfer method and/or cell block method for immunochemical staining.
Study Design: This study included 5 patients with MPM diagnosed only by effusion cytology in the past 4 years in Tama-Nagayama hospital. These patients were considered as having mesothelioma by pleural effusion cytology. Furthermore, immunochemical staining was carried out for a definitive diagnosis using the cell transfer method and/or the cell block method. Tumor cells were immunostained using multiple antibodies (calretinin, CK5/6, D2-40, thrombomodulin, mesothelin, CEA, MOC31, EMA, E-cadherin, and p53 protein).
Results： Calretinin was positive in 5/5 patients (100%), CK5/6 in 5/5 (100%), D2-40 in 5/5 (100%), mesothelin in 5/5 (100%), thrombomodulin in 4/5 (80%), CEA in 0/5 (0%), MOC31 in 0/0(0%), EMA in 5/5 (100%), p53 protein in 5/5 (100%) and E-cadherin in 4/5 (80%), As a result, the five patients were definitively diagnosed as having MPM. EMA, p53 protein and E-cadherin were useful for discrimination of MPM and reactive mesothelial cells. Histological examination was finally done, and five patients were diagnosed as mesothelioma.
Conclusions: Good immunochemical staining results were obtained, enabling the definitive diagnosis of mesothelioma using the cell transfer method and/or cell block method with pleural effusion. So, we would like to emphasize that the definitive diagnosis of mesothelioma is possible only by pleural effusion cytology.

WS01-3

Diagnostic significance of *p16^{INK4A}* loss in molecular cytology of early malignant pleural mesothelioma

Tohru Tsujimura[1], Ayuko Sato[1], Ikuko Torii[1], Tatsuki R. Kataoka[1], Takashi Nishigami,[1], Yoshie Yoshikawa[2], Tomoko Hashimoto-Tamaoki[2], Seiki Hasegawa[3], Takashi Nakano[4]

[1]Department of Pathology, Hyogo College of Medicine, Japan, [2]Department of Genetics, Hyogo College of Medicine, [3]Department of Thoracic Surgery, Hyogo College of Medicine, [4]Division of Respiratory Medicine, Department of Internal Medicine, Hyogo College of Medicine

Aims: Pleural effusion is frequently observed in patients with malignant pleural mesothelioma (MPM), and cytological analysis of pleural effusion provides valuable information for diagnosis of MPM. Differential diagnosis of early MPM and reactive mesothelium (RM) is very important to determine therapeutic strategy, but cytological discrimination between early MPM and RM is often difficult. To resolve this issue, it is necessary to find a molecular marker for cytological diagnosis of early MPM. The p16^{INK4A} gene has been reported to be frequently deleted in various malignant tumors including MPM in advanced stages. In this study, we examined whether analysis of p16^{INK4A} gene abnormalities is useful to discriminate between early MPM and RM in pleural effusion cytology.
Methods: Cases of advanced MPM, early MPM, and RM were selected based on histopathological examination of pleural tissues obtained by video-assisted thoracoscopic surgery. Papanicolaou smear specimens of pleural effusion were prepared from the patients, and mesothelial lineage cells (i.e., mesothelioma cells or mesothelial cells) and inflammatory cells were microdissected. Genomic DNA of mesothelial lineage cells was analyzed for the p16^{INK4A} gene by real-time PCR using genomic DNA of inflammatory cells as a control.**Results:** The p16^{INK4A} gene was deleted in the advanced MPM cases, whereas it was retained in the RM cases. The p16^{INK4A} gene was also deleted in the early MPM cases examined in this study.**Conclusions:** The deletion of p16^{INK4A} gene is a molecular marker for diagnosis of early MPM as well as advanced MPM. The gene diagnosis involving the p16^{INK4A} locus in plural effusion cytology may be useful for the discrimination of early MPM and RM.

WS01-4

Cytopathology of desmoplastic malignant mesothelioma (DMM)

Sadayuki Hiroi[1], Susumu Tominaga[1], Sho Ogata[1], Kenshi Urata[2], Akira Hebisawa[2], Toshiaki Kawai[1]

[1]Department of pathology and Laboratory Medicine, National Defense Medical College, Japan, [2]Division of Clinical Laboratory, National Hospital Organization Tokyo Hospital

Background : DMM is rarely associated with a pleural effusion, and if there is an effusion it exfoliates very few cells into the fluid. We had 8 cases of DMM with cytologic samples, and 3 of these cases were cytologically detected as malignant. *Design* : To inform the accurate recognition of DMM, we examined clinicopathologic, cytopathologic, and immunohistochemical methods in 3 cases of DMM (including 1 autopsy case). *Results* : All 3 were males with a history of asbestos exposure (57-78 years). They presented with pleural effusion, cough, and dyspnea, and had abnormal findings on chest X-ray and CT scan. Cytological findings in pleural fluid were small numbers of large atypical spindle-to-polyhedral cells in small clusters. These cells had round nuclei and an abundant and spindle-shaped cytoplasm, and nuclear grooves and prominent nucleoli were seen. An imprint specimen showed almost all the same features as pleural effusion, atypical spindle cells with high nuclear/cytoplasmic ratios being detected at autopsy. The patients died at 27 and 22 months after operation, or 8 months after diagnosis. Microscopically, DMM was characterized by the presence, in at least 50% of the tumor, of dense collagenized tissue separated by atypical tumor cells arranged in a storiform or "patternless " pattern. Tumor cells were positive for calretinin, AE1/AE3, D2-40, and WT-1. *Conclusion* : Effusion in DMM is commonly positive for only a few malignant cells. DMM usually shows cytologic atypia, but with a poor cellular component. So, it may not be difficult to diagnose "malignancy ", but in cases with a poor cellular component, immunohistochemistry may be useful for diagnosis.

WS01-5

Correlation of pleural fluid cytological yield and visceral pleural invasion in patients with malignant pleural mesothelioma

Valentina Pinelli[1], Lama Sakr[2], P Roll[3], Laurent Greillier[4], Gian Pietro Marchetti[1], Herve Dutau[4], Andree Robaglia[3], P Cau[3], Gian Franco Tassi[1], Philippe Astoul[4]

[1]Pulmonary Division of Spedali Civili Brescia, Italy, [2]Jewish General Hospital, Montreal,Canada, [3]University La Timone, Marseille, France, [4]University La Mediterranee, Marseille, France

Background: Malignant pleural mesothelioma (MPM) is an aggressive malignancy arising from mesothelial cells lining the pleura. Most commonly, it presents as a unilateral pleural effusion. MPM usually develops on the parietal pleural surface and later spreads to the visceral pleura. Visceral pleural involvement entails a more advanced disease stage, and is therefore an important prognostic factor. Pleural fluid (PF) cytology is often the first diagnostic test, but it is positive in less than 30% of cases. No data are available with regard to the association of PF cytological yield with visceral pleural involvement. Aim: To assess whether pleural fluid cytological yield is related to the extent and pattern of visceral pleural invasion, as assessed on thoracoscopy. Methods: Medical records of all patients who underwent thoracoscopy for suspicion of MPE were included if they initially underwent a diagnostic thoracentesis prior to thoracoscopy, if visceral pleural appearance during thoracoscopy was clearly documented, and MPM confirmed on pleural tissue biopsy. Results: 75 patients were included. Forty-five patients had a positive PF cytology on thoracentesis (Group A), while 30 had a negative PF cytology (Group B). Thoracoscopy showed parietal pleural invasion in all subjects. Interestingly, 82% of patients with positive PF cytology on thoracentesis had visceral pleural involvement, while only 30% of those with negative PF cytology had visceral pleural invasion (p lower than 0.001). The pattern of visceral pleural invasion consisted of pleural masses, nodules or pleural thickening. No statistically significant difference was found with regard to the pattern of visceral pleural involvement among both groups. Conclusion:In MPM, PF cytological yield was significantly higher in patients with visceral pleural invasion on thoracoscopy. Positive PF cytology might therefore be associated with a more advanced disease and represent a poor prognostic indicator.

WS01-6

Differential diagnosis of malignant mesothelioma and reactive mesothelial cell proliferation for cytology and biopsy

Noriko Kimura[1], Nozomu Iwashiro[2], Osamu Wakabayashi[3], Fumiaki Yoshida[3], Eisei Jinushi[3], Yoshikazu Araya[3], Hiromitsu Domen[2], Masanori Ishizaka[2]

[1]Department of Clinical Research Pathology Section, National Hospital Organization Hakodate Hospital, Japan, [2]Department of Surgery, National Hospital Organization Hakodate Hospital, Hakodate, Japan, [3]Department of Respiratory Disease, National Hospital Organization Hakodate Hospital, Hakodate, Japan

To improve patients ´ prognosis with malignant mesothelioma (MM), it is necessary to detect patients in early stages. Both cytology and biopsy are useful and necessary tools to detect MM. Based on our case review and cytological features described in previous articles, we developed a scoring system for malignant mesothelioma (SSMM) of effusion cytology to distinguish MM cells from reactive mesothelial cells (RMC). The SSMM is based on characteristic features of mesothelial and malignant cells. The total achievable score is ten points: one point each is given for variety of cell size, cyanophilic cytoplasm with villosity/windows/bleb, sheet–like arrangement, mirror–ball–like cell clusters, nuclear atypia, and cannibalism, respectively. Further two points each are ascribed for acidophilic large nucleoli and multinucleated cells with more than eight nuclei. The total score for each of all 22 mesotheliomas was more than 5 points. On the other hand, all 20 RMC and the 50 metastatic carcinoma cases scored less than 3 points. SSMM is useful for the differential diagnosis of MM. Tissue specimens obtained by video–assisted thoracic surgery (VATS), and needle biopsy were compared to RMC those were mostly associated with emphysema. The characteristic features of epithelioid MM of biopsy specimens in early stages were trabecular or glandular arrangement of smaller tumor cells with plumped hyperchromatic nuclei, high N/C ratio, vertical proliferation from surface of the pleura, and scanty vascularity. On the contrary, RMC showed dispersed, non-organoid distribution of mesothelial cells having nuclei with less chromatin and several irregular–shaped nucleoli, and high vascularity. Ancillary use of immunocytochemistry, positive for CAM5.2, podoplanin (D2–40), calretinin, HBME–1, and negative for TTF–1 and CEA supported the diagnostic accuracy of MM. Immunohistochemistry of Ki67 and p53 was not necessarily useful to distinguish RMC and epithelioid MM except sarcomatoid MM. In conclusion, SSMM and pointed observation for biopsy are useful for accurate diagnosis.

WS02

Molecular oncogenesis

WS02-1 **Keynote Speaker**

Signalling transduction and survival pathways in mesothelial carcinogenesis

Luciano Mutti

Department of Medicine, Local Health Unit 11, Vercelli, Italy

Malignant Mesothelioma (MMe) is an aggressive cancer arising from mesothelial cells of the pleura, peritoneum, pericardium and tunica vaginalis testis.
The incidence of pleural MMe is increasing worldwide due to widespread asbestos exposure.
Other factor such as radiation exposure and exposure to other fibers with similar physical properties to asbestos, such as zeolite and erionite, simian-virus-40, genetic predisposition may be causative agents of MMe
Over the last few years pre-clinical research allowed to achieve a better understanding of the mechanisms by which these agents lead to MMe development and progression.

a) Genetic abnormalities (including methylation)
b) Abnormalities in apoptosis
c) Dysregulation of pathways involving growth factors and growth factor receptors
d) Alteration in proteasome complex machinery
e) Cell metabolism derangement
f) Hormonal influences
g) Tumour stem cells generation
h) miRNA gene and expression

All these aspects will be discussed with particular focus on their translational implications.

WS02-2 **Keynote Speaker**

Inactivation of LATS2 indicates frequent dysregulation of the Merlin-Hippo signaling pathway in malignant mesothelioma cells

Yoshitaka Sekido[1,2], Tetsuya Mizuno[1,3], Tetsuo Taniguchi[1,3], Makiko Fujii[1], Futoshi Ishiguro[1,3], Takayuki Fukui[4], Shinya Akatsuka[5], Yoshitsugu Horio[6], Toyoaki Hida[6], Yutaka Kondo[1], Shinya Toyokuni[5], Hirotaka Osada[1,2], Hideki Murakami[1]

[1]Division of Molecular Oncology, Aichi Cancer Center Research Institute, Nagoya, Japan, [2]Department of Cancer Genetics, Program in Function Construction Medicine, Nagoya University Graduate School of Medicine, Nagoya, Japan, [3]Department of Cardio-Thoracic Surgery, Nagoya University Graduate School of Medicine, Nagoya, Japan, [4]Department of Thoracic Surgery, Aichi Cancer Center Hospital, Nagoya, Japan, [5]Department of Pathology and Biological Responses, Graduate School of Medicine, Nagoya University, Nagoya, Japan, [6]Department of Thoracic Oncology, Aichi Cancer Center Hospital, Nagoya, Japan

Malignant mesothelioma (MM) is an aggressive neoplasm associated with asbestos exposure. Although MM shows frequent mutation of $p16^{INK4a}$/$p14^{ARF}$ and neurofibromatosis type 2 (NF2), a relatively small number of cancer-associated genes have been identified thus far. We carried out a genome-wide array-based comparative genomic hybridization analysis using 14 MM cell lines and identified multiple genomic abnormalities. Three cell lines showed overlapping deletion at the chromosomal region of 13q12. The deletion region harbors a single gene, Large tumor suppressor homolog 2 (LATS2), which encodes a serine/threonine kinase, a component of the Hippo signaling pathway. With 6 other MM cell lines, we performed a mutational analysis of LATS2 and found another cell line with a deletion and three cell lines with nonsense mutation or small deletion. Totally, 7 (35%) of 20 MM cell lines had an inactivating mutation of LATS2. To determine whether LATS2 has a tumor-suppressive activity, we transduced wild-type LATS2 in MM cells. Transduction of LATS2 inhibited cell proliferation of MM cells with LATS2 mutation, indicating LATS2 acts as a tumor suppressor in MM cells in vitro. Since the NF2 gene is genetically mutated in 40-50% of MMs and Merlin, which is encoded by NF2, is also thought to be involved in the Hippo pathway, our data indicate that Hippo pathway dysregulation is frequently observed in MM cells. Furthermore, our immunohistochemical analysis revealed activation of YAP, a transcriptional co-activator regulated by the Hippo signaling cascade, in over 70% of primary MM specimens. Thus, our results indicate that the inactivation of Merlin-Hippo signaling induces constitutive activation of YAP, which is important for MM cell proliferation.

WS02-3

Genomic alterations in asbestos-related lung cancer and malignant mesothelioma

Penny Nymark[1,2], Mohamed Guled[2], Eeva Kettunen[1], Pamela Lindholm[2], Ioana Borze[2], Ali Faisal[3], Leo Lahti[2,3], Antti Karjalainen[1], Kaisa Salmenkivi[2], Andrew Nicholson[4], Harriet Wikman[5], Sisko Anttila[1], Sakari Knuutila[2]

[1]Finnish Institute of Occupational Health, Finland, [2]Department of Pathology, Haartman Institute and HUSLAB, University of Helsinki and Helsinki University Central Hospital, Helsinki, Finland, [3]Helsinki Institute for Information Technology, Laboratory of Computer and Information Science, Helsinki University of Technology, Espoo, Finland, [4]Department of Histopathology, Royal Brompton Hospital, London, UK, [5]Department of Tumor Biology, University Medical Center Hamburg-Eppendorf, Hamburg, Germany

Malignant mesothelioma (MM) and an estimated 5-7% of all lung cancers are associated with a history of asbestos exposure.Over the past decades much data has been gathered on the genetic alterations in MM. Losses seem to be predominant in this type of cancer, e.g. in the chromosomal regions 3p21, 6q, 9p21, 13q, 14q, 17p13 and 22q. In lung cancer, using array comparative genomic hybridization (aCGH) we have been able to identify 18 chromosomal regions harbouring asbestos-related copy number alterations (CNA). Three of these regions (2p16, 9q33.1 and 19p13) were verified and characterized in detail with other methods in larger study populations. Common recurrent CNAs that have been identified in both MM and asbestos-related lung cancer, such as losses at 3p21, 9q, 17p13 and 19p13, could indicate potential breakpoint hotspots for asbestos induced DNA damage.Furthermore, studies profiling the miRNAome in both MM and asbestos-related lung cancer have been performed in our laboratory. MicroRNAs are an attractive method of profiling cancers, since they have proved to be very efficient in distinguishing between tumour histology, classifying undifferentiated tumours and predicting patient outcome. We have identified 21 miRNAs with differential expression in MM compared to normal mesothelium. Several of the miRNA's target genes, such as CDKN2A, NF2, RB1 and JUN, have been reported to be frequently deregulated in MM. In lung cancer, preliminary results indicate that 8 miRNAs may be associated with asbestos exposure in tumours with adenocarcinoma histology.Both MM and lung cancer have very dismal prognoses. The carcinogenic pathways are poorly understood and diagnosis as well as prognosis would greatly benefit from genetic biomarkers associated with early stages of the diseases. Furthermore, distinguishing between asbestos-related and non-related lung cancer could be of importance not only for diagnosis, prognosis and treatment, but also for the medicolegal aspects accompanying occupational diseases.

WS02-4

Deep sequencing of mesothelioma genome DNA reveals chromosomal rearrangement as the dominant mutation

Raphael Bueno[1], Assunta De Rienzo[1], Lingsheng Dong[1], Gavin Gordon[1], William Richards[1], Roderick Jensen[2], Lucian Chirieac[3], Steven Gullans[4], David Sugarbaker[1]

[1]Division of Thoracic Surgery and the International Mesothelioma Program, Brigham and Women's Hospital, and Harvard Medical School, USA, [2]Department of Biological Sciences, Virginia Tech, Blacksburg, VA 24060, USA, [3]Department of Pathology, Brigham and Women's Hospital Brigham and Women's Hospital, and Harvard Medical School, Boston, MA 02115, USA, [4]Excel Medical Ventures, Boston, MA USA

Malignant pleural mesothelioma (MPM) is an aggressive tumor linked to asbestos exposure. Previous cytogenetic and loss of heterozygosity (LOH) analyses have shown frequent deletions and gain of chromosomal regions in specific sites. In this study, whole genome analysis has been performed on a primary human MPM tumor using a combination of approaches: Illumina sequencing by synthesis and Roche/454 pyrosequencing. In particular, we sequenced MPM tumor genomic DNA (gDNA) and matched normal lung gDNA using Illumina paired-ends (PE) technology to generate 17.8 and 15.67 Gigabase pairs (Gbp) or 5.6X and 5.2X coverage of the respective genomes. In addition, Roche/454 sequencing of the tumor gDNA was performed to generate 10.8 Gbp or ~4X coverage extending the total coverage of the tumor genome to nearly 10X. When the MPM tumor's shotgun read density was analyzed across the human RefSeq genome, numerous chromosomal copy number variants were identified. However, substantial variability in the normal gDNA was also found. Interestingly, many more tumor-specific rearrangements than point mutations were discovered in the tumor gDNA at this depth of sequencing, resulting in novel, large-scale, inter- and intra-chromosomal deletions, inversions, and translocations. In particular, we were able to validate 30 different tumor-specific rearrangements: 6 of them were inter-chromosomal and 24 were intra-chromosomal. Fifteen rearrangements disrupted 17 genes. Furthermore, we identified 14 chromosomal regions exhibiting rearrangements resulting in DNA amplification in the tumor relative to the normal, including the DHFR gene. Finally, we found hundreds of previously unreported single nucleotide variants (SNVs). Of these, 83 heterozygous novel SNPs were identified: 3 were homozygous in the tumor only, presumably due to LOH, and 3 in NKX6-2, CDH8, and NFRKB were heterozygous point mutations. In conclusion, deep sequencing of MPM tumor uncovered many types of mutations, with DNA rearrangements representing the dominant type.

WS02-5

Integrated genomic analysis reveals BRCA1-associated Protein 1 (BAP1) as a commonly mutated gene in malignant pleural mesothelioma (MPM)

Matthew Bott, Marie Brevet, Barry Taylor, Shigeki Shimizu, Tatsuo Ito, Robert Delsite, Simon Powell, Christine Zhou, Adam Olshen, Ronglai Shen, Maureen Zakowski, Valerie Rusch, Marc Ladanyi

Memorial Sloan-Kettering Cancer Center, USA

Background: CDKN2A and NF2 are inactivated in most MPM, but other recurrent mutations have not been described. To identify additional driver genes, we did an integrated analysis of copy number and expression data, followed by focused sequencing. **Methods:** Array comparative genomic hybridization (aCGH) data (Agilent 244K arrays) and gene expression data (Affymetrix U133A arrays) were generated from 53 MPM. **Results:** The 3 most frequent losses were at 9p21 (CDKN2A), 22q (NF2), and 3p21. We selected for sequencing 25 genes based on analysis of minimal common regions of gain or loss and integration with expression data, including some previously known genes (e.g. NF2, P53, RB, but not CDKN2A). The most commonly mutated gene (12/53; 23%) was the BRCA1-associated Protein 1 (BAP1) gene at 3p21, encoding a de-ubiquitinating enzyme that interacts with the BRCA1 complex and other proteins. BAP1 mutations were then confirmed in MPM cell lines (4/13 mutated; 31%) and in an additional set of 68 MPM tumors (13 mutated; 19%). Overall, the 28 mutations include 6 nonsense, 5 missense, 9 frameshifting indels, and 8 mutations near splice sites (many associated with abnormal mRNA transcripts). About 30% of MPM show a loss of BAP1 and 42% show either loss or mutation. There was no correlation between BAP1 mutation, NF2 loss, or CDKN2A loss. Two findings suggest that BAP1 loss does not affect BRCA1 function. First, MPM cells with BAP1 loss show normal BRCA1/RAD51 complex formation upon radiation (10 Gy). Secondly, MPM cells with BAP1 loss do not show differential sensitivity to PARP inhibition. We are presently evaluating the effects of BAP1 mutation on downstream de-ubiquitination targets in MPM. **Conclusion:** BAP1 is a novel and frequent target of inactivating somatic mutations in MPM. The possible involvement of BAP1 in key processes regulated by ubiquitination could provide new target pathways for MPM treatment.

WS03

Tumor microenvironment & Oncogenesis

WS03-1 **Keynote Speaker**

Introduction to the tumour microenvironment session

Marie-Claude Jaurand

INSERM, UMR 674, Paris, Descartes, France

Carcinogenesis is a long-term mechanism progressing because of the occurrence of genomic abnormalities, failure of death processes, selective growth advantage and lack of immune recognition. Carcinogenesis involves cell and molecular changes, from a normal to a neoplastic state, consisting of damage to the genetic material, epigenetic modifications and interactions with the tumour microenvironment. Damage to the genetic material includes DNA alterations (base oxidation, insertion, deletion···) and chromosome mutations (translocation, loss of heterozygozity). Epigenetic modifications affect gene function by promoter hypermethylation, chromatin remodelling and changes in miRNA expression.

The tumour microenvironment is complex, consisting of diverse stuctures, cell types and factors. It is the site of inflammation, angiogenesis, a centre of inflammatory factors (reactive oxygen and nitrogen species, chemokines, cytokines, growh factors). In this context, tumour cells are surrounded and interact with extracellular matrix, blood vessels and a number of cells of different origin. There are infiltrates of inflammatory cells, including leucocytes and macrophages, immune and stromal cells are present, and bone marrow derived cells have been detected in the tumour cell environment. Chronic inflammatory reaction plays an important role in the recruitment of inflammatory cells and production of inflammatory mediators, which contribute to the survival and proliferation of tumour cells, as well as the enhancement of genetic instability. Interactions between tumour cells and extracellular matrix control the cell motility, migration and invasion. Although various immune effector cells are recruited to the tumour site, their anti-tumour functions are not efficient, largely in response to tumour microenvironment.

WS03-2

Extracellular PDGF−D promotes migration of malignant mesothelioma

Takahiro Yaguchi[1], Takashi Nakano[2], Tomoyuki Nishizaki[1]

[1]Division of Bioinformation, Department of Physiology, Hyogo College of Medicine, Japan, [2]Department of Thoracic Oncology, Hyogo College of Medicine, Hyogo, Japan

PDGF− β β receptors were abundantly expressed in malignant mesothelioma cells such as MSTO−211H, NCIH−2052, NCIH−2452, and NCIH−28 cells, and those cells secreted PDGF−D to an extent greater than that for non-malignant mesothelioma cells. PDGF−D accelerated the velocity of chemotaxis for malignant mesothelioma cells, and the PDGF−D effect was inhibited by UTI, an inhibitor of urokinase plasminogen activator bearing proteolytic processing from the inactive form of PDGF−D into the active form to activate PDGF-β β receptors. The PDGF−D effect was also prevented by knocking−down PDGF−β β receptors or the PI3 kinase inhibitor wortmannin. The results of the present study represent a PDGF−D/PDGF−β β receptor signal transduction pathway for malignant mesothelioma migration.

WS03-3

Epigenetic silencing of microRNA-34b/c plays a pivotal role in the pathogenesis of malignant pleural mesothelioma

Shinichi Toyooka[1], Takafumi Kubo[1], Kazunori Tsukuda[1], Masakiyo Sakaguchi[2], Takuya Fukazawa[3], Junichi Soh[1], Hiroaki Asano[1], Yasutomo Nasu[4], Takumi Kishimoto[6], Harvey Pass[7], Hideki Matsui[5], Nam-ho Huh[2], Shinichiro Miyoshi[1]

[1]Department of Cancer and Thoacic Surgery, Okayama University, Japan, [2]Department Cell Biology, Okayama University, Okayama, Japan, [3]Department of Gastroenterological Surgery, Transplant, and Surgical Oncology, Okayama University, Okayama, Japan, [4]Department of Urology, Okayama University, Okayama, Japan, [5]Department of Cellular Physiology, Okayama University, Okayama, Japan, [6]Department of Internal Medicine, Okayama Rosai Hospital, Okayama, Japan, [7]Division of Thoracic Surgery, Department of Cardiothoracic Surgery, NYU Langone Medical Center, New York, NY, U.S.A

Malignant pleural mesothelioma (MPM) is a highly invasive tumor with a dismal prognosis. Unlike other malignancies, mutations of TP53 are rare, and p53 expression is generally intact in MPM, even though MPM exhibits anti-apoptosis and cell cycle alterations, which suggests functional p53 deficiency. Altered expression of microRNA (miRNA) has been strongly implicated in human malignant tumors. The miR-34s are direct transcriptional targets of p53. We focused on the miR-34s status in MPM and examined the expression and methylation status of miR-34a and 34b/c in MPM. Aberrant methylation was found in 2 (33.3%) of 6 MPM cell lines and 13 (27.7%) of 47 primary MPMs in miR-34a and in all 6 MPM cell lines (100%) and 40 (85.1%) of 47 primary MPMs in miR-34b/c. Expression of miR-34a and 34b/c in all methylated cell lines was reduced and restored with 5-aza-2'-deoxycytidine treatment. Because epigenetic silencing was the major event in miR-34b/c, we investigated the role of miR-34b/c in 3 MPM cells (NCI-H28, NCI-H290, and NCI-H2052). miR-34b/c-transfected 3 MPM cells with physiological miR-34b/c expression exhibited anti-proliferation with G1 cell cycle arrest and the suppression of migration, invasion, and motility potential. The forced overexpression of miR-34b/c, but not p53, using an adenoviral vector showed a significant anti-tumor effect with the induction of apoptosis in MPM cells. Western blotting was done for c-MET, CDK4, CDK6, CCND1, CCNE2, Bcl-2, c-MYC, and E2F3 that had been reported as primary targets of miR-34s and confirmed that these proteins, especially c-MET, were down-regulated after miR-34b/c introduction. Altogether, our study demonstrates that epigenetic silencing of miR-34b/c by methylation plays a pivotal role in pathogenesis of MPM. Our study also might explain why p53 functions are impaired in MPM despite p53 itself is intact in the majority of MPM and provides new insights into the molecular pathogenesis of MPM.

WS03-4

Expression, mutation and functional analysis of paxillin in malignant pleural mesothelioma

Rifat Hasina[1], Ichiro Kawada[1], Geetanjali Kanade[1], Vidya Nallasura[1], Rajani Kanteti[1], Essam El-Hashani[1], Maria Tretiakova[2], Aliya Husain[2], Wickii Vigneswaran[3], Hedy Kindler[1], Ravi Salgia[1]

[1]Department of Medicine, The University of Chicago, USA, [2]Department of Pathology, The University of Chicago, Chicago, IL, USA, [3]Department of Surgery, The University of Chicago, Chicago, IL, USA

Paxillin is a focal adhesion protein that provides multiple docking sites at the plasma membrane for signaling molecules and helps form a structural link between the extracellular matrix and the actin cytoskeleton. Essential in actin filament assembly and focal adhesion formation, it is involved in migration, motility and key signal transduction pathways. We reported paxillin upregulation, amplification and mutation in a significant number of lung cancer patients. The gene was also amplified in some pre-neoplastic lung lesions. Of several frequently found point mutations, A127T enhanced cell proliferation, focal adhesion formation and colocalization with Bcl-2. We have now analyzed 50 epithelioid, 16 sarcomatoid, and one mixed malignant pleural mesothelioma samples and compared them to 40 normal adjacent lung parenchyma by immunohistochemistry. Automated cellular imaging calculated average staining intensity as an integrated optical density (IOD). Normal lungs had IOD of 45 for paxillin, epithelioid MPM had IOD of 268 and sarcomatoid 331, demonstrating that paxillin is highly overexpressed in both epithelioid as well as sarcomatoid mesothelioma. In ongoing screenings of mesothelioma patients, we have detected a proline to serine as well as other mutations in paxillin. Utilizing confocal live cell imaging, the biological effects of GFP tagged wild-type and mutant clones were systematically studied. Compared to wild-type, mutants confer a) enhanced focal adhesion formation, b) increased filopodia and lamellipodia c) increased mobility and d) increased cell displacement in transiently transfected HEK293 cells. We believe that paxillin is an important molecule in malignant mesothelioma, and its therapeutic potential needs to be explored further.

WS03-5

Formation of 8-nitroguanine, a DNA lesion in the lung of asbestos-exposed mice in relation to inflammation-related carcinogenesis

Yusuke Hiraku[1], Kiyoshi Sakai[2], Eiji Shibata[3], Michihiro Kamijima[4], Naomi Hisanaga[5], Ning Ma[6], Shosuke Kawanishi[7], Mariko Murata[1]

[1]Department of Environmental and Molecular Medicine, Mie University Graduate School of Medicine, Japan, [2]Nagoya City Public Health Research Institute, Nagoya, Aichi, Japan, [3]Department of Health and Psycological Medicine, Aichi Medical University School of Medicine, Nagakute, Aichi, Japan, [4]Department of Occupational and Environmental Health, Nagoya City University Graduate School of Medical Sciences, Nagoya, Aichi, Japan, [5]Center for Campus Health and Environment, Aichi University of Education, Kariya, Aichi, Japan, [6]Faculty of Health Science, Suzuka University of Medical Science, Suzuka, Mie, Japan, [7]Faculty of Pharmaceutical Sciences, Suzuka University of Medical Science, Suzuka, Mie, Japan

BACKGROUND: Asbestos is a potent carcinogen causing lung cancer and malignant mesothelioma. Chronic inflammation is considered to play a key role in asbestos-induced carcinogenesis. Reactive oxygen and nitrogen species generated under inflammatory conditions may contribute to carcinogenesis by causing DNA damage. 8-Nitroguanine is a mutagenic DNA lesion formed during inflammation. In this study, we examined 8-nitroguanine formation in human lung tissues and its association with asbestos exposure.
METHODS: We obtained autopsy and surgical specimens, including lung and tumor tissues of patients with malignant mesothelioma (n=9) and lung tissues of control subjects without asbestos-associated diseases (n=9). This study was approved by the Ethics Committee of Mie University School of Medicine. Fiber contents (chrysotile, amphiboles and non-asbestos fibers) in tissues were analyzed by transmission electron microscopy using a low-temperature ashing procedure. We performed immunohistochemistry to examine 8-nitroguanine formation using specific antibody produced by us (*Nitric Oxide* 2004). We evaluated the relative staining intensity in lung tissues, and statistically analyzed its correlation with fiber contents.
RESULTS: The fiber contents of chrysotile and amphiboles in the lung were significantly larger in mesothelioma patients than in control subjects ($p<0.05$, Mann-Whitney U test). The immunoreactivities of 8-nitroguanine and inducible nitric oxide synthase (iNOS) were observed in alveolar and bronchial epithelial cells, inflammatory cells and tumor cells. In control subjects, the staining intensity of 8-nitroguanine was significantly correlated with the content of amphiboles ($p<0.05$, Spearman's rank correlation), but not with those of chrysotile and non-asbestos fibers. Although 8-nitroguanine formation was apparent in mesothelioma patients, there was no significant correlation with fiber contents.
CONCLUSION: These results suggest that 8-nitroguanine formation primarily involves the generation of reactive oxygen and nitrogen species mediated by amphiboles. 8-Nitroguanine can be a potential biomarker to evaluate the exposure of amphiboles and the risk of asbestos-induced carcinogenesis.

WS03-6

Proteomic analysis of the mechanism of action of DuP-697 in mesothelioma cells

Vijay Agarwal[1,2,3], Victoria C Hodgkinson[1], Gina L Eagle[1], Lucy Scaife[1], Michael J Lind[1,2,3], Lynn Cawkwell[1,2]

[1]Cancer Biology Proteomics Group, Postgraduate Medical Institute, University of Hull, UK, [2]Hull York Medical School, Hull, UK., [3]Queens Centre for Oncology and Haematology, Castle Hill Hospital, Hull and East Yorkshire NHS Trust, Hull, UK.

Introduction: Activation of the cyclooxygenase (COX) pathway has been shown to play an important role in inflammation and carcinogenesis of various tumour types. Our previously published work has shown that COX2 is highly expressed in malignant pleural mesothelioma (MPM) samples (Eur J Cancer 41:1645-8; 2005). We have also shown that specific COX2 inhibitors, including DuP-697, have cytotoxic effects on MPM cells and also potentiate the cytotoxic effects of pemetrexed (Lung Cancer 67:160-5; 2010). However, little is known about the full mode of action of this agent. In this study we have used proteomic techniques to identify the mechanism of action of DuP-697 in MPM cells.Methodology: The MPM cell line MSTO-211H and the A549 lung cancer cell line were exposed to DuP-697 for 72 hours. Drug carrier only was added to control (untreated) cells. Extracted proteins from treated and untreated cells were analysed using the comparative proteomic methods of antibody microarray analysis and two dimensional gel electrophoresis/MALDI mass spectrometry (2DE/MS).Results: Antibody microarray analysis identified a total of 23 proteins which demonstrated a significant (>2 fold) difference in expression between treated and untreated cells. A further 8 proteins showing differential expression (>2 fold difference) were identified using 2DE/MS analysis. A significant number of proteins identified play an important role in apoptosis. These include IKKa, RIP, Bcl xL, Rififylin, BID, MDMX and p21 activated kinase. Further investigation of these proteins using immunoblotting is ongoing. Conclusion: Specific COX2 inhibitors may have a possible therapeutic role in MPM. DuP-697 has shown cytotoxic effects in mesothelioma cells and our proteomic analysis suggests that its mechanism of action may be exerted via the induction of apoptosis.

WS04

Peritoneal mesothelioma

—Clinical and translational research—

WS04-1 **Keynote Speaker**

Clinical management of diffuse malignant peritoneal mesothelioma

Paul Sugarbaker

Washington Cancer Institute, Washington, DC

Aims: In the past, diffuse malignant peritoneal mesothelioma (DMPM) has been regarded as a terminal condition. The length of the survival was dependent upon the aggressive versus indolent biology of the neoplasm, nevertheless cure was not considered as a reasonable expectation and the overall median survival was approximately one year.
Methods: A comprehensive literature review and a collection of pertinent data published on DMPM from the Washington Cancer Institute were used to construct this report.
Results: Recent publications from Bethesda MD, New York, Milan Italy, Lyon France, Paris France and Washington DC have shown a remarkable prolongation in the median survival of this group of patients with approximately half the patients alive at 5 years. These prolonged survivors were treated with an intensive local-regional treatment strategy that included cytoreductive surgery (CRS) with peritonectomy and hyperthermic intraoperative intraperitoneal chemotherapy (HIIC) and some patients with early postoperative intraperitoneal chemotherapy (EPIC). As larger numbers of patients have been treated, clinical features by which to select patients most likely to benefit from this approach have been identified. Also, as the experience in the management of patients receiving these treatments has increased, the morbidity and mortality associated with their management is being reduced.
Conclusions: A new standard of care involves cytoreductive surgery for removal of large disease deposits combined with perioperative intraperitoneal chemotherapy. Knowledgeable management uses aggressive local-regional treatments, selection by quantitative prognostic indicators, and incurs low morbidity and mortality.

WS04-4 **Keynote Speaker**

Traslation of new prognostic biomarkers and therapeutic targets in clinical practice

Marcello Deraco[1], Dario Baratti[1], Federica Perrone[2], Genny Jocollè[2], Marzia Pennati[3], Raffaella Villa[3], Silvana Pilotti[2], Elvira Mingrone[1], Shigeki Kusamura[1], Nadia Zaffaroni[3]

[1]Department of Surgery-Peritoneal Malignancy Unit, Fondazione IRCCS Istituto Nazionale dei Tumori, Italy, [2]Pathology, [3]Experimental Oncology and Molecular Medicine, Fondazione IRCCS Istituto Nazionale dei Tumori, Milano, Italy

Introduction: Diffuse Malignant Peritoneal Mesothelioma (DMPM) accounts for 10 to 30% of all Mesotheliomas. Considered as a fatal condition without treatment options for several decades, DMPM aroused, in recent years, the interest of the scientific community on biological and clinical areas of research. In historical case-series, standard therapy with palliative surgery and systemic or intraperitoneal chemotherapy is associated to a median survival of about one year, ranging from 9 to 15 months. During the last two decades, few specialized centers have developed an innovative treatment consisting on Cytoreductive Surgery (CRS) with peritonectomy procedures and by multivisceral resections to remove all the visible tumour. Microscopic residual disease is treated with Hyperthermic Intraperitoneal Chemotherapy (HIPEC) .
The Innovative Treatment: this combined approach modified the natural history of DMPM with a dramatic improvement in outcomes. The analysis of the most important experiences allow to the conclusion that median survival grew from 12 months with a sCT treatment to 53 months with CRS + HIPEC with 50% 5 years OS. The most significant prognostic factors, independently associated with improved survival in the multivariate analysis resulted: the epithelial subtype compared with the biphasic or sarcomatoid , the absence of lymph node metastasis, the completeness of cytoreduction scores and the treatment with HIPEC compared to other adjuvant treatment. At the analysis of pattern failure, small bowel is the site most commonly involved at recurrence while the residual tumor >2.5 mm vs no visible is the only independent risk factor for disease progression. Quality of life is satisfactory since 94% of patients have a resolution of ascites and related morbility and mortality acceptable with reasonable financial cost effectiveness.
Traslational Research: notwithstanding the significant improvement in survival with CRS and HIPEC, the poor prognosis subset of DMPM patients is still substantial thus making it mandatory to identify new therapeutic strategies. In this context, at the Fondazione IRCCS Istituto Nazionale Tumori of Milan, work has been done in recent years to identify and validate new terapeutic targets as well as strategies to predict the sensitivity to conventional drugs. This part of the study will deal with the generation of primary cultures directly obtained from DMPM surgical specimens with the aim to evaluate at the preclinical level the sensitivity of individual tumors to a panel of conventional drugs (including cisplatin/carboplatin, pemetrexed, gemcitabine, vinorelbine, and doxorubin/epidoxorubicin), singly administered or in combination, to provide the biological rationale for the design of tailored adjuvant CT treatment in patients who underwent CRS and HIPEC. In addition, the sensitivity profiles of individual cultures to a series of targeted agents (including the multitarget TKR inhibitor Sorafenib, as well as more selective inhibitors of EGFR, mTOR, IGFR and MET) will be tested. Such results, together with those emerged from the characterization of the molecular profile of the surgical specimens will provide the rational for the selection of a personalized CT treatment in patients who experienced recurrent disease.
Perspectives: during the last two decades, the combined treatment of CRS and HIPEC began the so called "Gold Standard" for DMPM and trough important efforts is today world wide available. Several studies, allow to clarify the biology and natural history of DMPM identifying new prognostic biological factors as well as new therapeutic targets. Anyway, there is still a critical need for novel investigational strategies aiming to demonstrate benefit of sCT in DMPM patients. With this aim, an important step is to translate and verify the impact on outcomes of the preclinical knowledge in the clinical practice. Next steps should be aimed at improving our understanding of the biological and molecular features of DMPM and integrate CRS + HIPEC with an individualized approach of sCT and molecular-targeted therapy based on molecular characterization and chemosensitivity profile on primary cultures. Efforts should be finally aimed to validate a staging system for DMPM taking account of the most important prognostic indicators.

WS04-2

Cancelled

WS04-3

Identification and validation of new prognostic biomarkers and therapeutic targets

Nadia Zaffaroni[1], Federica Perrone[2], Marzia Pennati[1], Raffaella Villa[1], Silvana Pilotti[2], Dario Baratti[3], Genny Jocollè[2], Shigeki Kusamura[3], Mingrone Elvira[3], Marcello Deraco[3]

[1]Departments of Experimental Oncology and Molecular Medicine, Fondazione IRCCS Istituto Nazionale dei Tumori, Italy, [2]Pathology, [3]Surgery-Peritoneal Malignancy Unit, Fondazione IRCCS Istituto Nazionale dei Tumori, Milano, Italy

Notwithstanding the significant improvement in survival with the advent of combined cytoreductive surgery and hyperthermic intraperitoneal chemotherapy (HIPEC), the poor prognosis subset of diffuse malignant peritoneal mesothelioma (DMPM) patients is still substantial thus making it mandatory to identify new biomarkers to stratify patients according to prognosis as well as novel terapeutic targets and strategies. In this context, at the Fondazione IRCCS Istituto Nazionale Tumori of Milan, work has been done in recent years to identify and validate new prognostic markers. Specifically, we investigated the prevalence and the prognostic relevance of the two known telomere maintenance mechanisms, telomerase activity (TA) and alternative lengthening of telomeres (ALT), in 44 DMPM specimens obtained from 38 patients who underwent a uniform treatment regimen consisting of cytoreductive surgery and HIPEC. ALT or TA alone was found in 18.2% or 63.6% of lesions, respectively, whereas 2 cases were ALT+/TA+. TA was prognostic for 4-year relapse (TA+ v TA-, hazard ratio, 3.30; 95% CI, 1.23-8.86; $P = 0.018$) and cancer-related death (TA+ v TA-, hazard ratio, 3.56; 95% CI, 1.03-12.51; $P = 0.045$), whereas ALT failed to significantly affect clinical outcome. Such data highlight telomerase as a possible new prognostic marker in the disease.

As far as the search for new therapeutic targets is concerned, we assessed the expression of survivin and other members of the inhibitors of apoptosis proteins (IAP) family (IAP-1, IAP-2 and X-IAP) in a series of 32 DMPM surgical specimens by immunohistochemistry. Survivin expression was observed in 29 (91%) specimens, whereas the positivity rate for the other IAPs ranged from 69% to 100%. To functionally validate survivin as a possible therapeutic target, a DMPM cell line (STO) established in our laboratories was transfected with a small-interfering RNA (siRNA) targeting survivin mRNA. Survivin knockdown induced a time-dependent decline in DMPM cell growth and an enhanced rate of spontaneous and drug (cisplatin, doxorubicin)-induced apoptosis, with a concomitant increase in the catalytic activity of caspases. Such findings indicate that survivin, as well as other IAPs, is largely expressed in clinical DMPM and suggest that strategies aimed at down-regulating survivin may provide a novel approach for the treatment of the malignancy.

More recently, we assess the activation profile of selected receptor tyrosin kinases (RTK) and their downstream effectors in a series of 20 cryopreserved DMPM surgical specimens to discover targets for drug inhibition. We found the expression/phosphorylation of EGFR and PDGFRB in most of the tumors, and PDGFRA activation in half. The expression of the cognate ligands TGF-α, PDGFB and PDGFA in the absence of RTK gene mutation and amplification suggested the presence of an autocrine/paracrine loop. There was also evidence of EGFR and PDGFRB co-activation. RTK downstream signalling analysis demonstrated the activation/expression of ERK1/2, AKT and mTOR, together with S6 and 4EBP1, in almost all of the DMPM. We also made a complementary analysis of the cytotoxic effects of some kinase inhibitors on the proliferation of STO cell line. In vitro cytotoxicity studies showed STO cells to be sensitive to sequential treatment with RAD001 and sorafenib. These data highlight the ligand-dependent activation and co-activation of EGFR and PDGFRB, as well as a connection between these activated RTKs and the downstream mTOR pathway, thus supporting the role of combined treatment with RTK and mTOR inhibitors in DMPM.

WS05

Apoptosis and mesothelioma

WS05-1 **Keynote Speaker**

Apoptosis and mesothelioma

Dean Fennell

Center for Cancer Research and Cell Biology, The Queen's University of Belfast, UK

Progress in developing effective therapies for mesothelioma have been slow, in part due to the empirical approaches adopted historically in clinical trials. Although advances in first line therapy have led to a standard care, with the first randomised studies, there still remains a lack standard therapy in the relapsed setting. Drug resistance is a major problem, and accounts for the low or absence responses associated with conventional chemotherapy. The core cell death machinery when harnessed effectively for therapy, can improve survival in other solid cancer. In mesothelioma the essential components of this machinery to induce cell death, at least in preclinical models of mesothelioma, are present and functional, however they are normally restrained. Mitochondrial activation of apoptosis can be activated however by BH3 pepidomimetics in clinical development; repression of caspases, the demolition machinery involved in mediating apoptosis, is suppressed by inhibitors of apoptosis (survivin, cIAP2) - now targets for specific small molecules entering clinical trials. The extrinsic pathway may be a particularly important target for therapy, either through ligand driven apoptosis by tumour necrosis factor related apoptosis inducing ligand, or via degradation of the antiapoptosis protein cFLIP. Resistance to these new agents will inevitably limit their efficacy in some individuals; efforts to personalise treatment will therefore be crucial for their success. In non-small cell lung cancer, the paradigm of blocking addiction to growth factor tyrosine kinases to activate apoptosis and dramatic tumour responses is well established, eg. the unleashing of the BH3 only protein BIM by inhibition of somatically mutated epidermal growth factor receptor. However, the spectrum of possible somatic mutations in the kinome of mesothelioma has not yet been fully explored. Discovery and exploitation of such somatic vunerabilities could bring effective therapy in the future. Similarly identification of strategies for inducing synthetic lethality via apoptotic (and non-apoptotic) pathways will be discussed.

WS05-2 **Keynote Speaker**

The Bcl-2 repertoire of mesothelioma spheroids underlies acquired apoptotic multicellular resistance

Dario Barbone[1,2], Nikita Kolhatkar[1], Jeremy Ryan[3], Alexander Chacko[2], David Sugarbaker[4], Raphael Bueno[4], Anthony Letai[3], Dean Fennell[2], Courtney Broaddus[1]

[1]Lung Biology Center, SFGH, UCSF, USA, [2]Centre for Cancer Research and Cell Biology, Queens University Belfast, Ireland, [3]Dana-Farber Cancer Institute, Boston, MA USA, [4]Brigham and Women's Hospital, Harvard Medical School, Boston, MA USA

The study of the molecular mechanisms underlying resistance to apoptosis is key to improving existing therapy for cancer. While cancer cells attain a chemoresistant phenotype on a single cell (unicellular) level, they acquire further resistance to apoptosis when they form three-dimensional (3D) structures (multicellular resistance). In vitro 3D cell cultures (multicellular spheroids) have emerged as a valuable platform to study the acquired multicellular resistance of cancer. Bortezomib, a proteasome inhibitor, while effective as a single agent in multiple myeloma, has been generally ineffective in solid tumors such as mesothelioma. Mesothelioma cells acquire a marked multicellular resistance to Bortezomib when grown as spheroids compared to monolayers. Interestingly, the Bcl-2 repertoire of spheroids was different than monolayers, both at baseline and after bortezomib. The most relevant difference was the lack of upregulation of Noxa by bortezomib in spheroids compared to monolayers. Restoration of Noxa by a cell-permeable NoxaBH3 peptide bypassed multicellular resistance to bortezomib. In addition, ABT-737, an inhibitor of the anti-apoptotic proteins Bcl-2/XL/w, also bypassed the resistance acquired by cells in 3D, a finding that was also confirmed in 3D ex-vivo cultures. In conclusion, we find that the Bcl-2 repertoire of spheroids accounts for multicellular resistance and that its manipulation may be a successful adjunct to bortezomib therapy of solid tumors.

WS05-3

Arsenic trioxide induces apoptosis through JNK and ERK in human mesothelioma cells

Ryoji Eguchi[1], Yoshihiro Fujimori[1,2], Chiharu Tabata[1,3], Kouzo Kuribayashi[1,3], Kazuya Fukuoka[1,3], Takashi Nakano[1,2,3]

[1]Department of Thoracic Oncology, Hyogo College of Medicine, Japan, [2]Cancer Center, Hyogo College of Medicine, Hyogo, Japan, [3]Division of Respiratory Medicine, Department of Internal Medicine, Hyogo College of Medicine, Hyogo, Japan

Malignant mesothelioma is an aggressive tumor of serosal surfaces which is refractory to current treatment options. Arsenic trioxide (As2O3) is used clinically to treat acute promyelocytic leukemia, and also to inhibit proliferation of several solid tumors including hepatoma, esophageal and gastric cancer in vitro. Here we found that As2O3 inhibited proliferation of a mesothelioma cell line, NCI-H2052. As2O3 induced apoptosis of NCI-H2052 cells, which was accompanied by activation of caspase-3, JNK1/2 and ERK1/2. zVAD(OMe)-fmk, a broad-spectrum caspase inhibitor, inhibited As2O3-induced apoptosis and activation of caspase-3, but not JNK1/2 and ERK1/2. Small interfering RNAs (siRNAs) targeting JNK1 suppressed As2O3-induced apoptosis and caspase-3 activation more significantly than JNK2 siRNA. JNK1 siRNA inhibited As2O3-induced JNK2 activation and JNK2 siRNA inhibited As2O3-induced JNK1 activation. JNK1 siRNA, but not JNK2 siRNA, inhibited As2O3-induced ERK1/2 activation. JNK2 siRNA together with PD98059, a specific MAPK/ERK inhibitor, inhibited As2O3-induced apoptosis to a similar extent as JNK1 siRNA. These results indicated that As2O3 induces apoptosis of NCI-H2052 cells through mainly JNK1/2 activation, and that ERK1/2 is involved in As2O3-induced apoptosis when JNK1/2 are inactivated.

WS05-4

BRCA1 expression is required for efficacy of vinorelbine in malignant mesothelioma

Sara Busacca[1], Jennifer Quinn[1], Dean Fennell[1], Ken Byrne[2], Kathy Gately[2]

[1]Center for Cancer Research and Cell Biology, The Queens University Belfast, UK, [2]St James Hospital Dublin

Background Malignant mesothelioma is an aggressive tumour refractory to current therapeutics options and this may be due to intrinsic apoptosis resistance. Spindle poisons have known activity in mesothelioma, in particular a phase II trial with the third generation vinca alkaloid vinorelbine was conducted, and moreover a cytotoxic effect in combination with gemcitabine and cisplatin was demonstrated. A number of studies have correlated BRCA1 deficiency with down-regulation of several cell cycle regulatory proteins and spindle checkpoint. Interestingly, it was demonstrated that BRCA1 increases sensitivity to microtubule targeting agents by activating the spindle checkpoint. Results The cytotoxic effect of vinorelbine was studied in a panel of 9 mesothelioma cell lines and dose-response curves were generated. The impact of vinorelbine treatment on cell cycle progression was also investigated. Our data demonstrate a good correlation between BRCA1 expression and sensitivity of mesothelioma cells to vinorelbine. Furthermore, in H226 cells a low level of BRCA1 and a low expression of PTEN were correlated with a good response to the PARP inhibitor AZD2281. We then investigated the impact of the modulation of BRCA1 expression on sensitivity. The downregulation of BRCA1 expression by small interfering RNA (siRNA) mediated knockdown in E58 resulted in reduced sensitivity to vinorelbine. The overexpression of BRCA1 in H226 induced an increased response rate to treatment. These data suggest a correlation between BRCA1 expression and the activation of the mitotic spindle checkpoint in mesothelioma cells. Data obtained in BAX/BAK double negative cells suggest that vinorelbine mediates toxicity irrespective of a functional mitochondrial apoptosis pathway.Conclusion Our data show a strong correlation between BRCA1 expression and response of mesothelioma cells to vinorelbine, suggesting that BRCA1 may function as a biomarker for malignant mesothelioma.

WS05-5

Malignant mesothelioma resists against chemotherapies via mitophagy

Xiaobo Cao, Chander Peddaboina, Daniel Jupiter, Philip Rascoe, William Smythe

Department of Surgery, Scott & White Memorial Hospital and Clinic; the Texas A&M University System, Health Science Center, USA

Malignant mesothelioma (MM) has been shown to be unresponsive to conventional therapies due to resistance to apoptosis. In MM, autophagy represents an adaptive survival mechanism to clear damaged organelles and survive bioenergetics stress caused by chemotherapy. The objective of this research is to define the molecular autophagy mechanisms by which mesothelioma resists against chemo-induced apoptosis. We provide evidence that HDAC inhibitors induced apoptosis and autophagy in MM. Autophagy occurred much earlier than apoptosis. Reducing Beclin-1 resulted in increased apoptotic cell death, while reducing bak and bax increased the autophagy population. To determine whether mitophagy occurred after SAHA treatment, I45 EGFP-LC3; cell were transfected with mito-red cDNA and subsequently exposed to a SAHA. The overlapping of bright green (autophagy) and mito-red labeled mitochondria observed by confocal microscopy indicates the induction of mitophagy. Our confocal microscopy study further demonstrated that fissed mitochondria were the target of mitophagy. Inhibiting mitochondria fission decreased mitophagy population and increased apoptosis in MM.To study whether Nix is required for SAHA-induced mitophagy, cell lines of I45 EGFP-LC3; (Nix positive) were transfected with Nix siRNA and control siRNA prior to SAHA exposure. Flow cytometry analysis was utilized to quantify mitochondria following SAHA exposure. Nix positive I45 cells (control siRNA) demonstrate reduced numbers of mitochondria (presumably due to mitophagy) when compared with Nix negative I45 cells (Nix siRNA). Attenuating Nix expression with siRNA reduced the autophagy population and sensitized cancer cells to chemotherapy. Co-localization of SQSTM1/p62 (autophagy receptor protein) and Nix at mitochondria level after SAHA treatment was documented by confocal fluorescent microscopy. Elevation of SQSTM1/P62 in mitochondria fraction observed via i.p. western blotting further confirmed a potential link exists between mitochondria and autophagosome formation. All these suggest the protective role of autophagy in mesothelioma after chemotherapy. Our results show that mitophagy protect mesothelioma via removing damaged mitochondria.

WS05-6

c-FLIP, a critical target for histone deacetylase inhibitors in malignant pleural mesothelioma

Jane Hurwitz, Patrick Johnston, Dean Fennell, Daniel Longley

Center for Cancer Research and Cell Biology, The Queen's University Belfast, UK

Resistance to apoptosis is a key mechanism underlying the failure of chemotherapy in malignant pleural mesothelioma (MPM). The caspase 8 inhibitor c-FLIP blocks death receptor signalling, and has previously been shown to be important in regulating apoptosis and drug resistance in several cancers. Here, we investigated the role of c-FLIP in regulating the response of MPM cells to the histone deacetylase (HDAC) inhibitor Vorinostat, an agent which is currently under evaluation in a phase III clinical trial in the relapsed setting.Three mesothelioma cell lines were studied: REN, E58 and H28. The IC50 doses of Vorinostat were found to be in the low micromolar range, as determined by MTT assay. c-FLIP was down-regulated at the protein and mRNA level after exposure to Vorinostat in a dose-dependent manner, with potent down-regulation observed at the IC50 dose. Vorinostat-induced down-regulation of c-FLIP correlated with caspase 8 activation and induction of apoptosis. Importantly, apoptosis induced by Vorinostat was significantly reduced in FLIP overexpressing cell lines, while siRNA-mediated silencing of caspase 8 and the key death receptor adapter protein FADD were found to inhibit Vorinostat-induced cell death. Furthermore, siRNA-mediated silencing of c-FLIP was found to be sufficient to activate caspase 8 and induce apoptosis in the mesothelioma cell lines. These results are consistent with c-FLIP down-regulation being a major mechanism of Vorinostat-induced apoptosis in mesothelioma. Moreover, Vorinostat does not affect expression of other proteins involved in apoptotic pathway, such as Mcl-1, Bcl-2, Bcl-XL, BAK, and XIAP. c-FLIP is down-regulated at both a transcriptional and post-transcriptional level in response to Vorinostat. This appears to be a major mechanism leading to apoptosis induction by this agent in mesothelioma cell lines and suggests that FLIP, caspase 8 and other death receptor signalling molecules may be potential biomarkers of response to Vorinostat in mesothelioma.

WS06

Immunotherapy: bench to bedside-I

WS06-1

Chemotherapy alters cross-presentation of tumour antigen at the effector site

Alison McDonnell[1], Amy Prosser[2], Anna Nowak[1,2], Richard Lake[1,2], Bruce Robinson[1,2], Andrew Currie[2]

[1]School of Medicine and Pharmacology, The University of Western Australia, Australia, [2]National Centre for Asbestos Related Diseases, Perth, Western Australia, Australia

Cross presentation defines the unique capacity of an antigen presenting cell (APC) to present exogenous antigen via MHC class I molecules to CD8+ T cells. This process serves a critical role in host anti-tumor immunity. Dendritic cells (DC) are specialised cross-presenting cells; however there is little understanding of how systemic chemotherapy, a common modality in cancer treatment, effects their capacity to prime tumour-specific CTL. In this study we have examined the cross-presentation of a marker tumour antigen (transfected HA) expressed by the murine malignant mesothelioma (MM) line, AB1-HA. We found that MM antigen is constitutively cross-presented in the draining lymph node throughout disease progression by immature DCs. Interestingly, while tumour-infiltrating DCs (TiDC) fail to cross present, systemic chemotherapy using the apoptosis inducing false nucleotide agent, gemcitabine, which primes for anti-tumor immunity, reverses the defect in antigen cross presentation of tumor DCs. Further analysis revealed that these TiDC were not enhanced in their expression of MHC or co-stimulatory molecules and their capacity to acquire particulate antigen in situ was not enhanced. Instead, gemcitabine chemotherapy reduced the proportion of $CD11c^{hi}CD11b^{+}Gr1^{int}$ DCs infiltrating the tumour site. Thus systemic gemcitabine chemotherapy acts to reverse the refractory state of TiDCs by altering the tumour microenvironment. These data suggest that local cross-presentation within MM tumors may be essential for effective anti-tumor immunity, and may have important implications for anti-cancer therapy, particularly the use of immunotherapy in conjunction with tumor apoptosis-inducing therapy.

WS06-2

Novel internalizing human single chain antibodies targeting both common and treatment-resistant forms of mesothelioma

Scott Bidlingmaier, Yong Wang, Xiaodong Zhu, Jinjing Feng, Xiaoju Zhang, Yang Su, Dario Barbone, Feng An, Jiang He, Courtney Broaddus, Bin Liu

UCSF Comprehensive Cancer Center, University of California at San Francisco, USA

The goal of this research is to develop human antibody-based therapy and imaging for mesothelioma. Mesothelioma is incurable and has three main subtypes: epithelioid (common), sarcomatoid (difficult to treat), and mixed. Currently, very few cell surface antigens have been identified that are overexpressed by all types of mesothelioma. For example, the mesothelin molecule has been shown to be a useful marker for epithelioid mesothelioma, but it is not expressed by sarcomatous mesothelioma, which is the most difficult type to treat.

We aim to identify human antibodies targeting all types of mesothelioma. We further aim to identify novel antibodies with a therapeutically desired intracellular delivery function. To this end, we selected a 500-million member phage antibody display library under internalizing conditions on live epithelioid and sarcomatoid mesothelioma cells, and identified a panel of rapidly internalizing human single chain antibodies (scFvs) that target both types of mesothelioma cells. These internalizing scFvs mediate efficient and targeted intracellular delivery of small molecule drug payloads to both epithelioid and sarcomatous mesothelioma cells in vitro. In addition, a multi-modality imaging study with a technetium-labeled scFv showed that the scFv targets efficiently mesothelioma organotypic xenografts in vivo.

To identify tumor antigens bound by these scFvs, we created a yeast surface displayed human cDNA library and screened by flow cytometry the entire human proteome for antibody binding. We identified MCAM as the target antigen for one of our scFvs. Immunohistochemistry analysis of mesothelioma tissue microarrays confirmed that MCAM is widely expressed by epithelioid, sarcomatous and mixed types of mesothelioma tumor cells in situ but not by normal mesothelial cells. Thus, we have identified a cell surface antigen expressed by all types of mesothelioma, making MCAM a candidate for therapeutic targeting. We are now applying the same antigen identification strategy to the entire panel of our novel scFvs.

WS06-3

Anti-HM1.24 antibodies induce antibody-dependant cellular cytotoxicity against mesothelioma cells

Jun Huang[1], Yasuhiko Nishioka, Shinji Abe[2], Yuki Morita[2], Shuji Ozaki[3], Vinod K. Verma[1], Masatoshi Kishuku[2], Kazuo Minakuchi[2], Yoshitaka Sekido[4], Toshio Matsumoto[3], Saburo Sone[1]

[1]Department of Respiratory Medicine and Rheumatology, Institute of Health Biosciences, The University of Tokushima Graduate School, Japan, [2]Department of Pharmacy, Tokushima University Hospital, Tokushima, Japan, [3]Department of Medicine and Bioregulatory Science, Institute of Health Biosciences, The University of Tokushima Graduate School, Tokushima, Japan, [4]Division of Molecular Oncology, Aichi Cancer Center Research Institute, Nagoya, Japan

Mesothelioma is an aggressive tumor with increasing incidence worldwide. Despite certain advances in conventional regimes (surgery, radiotherapy, chemotherapy, etc.), the prognosis of patients with mesothelioma remains poor. This provides a strong imperative for development of novel therapeutic modalities. We have previously reported that HM 1.24, originally identified as a cell surface protein that is over expressed on multiple myeloma, is a useful target against lung cancer in antibody-based immunotherapy. In the present study, we examined the expression of HM1.24 antigen in mesothelioma cell lines by flow cytometry and the possibility of immunotherapy with anti-HM1.24 antibody (Chugai Pharmaceutical Co., Ltd.) to induce antibody-dependent cellular cytotoxicity (ADCC) in vitro. The activity of ADCC was assessed by ^{51}Cr-release assay. 67% (10/15) of mesothelioma cells were positive for HM1.24 expression. The high level of HM1.24 antigen expression was observed in 33% (5/15) of all cell lines. Mouse anti-HM1.24 antibody effectively induced complement-dependent cytotoxicity (CDC) against HM1.24 positive mesothelioma cells. Moreover, human MNC (mononuclear cells) exhibited effective ADCC against HM1.24 positive cells induced by chimeric anti-HM1.24 antibody in a time-and dose-dependent manner. It was observed that there was positive correlation between the effector-to-target ratio (E/T ratio) and the ADCC activity of human MNC. These observations suggest the potential activity of anti-HM1.24 mAb, in novel biological therapy for patients with mesothelioma.

WS06-4

Tumor cell repopulation is inhibited by regulatory T cell depletion between cycles of chemotherapy in a murine mesothelioma model

Licun Wu, Zhihong Yun, Tetsuzo Tagawa, Katrina Rey-McIntyre, Masaki Anraku, Marc de Perrot

Latner Thoracic Surgery Research Laboratories and Division of Thoracic Surgery, Toronto General Hospital, University Health Network, University of Toronto, Canada

Aim of study: Malignant pleural mesothelioma is a highly aggressive cancer with poor prognosis. We have previously demonstrated that Treg depletion can impact tumor microenvironment when combined with chemotherapy. The aim of this study is to analyze the impact of Treg depletion on tumor cell repopulation during cycles of chemotherapy in a murine mesothelioma model. Methods: Tumor-bearing mice were treated with chemotherapy once weekly to mimic clinical settings, and with PC61 to cause Treg depletion after each cycle of chemotherapy. Tumor cell repopulation was evaluated by BrdU labeling indix with immunohistochemistry and flow Cytometry, and Ki67 gene expression was determined by real-time RT-PCR. The proportion of CD4+ CD25+Foxp3+ Tregs, CD4+ and CD8+ T cells in the tumor, spleen, draining lymph node and peripheral blood from tumor-bearing mice were determined by using flow cytometry, and gene expression of activated T cell-related cytokines were quantified by ELISA and RT-PCR. Results: Tumor growth delay was achieved by cisplatin followed by PC61 or cyclophosphamide. The BrdU labeling index indicated that tumor cell repopulation between cycles of cisplatin was significantly inhibited by PC61. The CD4+CD25+Foxp3+ Tregs in tumor and lymphoid organs were almost completely depleted, whereas the total CD4+ or CD8+ T cells did not change significantly. PC61 following chemotherapy resulted in an increase of gene expression of IFN-γ, granzyme B, perforin and IP-10, thus leading to tumor cell lysis in CTL assay. However, cell killing induced by cyclophosphamide combined with cisplatin was due to cytotoxicity rather than specific immune response. Conclusion: Administration of Treg depletion could dramatically inhibit tumor cell repopulation between cycles of chemotherapy, thus might be a potent approach to treatment of mesothelioma.

WS06-5

Gene therapy for malignant mesothelioma using urokinase-targeted oncolytic Sendai virus

Yosuke Morodomi[1], Yoshikazu Yonemitsu[2,3], Tokujiro Yano[1], Hiroaki Kinoh[4], Tsukihisa Yoshida[1], Kensaku Ito[1], Yasunori Shikada[1], Riichiroh Maruyama[1], Kumi Yoshida[2,3], Yasuji Ueda[4], Makoto Inoue[4], Mamoru Hasegawa[4], Yoshihiko Maehara[1]

[1]Department of Surgery and Science, Graduate School of Medical Sciences, Kyushu University, Japan, [2]R&D Laboratory for Innovative Biotherapeutics Graduate School of Phermaceutical Sciences, Kyushu University, [3]Operating Unit for Clinical Trials of Gene Therapy Department of Surgery and Science, Graduate School of Medical Sciences, Kyushu University, [4]DNAVEC Corporation, Tsukuba, Japan.

Background: The high malignancy of malignant pleural mesothelioma (MPM) demands novel therapeutic strategies. Malignant tumors that are potentially invasive and capable of metastasis, including MPM, frequently express urokinase–type plasminogen activator or its receptor. We have recently developed the "BioKnife "– a novel type of oncolytic Sendai virus vector specifically targeted to the urokinase–type plasminogen activator. Presently, the potential of this vector for the treatment of mesothelioma was evaluated.Methods:Orthotopic mouse models were established by independently administrating the human MPM cell lines MSTO–211H and NCI–H226 into the pleural cavity of immunodeficient nu/nu mice. Oncolytic vectors expressing green fluororescent protein (GFP) were administered as a bolus or repeatedly (three or six times) to the pleural cavity of the MPM–bearing mice. To examine the effects of antitumor activity in a single individual, MPM–bearing mice that stably expressed luciferase in the tumor were established and luciferase activity was measured with an IVIS® imaging system before and after therapy. Results:Both human MPM cells already exhibited multiple tumor nodules in the pleural cavity on day 7, and all tumor–bearing mice died within 50 days (MSTO–211H) or 140 days (NCI–H226). Repeated administration of Bioknife–GFP significantly prolonged survival of mice bearing MSTO–211H and NC–H226 ($p<0.001$). GFP expression occurred almost exclusively in the tumors and only rarely in normal tissues. An immunofluorescent study revealed extensive TUNEL–positive cells in the tumors treated with BioKnife–GFP. Imaging of luciferase *in vivo* revealed a decreased enzyme activity in the treatment group and an increased activity in the control group.Conclusions:The BioKnife is considered to be an effective antitumor agent in the treatment of MPM in orthotopic mice. It may also be potentially valuable as a novel therapeutic agent to treat MPM in a clinical setting.

WS06-6

mRNA electroporated T-cells bearing mesothelin targeted chimeric antibody receptor have anti-tumor effect in a murine mesothelioma model

Edmund Moon[1], Yangbing Zhao[2], Carmine Carpenito[2], Lou Aliperti[1], Veena Kapoor[1], Daniel Sterman[1], Carl June[2], Steven Albelda[1]

[1]Thoracic Oncology Research Group, University of Pennsylvania, USA, [2]Department of Pathology and Laboratory Medicine, University of Pennsylvania, Philadelphia, PA, USA

Rationale: T cells with chimeric antigen receptors (CARs) are a promising treatment for cancer. We have shown that mesothelin directed CARs have strong activity in vitro/vivo and are planning a clinical trial. However, permanent CAR expression in T cells carries safety risks due to normal tissue expressing mesothelin. To screen for mesoCAR T cell toxicity we explored the possibility of using transient CAR expression using mRNA electroporated T cells (mETs).Methods: 56 days after IP injection with a mesothelioma cell line expressing luciferase into SCID/NOD/G2KO mice, 30 mice were randomized into three IP treatment groups - saline, control mETs (CD19), and mesoCAR mETs (mesoCAR). 6 IP doses of mETs were given over 2 weeks with an additional 8 IP doses when tumor recurred. Bioluminescence and survival were followed. The T cells were autologous (i.e. cryopreserved PBMC from the same patient.) Results: After 6 T cell doses, tumor bioluminescence decreased in mesoCAR mice compared to control mETs and saline mice ($p<0.001$). Although we observed disease stability and even "cures" by imaging in mesoCAR mice, tumor eventually recurred. Despite an additional 8 doses, tumor burden in mesoCAR mice eventually approached that of control mice. The 50% median survival was greater in the mesoCAR mice (73 days) compared to the CD19 (62 days) and saline mice (36 days) ($p<0.05$).Conclusions: 1) Repeatedly dosed mesoCAR mETs have anti-tumor effect that abrogates with subsiding mesoCAR expression.2) mesoCAR mETs are a feasible first step in testing safety in the clinical setting.

WS06-7

Complete and sustained tumor regression of human malignant mesothelioma xenografts in athymic mice following local injection of midkine promoter-regulated oncolytic adenovirus

Shuji Kubo[1], Yoshiko Kawasaki[1], Norie Yamaoka[1], Yunfeng Xu[1], Hideyuki Yamamoto[1], Masatoshi Tagawa[2], Noriyuki Kasahara[3], Nobuyuki Terada[4], Haruki Okamura[1]

[1]Laboratory of Host Defenses, Institute for Advanced Medical Sciences, Hyogo College of Medicine, Japan, [2]Division of Pathology and Cell Therapy, Chiba Cancer Center Research Institute, Chiba, Japan, [3]Department of Medicine, David Geffen School of Medicine, University of California Los Angeles, USA, [4]Department of Pathology, Hyogo College of Medicine, Nishinomiya, Japan.

Malignant mesothelioma is highly aggressive and generally non-curative. Therefore, new treatment paradigms are urgently needed. We have investigated the use of transcriptionally targeted oncolytic adenovirus as a novel therapeutic approach for this malignancy.
We found that midkine (Mdk), a developmentally important heparin-binding growth factor, was significantly increased in six mesothelioma cell lines tested by quantitative RT-PCR, but was low or undetectable in normal cells. Mdk promoter is also highly activated in mesothelioma cells by luciferase reporter assay. On this basis, we constructed a conditionally replicating adenovirus (CRAd), in which the adenoviral E1 gene is driven by the Mdk promoter and is armed with the HSV-thymidine kinase (TK) suicide gene, and which also carries the enhanced green fluorescent protein marker gene. This oncolytic adenovirus, designated CRAd-Mdk-E1-iresTK, was seen to efficiently replicate, produce viral progeny, and spread in multiple established mesothelioma cell lines. Tumor-selective lytic spread of CRAd-Mdk-E1-iresTK was observed to mediate efficient killing of these mesothelioma cells, and its cytocidal effect was significantly enhanced by treatment with the prodrug ganciclovir. Finally, intra-tumoral injections of the CRAd-Mdk-E1-iresTK caused complete regression of human mesothelioma xenografts in athymic mice. In vivo imaging demonstrated intratumoral spread of CRAd-Mdk-E1-iresTK-derived fluorescence signals, which then vanished after tumor eradication.
In conclusion, the Mdk promoter is a feasible tumor-specific promoter for transcriptional targeting, and Mdk promoter-driven CRAd might be a promising general strategy for oncolytic virotherapy of Mdk-upregulated cancers.

WS07

Immunotherapy: bench to bedside-II

WS07-1

Antibody-dependent cellular cytotoxicity mediated by cetuximab against mesothelioma cells

Hiroki Chikumi[1], Jun Kurai[1], Naoki Kinoshita[1], Masaki Nakamoto[1], Shingo Matsumoto[1], Tadashi Igishi[1], Hironobu Hamada[3], Seiji Yano[2], Eiji Shimizu[1]

[1]Division of Medical Oncology and Molecular Respirology, Department of Multidisciplinary Internal Medicine, Tottori University, Japan, [2]Division of Medical Oncology, Cancer Research Institute, Kanazawa University, [3]Department of Integrated Medicine and Informatics, Ehime University Graduate School of Medicine

Purpose Epidermal growth factor receptor (EGFR) is expressed on the cell surface of mesothelioma and thought to be a possible new therapeutic target. Cetuximab is a chimeric mouse-human antibody targeting the extracellular domain of EGFR and now approved in colorectal cancer and head and neck cancer. Cetuximab directly inhibits the growth of the cancer cells by inhibiting EGFR activation or by inducing receptor internalization and degradation. In addition, we have recently reported the significance of an immunological anti-cancer mechanism called antibody-dependent cellular cytotoxicity (ADCC) in colon and lung cancer cells. However, no publish studies have focused on the immunological activity of cetuximab against mesothelioma cells. In this study, we investigated the biological activity of cetuximab against a panel of mesothelioma cells with respect to ADCC activity. **Methods** EGFR expression of mesothelioma cells was measured by quantitative flow cytometirc analysis. The direct effect of cetuximab was estimated by MTS assay. The ADCC activity of cetuximab was assessed by a 4h-^{51}Cr release assay. The in vivo effect of cetuximab against mesothelioma was estimated using mouse-xenograft model.**Results** Although cetuximab did not show any growth inhibitory effect against mesothelioma cells with MTS assay, it exhibited significant ADCC activity against these cells. A logarithmic correlation was observed between the number of EGFR expressions and ADCC activity and this activity was enhanced by interleukin-2 (IL-2). Cetuximab significantly inhibited intrathoracic mesothelioma growth in the mice, and this inhibition was enhanced by the IL-2 administration to the thoracic cavity.**Conclusions** These observations suggest the possibility of a novel and effective therapy against mesothelioma by using cetuximab and its ADCC activity.

WS07-2

Expression and regulation of B7-H3, a new member of the B7 family of immunoregulatory receptors, in human mesothelial and mesothelioma cells: immunotherapeutic implications

Luana Calabro[1], Luca Sigalotti[2], Ester Fonsatti[1], Anna Maria Di Giacomo[1], Riccardo Danielli[1], Alessia Covre[2], Luciano Mutti[3], Pier Giorgio Natali[4], Michele Maio[1,2]

[1]Medical Oncology and Immunotherapy, Department of Oncology, University Hospital of Siena, Italy, [2]Cancer Bioimmunotherapy Unit, Department of Medical Oncology, Centro di Riferimento Oncologico, Istituto di Ricovero e Cura a Carattere Scientifico, Aviano, Italy, [3]Lab of Clinical Oncology, Hospital of Vercelli, Vercelli, Italy, [4]CINBO Laboratories, University of Chieti, Chieti, Italy.

No treatment prolongs the survival of malignant mesothelioma (MM) patients. Since MM elicits anti-tumor immune responses, immunotherapy represents a promising strategy for its control. Immunomodulatory antibodies against components of the B7 family of immunomodulatory molecules that regulate T cell activation are being investigated in human malignancies including MM. The expression of B7-H3, a new component of the B7 family was investigated in primary cultures of human mesothelial cells (HMC) and in MM cell lines by flow cytometry and molecular analyses, and in MM tissues by immunohistochemistry. The role of DNA hypomethylating agents in modulating levels of B7-H3 expression in MM cells was also studied.Reverse transcriptase-polymerase chain reaction (RT-PCR) demonstrated that B7-H3 mRNA was consistently detectable in mesothelial and MM cells investigated; however, real-time quantitative RT-PCR analyses showed highly heterogeneous levels of B7-H3 mRNA among investigated MM cells. The analysis of B7-H3 protein expression indicated that comparable levels of B7-H3 were expressed on both cell types. Treatment with the DNA hypomethylating agent 5-aza-deoxycytidine did not significantly affect the expression of B7-H3 mRNA in MM cells. In vivo, while B7-H3 was expressed in all 13 tumor biopsies of the epithelial variant, with high levels in 58% of cases, it was rarely detectable in spindle type MM in which 1/5 biopsies weakly expressed B7-H3. These findings suggest that B7-H3 is a promising target for new immunotherapeutic strategies in MM, with particular emphasis in the epithelial variant.

WS07-3

Oncolytic activity of measles virus against mesothelioma: potential role of the immune system

Nicolas Boisgerault[1], Jean-Baptiste Guillerme[1], Daniel Pouliquen[1], Frederic Tangy[2], Marc Gregoire[1]

[1]INSERM U892, Cancer Research Center, Nantes, France, [2]Institut Pasteur, CNRS-URA 3015, Viral Genomics and Vaccination Laboratory, Paris, France

Cancer virotherapy has recently emerged as a hopeful alternative therapeutic strategy in the aim of better responding to the diversity of cancerous pathologies. This approach is based on the preferential tropism of certain viruses for tumor cells; as an example, such property is exhibited naturally by an attenuated vaccine strain of measles virus (MV). Oncolytic MV targets CD46 complement regulatory molecule that is overexpressed in numerous cancers and especially in mesothelioma. We previously showed that MV was able to efficiently infect and kill mesothelioma tumor cells while other studies had already shown similar results against lymphoma, multiple myeloma, glioma, ovarian, prostate and breast cancers. We also demonstrated that infected mesothelioma cells were able to induce spontaneous maturation of myeloid dendritic cells and subsequently to prime mesothelin-specific T CD8 response. More recently, we found that MV exhibited oncolytic properties in vivo in mice model against human melanoma, lung adenocarcinoma and colorectal adenocarcinoma. Similar experiments are currently in progress concerning mesothelioma and our preliminary results are very encouraging. Interestingly, we found that MV induces an immunogenic apoptosis of mesothelioma cells associated with HSP70 induction, calreticulin translocation to cell surface and HMGB1 release in vitro. These molecules are expected to play an essential role in the activation of the adaptive immune response by acting directly on dendritic cells. The immune side of cancer virotherapy remains poorly documented, but it offers exciting outlooks in order to combine direct viral oncolysis with long-term protection by enhancing a potential cancer-specific immune memory.

WS07-4

Fiber-modified replication-competent adenoviruses powered by transcriptional regulatory region produced anti-tumor effects on mesothelioma and the differential cytotoxicity is linked with the replication and host cell mechanisms

Masatoshi Tagawa[1], Kiyoko Kawamura[1], Yuji Tada[2], Makako Yamanaka[2], Shinya Okamoto[3], Shan Yang[1], Suguru Yamauchi[1], Hiroshi Kobayashi[3], Shuji Kubo[4], Yuichi Takiguchi[2], Koichiro Tatsumi[2], Hideaki Shimada[5], Kenzo Hiroshima[6]

[1]Division of Pathology and Cell Therapy, Chiba Cancer Center Research Institute, Japan, [2]Department of Respirology, Graduate School of Medicine, Chiba University, Chiba, Japan, [3]Department of Biochemistry, Graduate School of Pharmaceutical Science, Chiba University, Chiba, Japan, [4]Laboratory of Host Defenses, Institute for Advanced Medical Sciences, Hyogo College of Medicine, Nishinomiya, Japan, [5]Department of Surgery, School of Medicine, Toho University, Tokyo, Japan, [6]Department of Pathology, Tokyo Women's Medical University Yachiyo Medical Center, Chiba, Japan

We prepared recombinant type 5 adenoviruses (Ad5) in which the E1A expression was regulated by an exogenous regulatory region of a gene, whose expression is up-regulated in mesothelioma cells. The Ad preferentially proliferated in mesothelioma cells and produced cytotoxic effects. Infectivity of Ad5 to cells is primarily mediated by the interaction between their fibers-knob regions and the coxsackievirus and adenovirus receptor (CAR) on target cells. Down-regulated CAR expression, often found in human mesothelioma, hampered Ad5-mediated gene transfer and the Ad5-mediated cytotoxicity. We then replaced the fiber-knob structure with that of type 35 Ad, which use CD46 molecules as their cellular receptors. The chimeric Ad5 with the same E1A transcriptional unit and the substituted fiber-knob structure infected mesothelioma better than Ad5 and subsequently achieved greater anti-tumor effects to mesothelioma in particular those with CAR-low expression. Subsequent investigations also demonstrated that the cytotoxicity was not only correlated with the infectivity but dependent on cell types tested. We examined possible mechanisms with two representative cells, Ad sensitive and insensitive cell lines with similar infectivity. The sensitive cells produced greater E1A and the late viral protein, and better viral progenies than the insensitive cells. Several cellular protein expressions including NF-1, TFIID, B23 and Topo-1, all of which are associated with Ad DNA replication, were not directly linked with the cytotoxicity except NF-1. Type I interferon and anti-viral molecules, MxA and 2,5-OAS, were constitutively expressed in the insensitive but not the sensitive cells. These data suggest that cytotoxicity of the Ad is also attributable to viral replication activities and host defense mechanisms.

WS07-5

Replication-competent E1B-55kDa deleted adenoviruses induce p53 up-regulation and apoptosis with mitosis arrest in mesothelioma cells

Yuji Tada[1], Makako Yamanaka[1], Kiyoko Kawamura[2], Shinya Okamoto[3], Shan Yang[2], Suguru Yamauchi[2], Hiroshi Kobayashi[3], Yuichi Takiguchi[1], Koichiro Tatsumi[1], Hideki Shimada[4], Kenzo Hiroshima[5], Masatoshi Tagawa[2]

[1]Department of Respirology, Graduate School of Medicine, Chiba University, Japan, [2]Division of Pathology and Cell Therapy, Chiba Cancer Center Research Institute Chiba, Japan, [3]Department of Biochemistry, Graduate School of Pharmaceutical Science, Chiba University, Chiba, Japan, [4]Department of Surgery, School of Medicine, Toho University, Tokyo, Japan, [5]Department of Pathology, Tokyo Women's Medical University Yachiyo Medical Center, Chiba, Japan

E1B-55kDa molecules of adenoviruses (Ad) inhibit p53 functions and Ad deficient of the molecules (Ad-delE1B55) proliferate preferentially in p53-mutated cells rather than in p53 wild-type cells. The mechanism of Ad-delE1B55-mediated anti-tumor effects remains poorly understood but a number of previous studies, although empirically, showed the elevated cytotoxicity to tumors cells. We then examined the possible cytotoxicity to human mesothelioma cells, most of which possess the wild-type p53 gene but lack p14/p16 genes. Ad-delE1B55 inhibited proliferation of 5 kinds of mesothelioma cells with the wild-type p53 gene and the suppressed growth levels in respective cells were in part correlated with the Ad infectivity. Ad-delE1B55 up-regulated p53 protein expression and induced the phosphorylation at Ser 15 and Ser 46 in the infected cells, and subsequently the MDM2 expression increased. Expression levels of p21 and p27 were down-regulated and caspase-8, -9 and -3 were cleaved although Fas and FADD expressions were not modulated by the Ad-delE1B55. These data collectively indicate that Ad-delE1B55 activate p53-mediated apoptosis pathways and imply possible activation of mitochondrial-mediated apoptosis. Cell cycle analyses however showed not only increased sub-G1 fractions in Ad-delE55-infected mesothelioma cells but also a greater fraction that had more than 4 N. Expression levels of MAD2, which inhibits Cdc20 and chromosome segregation, was however unchanged. The combinatory use of Ad-delE1B55 with an anti-cancer agent produced additive cytotoxic effects. Intrapleural injection of Ad-delE1B55 into mice that were inoculated with mesothelioma cells inhibited the tumor growth in vivo settings. These data suggest that Ad-delE1B55 is a possible therapeutic agent for mesothelioma.

WS08

Immunotherapy: bench to bedside-III

WS08-1

A phase I clinical trial of two-dose intrapleural IFN-α gene transfer for malignant pleural mesothelioma

Edmund Moon[1], Andrew Haas[1], Adri Recio[1], Joseph Friedberg[2], Richard Carroll[3], Anil Vachani[1], Sharyn Katz[4], Steven Albelda[1], Daniel Sterman[1]

[1]Thoracic Oncology Research Group, University of Pennsylvania, USA, [2]Department of Thoracic Surgery, University of Pennsylvania, [3]Abramson Family Cancer Research Institute, University of Pennsylvania, [4]Department of Radiology, University of Pennsylvania

Previously conducted Phase I trials studying the effects of Ad.IFN-β (supplied by Biogen Idec) in malignant pleural effusions and malignant pleural mesothelioma (MPM) showed it to be safe/well tolerated, effective in local gene transfer and inducing a humoral immune response, and associated with good anecdotal clinical responses. We present data from the most recent bridging study involving intrapleural Ad.IFN-α2b (supplied by Schering-Plough/Merck) instillation in MPM patients to see if Ad.IFN-α2b is safe/well-tolerated, there is an advantage to shortening the dose interval, there is humoral anti-tumor immunity induction, and there is a clinical response.7 MPM subjects who progressed through prior anti-neoplastic therapy or had refused therapy received 1e12 Ad.IFN-α2b viral particles (vp) (1st cohort) or 3E11 Ad.IFN-α2b vp (2nd cohort after dose de-escalation) intrapleurally on Days 1 and 4. Subjects had pre/post serum and pleural fluid sampling to measure IFN-α2b levels (ELISA) and Ad neutralizing antibody (NAb) level. Western Blot was performed to study anti-tumor humoral responses. Clinical follow-up included routine clinical examinations and CT/PET imaging.No DLT/SAEs observed in any patient. All patients developed significant Ad NAb levels by day 7 after the 2nd vector dose. All patients had detectable IFN-a2b levels in their serum/pleural fluid after each dose. Peak IFN-a2b pleural fluid levels after dose 1 was 150.82ng/ml to 1906.79ng/ml (1st cohort) and 11.09ng/ml to 127.75ng/ml (2nd cohort) . Immunoblots revealed anti-tumor humoral responses using pre/post gene transfer patient serum. Pre/post FDG-PET imaging in some subjects revealed significant intrapleural tumor regression. At 3 month follow up, 3 had stable disease, 2 had progressive disease, and 1 had a mixed response. At 6 month follow up, 1 had stable disease, 2 had progressive disease, and 1 had partial response. A small Phase 2 trial combining Ad.IFN with first or second-line chemotherapy for patients with MPM.

WS08-2

Systemic and tumour-associated T cells specific for 5T4 and other tumour antigens in mesothelioma and the design of a clinical trial with TroVax(R)

Zsuzsanna Tabi[1], Jason F Lester[2], Richard Harrop[3], Malcolm D Mason[1], Saly Al-Taei[1]

[1]Cancer Immunology Research Group, Department of Oncology, School of Medicine, Cardiff University, UK, [2]Velindre NHS Trust, Whitchurch, Cardiff, [3]Oxford BioMedica UK Ltd., Oxford, UK

We have identified 5T4, a 72 kDa oncofetal antigen, by flow cytometry on mesothelioma cells present in pleural fluid (7/7) and on 11/11 cell lines established from mesothelioma biopsies. Preliminary results of western blotting confirmed the presence of 5T4 in frozen tumour tissues (13/16). 5T4 is a common tumour antigen, also present on colorectal, renal and prostate cancer cells, and a vaccine, encoding for 5T4 in an attenuated vaccinia vector (TroVax®)is undergoing clinical trials in these cancers. We have established an immunomonitoring cytokine flow cytometry protocol to detect antigen-specific T cell responses in the blood and pleural fluid of mesothelioma patients. CD8+ and CD4+ T cell responses were detected against peptides, derived from the 5T4 antigen, both in the peripheral blood and pleural fluid of patients. T cell responses were also detected against peptides derived from folate receptor-alpha, and a group of tumour antigens including MUC-1, NY-ESO1, MAGE1, p53 and Her-2. The frequency of T cell responses to common viral peptides (cytomegalovirus, EBV and influenza) were comparable to that in healthy controls, confirming systemic immunocompetence of mesothelioma patients. The frequencies of anti-tumour or anti-viral T cell responses were not consistently different in the pleural effusion than in peripheral blood, although the specificity pattern of anti-tumour responses can be different between these sites indicating either specific local T cell activation or selective recruitment/retention of certain T cells at the tumour site. Taken together, mesothelioma patients have systemic and tumour-associated T cells specific for tumour antigens, indicating immune engagement against the tumour and also immune competence of patients. These observations indicate that mesothelioma patients are likely candidates for immunotherapies. We are in the process of setting up a phase I/II clinical trial in mesothelioma patients with TroVax® cancer vaccine in combination with the standard pemeterexed /cisplatin chemotherapy.

WS08-3

Intratumoral immune stimulation combined with reduction of immunosuppressive factors prevents disease progression after debulking surgery in malignant pleural mesothelioma

Jarrod Predina, Louis Aliperti, Veena Kapoor, Aaron Blouin, Natalie Ridge, Daniel Sterman, Steve Albelda, Sunil Singhal

University of Pennsylvania School of Medicine, USA

Background: Surgical debulking is an established therapy for malignant pleural mesothelioma (MPM), however, the majority of patients die within 2 years due to disease progression. We hypothesized that immune surveillance of recurrent disease fails because of inadequate post-operative anti-tumor immune responses and persistently elevated systemic tumor immunosuppression. Our goal was to improve the anti-tumor immune response by simultaneously stimulating cytotoxic T lymphocytes and reducing suppressive immune factors. Materials and Methods:MPM (AB12 and AE17) cells were injected subcutaneously into the flanks of immunocompetent syngeneic mice (n=114) in several experiments. Two models of surgical debulking were evaluated: one using a positive margin and one a rechallenge approach. Neoadjuvant intratumoral immune stimulation was performed utilizing immuno-gene therapies such as Ad.HSVtk and Ad.IFNalpha. T regulatory cell (Treg) depletion was accomplished using anti-CD4 antibodies, and TGFbeta activity was blocked with a neutralizing antibody. Mice were monitored for recurrence and overall survival. Leukocyte populations were characterized prior to and following surgery using flow cytometry and functional assays. Results:Tumors recurred rapidly in all animals after surgery. When surgery was combined with neoadjuvant intratumoral immunostimulation (Ad.HSVtk, Ad.IFNalpha) with reduction of suppressive factors (Tregs, TGFbeta), the time to tumor recurrence was significantly prolonged compared to controls with surgery alone. Trimodal approaches combining surgery, CD8+ T lymphocyte stimulation and reducing suppressive factors had the most favorable prolongation of disease recurrence. As many as 40% of animals were cured of all disease burden in several combined therapies. Conclusions:Our data suggests that combining immunotherapy approaches (improving anti-tumor CTL activity and reducing tumor derived immunosuppression) with debulking surgery prolongs time to disease progression in murine models. Application of similar approaches in patients (clinical trials with Ad.INF and anti-TGFbeta antibody are currently underway in our institution) could have a similar beneficial effect.

LS

Treatment and prophylaxis of MRSA infections in patients with thoracic cancer

Yoshio Takesue
Department of Infection Control and Prevention, Hyogo College of Medicine, Japan

1. **Bacteriology of thoracic empyema:** Pleural infection has a significant morbidity and mortality of over 15%. In addition, up to 40% of patients with complicated pleural infection require surgical intervention. In community-acquired thoracic empyema, the most commonly isolated pathogen was *Streprococcus* spp. By contrast, hospital-acquired infection includes more *Staphylococcal* (MRSA 28%), *Enterococcus* and Enterobacteriaceae infections. Routine bacterial culture from pleural fluids often negative. The majority of patients are therefore treated with empirical antibiotics. Empirical antibiotic therapy for hospital-acquired thoracic empyema should be effective against these multidrug-resistant organisms including MRSA
2. **Vancomycin (VCM) minimum inhibitory concentration (MIC) creep in MRSA:** There is growing concern that VCM may provide suboptimal therapy for severe MRSA infections and it has been suggested that this may be related to increases in MICs. These MIC increases over time were not reliably detected by percentage susceptibility as they occurred below the susceptibility breakpoint. Recent MIC creep in VCM has prompted guidelines to recommend a VCM target trough of 15 to 20 μg/mL. In addition, a reduction in the efficacy of VCM against MRSA strains with a high MIC (2 μg/mL) has been described. Independent predictors of mortality in multivariate analysis in patients with MRSA bacteremia included the receipt of empirical VCM and having an isolate with a vancomycin MIC of 2 μg/mL. Therapy with alternatives to VCM such as linezolid should be considered for invasive MRSA infections caused by these strains.
3. **Impact of rapid screening tests to prevent postoperative MRSA infections:** PCR based systems are now available that detect MRSA within 1 day. It is hypothesized that rapid detection of MRSA carriers will lead to faster implementation of control procedures, reducing the endogenous infections and transmission of MRSA to other patients. Recently it was reported that rapid identification of *S.aureus* nasal carriers by means of a real-time PCR assay, followed by treatment with mupirocin nasal ointment and chlorhexidine soap, reduced the risk of surgical site infections (SSI).
4. **Preoperative skin cleansing with 2% chlorhexidine-alcohol:** Since the patient's skin is a major source of pathogens that cause SSI, optimization of preoperative skin antisepsis may decrease postoperative infections. Preoperative skin cleansing with 2% chlorhexidine-alcohol was reported to be more protective against both superficial incisional infections and deep incisional infections than is povidone-iodine.

LS01-1

Histone Deacetylase Inhibitors in Malignant Pleural Mesothelioma: Pre-clinical Rationale and Clinical Trials

Lee Krug
Memorial Sloan Kettering Cancer Center, USA

Malignant pleural mesothelioma (MPM) is a rare and aggressive cancer that is increasing in incidence. Because the majority of patients present with advanced disease, mortality is high, and median overall survival is <12 months. Current treatment options are limited and largely ineffective for prolonging survival. Thus, there is an urgent need for effective treatments for patients with MPM.
Epigenetic regulation of tumor suppressor genes has emerged as an important mechanism in cancer, with histone acetyltransferases and deacetylases playing an important role in the regulation of chromatin condensation and gene transcription. An increasing body of evidence has demonstrated the effectiveness of histone deacetylase (HDAC) inhibition in MPM cell lines and mouse xenograft models. Although in vitro data have focused on apoptosis, HDAC inhibitors have also been shown to regulate the acetylation of signaling intermediates and transcription factors, thereby promoting cell cycle arrest and inhibition of angiogenesis.
Activity has also been observed in early-phase clinical trials in patients with MPM. Taken together, the results of these efforts have led to a phase III, multicenter, randomized, placebo-controlled study (Vantage 014) evaluating vorinostat (a first-in-class HDAC inhibitor approved in 2006 for the treatment of advanced cutaneous T-cell lymphoma) plus best supportive care in patients with advanced MPM for whom prior chemotherapy with pemetrexed and either cisplatin or carboplatin has failed. Study endpoints include overall survival, objective response rate, progression-free survival, and patient-reported outcome measures; molecular profiling studies are also being conducted in an effort to identify patients most likely to benefit from vorinostat treatment.
In summary, several lines of evidence support the clinical evaluation of HDAC inhibitors in patients with MPM. Hopefully, the results of Vantage 014 and other studies will demonstrate a clinical benefit with treatment, providing additional therapeutic options for patients with MPM and filling an urgent unmet medical need.

LS01-2

Apoptosis regulation & drug resistance in mesothelioma: Future treatment strategies

Dean Fennell

Cancer Research UK Clinician Scientist Consultant and Reader in Thoracic Medical Oncology Belfast Cancer Research UK Centre Northern Ireland

New, more effective strategies for treating mesothelioma are urgently needed if survival rates are to improve. Systemic therapies achieve disease control in part by inducing tumour cell kill, as well as slowing proliferation. Evasion of apoptosis is both a hallmark of cancer and a characteristic of mesothelioma that has historically, limited clinical success, as reflected in low response rates particularly in the relapsed setting. However, preclinical evidence suggests that in common with other more common cancers, mesotheliomas exhibit a functional core death machinery which can be targeted. This can be exploited pharmacologically and will be be discussed. Opportunities include 1) utilisation of known and novel putative biomarkers to identify patients likely to be resistant to conventional therapies or conversely, hypersensitive to novel therapies,eg, those that exploit synthetic lethality 2) direct targeting of the caspase executioner pathway, using death receptor or epigenetic therapy, and 3) targeting vital survival pathways both empirically and rationally to trigger mesothelioma apoptosis via the mitochondrial pathway.

LS02

Adverse respiratory complications of cancer chemotherapy: Recognition and intervention

Daniel Sterman

Section of Interventional Pulmonology and Thoracic Oncology, Pulmonary, Allergy, & Critical Care Division, University of Pennsylvania Medical Center, USA

As many as 10% of patients receiving chemotherapeutic agents will develop an adverse drug reaction in their lungs. The most common drugs resulting in lung toxicity are Bleomycin, Methotrexate, Carmustine, Busulfan, and Cyclophosphamide. Chemotherapeutic drugs can result in four main types of lung reaction: interstitial pneumonitis and fibrosis, hypersensitivity reaction, acute respiratory distress syndrome, and bronchiolitis obliterans organizing pneumonia [BOOP/COP]. The high-resolution CT findings of chemotherapeutic drug–induced lung disease reflect the histologic findings. Interstitial pneumonitis and fibrosis result in ground-glass opacities, focal areas of consolidation, and irregular linear opacities that tend to involve the lower zones of the lungs. This is the most consistent finding with cytotoxic chemotherapeutic agents, particularly Bleomycin.

Many of the novel agents used to treat thoracic malignancies in the present day can also cause pulmonary toxicity. Paclitaxel and other taxanes have been described to cause inflammatory pneumonitis, as has Gemcitabine. These are generally reversible with corticosteroids and drug withdrawal. There have even been occasional reports of inflammatory pneumonitis secondary to Pemetrexed, including in treatment of malignant mesothelioma.

In the case of the oral epidermal growth factor tyrosine kinase inhibitors (EGFR-TKIs), there have rare, been but statistically significant occurrences of interstitial lung disease (ILD). These typically manifest as an acute onset of dyspnea, usually over a 24-48 hour time frame resulting from an acute inflammation of lung tissue. In studies of Gefitinib, ILD rates occur in about 0.3%-1% of the Unites States population, and about 2% of the Japanese patients. ILD rates in the BR.21 Erlotinib trial were less than 1%. There have also been case reports of pulmonary toxicity from Cetuximab, a monoclonal antibody directed against the EGFR receptor.

Pulmonary toxicity related to Bevacizumab (Avastin) has also been of significant concern, with episodes of massive hemoptysis with treatment of squamous cell carcinoma of the lung, and an increased risk of thromboembolic disease in patients with lung and other primary malignancies.

LS03

Malignant Mesothelioma

Bruce Robinson
National Centre for Asbestos Related Diseases, University of Western Australia, Australia

Malignant mesothelioma is an aggressive tumor of serosal surfaces such as pleura and peritoneum. The incidence of mesothelioma is increasing, including in Japan, likely owing to asbestos exposure, with which it is associated . The economic burden from mesothelioma is predicted to cost > $300 billion.

Advances in diagnosis: The most usual *pathological* diagnostic problem is the differentiation of mesothelioma from reactive mesothelium, and sometimes also from adenocarcinoma. Cytological evidence of mesothelioma in pleural/ascitic relies on immunohistochemical markers, the best of which include calretinin or WT1 (to determine if the tissue is mesothelial) then epithelial membrane antigen (EMA) to determine if it is malignant. Tumor biopsy is often needed. *Positron Emission Tomography* (PET) is useful to distinguish benign from malignant pleural masses, for identifying extrathoracic disease and for predicting response to therapy. *The best biomarkers* for serum and effuson fluid is SMRP, is a soluble form of mesothelin. DNA microarray analysis of a small number of genes discriminates between mesothelioma and lung cancer.

Advances in therapy:*Surgery* is useful for palliation but the role of debulking surgery versus radical resection (extra-pleural pneumonectomy), is uncertain. *Pemetrexed or gemcitabine* plus cisplatinum improve overall median survival with objective responses. *Imatinib, gefitinib* and *anti-angiogenic agents* have not proven useful. Intensity modulated radiotherapy (IMRT) is used in some centres. *Gene therapy* and *immunotherapy* remain experimental.

New methods of diagnosis and treatment have only arisen because of concerted laboratory and clinical studies, often done by international collaboration.

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LS04

Pulmonary Fibrosis and Asbestos

Françoise Galateau-Sallé
MESOPATH center- INSERM ERI3-Department of Pathology CHU Caen 14 033 Caen cedex. France

The American Thoracic Society (ATS) consensus statement published in 2000, linked Usual Interstitial Pneumonia (UIP) to Idiopathic Interstitial Fibrosis (IPF). The definition of IPF is a chronic fibrosing interstitial pneumonia associated with the histological appearance of UIP on surgical lung biopsy. The final diagnosis is based on clinical-radiological-pathological approaches. In March 2010, the Asbestosis Committee (an international committee of North American, European, and Australasian pathologists) of the college of the American Pathologists and Pulmonary Pathology Society defined asbestosis as diffuse pulmonary fibrosis caused by the inhalation of asbestos fibers. All type of asbestos have been implicated with a dose-response relationship between the concentration of fibers in the lung and the severity of the fibrosis. The diagnosis of asbestosis is usually based on a previous history of heavy asbestos exposure, clinical-radiological findings (end-inspiratory crackles and presence of linear diffuse opacities predominant in the lower zones on chest X-ray/CT scan). The disease may progress even after exposure has ceased but it is relatively slow. Histologic diagnosis is not mandatory, but histology is most useful when the clinical and radiological background are atypical or when asbestos exposure is equivocal and a surgical biopsy is carried out. The major differential diagnosis is between asbestosis and other interstitial disorders, particularly IPF. Histologically, fibrosis in asbestosis is always paucicellular, lacks inflammation and does not usually show fibroblastic foci. Early asbestosis is a fibrosing process limited to the walls of alveoli around the bronchioles that later extends outward to link fibrosis extending from adjacent bronchioles. Asbestosis is most severe subpleurally and is characterized by a lower lobe and peripheral distribution. The mechanisms underlying asbestos-induced lung injury are not totally understood, even if the critical determinants of fiber bioactivity has been extensively investigated during the last two decades. There is some evidence showing that alveolar epithelial cell apoptosis is a crucial pathological event leading to pulmonary fibrosis. In addition several other mechanisms have been reported including asbestos–induced ROS production and DNA damage, or asbestos- induced mitochondrial dysfunction, or intrinsic apoptosis and p53 activation. It is of major importance to decipher the molecular basis for asbestos related-lung diseases and IPF in order to develop novel treatment strategies.

S01

Epidemiology

S01-3 **Keynote Speaker**

Mesothelioma, a global panoramic view

Ken Takahashi

University of Occupational and Environmental Health, Japan

More than 92,000 mesothelioma deaths (C45, ICD-10) have been recorded in the WHO Mortality Database from 1994-2008. An additional 330+ cases have been recorded as malignant neoplasm of the pleura (163, ICD-9). These deaths occurred in 56 countries, mostly the developed countries of the West. We estimate the 15-year cumulative mesothelioma during 1994-2008 in the 56 countries to be 174,300. The leading countries are the USA (36,600), the UK (28,400), Italy (18,500), Germany (16,000) and France (12,400).
In the above countries diagnosing, recording and reporting mesothelioma, the crude mortality rate (CMR) and age-adjusted mortality rate (AAMR) for mesothelioma are 6.7 and 5.1 per million, respectively. The mean age at death is 69.6 yr (SD 11.6 yr) which is only slightly higher for females. By anatomical site, the pleura (41%) and unspecified (43%) far outnumber the peritoneum (4.5%) and pericardium (0.3%) although the peritoneum is more than twice common among females (7.8% in females vs. 3.6% in males). The male to female (M/F) ratio for all mesothelioma is 3.6.
There is a statistically significant positive linear relation between the log-transformed national cumulative numbers and the log-transformed cumulative asbestos use (in metric tons) with adjusted $R^2=0.83$ ($p<0.0001$) in the group of 56 countries. This relationship can be used to "predict" the number of unreported mesothelioma cases in the 33 countries with no mesothelioma data but which have recorded asbestos use. These countries are primarily developing countries of the non-West. We predict this number to be 38,900 (95%CI = 36,700-41,100). Therefore, globally, one case for every 4 to 5 reported cases of mesothelioma, possibly occurred but was overlooked.
The 89 (56+33) countries analyzed here represent 83% of the world's population.

S01-4 **Keynote Speaker**

Epidemiology of mesothelioma in Egypt and Mediterranean countries

Rabab Gaafar

Department of Medical Oncology, National Cancer Institute, Cairo University, Egypt

Mesothelioma remains a universally fatal disease of increasing incidence worldwide. Asbestos exposure is the most common risk factor linked to MPM. The Simian virus (SV40) has been implicated as a potential etiologic factor. There is substantial interest in this disease on the part of the medical community and the general public, because millions of people have been exposed to asbestos fibers, and many articles about the dangers of asbestos have appeared in the press.There is substantial concern that the increased use of asbestos in developing countries may result in an increase in the number of cases of malignant mesothelioma for many decades to come unless strong occupational health controls are put in place.In Egypt, the incidence of MPM is rising dramatically and according to the National Cancer Institute (NCI) hospital-based registry, an increase in frequency from 0.47% during 2001 to 1.3 during 2003 has been observed. The median age has been reported to be below 50 years with an almost equal distribution among males and females. There are around 14 factories utilizing asbestos are in Egypt, and what makes the situation peculiar is the presence of these factories in residential areas. Residents of such residential areas like "Shobra El-Khaymah "and "Hadayek Helwan "remain at a high risk for both environmental as well as occupational asbestosis. - Despite technical difficulties, the process of replacing asbestos with safer materials, especially in small informal workshops, is essential to prevent further release of asbestos into the environment.Differences between East Mediterranean countries and Western countries will be highlighted. Management of asbestos-related disease in the workplace requires collaboration between workers and unions and companies (responsible for engineering controls), reinforced by appropriate government regulations and by community support.

S01-5 **Keynote Speaker**

The epidemiology of 3 asbestos ores and 3 disease profiles in a South African compensation database

Jim teWaterNaude

Asbestos Relief Trust, South Africa and School of Public Health, University of Cape Town, South Africa

Introduction
Two Trusts were set up to compensate people who had contracted asbestos-related diseases as a direct result of past mining of all three commercial types of asbestos in rural areas of South Africa.

Method
Starting in 2004, claims handlers were commissioned to actively find ex-miners, and get them to local accredited general practitioners and hospitals for chest radiography, spirometry, medical examination, and other tests where needed. Completed files were couriered to a panel of radiologists and occupational medicine specialists, who read the radiographs according to the ILO system, and adjudicated the case for compensation, or requested new tests for reassessment when done.

Results
Of 15 731 claimants, the results of 12 346 occupational claimants were usable for this analysis. 84.1% were male and 1238, 9624 and 1484 respectively had predominantly amosite, crocidolite and chrysotile exposure, working for respective medians (IQRs) of 8 (4-15), 2 (1-5) and 9 (4-15) years. Median age at presentation was 52 years (IQR 46-61), with age at first exposure being 22 years (19-28), and latency (time since first exposure) 28 years (23-32), with no differences by asbestos type.

Overall 34.8% of claimants (95% CI 33.9 - 35.6) had asbestos related pleural disease or asbestosis being 55.5%, 33.1% and 27.9% respectively for amosite, crocidolite and chrysotile. Asbestosis (1/0 or greater) was respectively found in 59.8%, 30.4% and 36.9%, and pleural disease in 67.9%, 27.6% and 17.4% respectively. In adjusted logistic regression, years worked and latency gave increased odds of disease of 6% and 5% per year respectively, with the toxicity being amosite>>croci dolite>chrysotile.

There were 238 mesotheliomas and 70 lung cancers found, with only one of these not exposed to crocidolite.

Discussion
Despite the inherent biases of a compensation database, amosite is clearly the most fibrogenic, and crocidolite clearly the most mesotheliomagenic.

S01-1

Global epidemiology of malignant pediatric mesothelioma

Sergey Kashanskiy[1], Nicolas Andre[2]

[1]The laboratory of occupational medicine, Yekaterinburg Medical Research Center, Russia, [2]Hopital pour enfants de La Timone, Marseille, France

Object: definition of approaches for the studying of the epidemiology of malignant pediatric mesothelioma (MPM), the rare nosological form. Methods: authors realized the monitoring in different databases of publication with the term mesothelioma. In result it was chosen 414 publications where the upper age limit of patients was 20 years inclusively. The cases were analyzed by country, year of diagnosis, sex, age at the date of diagnosis, localization, histological type, asbestos exposure. Results: We collected 499 cases of MPM, published from 1880 to 2010 on medical literature in 46 countries in 17 languages. Majority of cases were registered in Europe (31.5%) and USA (29.3%). The quantity of published cases of MPM in 20th century raises exponentially (R2=0.922) and in the beginning of 21th century it reached 10 cases in the year all around the World. In 58.7% of cases MPM localized in pleura, in 22.2% - in peritoneum, in 7.6% - in pericardium and in 5.8% - in tunica vaginalis testis, in two cases - in ovary and in one case - in mediastinum. Morbidity rate on sex (boys:girls): 1.2/1, but in peritoneal mesothelioma: 1:2.1. The average age is 12.6 years (ranged from 16 days to 20 years). The most popular histological type is epithelial (63%).In 181 (36.3%) cases there wasn't asbestos exposure, and in 309 (61.9%) cases asbestos wasn't considered as etiological factor. Only in 9 (1.8%) cases there was exposure to natural and synthetics fiber minerals. Cartographical analysis of frequency distribution of cases didn't show abundant number of MPM in regions, where there are mines and enterprises on extraction and processing of asbestos. Conclusion: The quality of published in medical literature cases allows conducting large-scale retrospective clinical epidemiological national and international studies of MPM.Please share your experience with us: skashansky@yandex.ru

S01-2

Epidemiologic and clinicopathologic analysis of malignant mesothelioma in Korea during past four years (from 2006 to 2009)

Soon-Hee Jung[1,8], Minseob Eom[1], Hyoung-Ryoul Kim[2], Sang-Baek Koh[3], Suk-Joong Yong[4], Myoung Ja Chung[5,8], Chang Hun Lee[6,8], Ji Sun Song[7,8], the Members of the Cardiopulmonary Study Group of the Korean Society of Pathologists[8]

[1]Department of Pathology, Yonsei University Wonju College of Medicine, Korea, [2]Department of Preventive Medicine and Industrial Medical Center, Catholic University College of Medicine, Seoul, Korea, [3]Department of Pathology, Chonbuk National University Medical School, Jeonju, Korea, [4]Department of Pathology, Pusan National University College of Medicine, Busan, Korea, [5]Department of Pathology, Kwandong University College of Medicine, Goyang, Korea, [6]The Cardiopulmonary Study Group of the Korean Society of Pathologists, [7]Department of Pathology, Kwandong University College of Medicine, Goyang, Korea, [8]The Cardiopulmonary Study Group of the Korean Society of Pathologists

Backbground: This study estimated the magnitude of malignant mesothelioma in Korea and its clinicopathologic features. We collected 244 cases through the Korea malignant mesothelioma surveillance system (KMMS) from 2006 to 2009. Method: Using the clinical information, epidemiologic survey and pathological evaluations collected by the members of the cardiopulmonary study group of the Korean Society of Pathologists, the patients' age, sex, occupation history, tumor sites, specimen type with diagnostic method, and histopathologic subtype during past four years were analyzed and compared with previous 5 year results. Results: Patients were 159 men (65%) and 85 women (35%). The average age of the patients was 60 years. Among 70 cases with available occupational history, 31 (44%) were related with asbestos exposure occupation. The distribution of sites was pleura (71%), peritoneum (27%), and pericardium (3%). Twenty patients were received a radical operation including extrapleural pleuropneumonectomy and others were diagnosed by the pathologic examination of biopsy, excision, or cytologic specimen. The pathologic subtypes were epithelioid (69%), biphasic (16%), sarcomatous (10%), and desmoplastic (3%), and variants (1%) in order. The epidemiologic and clinicopathologic data of past four years were not so different with that of previous 5 years data. However, we could estimate the accuracy rate (68%) of reporting cases of malignant mesothelioma after comparing the data of Health Insurance Institute with the pathologic review of those cases in the university hospital of Seoul (one) and Pusan (four) Area. According to this rate, about 100 cases of malignant mesothelioma could be occurred every year in Korea. Conclusion: The number of malignant mesothelioma patients and the relation rate with occupational asbestos exposure were relatively low. The surveillance system of malignant mesothelioma in Korea supplemented by an active surveillance system using death certificate data, cancer registry data, and central surveillance system by the government support could be helpful to understand the more exact status of malignant mesothelioma of Korea.

S02

Clinical tumor markers: the state of the art

S02-5

Keynote Speaker

Prognostication of mesothelioma: Isolation without integration

Harvey Pass, Anil Wali, Margaret Huflejt

Division of Thoracic Surgery and Thoracic Oncology, Department of Cardiothoracic Surgery, NYU Langone Medical Center, USA

Malignant Pleural Mesothelioma (MPM) is an unpredictable neoplasm, and therapy for this malignancy is far from standard. Surgery, chemotherapy, and radiation therapy are all used for the disease, yet determining who should have what therapy, and in what combination, cannot be determined as witnessed by the rates of attrition on multimodality therapy initiatives. Probably the most controversial aspect of the treatment is whether to put a patient through an operation with a 2-5% mortality and a 50% morbidity, only to see the patient fail within a period of 8-21 months depending upon how data is subgrouped for maximum "apparent benefit." The goal must be to be able to "select" patients accurately whose tumor biology will allow them to be in a category of patients for whom potentially high risk/morbid procedures should be performed because their disease is more amenable to such treatments. For patients whose tumors are so aggressive that their survival time does not justify such radical interventions, non-surgical options should be explored. This lecture will summarize the state of the art with regard to proteomic, immunohistochemical, genomic, epigenomic, glycomic, and microRNA prognostication of "potentially" resectable patients, and emphasize the lack of coordination between platforms and sites in order to both validate and improve the accuracy of such prognostic profiles.

S02-1

Circulating microRNAs as markers of malignant mesothelioma

Glen Reid[1], Lyn Schedlich[1], Michaela Kirschner[1], Nicola Armstrong[2], Alsion Reid[3], Nico van Zandwijk[1], Bill Musk[4]

[1]Asbestos Diseases Research Institute, Bernie Banton Centre, University of Sydney, Australia, [2]Peter Wills Bioinformatics Centre, Garvan Insitute of Medical Research, Darlinghurst NSW 2010, Australia, [3]Laboratory for Cancer Medicine, Western Australian Institute for Medical Research, University of Western Australia, Crawley WA6009, Australia, [4]School of Population Health, The University of Western Australia, Crawley WA6009, Australia

Malignant mesothelioma (MM) is often diagnosed in the later stages of the disease, due to the lack of a reliable early marker. Recent studies have shown that tumour cells secrete exosomes containing miRNAs into the circulation and the levels of these secreted miRNAs is linked to tumour load. The aim of this study is to characterise the miRNA content of exosomes derived from MM cells in order to identify candidate markers for early detection of MM. As a first step, exosomes were isolated by immunopurification from the conditioned medium (CM) of ATCC and primary MM cell lines. The presence of exosome-derived Hsa-miR-16 was detected by RT-qPCR, normalised against spiked-in *C. elegans* miRNAs and quantified with synthetic Hsa-miR-16 as standard. Hsa-miR-16 was detected in the CM derived from all cell lines at 1.1 to 4.9 x 10^7 copies/μl. After optimising RNA isolation procedures, Hsa-miR-15b, Hsa-miR-16 and Hsa-miR-24 were detected by RT-qPCR at comparable levels in the plasma of three normal individuals, at 3.7x10^5, 1.9x10^7 and 1x10^5 copies/μl, respectively. Initial results of experiments comparing exosomal and cellular miRNA profiles of MM cells, as well as those from plasma samples from MM patients will be presented. The candidate miRNAs identified will be assessed for their suitability as biomarkers of early disease using plasma samples from MM patients in the Asbestos Review Program, which have been collected yearly since 1990. This approach will allow the miRNAs found at high levels at time of diagnosis to be tracked over the previous years, linking appearance of miRNAs to tumour progression. The identification of plasma miRNAs associated with early stage MM has the potential to lead to improvements in treatment outcome for patients.

S02-2

Glycomics identifies diagnostic biomarkers and immuno-therapeutic targets of malignant mesothelioma

Margaret Huflejt[1], Marko Vuskovic[2], Naga Sri Samavedam[1], Nicolai Bovin[3], Harvey Pass[1]

[1]Department of Cardiothoracic Surgery, New York University, School of Medicine, USA, [2]Department of Computer Science, San Diego State University, San Diego, CA, [3]Shemyakin-Ovchinnikov Institute of Bio-organic Chemistry, Russian Academy of Sciences, Moscow, Russia

Malignant mesothelioma (MM) is a cancer usually caused by past exposure to asbestos, and is the single-biggest environmental carcinogen exposure-related health disaster in modern times, claiming over 200,000 lives annually worldwide. While a median survival is 8-18 months, recent studies show that patients with Stage I MM will have median survivals approaching 35 months, and it is therefore expected that the biomarkers detecting MM very early would make a major difference in therapeutic benefits, survival and overall quality of life.
Every cell in a body is coated by complex glycans attached to cell surface proteins and lipids. These elaborate complex macromolecules are generated in a process called glycosylation that involves synchronized activities of hundreds of genes and gene products. Genetic damage occurring during malignant transformation results in massive changes in cell surface and tissue glycosylation, these abnormalities are detected by immune system and multiple anti-glycan autoantibodies (AGAs) are generated.
We have developed printed glycan array (PGA) consisting of hundreds of glycans that detects a robust panel of anti-glycan autoantibodies (AGAs) in sera of MM- and asbestos-exposed at-risk patients, and defines putative AGA-based diagnostic and prognostic signatures of MM. We have recently identified also an extensive genetic damage leading to abnormal glycosylation of MM cells, and this information allows us to design and synthesize MM-specific glycans expected to improve a diagnostic and prognostic power of serum immunoprofiling and to develop a sensitive clinical screening test for early detection of MM and MM risk. A multitude of revealed abnormal MM cell surface glycans and a wealth of information about changes in immune system as a result of exposure to asbestos and MM development obtained through individual serum immunoprofiling offer now unique possibilities for the development of preventive and MM-targeting immuno-therapies.

S02-3

Malignant pleural mesothelioma: Potential biological role of fibroblast growth factor (FGF) -9

Ai Ling Tan[1], Mulugeta Worku[2], Helen E. Davies[3], Eleanor Mishra[3], Ross Sadler[3], Jeanette Creaney[1], Sally Lansley[1], Robert Davies[3], Gary Lee[1]

[1]Department of Medicine and Pharmacology, University of Western Australia and Sir Charles Gairdner Hospital, Australia, [2]University College London, UK, [3]University of Oxford, UK

RATIONALE: The global incidence of malignant mesothelioma (MM) continues to rise. There is no effective treatment for MM and there is an urgent need to search for proteins important in the pathogenesis of MM.
METHODS/RESULTS: Pleuroscopic tissue biopsies (n=49) were profiled using cDNA (Affymetrix) microarrays. Data were analysed with GeneSpring software and revealed FGF-9 (formerly called glia-activating factor) as a novel candidate gene not previously associated with MM. FGF-9 was up-regulated (median 17 fold) in MM over metastatic pleural carcinomas and benign pleuritis. This was validated in a second cohort of pleural biopsies. In addition, pleural fluid FGF-9 levels in MM patients (n=43) was 7.2 fold and 4.6 fold higher than those in pleural effusions from metastatic pleural cancers (n=137) and benign pleuritis (n=103), $p<0.05$ for both. FGF-9 levels were significantly higher in the pleural fluid than in corresponding blood samples in 35 MM patients, further confirming the pleural origin of FGF-9. High protein expression of FGF-9 and its four receptors (FGFR1-4) was detected by immunofluorescence in eight human and six murine MM cell lines. Immunohistochemistry on human and murine MM tissues showed cytoplasmic and nuclei staining of FGF-9 in the tumor cells. FGF-9 is important in the pathobiology of MM. FGF-9 potently induces time- and dose-dependent proliferation of both human and murine MM cells up to 2 fold (at 100 ng/mL) over serum-free control. FGF-9 also induces a dose- and time- (from 0 to 72 hrs) dependent release of IL-8 (or MIP-2), VEGF and MCP-1 from human and murine MM cells by 3, 2 and 2.5 fold respectively over serum-free treated controls (at 100 ng/mL). In addition, FGF-9 induces MM cell invasion (Matrigel assay) *in vitro*.
CONCLUSION: MM produces high levels of FGF-9 which potently induces MM cell proliferation and cytokine release.

S02-4

Diagnostic markers of mesothelioma: A systematic review

Jacobus Burgers[1,5], Sjoukje van der Bij[2], Eva Schaake[1], Erik Koffijberg[2], Bas A.J.M. de Mol[3,4], Karl G.M. Moons[2]

[1]Department of Thoracic Oncology, The Netherlands Cancer Institute, The Netherlands, [2]Julius Center for Health Sciences and Primary Care, University Medical Center, Utrecht, [3]Institute of Asbestos Victims, The Hague,, [4]Cardiothoracic Surgery, Academic Medical Centre, Amsterdam,, [5]on behalf of NVALT mesothelioma working party

Many biomarkers have been evaluated in the diagnosis of MM. Nevertheless, it remains unclear which marker has superior diagnostic performance. We performed a systematic literature review on the diagnostic accuracy of markers in patients suspected for MM.Materials and Methods: Medline and Embase were searched(last update December 2009). Eligible were original papers, describing markers in body fluids, with a pathologically confirmed diagnosis, a minimum sample size of 10 and sufficient data to construct a 2x2-contingency table. Data were extracted by two independent reviewers, assessing the methodological quality of the studies using QUADAS. Results and conclusion: The search yielded 1642 hits. 307 qualified for full text assessment, resulting in 82 eligible papers. The quality of the papers was suboptimal. Only one study was prospectively designed including patients suspected for mesothelioma. Most papers had either a case-control design (n=70) or a cohort of patients with effusions (n=9). As such 88% of the studies was hampered by a partial verification bias. The most common flaws in the study design included a non-representative patient sample (mesothelioma patients instead patients suspected to have mesothelioma; morethan 95%) and an unknown delay between the index and marker test (100%). SMRP, Ber-EP4, calretinin, CEA and EMA were most frequently studied, with a reported accuracy up to 100% in order to discriminate mesothelioma from other malignancies or non-malignant conditions. Due to the potential biases introduced by the almost invariably inadequate study design these results need to be interpreted with caution.

S03

Biology
Pathogenesis

S03-1

The expression of DPP10, a novel chimera gene identified by second generation sequencing of tumor genome, is associated with outcome in mesothelioma

Assunta De Rienzo[1], Lingsheng Dong[1], Melissa Coleman[1], William Richards[1], Beow Yeap[2], Gavin Gordon[1], David Sugarbaker[1], Raphael Bueno[1]

[1]Division of Thoracic Surgery and the International Mesothelioma Program, Brigham and Women's Hospital, and Harvard Medical School, USA, [2]Department of Medicine, Massachusetts General Hospital and Harvard Medical School, Boston, MA, USA

The dipeptidyl-peptidase 10 (DPP10) protein is a member of the S9B serine protease subfamily. It is located on the long arm of chromosome 2 (2q12.3-2q14.2), and extends over 1 Mb of genomic DNA. DPP10 binds specific voltage-gated potassium (K+) channels modulating their expression and biophysical properties. As member of the "DPP-IV activity and/or structure homologues" (DASH) family, it has been related to carcinogenesis. In DPP10, the serine residue critical to the active site of other DPP family members is replaced by a glycine residue resulting in loss of activity. Recently, we sequenced the genome of a malignant pleura mesothelioma (MPM) tumor and matched normal tissue using a combination of sequencing-by-synthesis and pyrosequencing methodologies to 9.6X depth of coverage. Read density analysis displayed significant aneuploidy and numerous mutations at all levels. One was a large deletion within the DPP10 gene (exons 4-25) and produced the expected truncated fusion transcript uniquely in the tumor's transcriptome. We examined DPP10 expression using reverse transcriptase (RT)-PCR in 56 additional MPM samples. DPP10 transcript was detected in 31 of 56 (55%). In the samples expressing DPP10, all the 26 DPP10 exons were further analyzed by RT-PCR reveling truncated DPP10 transcripts in 7 samples. Next, we correlated DPP10 expression with clinical features in the patients expressing DPP10. Surprisingly, we found that patients with tumors expressing any DPP10 transcripts had statistically significant better overall survival than patients whose tumors lacked DPP10 expression (22 months versus 8 months median survival; P=0.004). Interestingly, the analysis of 10 MPM cell lines revealed that only one MPM cell lines expressed DPP10, and its transcript was truncated. This study shows that DPP10 is mutated in a subset of MPM tumors and that its expression is correlates with survival. Further studies are in progress to investigate the role of DPP10 in MPM.

S03-2

Next-generation transcriptome sequencing identifies novel fusion transcripts in malignant pleural mesothelioma

Lingsheng Dong[1], Assunta De Rienzo[1], Roderick V. Jensen[2], Melissa H. Coleman[1], Gavin J. Gordon[1], Yanlong Xu[3], David J. Sugarbaker[1], Raphael Bueno[1]

[1]Division of Thoracic Surgery and the International Mesothelioma Program, Brigham and Women's Hospital, and Harvard Medical School, USA, [2]Department of Biological Sciences, Virginia Tech, Blacksburg, VA, USA, [3]Enterprise Research IS and Informatics, Brigham and Women's Hospital and Partners Healthcare Inc., Boston, MA, USA

A fusion transcript is a hybrid derived from two separate genes. It is often caused by chromosomal abnormalities and is frequently involved in carcinogenesis. Based on previously published whole-transcriptome shotgun pyrosequences of 4 malignant pleural mesotheliomas (MPM), 1 lung adenocarcinoma, and 1 normal lung, we identified putative "expressed sequences" chimeras using a customized bioinformatics pipeline, and further validated them using PCR. Briefly, the transcriptome sequence reads from the six samples were mapped to the AceView transcriptome sequence database using NCBI Blast software. A Perl script was run in AceView database to identify read sequences not matching a single gene, but mapping to two different genes. We initially identified 326 candidate chimeras supported by at least one read. Forty-six were related to genes involved in cancer or chimeras, and 12 were further analyzed by PCR. Churc1-FNTB fusion transcript was identified as novel transcription-induced chimera (TIC) in one MPM sample. Furthermore, Churc1-FNTB was analyzed in an additional 52 mesothelioma and 20 normal samples and found to be expressed in most of both sample types. We focused, then, on the fusion reads mapping to two different chromosomes, with fusion breakpoints at least 5 bp away from the exon-exon boundary and unique to the MPM samples. Ten fusion reads were identified and only two were confirmed by PCR. However, the two chimeras were present in both normal and tumor cDNA from the same patient indicating that the fusion transcripts are not tumor-specific. We conclude that our bioinformatics pipeline is a powerful high-throughput tool to identify fusion transcripts in whole-transcriptome shotgun sequences in cancer.

S03-3

Specific syndecan-1 domains regulate mesothelioma cell growth and migration

Fang Zong[1], Eleni Fthenou[2], Filip Mundt[1], Elena Ferola[3], Ilona Kovalszky[4], Laszlo Szilak[4], Roberto Perris[5], Georgios Tzanakakis[2], Anders Hjerpe[1], Katalin Dobra[1]

[1]Department of Laboratory Medicine, Karolinska Institutet, Sweden, [2]Department of Histology, University of Crete, 71110 Heraklion, Greece, [3]Department of Experimental Oncology 2, The National Cancer Institute IRCCS-CRO, Aviano, Pordenone, Italy, [4]1st Institute of Pathology and Experimental Cancer Research, Semmelweis University Budapest, Hungary, [5]Department of Genetics, Microbiology and Anthropology, University of Parma, Italy

Syndecan-1 is a transmembrane proteoglycan involved in many biological processes including cell proliferation, adhesion and migration. It consists of extracellular, transmembrane and cytoplasmic domains which may all participate in ligand interaction and signal transduction. To explore the role of distinct syndecan-1 domains, we transfected mesothelioma cells with a full-length syndecan-1 construct and three truncated variants: 78 lacking the extracellular domain with the exception of the juxtamembrane DRKE sequence proposed to be essential for oligomerization; 77 lacking the whole extracellular domain; and RMKKK being a potential nuclear localization signal within the cytoplasmic domain. Syndecan-1 and FGF-2 share a tubulin-mediated transport route and co-localize with heparanase in the nucleus. For the first time, we proved that the RMKKK sequence of syndecan-1 is sufficient for the nuclear translocation of syndecan-1 and thus serves as a nuclear localization signal. Overexpression of syndecan-1 influences the expression profile of the other syndecan family members; in particular down regulates syndecan-2. Both full-length and truncated syndecan-1 constructs decrease mesothelioma cell proliferation in two ways: the full-length syndecan-1 prolongs the S phase of the cell cycle, whereas the extracellular truncated variants 77 and RMKKK prolong the G0/G1 phase. Syndecan-1 decreases migration and motility, but enhances cell adhesion. Distinct protein domains have differential effects; the extracellular domain is more important for promoting cell adhesion, while the transmembrane and cytoplasmic domains are sufficient for inhibition of cell migration. Cell behavior seems to depend also on the nuclear localization of syndecan-1. A gene microarray analysis showed that many cell adhesion/migration-related genes are regulated by syndecan-1 overexpression. These results address the importance of nuclear translocation, and the functional protein domains, thereby providing new insights into the role of syndecan-1 in tumor progression. A better understanding of the mechanisms behind these functions could make this family of PGs a potential target for future therapy.

S03-4

Mesothelin expression promotes invasive phenotype and matrix metalloproteinase secretion in pleural mesothelioma

Elliot Servais, Christos Colovos, Stefan Kachala, Luis Rodriguez, Kyuichi Kadota, Valerie Rusch, Michel Sadelain, Prasad Adusumilli

Memorial Sloan-Kettering Cancer Center, USA

Introduction: Mesothelin, a cell-surface antigen overexpressed by epithelioid and biphasic malignant pleural mesothelioma (MPM), is known to be a poor prognostic factor. The goal of this study is to investigate the biological role of mesothelin and its significance as a marker of locoregional aggressiveness.
Methods: Human biphasic MPM (MSTO-211H) and murine epithelioid MPM (AB-12) cells were transduced to stably overexpress mesothelin. The influence of mesothelin overexpression on MPM cell proliferation, migration/invasion, matrix metalloproteinase (MMP) secretion, and gene expression profile was investigated *in vitro* by serial counting, Boyden chamber, quantitative multiplex assays, and Illumina microarray with gene set enrichment analysis (GSEA), respectively. The effect of mesothelin-expression was assessed *in vivo* in both immunodeficient SCID-beige and immunocompetent BALB/c orthotopic MPM mouse models by systematic evaluation of locoregional invasion into chest wall and diaphragm by histology as well as mesothelin and MMP-9 co-expression by immunohistochemistry (IHC).
Results: Mesothelin expression was quantified *in vitro* by flow cytometry, western blot, and cell supernatant soluble mesothelin-related peptide (SMRP) and *in vivo* by serum SMRP. *In vitro*, mesothelin expression: (a) increased migration ($p<0.0001$) and invasion ($p=0.02$) of MPM cells without affecting cell proliferation or morphology, (b) increased MMP-2 ($p<0.001$) and MMP-9 ($p<0.001$) secretion, and (c) upregulated MMP activation gene sets (false discovery rate <0.01) as revealed by GSEA. *In vivo*, mesothelin overexpression: (a) significantly decreased survival in orthotopic MPM mice ($p<0.001$), which correlated with SMRP levels ($p<0.05$), and (b) was associated with increased local tumor invasion with a leading invasive edge demonstrating co-localization of mesothelin and MMP-9 as evident by histology and IHC.
Conclusions: Mesothelin overexpression promotes a more invasive phenotype, is associated with increased matrix metalloproteinase secretion, and decreases survival in MPM. Our data provide evidence that mesothelin is a key factor for the locoregional aggressive behavior of epithelioid and biphasic MPM.

S03-5

Transforming growth factor beta signaling in malignant mesothelioma growth and collagen production

Bahareh Badrian[1], Gary Lee[1], Robin McAnulty[2], Steven Mutsaers[1]

[1]Lung Institute of Western Australia, Department of Medicine and Pharmacology, Sir Charles Gairdner Hospital, Australia, [2]Centre for Respiratory Research, University College London, London, UK

Introduction: Malignant Mesothelioma (MM) is an aggressive cancer with a very poor prognosis. Interactions of the components of the extracellular matrix (ECM) are now known to be important for the growth and regulation of cancer cells. TGFβ is an important regulator of the ECM and in particular collagen. Previous data in our laboratory has shown that blocking TGFβ signaling by using TGFβ antibodies inhibits collagen production and MM growth. Aim: to determine the signaling pathways downstream of TGFβ that are important in the regulation of collagen expression in MM. Methods: Components of the TGFβ pathway were inhibited by use of chemical inhibitors and overexpression of the endogenous inhibitor Smad7 in control and MM cell lines. Collagen levels were measured by realtime PCR. Results: Collagen regulation is thought to occur through the classic Smad2/3 signaling pathway. Our data show that Smad7 overexpression does not inhibit TGFβ induced collagen production in the MM cell lines investigated. However, a chemical inhibitor for the TGFβ receptor which inhibits all TGFβ signalling, effectively inhibited collagen stimulation. Therefore, the regulation of collagen does not appear to involve Smad2/3 in MM. It was shown that Smad2/3 are expressed, phosphorylated and activated by TGFβ in the MM cell lines. Our results suggest that downstream components of this signalling pathway may be altered in MM. Specifically we show that nuclear import of Smad4, which is important for signalling via the Smad2/3 pathway, is not induced by TGFβ in MM. Conclusions: Collagen is not regulated by the Smad2/3 signalling pathway in MM as previously thought, and this may be due to the altered function of Smad4. These results are important for understanding the growth and regulation of MM.

S03-6

Genome-wide profile of pleural mesothelioma versus parietal pleura: Confirmation of the gene portrait of mesothelioma

Oluf Roe[1], Endre Anderssen[2], Helmut Sandeck[3], Erik Larsson[4], Mark Ladanyi[5]

[1]Dept. of Oncology, St. Olavs Hospital, University Hospital of Trondheim, Norway, [2]Harvard Medical School, Boston, USA, [3]Dept. of Laboratory Medicine, Childrens and Womens Health, St. Olavs Hospital, University Hospital of Trondheim, Norway, [4]Dept. of Laboratory Medicine, Childrens and Womens Health, Norwegian University of Science and Technology, Trondheim, Norway, [5]Memorial Sloane Kettering Cancer Center, NY, USA

Background: Malignant pleural mesothelioma predilection site is the parietal pleura. Genome-wide profiling of mesothelioma versus parietal tissue could thus reveal novel genes and pathways explaining its aggressive phenotype.**Methodology and Principal Findings:** Our recently published genome-wide analysis of well-characterised tissue from mesothelioma patients versus normal parietal pleural samples from non-cancer patients by Affymetrix oligoarray U133 Plus 2.0 (38 500 genes) suffers from small sample size (Roe et al, PlosOne, 2009). We sought to validate the findings by replacing our mesothelioma samples with the published dataset from Memorial Sloan Kettering Cancer Center (MSKCC) of 89 mesotheliomas of epithelial and biphasichistological types analysed by the U133A (14 500 genes) and perform the same statistical analyses .There were some differences between the two case cohorts that could be technical due to different RNAextraction protocols, RNA quality, laser intensity and detector sensitivity. In spite of this, there was a highconcordance on identical genes, pathways and gene ontology. Among the 784 overexpressedgenes identified, 368 (47%) were identical with our material and among the 667 down-regulated genes 524 (79%) were identical. Several genes of the "salvage pathway" were overexpressed including TYMS, encoding thymidylate synthase, the main target of the most active drug against mesothelioma, pemetrexed. DNA-repair genes, mainly of homologous recombination were overexpressed.Circadian rhythm genes were expressed in favour of cell division. Cytokine-cytokine interaction, T-cell andB-cell receptors signalling pathways as well as leukocyte transendothelial migration were down-regulated. **Conclusions:** Genome-wide microarray on mesothelioma versus normal parietal pleura was validated comparing the results from a six-sample dataset with an 89-sample dataset from MSKKC. The main conclusions drawn from our previous study remain, reflecting some important features of mesothelioma biology that could be a base for exploring new treatment targets.

S04

Biomarkers

S04-1 **Keynote Speaker**

Soluble biomarkers for Mesothelioma – beyond diagnosis

Jenette Creaney[1,2], A. Segal[3], A.W. Musk[1,4], N. de Klerk[1,5], S.J. Skates[6], B.W.S. Robinson[1,2,4]

[1]National Research Centre for Asbestos Related Diseases, UWA, Australia, [2]The Australian Mesothelioma Tissue Bank, Sir Charles Gairdner Hospital, WA, [3]PathWest Laboratory Medicine, WA, [4]Department of Respiratory Medicine, Sir Charles Gairdner Hospital, WA, [5]Biostatistics and Epidemiology Unit, Telethon Institute for Child Health Research, Subiaco, WA, Australia, [6]Biostatistics Center, Massachusetts General Hospital, USA

The asbestos-related tumour malignant mesothelioma represents a significant clinical challenge. Not only can this tumour be difficult to diagnose but treatment options are limited. Recently there has been increased interest in the role of biomarkers in mesothelioma; for screening of asbestos-exposed individuals, as an aid for diagnosis, and as an alternative to radiological methods of monitoring patients' response to therapy.
A resurgence of interest in mesothelioma biomarkers followed the finding in 2003 that soluble mesothelin was elevated in these patients. Follow-up studies have demonstrated that mesothelin is elevated in over 80% of patients with advanced disease, in approximately 50% at diagnosis and 15% before diagnosis. Whilst mesothelin has a strong positive predictive value for mesothelioma, the lack of sensitivity at diagnosis has fuelled the search for complementary markers. However, studies world-wide on a range of soluble markers including osteopontin, MPF, hyaluronic acid, CA125, CA15-3 and others have failed to improve upon diagnostic accuracy. Therefore the search is on to discover novel biomarker(s) for this disease using a variety of genomic, proteomic and immunologic approaches.

S04-2

Sequential gene expression ratio-based diagnostic tests distinguish malignant pleural mesothelioma from other thoracic malignancies

Assunta De Rienzo[1], Roderick Jensen[2], Michael Lombardi[1], Lingsheng Dong[1], Melissa Coleman[1], William Richards[1], Gavin Gordon[1], David Sugarbaker[1], Raphael Bueno[1]

[1]Division of Thoracic Surgery and the International Mesothelioma Program, Brigham and Women's Hospital, and Harvard Medical School, USA, [2]Department of Biological Sciences, Virginia Tech, Blacksburg, VA 24060, USA

Establishing the pathological diagnosis of malignant pleura mesothelioma (MPM) usually requires a lengthy procedure including immunohistochemistry panels. Innovations that speed up and automate the process may improve clinical care. Previously, we developed an accurate gene expression ratio test to distinguish MPM from lung adenocarcinoma (ADCA). Herein, we extended the use of the gene ratio algorithm to discriminate MPM from all the other potentially confounding diagnoses. We performed microarray analysis on114 specimens including MPMs, and a spectrum of all the other malignancies and benign tissues in the differential diagnosis of MPM using Illumina whole genome microarrays. Our goals were to develop molecular signatures for the differential diagnosis of MPM and to obtain clues via differential gene expression to pathways uniquely relevant in MPM. We initially generated gene expression ratio-based diagnostic tests to discriminate MPM from normal pleura, and from sarcoma using the same rules previously established by our group to identify diagnostic and predictive tests in MPM. When the 3 diagnostic tests (MPM vs. ADCA, MPM vs. normal pleura, and MPM vs. sarcoma) were sequentially applied to the expression profiling data, 39 of 40 (98%) MPM were successfully discriminated from all the other samples. Next, we validated our tests in the 114 samples by Real Time-PCR. All the MPM samples were correctly identified; however, 9 different tumors, mostly renal cell carcinomas and thymomas, were classified as MPM. We developed additional diagnostic tests for these diagnoses. We also determined that sequential combination of binary diagnostic tests, much as those used in pathology, were more accurate than a single diagnostic signature. In this study, we show that molecular signatures using expression ratio tests may be applied for the comprehensive differential diagnosis of MPM.

S04-3

An *in vivo* platform for biomarker validation in malignant pleural mesothelioma

Elliot Servais, Kei Suzuki, Martin Fleisher, Camelia Sima, Christos Colovos, Luis Rodriguez, Valerie Rusch, Michel Sadelain, Prasad Adusumilli

Memorial Sloan-Kettering Cancer Center, USA

Introduction: Validation of tumor biomarkers in malignant pleural mesothelioma (MPM) is constrained by rarity of disease, heterogeneous histologies and therapeutic interventions, and poor survival. We have characterized a novel orthotopic mouse model for investigating MPM serum biomarkers. Utilizing this in vivo platform, we evaluated the performance efficacy of biomarkers currently under clinical investigation - soluble mesothelin-related peptide (SMRP) and osteopontin (OPN).
Methods: Orthotopic MPM mice biologically recapitulating human disease were established by intrapleural injection of MPM cells secreting SMRP and OPN. Quantitative bioluminescence imaging (BLI) and volumetric MRI monitored tumor burden and progression and were compared to serial SMRP and OPN levels. To evaluate SMRP for therapeutic response monitoring, MPM mice were treated with: (a) Cisplatin, (b) isolated thoracic radiation, (c) chemoradiation, or (d) no treatment. We further investigated SMRP performance efficacy in detecting dose-dependent therapy response and recurrence in MPM mice treated with mesothelin-targeted therapy.
Results: SMRP correlated with tumor burden in MPM mice confirmed by BLI ($r=0.92$, $p<0.0001$) and MRI ($r=0.87$, $p<0.0001$); correlation remained in MPM tumors with low-level mesothelin expression. SMRP was more sensitive in early detection of tumor progression than either MRI or BLI. In contrast, serum OPN levels did not correlate with tumor burden. Following chemoradiation, SMRP decreased compared to control mice ($p=0.03$) and levels predicted survival (HR=4.5, 95%CI 1.53-13.1). In response to mesothelin-targeted therapy, SMRP levels correlated in a dose-dependent manner and predicted both survival and tumor relapse.
Conclusions: We have developed an accurate, reproducible platform for investigating serum biomarkers in a clinically-relevant MPM mouse model allowing noninvasive, quantitative tumor bioimaging as a confirmatory tool. In this model, serum SMRP, but not osteopontin, accurately reflects tumor progression, therapy response, recurrence, and survival. Our well-characterized mouse model facilitates the cost-effective, rapid validation of multiple candidate biomarkers for clinical application, particularly in rare diseases such as MPM.

S04-4

Circulating endothelial cells (CECs) in the diagnosis of malignant pleural mesothelioma (MPM)

Fumihiro Tanaka[1], Kazue Yoneda[1], Nobuyuki Kondo[1], Masaki Hashimoto[1], Teruhisa Takuwa[1], Seiji Matsumoto[1], Yoshitomo Okumura[1], Kazu Shiomi[1], Ayuko Sato[2], Tohru Tsujimura[2], Noriaki Tsubota[3], Kozo Kuribayashi[4], Kazuya Fukuoka[4], Takashi Nakano[4], Seiki Hasegawa[1]

[1]Department of Thoracic Surgery, Hyogo College of Medicine, Japan, [2]Division of Molecular Pathology, Department of Pathology, Hyogo College of Medicine, [3]Department of Thoracic Oncology, Hyogo College of Medicine, [4]Division of Respiratory Medicine, Department of Internal Medicine, Hyogo College of Medicine

Backgrounds: Circulating endothelial cell (CEC) is a potential surrogate of tumor angiogenesis, and can be a clinically promising diagnostic marker in malignant tumors with aggressive angiogenic behavior such as malignant pleural mesothelioma (MPM). As CECs in the peripheral blood were easily and reliably captured with an anti-CD105 antibody by using a semi-automated system (C "ellSearch) ", we prospectively evaluated the diagnostic performance of CEC in MPM.Methods: Patients who presented at our institute with suspicion or diagnosis of MPM were eligible. CEC-count in 4.0mL of peripheral blood sampled from each patient was quantitatively evaluated with the "CellSearch " system without knowledge of final diagnosis.Results: Among 144 eligible cases, 91 were finally diagnosed as MPM and 53 as nonmalignant diseases (NM). The mean CEC-count was significant higher in MPM than NM (91 and 53, respectively; p=0.031), and a receiver operating characteristics (ROC) curve analysis provided a significant diagnostic value in discrimination between MPM and NM with the are under curve (AUC-ROC) of 0.782 (95% confidence interval, 0.675 to 0.888; p0<.01). The sensitivity and specificity of the CEC-count for the diagnosis of MPM were 54% and 86%, respectively, when the cut-off value of 50 was employed. In addition, the mean CEC-count was increased along with tumor progression (42 for stage I, 75 for stage II, 68 for stage III, and 117 for stage IV case), and stage IV MPM cases showed a significant higher mean CEC-count than stage I-III cases (117 and 67, respectively; p0<.05).Conclusions: CEC-count is potentially useful clinical marker in the diagnosis of MPM, and also can be a marker of therapeutic effect. Acknowledgement: This study supported by "The Special Coordination Funds for Promoting Science and Technology from the Japanese Ministry of Education, Culture, Sports, Science, and Technology"

S04-5

ERCC1: with our prognostic or predictive impact for malignant pleural mesothelioma

Isabelle Opitz[1], Alex Soltermann[2], Michaela Tutic[1], Svenja Thies[2], Niklaus Schaefer[3], Holger Moch[2], Rolf Stahel[3], Walter Weder[1]

[1]Division of Thoracic Surgery, University Hospital Zurich, Switzerland, [2]Institute for Surgical Pathology, Department Pathology, University Hospital Zurich, Zurich, Switzerland, [3]Clinic and Polyclinic of Oncology, University Hospital Zurich, Zurich, Switzerland

Background: Expression of the excision repair cross-complementation group 1 (ERCC1) protein predicts response to platinol-based chemotherapy and survival in lung cancer patients. The relevance of ERCC1 expression in Malignant Pleural Mesohtelioma (MPM) was assessed in a set of patients treated with induction chemotherapy followed by extrapleural pneumonectomy (EPP).Patients and Methods: From May 1999 to January 2010, 167 were intended to be treated with induction chemotherapy (40% cisplatin/gemcitabine; 60% cisplatin/pemetrexed) followed by extrapleural pneumonectomy (EPP). Response to chemotherapy according to modified RECIST criteria was available for 89 patients. One TMA with tumour of 126 MPM patients who underwent induction chemotherapy followed by EPP was constructed (post-CTX). Another TMA with 110 patients where pre-chemotherapy biopsies (pre-CTX) were available was constructed. ERCC1 expression was assessed and correlated to prospectively documented data. The influence on overall survival (OAS) and response to chemotherapy was evaluated. Results: ERCC1 was expressed in >90% of the pre-and post CTX biopsies. The expression score changed from a median score of 2.8 in the pre-CTX- to a median score of 2 in the post-CTX biopsies. There was no correlation between ERCC1 expression and the response to chemotherapy assessed by modified RECIST criteria. The median overall survival of all 167 patients was 19 months, of the 116 patients undergoing EPP 22 months. Neither pre- nor post CTX ERCC1 - and also not the ERCC1 change of expression global score showed significant influence on OAS.Conclusion: The prognostic role of ERCC1 expression for OAS was not confirmed in mesothelioma patients treated with induction chemotherapy followed by EPP. A predictive role for response to chemotherapy was not proven.

S04-6

Immunohistochemical detection of ERCC1 and class III β-tubulin in 54 malignant pleural mesotheliomas treated with cisplatin and vinorelbine: Association with treatment response and survival

Zarah Zimling[1], Cecilia Bech[2], Jens Sorensen[2], Eric Santoni-Rugiu[1]

[1]Department of Pathology, National University Hospital, Denmark, [2]Department of Oncology, National University Hospital, Copenhagen, Denmark

Aim: To correlate ERCC1 and class III β-tubulin expression with clinical outcome in malignant pleural mesotheliomas (MPMs) treated with cisplatin and vinorelbine.**Background:** DNA repair mechanisms are important in the resistance to platinum-based chemotherapy, by removing platinum-induced cross-links in the DNA strands. The excision repair cross-complementation group 1 enzyme (ERCC1) plays a rate limiting step in this process. Several studies have linked ERCC1 expression to cisplatin resistance in different cancers.One of the supposed cellular mechanisms leading to resistance towards tubulin-binding agents is the overexpression of class III β-tubulin, which is insensitive to the suppression of microtubule dynamics induced by tubulin-binding agents. This relationship to a resistant phenotype has been proposed for some of the major cancers.Few data, though, exist regarding the baseline expression of these biomarkers in MPM and their association with outcome.**Patients and methods:** Fifty-four consecutive patients were enrolled between February 2003 and September 2006 into a phase II trial with cisplatin and vinorelbine. The formalin-fixed paraffin-embedded bioptic tumor specimens from these MPM patients were retrospectively evaluated for ERCC1 and class III β-tubulin expression by immunohistochemistry (IHC) using an H-score. The cut-off point was chosen as the median value of the H-scores to separate positive (H-score > median) from negative (H-score <= median) tumors. **Results** Fifty patients had enough tumor tissue for IHC. For ERCC1 the median H-score was 2 yielding 20 positive and 30 negative tumors.For class III β-tubulin the median H-score was also 2, resulting in 10 positive and 40 negative tumors. At present no correlation is made with outcome, since we are currently doing a last round of follow up, but these will be presented at the IMIG conference.

S05

Novel targets

S05-1 **Keynote Speaker**

Anti-Mesothelin Immunotoxin SS1P Plus Cisplatin And Pemetrexed For First Line Treatment of Mesothelioma

Raffit Hassan, Elad Sharon, Ira Pastan

Laboratory of Molecular Biology, Center for Cancer Research, National Cancer Institute, National Institutes of Health, USA

Background: SS1P is a recombinant immunotoxin targeting mesothelin, a cell surface glycoprotein that is highly expressed in many cancers including malignant mesothelioma (MM). The safety and maximum tolerated dose (MTD) of SS1P have been established. Based on preclinical studies that show remarkable synergy when SS1P is given with chemotherapy we initiated a clinical trial of SS1P with pemetrexed and cisplatin for frontline treatment of pleural MM. **Methods:** Eligible patients had received no prior treatment, stage III / IV disease, ECOG 0-2, and good organ function. Patients were given Pemetrexed 500 mg/m2; Cisplatin 75 mg/m2 every 3 weeks for 6 cycles. SS1P dose escalation was: 25 μg/kg; 35 μg/kg; 45 μg/kg and 55 μg/kg on days 1, 3 and 5 of cycle 1 and 2. CT scans were performed every 2 cycles and every 3 months after treatment completion to assess response. **Results:** Thirteen patients (11 males, 2 females) have been treated. Five at 25 μg/kg; 3 at 35 μg/kg; 4 at 45 μg/kg and 1 at 55 μg/kg. Two patients treated at 25μg/kg withdrew after only 1 cycle and this cohort was expanded to 5 patients. One patient treated at 55μg/kg had Grade 3 fatigue that was considered a DLT. Adverse events that were possibly related to SS1P included Grade 3 hypoalbuminemia (n=3) and pain (n=2) and Grade 2 toxicities of hypoalbuminemia (n=5), edema (n=3), pain (n=2) and fatigue (n=6). Five patients had a partial response (lasting 16 mo; 11 mo; 9+ mo; 6 mo; 6 mo), 2 had stable disease (23+ mo; 6 mo) and 4 had progressive disease. **Conclusions:** SS1P given in combination with Pemetrexed and Cisplatin is well tolerated. Benefit was noted in 7 of 11 evaluable patients (5 partial responses and 2 stable disease). Expansion is ongoing at the MTD of 45 μg/kg.

S05-2

Activity and resistance to the pan-BCL-2 antagonist Obatoclax in malignant pleural mesothelioma

Niall McTavish, Francis McCoy, Ian Paul, Sara Busacca, Daniel Longley, Dean Fennell

Centre for Cancer Research and Cell Biology, Queens University, UK

Background. Resistance to apoptosis is a common characteristic of mesothelioma and contributes to multidrug resistance, a particular problem in the clinical setting upon relapse following conventional chemotherapy. Overexpression of the prosurvival BCL-2 family of proteins contributes to anti-apoptosis and are highly expressed in mesothelioma. MCL-1 is one of the most commonly ampflied genes at 9q21.2 in human cancer and this locus is amplified in mesothelioma. Obatoclax is an inhibitor of the prosurvival BCL-2 family including MCL-1. **Results.** On target activity was demonstrated by MCL-1 immunoprecipitation. After 1 μM obatoclax Bak dissociated from Mcl-1 at early timepoints. In a panel of 10 mesothelioma cell lines obatoclax exhibited cytotoxicity associated with reduction in cell viability. MCL-1 expression was not correlated with sensitivity. Obatoclax toxicity was associated with activation of the intrinsic apoptosis pathway evidenced by capsase 9 and PARP cleavage.In Bax/Bak shRNA silenced cells deficent in a functional mitochondrial apoptosis pathway, obatoclax induced apoptosis was abolished, but not lethality suggesting additional non-mitochondrial mechanisms of toxicity. Obatoclax reduced clonogenic survival however a logfold range of sensitivity was identified in the cell lines panel. Resistant cells failed to undergo apoptosis and committed to cell death at later timepoints compared with sensitive cell lines. In addition, on target activity was diminished or absent in resistant cells. Genetic profiling of obatoclax resistant versus sensitive cell lines is ongoing in an effort to determine putative resistance biomarkers. Activity *in vivo* was evaluated using Ren and MSTO-211 xenografts. Obatoclax treatment (8mg/kg) of tumours exhibits significantly reduced tumour growth compared to control tumours.
Conclusion. Obatoclax exhibits on target inhibition indicating its activity as a prosurvival BCL-2 antagonist. It is active in both *in vitro* and *in vivo* and evaluation in relapsed mesothelioma is planned. Mechanisms underlying resistance to obatoclax are being delineated to enable putative biomarker identification for patient stratification.

S05-3

Polycomb repressive complex-2 is a novel target for mesothelioma therapy

Clinton Kemp[1], Mahadev Rao[1], Julie Hong[1], Mary Zhang[1], Haresh Mani[2], Patricia Fetsch[2], Taylor Ripley[1], Aarti Mathur[1], Victor E. Marquez[3], Maocheng Yang[1], David Schrump[1]

[1]Thoracic Oncology Section, Surgery Branch, Center for Cancer Research, National Cancer Institute, National Institutes of Health, USA, [2]Laboratory of Pathology, Center for Cancer Research, National Cancer Institute, National Institutes of Health, Bethesda, MD, USA, [3]Laboratory of Medicinal Chemistry, Center for Cancer Research, National Cancer Institute, National Institutes of Health, Bethesda, MD, USA

Polycomb group proteins are global epigenetic gene silencers, which play key roles in the maintenance of stem cell pluripotency, multicellular development, and tumorigenesis. Polycomb repressive complex-2 (PRC-2) contains three core proteins: enhancer of zeste 2 (EZH2), suppressor of zeste 12 (SUZ12), and embryonic ectoderm development (EED). PRC-2 mediates trimethylation of lysine 27 on histone H3 (H3K27Me3), a repressive chromatin mark which contributes to epigenetic silencing of tumor suppressor genes during malignant transformation. In the present study, Affymetrix microarrays, quantitative RT-PCR (qRT-PCR), gel-based RT-PCR, and western blot techniques were used to examine PRC-2 expression in a panel of malignant pleural mesothelioma (MPM) lines (NCI-SB-MES1-4, H28, H2052, H2452), and two normal mesothelial cell cultures (NCI-SB-NMES1, LP9). This analysis revealed significant up-regulation of EZH2 and EED in MPM lines; EZH2 and EED over-expression coincided with increased H3K27Me3 levels in these cells. Additional qRT-PCR, RT-PCR, and immunohistochemistry experiments utilizing 20 primary MPMs and tissue micro-arrays containing 28 MPMs and 17 peritoneal mesotheliomas revealed over-expression of EZH2 in ~85% of mesotheliomas compared to 12 normal mesothelia specimens. Knockdown of EZH2 or EED decreased global H3K27Me3, and diminished proliferation and migration of NCI-SB-MES1, NCI-SB-MES2, H28, and H2452 MPM cells ($p<0.05$). Furthermore, knockdown of EZH2 or EED decreased clonogenicity ($p<0.05$), and tumorigenicity ($p<0.05$) of NCI-SB-MES1; the effects of EED knockdown were more pronounced than EZH2 knockdown in these cells. DZNep, a novel inhibitor of polycomb expression, mediated dose-dependent depletion of EZH2, EED, and H3K27Me3, and inhibited proliferation ($p<0.05$), migration ($p<0.05$), clonogenicity ($p<0.05$), and tumorigenicity ($p<0.05$) of MPM cells. Additional experiments are underway to define the mechanisms by which inhibition of PRC-2 expression mediates growth arrest in MPM cells. Collectively, these data demonstrate that aberrant expression of PRC-2 contributes to the malignant phenotype of MPM, and suggest that PRC-2 may be a novel target for mesothelioma therapy.

S05-4

Inhibition of mTOR by temisolimus is active against malignant mesothelioma *in vitro* and *in vivo* and synergizes with chemotherapy

Mir Hoda[1,2], Amir Mohamed[2], Bahil Ghanim[1], Balazs Hegedus[1], Judit Berta[1], Ulrike Setinek[2], Michael Grusch[2], Martin Filipits[2], Michael Micksche[2], Walter Klepetko[1], Walter Berger[2]

[1]Division of Thoracic Surgery, Department of Surgery, Medical University of Vienna, Austria, [2]Institue of Cancer Research, Department of Internal Medicine I, Medical University Vienna, Vienna, Austria

PURPOSE: Human malignant mesothelioma is an asbestos-related malignancy characterised by frequent resistance against chemo- and radiotherapy. Inhibition of the mammalian target of rapamycin (mTOR) represents a novel anticancer strategy widely unexplored in case of mesothelioma. Thus we set out to clarify the feasibility of mTOR inhibition by the small-molecule inhibitor temsirolimus as an anti-mesothelioma strategy. EXPERIMENTAL DESIGN and RESULTS: Analysis of tumor specimens obtained from mesothelioma patients (N=70) frequently revealed strong immunoreactivity for phosphorylated mTOR (p-mTOR) in the malignant cell compartment while adjacent normal tissues remained generally unstained. Accordingly all mesothelioma cell lines and primary cell cultures analysed (N=8) harboured activated mTOR which was further confirmed by hyper-phosphorylation of the downstream targets pS6K, S6 and 4EBP. Temsirolimus blocked this pathway activation and exerted a cytostatic effect against all mesothelioma cell lines in vitro. Notably, mTOR inhibition also blocked self-renewal of mesothelioma-derived cancer stem cells in spheroid cultures. Mesothelioma cells resistant against the standard treatment component cisplatin tended to be hypersensitive against temsirolimus. Induction of cisplatin-resistance by drug selection led to upregulation of sensitivity against the mTOR inhibitor. Accordingly, cisplatin and temsirolimus exerted synergistic growth-inhibitory and autophagy-inducing activities against mesothelioma cell lines in vitro. Finally, temsirolimus was highly active as single agent against orthotopic, chemo-sensitive and chemo-resistant mesothelioma xenograft models synergising with chemotherapy. CONCLUSION: The mTOR inhibitor temsirolimus is active against mesothelioma models in vitro and in vivo and synergises with chemotherapy. These data suggest mTOR inhibition as promising novel therapeutic strategy against malignant mesothelioma.

S05-5

The role of the hedgehog pathway in the development of maglignant mesothelioma

Steven Mutsaers[1,2], HM Cheah[1], E Ngoh[1,2], Genovesi L[1], N Watkins[3], YC Lee[1], C Thomas[2]

[1]Lung Institute of Western Australia and Center for Asthma, Allergy and Respiratory Research, Department of Medicine, University of Western Australia, Australia, [2]PathWest Laboratory Medicine WA, 3Monash University, Vic, Australia

Increasing evidence supports an association between aberrant activation of key proteins within the Hedgehog (HH) signalling pathway and a number of human cancers. The role of the HH pathway in MM is unknown. We hypothesise that MM growth is regulated through the HH pathway. To test this hypothesis, the mRNA expression levels of key components of the HH pathway Sonic (SHH), Indian (IHH), Patched (PTCH-1), Smoothened (SMO) and GLI-1, were measured by real time PCR in mouse and human primary mesothelial cells and MM cell lines. All cells expressed mRNA for PTCH, SMO and IHH. The expression of PTCH and SMO was higher in malignant compared with normal mesothelial cells. All but one cell line expressed GLI-1 with the expression greater in the malignant compared to normal cells. SHH was not expressed in these cells, suggesting that in MM, HH pathway activation is predominantly paracrine driven. To further investigate the pathway, the cell lines expressing the most and least GLI-1 were used to make tumours in mice. The mRNA expression of HH pathway genes were examined in mouse tumours by PCR. All pathways genes examined were identified in both mouse tumours. SHH pathway protein expression and distribution were examined in the tumours by immunohistochemistry. The tumours stained positive for SHH, with strongest staining at the edge of the tumour in areas of large numbers of stromal cells, further supporting a paracrine role for HH signalling in MM. Staining was also present for IHH, PTCH-1, SMO and GLI-1. In conclusion, the mRNA of key HH pathway genes are expressed in in vitro mouse MM cell lines as well as in vivo mouse MM tumours, supporting a role for the HH pathway in MM.
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S05-6

Y-Box-binding protein 1: A potential therapeutic target in malignant mesothelioma

Michaela Kirschner, Lyn Schedlich, Yuen Yee Cheng, Nico van Zandwijk, Glen Reid

Asbestos Diseases Research Institute, Bernie Banton Centre, University of Sydney,Australia

Due to a significant genetic heterogeneity and intrinsic drug resistance, the prognosis for malignant mesothelioma (MM), an asbestos-related disease, has remained poor. Therefore, the identification of novel therapeutic targets for MM treatment is an important unmet need.The Y-Box-binding protein 1 (YBX-1), a DNA- and RNA-binding protein involved in transcriptional and translational regulation of many oncogenes and tumour suppressor genes, could be one of those novel targets. Being overexpressed in various cancers (e.g. NSCLC, breast) its nuclear expression has been shown to be associated with late stages of disease and a poor prognosis. Furthermore, YBX-1 is associated with the RNA-induced silencing complex (RISC) which is important for the biogenesis of microRNAs.We have used RNA interference to investigate the effects of YBX-1 knockdown in established ATCC MM cell lines as well as primary lines. Preliminary results indicate a possible subtype-specific effect on cell growth. Although in all cell lines transfection with YBX-1 siRNA leads to a reduction of YBX-1 mRNA expression of 80-90 %, the effect on cell growth is variable. 4 days after transfection the growth of the biphasic cell lines MSTO-211H and MM05 is reduced by 50-80 %, but only one of the epithelioid cell lines used, H2452, showed similar effects, while there was no growth inhibition in H28 and H2052 or the desmoplastic MM04 cell line. Experiments to determine chemosensitising effects of targeting YBX-1 in these lines will also be presented.Further experiments to identify the miRNAs and non-coding RNAs affected by YBX-1 knockdown have been carried out with NCode arrays. These experiments have identified ncRNAs and miRNAs expressed in MM lines and preliminary data suggest changes following YBX-1 knockdown. With effects on cell growth and the development of a specific inhibitory peptide, YBX-1 represents a potential therapeutic target in MM.

S06

First-line treatment

S06-6 **Keynote Speaker**

First line chemotherapy for malignant pleural mesothelioma

Jan van Meerbeeck

Department of Respiratory Medicine and Thoracic Oncology Ghent University Hospital, Belgium

In the past decades many phase II studies have been performed to select drugs with a potential activity against MPM. Unfortunately this approach did not result in a significant breakthrough and only a limited group of drugs were identified that exerted some activity. Doxorubicin, cisplatin and methotrexate were considered the most effective single agents [1]. A 3-armed randomised study was initiated in the UK that compared the efficacy of 2 different chemotherapy regimens – one platinum combination and one single agent 3rd generation drug- with best supportive care [2]. The study was prematurely stopped due to a slow accrual and was hence insufficiently powered to show a survival difference, even after pooling the results of both chemotherapy arms, but a positive trend favouring the vinorelbine single agent treatment was observed. Based on other reviews [1,3], the choice of the comparative chemotherapy cannot be considered to be optimal in this study. The decision to administer chemotherapy should be discussed with the patients and relatives, because of its palliative intent. In an individual patient meta-analysis of 1205 patients with landmark correction at 62 days, response to chemotherapy was found to be predictive for survival, irrespective of whether therapy contains platinum or not [4].

Two international randomised studies ([6,7] suggested that a combination chemotherapy including cisplatin and an antifolate, either pemetrexed or raltitrexed, increases survival compared to single agent cisplatin. The median survival observed in both studies showed an improvement of 2.6-2.8 months for the combination therapy arm. The results of the median survival in patients treated with cisplatin only are also above those usually reported in the literature (7 to 9 months) for active supportive care, confirming a modest single agent activity of cisplatin. Both trials showed a significant increase in response rate and no deleterious impact on quality of life. Based on these two randomised phase III trials, it is now generally accepted to treat patients with MPM with a combination of an anti-folate with platinum. When pemetrexed is used, folic acid and vitamin B12 supplementation are required to reduce the haematological toxicity. Unfortunately about 80% of the patients have recurrent disease within 2 years of follow-up. Other cisplatin-based combinations have also produced interesting response rates of 20-30% in phase 2 studies: etoposide, epirubicin, gemcitabine, vinorelbine or methotrexate[1,3]. As in non-small cell lung cancer (NSCLC), opinions differ as to the interchangeability of cisplatin and carboplatin, the combination of the latter drug with pemetrexed showing outcome data similar to the ones obtained with the former [8,9].

Only one study has addressed the question when to start chemotherapy treatment in patients with MPM. O'Brien et al. investigated if early administration was better than waiting until symptoms progression urged both the patient and clinician to start treatment with chemotherapy [10]. When a treatment with chemotherapy was started immediately, the median survival increased from 10 to 14 months with a 1-year survival of 66% versus 36%). The limited sample size of the study precluded a statistical significance. However, taking into account the data from numerous other tumour types, it is now considered not to be of any benefit for the patient to delay the start of treatment until progression of symptoms, unless the patient or other reasons indicate so.

The duration of treatment has attracted attention in MPM as soon as pemetrexed containing combination chemotherapy was introduced. In a few cases a delayed response to treatment was observed and the relatively low toxicity has allowed to treat patient beyond 4-6 courses. Ongoing responses have been the reason for a study showing that the number of courses of chemotherapy could be extended up to 12 in selected patients [11]. Not all patients received cisplatin in these cases but pemetrexed was well tolerated as single agent. Unfortunately, the study was neither designed nor powered for a change in practice and selection of patients could have influenced the outcome. In retrospect, 53% of patients in the cisplatin-pemetrexed arm of the registration trial received 6 cycles, whilst in the EORTC trial, the median number of cisplatin -raltitrexed cycles was 5. As in NSCLC, it is now recommended to consider prolonged treatment with the anti-folate and platinum for a minimum of 4 courses and in case of "delayed" responses to continue to 6 or more courses, whenever tolerance is kept. In case disease stabilization is observed as best response, a maximum of 6 courses is recommended. Patients progressing early should be switched to another (palliative) treatment.

Of the newer antifolates, only praletrexate has been tested in mesothelioma sofar [12], although the mechanism of action, transport in the cell and metabolism of nolatrexed, previtrexed, PT 523 and AG 2037 are similar to raltitrexed and pemetrexed [13,14]. Nolatrexed was designed using molecular modelling techniques and a high resolution crystal structure of thymidilate synthase, a key enzyme in the folate metabolism [15]. Its development was halted after a negative phase 2 trial in hepatocellular carcinoma.

The same applies for the platinum analogues picoplatin, satraplatin, lobaplatin and nedaplatin [16], although their benefit is more likely to result from a lower toxicity and not from a higher activity in MPM. Of the newer 3rd generation cytotoxic agents, only vinflunine has shown some comparable activity [17]. None of the epothilones have yet been tested, although the low activity of taxanes in MPM is not promising. Amrubicin, a doxorubicin analogue with topo-isomerase-1 activity is a promising drug, in view of its activity in small cell lung cancer and the known activity of other anthracyclines in mesothelioma.

As in NSCLC, it might hence be expected that the outcome with chemotherapy will plateau and that further improvement in advanced MPM is to be expected from drugs concentrating on mesothelioma specific targets - MTAP gene, folate receptor, VEGF, apoptotic pathways – or from selecting patients expressing one or more of these biomarkers.

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S06-1

Inoperable malignant pleural mesothelioma: Phase II study of 1st line treatment with carboplatin and vinorelbine

Jens Sorensen[3], Cecilia Bech[1], Peter Sorensen[2], Hannah Frank[3]

[1]Dept. Oncology, FinsenCentre, National Universiyty Hospital, Copenhagen, Denmark, [2]Dept. Oncology, Odense University Hospital, [3]Department of Oncology, Finsen Centre, National University Hospital, Denmark

Purpose: Platinum-based combination chemotherapy improve survival and quality of life in Malignant Pleural Mesothelioma (MPM). Vinorelbine (VNB) is also among the most active drugs. Thus, the combination of Carboplatin with VNB was explored.Methods: Previously untreated inoperable MPM patients (pts) in performance status (PS) 0-2, normal renal function, no major comorbidity, and no upper age limit received Carboplatin AUC 5 and VNB 25 mg/m2 i.v. day 1 and VNB 80mg/m2 p.o. day 8 q. 3 weeks for 4-6 courses. CT-scans were done initially and for every 2 courses. Modified recist criteria were used for response assessment. The study was approved by the National Health Authorities and the regional ethical committees. Pts gave written informed consent. Results: Median age among 47 pts included was 66 years (range 42-79), there were 89% males, 59% had epithelial subtype, 55% had IMIG stage stage IV, and PS 1 and 2 occurred in 66% and 11%, respectively. Median no. of courses were 4 (range 1-6) and median time on treatment was 15 weeks. Toxicity was modest, only grade 4 toxicity encountered was leucopenia (8.5 % of pts). There were 3 episodes of febrile leucopenia (7%), no bleeding episodes and no toxic deaths. Dose reductions were done in 13 pts (39%). Partial remission occurred in 13 pts (28%) and Complete remission in one patient (2%). Medians of Progression Free Survival was 32 wks (range 6-215 wks) and Overall Survival was 54 weeks (range 6-215 wks). Conclusions: This regimen of Carboplatin with VNB i.v. and p.o. was feasible and safe in this population of MPM pts including poor prognostic and frail pts in PS2 and age above70 years. The activity is similar to that of other regimens combining platinum with drugs such as Pemetrexed, Raltitrexed, Gemcitabine, or Epirubicin in such patient populations..

S06-2

Anti-mesothelin immunotoxin SS1P plus cisplatin and pemetrexed for first line treatment of mesothelioma

Raffit Hassan, Elad Sharon, Ira Pastan

Laboratory of Molecular Biology, Center for Cancer Research, National Cancer Institute, National Institutes of Health, USA

Background: SS1P is a recombinant immunotoxin targeting mesothelin, a cell surface glycoprotein that is highly expressed in many cancers including malignant mesothelioma (MM). The safety and maximum tolerated dose (MTD) of SS1P have been established. Based on preclinical studies that show remarkable synergy when SS1P is given with chemotherapy we initiated a clinical trial of SS1P with pemetrexed and cisplatin for frontline treatment of pleural MM. **Methods:** Eligible patients had received no prior treatment, stage III / IV disease, ECOG 0-2, and good organ function. Patients were given Pemetrexed 500 mg/m2; Cisplatin 75 mg/m2 every 3 weeks for 6 cycles. SS1P dose escalation was: 25 μg/kg; 35 μg/kg; 45 μg/kg and 55 μg/kg on days 1, 3 and 5 of cycle 1 and 2. CT scans were performed every 2 cycles and every 3 months after treatment completion to assess response. **Results:** Thirteen patients (11 males, 2 females) have been treated. Five at 25 μg/kg; 3 at 35 μg/kg; 4 at 45 μg/kg and 1 at 55 μg/kg. Two patients treated at 25μg/kg withdrew after only 1 cycle and this cohort was expanded to 5 patients. One patient treated at 55μg/kg had Grade 3 fatigue that was considered a DLT. Adverse events that were possibly related to SS1P included Grade 3 hypoalbuminemia (n=3) and pain (n=2) and Grade 2 toxicities of hypoalbuminemia (n=5), edema (n=3), pain (n=2) and fatigue (n=6). Five patients had a partial response (lasting 16 mo; 11 mo; 9+ mo; 6 mo; 6 mo), 2 had stable disease (23+ mo; 6 mo) and 4 had progressive disease. **Conclusions:** SS1P given in combination with Pemetrexed and Cisplatin is well tolerated. Benefit was noted in 7 of 11 evaluable patients (5 partial responses and 2 stable disease). Expansion is ongoing at the MTD of 45 μg/kg.

S06-3

Phase II study of the combination of bevacizumab plus pemetrexed and carboplatin as first-line therapy in patients with malignant pleural mesothelioma (MPM)

Giovanni Ceresoli[1], Paolo Zucali[2], Manlio Mencoboni[3], Fabio De Vincenzo[2], Mario Botta[4], Francesco Grossi[5], Paolo Bidoli[6], Emilio Bajetta[7], Andrea Ardizzoni[8], Adolfo Favaretto[9], Matteo Simonelli[2], Elena Lorenzi[2], Letizia Gianoncelli[2], Arturo Chiti[2], Armando Santoro[2]

[1]Oncology Unit, Cliniche Humanitas Gavazzeni, Italy, [2]Department of Oncology, Istituto Clinico Humanitas IRCCS, Rozzano (Milan), Italy, [3]Department of Oncology, Ospedale Villa Scassi; Sampierdarena, Genova, Italy, [4]Department of Oncology, Ospedale S. Spirito, Casale Monferrato, Italy, [5]Department of Oncology, Istituto Nazionale per la Ricerca sul Cancro, Genova, Italy, [6]Department of Oncology, Ospedale San Gerardo, Monza, Italy, [7]Department of Oncology, Fondazione IRCCS Istituto Nazionale Tumori, Milan, Italy, [8]Department of Oncology, Azienda Ospedaliera Universitaria, Parma, Italy, [9]Department of Oncology, Azienda Ospedaliera di Padova, Padova, Italy

Background: The combination of carboplatin and pemetrexed is active in MPM patients (pts). Vascular endothelial growth factor (VEGF) is highly expressed in MPM; in pre-clinical models, anti-VEGF antibodies were shown to decrease MPM cells growth. The aim of this study was to assess the activity of bevacizumab in combination with carboplatin/pemetrexed (BPC regimen) as first-line therapy in MPM pts.**Patients and Methods**: Chemotherapy-naive pts, not candidates for curative surgery, received pemetrexed 500 mg/m2 and carboplatin AUC5, followed by bevacizumab 15 mg/kg, administered intravenously every 21 days. All patients received vitamin supplementation. Main endpoint of the study was progression-free survival (PFS).**Results**: This multicenter, open label phase II study was designed to enrol 77 pts. The accrual was reached on September 2009 and data of 71 pts are available for a preliminary analysis. Pts characteristics were: M/F 46/25, median age 68 (range 40 to 78), EORTC prognostic score good/poor 61/10. Histology was epithelial in 57 pts, sarcomatoid or biphasic in 10, and not specified in 4. A partial response was achieved in 28/71 pts, for a response rate of 39%. 32 pts (45%) had stable disease. With a median follow-up of 10.6 months, 42 pts had progressed and 33 pts died. Median PFS and overall survival were 6.9 and 14.3 months, respectively. Haematological toxicity was low, with grade 3/4 neutropenia observed in 5 pts (7%), without febrile neutropenia. Non-haematological toxicity was generally mild; however, 3 cases of bowel perforation, possibly related to treatment, were observed. **Conclusion**: First-line treatment with BPC regimen in MPM pts is feasible, with acceptable toxicity, although bevacizumab-related adverse effects must be strictly monitored. However, according to this preliminary analysis, the addition of bevacizumab to pemetrexed-based chemotherapy did not seem to improve survival outcomes. Subset analysis is ongoing, and updated results will be presented at this meeting.

S06-4

IFCT-GFPC-0701 MAPS trial, a multi-center randomized phase II-III trial of pemetrexed - cisplatin with or without bevacizumab in patients with malignant pleural mesothelioma: preliminary results of the phase II trial

Arnaud Scherpereel[1,10], Julien Mazieres[2], Philippe Astoul[3], Jacques Margery[4], Isabelle Monnet[5], Armelle Lavole[6], Marie-Paule Lebitasy[8], Michel Andre[8], Denis Moro-Sibilot[7], Genevieve Marchandise[10], Rebecca Rouveau[8], Gerard Zalcman[9]

[1]Pulmonary and Thoracic Oncology, University Hospital (CHRU) of Lille, France, [2]University Hospital Larrey, Toulouse, France, [3]University Hospital Sainte-Marguerite, Marseille, France, [4]HIA Percy, Clamart, France, [5]CHI, Creteil, France, [6]Tenon University Hospital, Paris, France, [7]University Hospital, Grenoble, France, [8]IFCT, Paris, France, [9]CHU Cote de Nacre, Caen, France, [10]Unit INSERM U1019, Pasteur Institute of Lille, Lille, France

Background: MPM median OS does not exceed 13 months with pemetrexed/CDDP doublet. A phase II trial of gemcitabine/CDDP± bevacizumab led an appealing 15.6 months median OS in the bevacizumab arm. French Intergroup aimed to test pemetrexed/CDDP with bevacizumab (PCB), in a randomized phase II-III trial, including soluble and tissue markers assessment.Methods: Eligible chemonaive patients had unresectable histologically-proved MPM, PS 0-2, no thrombosis, nor bleeding. Phase II primary endpoint: % of non-progressive patients at 6 months. Patients received pem 500mg/m2, CDDP 75mg/m2 (PC), at D1, with (arm B) or without bevacizumab (arm A), 15 mg/kg Q21D, for 6 cycles. Arm B non-progressive patients received bevacizumab maintenance therapy until progression or toxicity. Results: 111 patients were enrolled within 24 months in 53 centers. Median age: 64.3 (34.7-75.5), M:F= 80:31, epithelioid:sarcomatoid/mixt= 90:21, WHO PS 0:1:2= 49:57:5. In the first 71 assessable patients (1 non eligible), there were 25/34 patients (73.5%) with DC (1 CR, 15 PR, 9 stable disease) in Arm B vs. 16/37 (43.2%) in arm A (p=0.010). There was no significant difference in grade 3/4 toxicity between the arms. No significant difference for soluble biomarkers data between A and B arms was found at the time of inclusion. Preliminary analysis on blood biomarkers data in 111 patients showed a significant decrease of VEGF level in both arms, but more markedly induced by bevacizumab in Arm B patients compared to Arm A (p<0.001). It also suggested a potential predictive value of initial level of serum mesothelin in both arms and of initial VEGF level in Arm A only, whereas osteopontin and endocan assays did not show any value.Conclusions: PCB chemo was well-tolerated and feasible. The statistical endpoint of the phase II study was reached. Updated toxicity data, PFS, DCR at 6 months and markers assessment will be presented at the meeting.

S06-5

Safety and effectiveness of pemetrexed in patients with malignant pleural mesothelioma based on all-case drug-registry study

Kenichi Hosokawa[1], Shinichi Nishiuma[1], Simon Voss[2], Shoji Kudoh[3], Takashi Nakano[4]

[1]Eli Lilly Japan, Japan, [2]Eli Lilly & Co, UK, [3]Fukujuji Hospital, Kiyose, Japan, [4]Division of Respiratory Medicine, Department of Internal Medicine, Hyogo College of Medicine, Nishinomiya, Japan

Background: Pemetrexed/cisplatin regimen is the only approved chemotherapeutic regimen for malignant pleural mesothelioma (MPM). At the time of launch, limited safety information, including drug-induced interstitial lung disease (ILD), was available. The purpose of this postmarketing all-case registry study was to investigate the safety and effectiveness of pemetrexed in patients with MPM.
Methods: From February 2007 to May 2008, every histologically-proven MPM patient to be treated with pemetrexed in Japan was registered to this study to monitor its safety and effectiveness. Supply of pemetrexed was restricted to institutes with experienced medical oncologists based on predetermined criteria.
Results: Of 953 patients registered, data from 903 patients were analyzed. Most patients were male, with median age of 65 years, and 68.5% had a history of asbestos exposure. More than 90% of patients received the first cycle of pemetrexed/cisplatin combination treatment. Median treatment cycle was 4.0. Treatment-associated death was reported in 0.8% of patients. Most common (≥10%) side effects included leucopenia, neutropenia, anemia, nausea, vomiting, anorexia, thrombocytopenia, constipation, and lymphopenia. Serious side effects occurring in >5% of patients were neutropenia, anemia, and leucopenia. Incidence of ILD associated with pemetrexed was 0.9%. The frequency of ILD in patients with pre-existing asbestosis was higher than that in patients without it. Of the 835 evaluable patients for tumor response, 9 patients achieved CR (1.1%) and 217 showed PR (26.0%), with the overall response rate was 27.1% [95% confidence interval (CI): 24.1%-30.2%]. The six-month survival rate estimated by Kaplan-Meier method was 75.9%.
Conclusions: This large-scale all-case registry study appeared to have enrolled a major portion of Japanese MPM patients. The treatment using pemetrexed was generally well tolerated and showed safety profiles comparable to prior clinical trials and resulted in acceptable effectiveness.

S07

SMRP & MPF

S07-1

Soluble mesothelin related protein (SMRP) in malignant pleural mesothelioma (MPM)

Rabab Gaafar, Ibrahim Abdelsalam, Ola Khorshid, Saad Elguindy, Inas Elattar, Abeer Ashmawy, Hatem Abdelazim

Department of Medical Oncology, National Cancer Institute, Cairo University, Egypt

Background and purpose: Building-up evidences suggests that SMRP carries a diagnostic and a prognostic value in MPM. Egypt suffers endemic asbestosis and thus this study was conducted to evaluate the value of using SMRP in diagnosing patients with MPM and to correlate this marker with known clinico-pathological prognostic factors.Material and Methods: In the period from January 2006 till March 2008, serum samples were obtained from MPM presenting to the Egyptian National Cancer Institute, Cairo University. Serum samples were provided from patients with breast cancer and from healthy individuals to function as controls.The SMRP was assayed by ELISA using MESOMARK and correlations were made with different clinico-pathological prognostic parametersResults: 83 patients (50 MPM, 33 breast cancer) and 22 healthy individuals were examined in this study. Serum SMRP levels were not different between patients with breast cancer and healthy controls ($p>0.05$). However, there was a significant difference between MPM patients and the other two groups ($p<0.0001$). ROC analysis showed an AUC = 0.765 for differentiating between the controls and MPM with a best statistical cut-off of 7.22nM/L (sensitivity=66%, specificity=70.9%).The mean SMRP concentrations were significantly higher in patients with advanced disease ($p=0.038$), poor performance status ($p=0.017$) and high alkaline phosphatase ($p=0.015$). Mean SMRP concentrations were also higher in males, elderly patients, asbestos-exposed patients, epithelioid subtypes and patients with high platelet and leucocytic counts. However, these differences did not reach statistical significance.Conclusion: This study confirms that SMRP is of considerable sensitivity and specificity in MPM patients. Higher levels are frequently seen in patients with high tumor burden, which could be helpful in monitoring response to therapy.

S07-2

Soluble mesothelin and megakaryocyte potentiating factor: Prognostic value and impact of covariates on the diagnostic performance

Kevin Hollevoet[1], Kristiaan Nackaerts[2], Olivier Thas[7], Joel Thimpont[3], Paul Germonpre[4], Lionel Bosquee[5], Paul De Vuyst[6], Eliane Kellen[2], Yoshiro Kishi[8], Joris Delanghe[9], Jan van Meerbeeck[1]

[1]Department of Respiratory Medicine, Ghent University Hospital, Belgium, [2]Department of Respiratory Medicine, University Hospital Gasthuisberg, Leuven, Belgium, [3]Occupational Diseases Fund, Brussels, Belgium, [4]Department of Respiratory Medicine, Antwerp University Hospital, Antwerp, Belgium, [5]Department of Respiratory Medicine, CHU Sart Tilman, Liege, Belgium, [6]Department of Respiratory Medicine, Erasme Hospital ULB, Brussels, Belgium, [7]Department of Applied Mathematics, Biometrics and Process Control, Ghent University, Ghent, Belgium, [8]Department of Research and Development, Ina Institute, Medical & Biological Laboratories, Co. Ltd., Nagano, Japan, [9]Department of Clinical Chemistry, Ghent, University Hospital, Belgium

Background: Soluble mesothelin (SM) and megakaryocyte potentiating factor (MPF) are serum biomarkers of mesothelioma. While their diagnostic performance is increasingly assessed, the presence and impact of covariates, and the prognostic value of both biomarkers, have been undervalued. **Methods:** 582 individuals were included in a multicenter prospective case-control study. Cases consisted of 101 malignant pleural mesothelioma patients, and controls included 471 participants: healthy (n=101) and asbestos-exposed individuals (n=214), patients with benign respiratory disease (n=78), lung cancer (n=69), and non-respiratory epithelial cancer (n=19). SM and MPF levels, assayed with the Mesomark and Human MPF ELISA kit, respectively, and personal characteristics were inputted in regression analyses. In asbestos-exposed individuals and mesothelioma patients, asbestos exposure was quantified using standardized questionnaires. **Results:** When differentiating mesothelioma patients from controls, receiver operating characteristic (ROC) curve analysis revealed an area under curve of 0.87 for SM and 0.84 for MPF. In controls, multiple linear regression identified age, glomerular filtration rate (GFR) and BMI as covariates of SM and MPF (R^2_{SM}=12%, R^2_{MPF}=14%). In the mesothelioma patients, GFR and tumor stage predicted biomarker levels (R^2_{SM}=10%, R^2_{MPF}=13%). ROC regression analysis showed that age, GFR and mesothelioma tumor stage had an impact on the diagnostic performance of the biomarker levels. Multivariate Cox regression analysis revealed that performance status, tumor stage and histology were prognostic factors, in contrast to SM and MPF. No association between asbestos exposure and biomarker levels was found. **Conclusions:** The diagnostic performance of SM and MPF was equivalent. Age, GFR, BMI and tumor stage explained only a limited portion of the between-subject variability in biomarker levels. Yet, ROC regression revealed that a subset of these covariates had a significant impact on the diagnostic performance of SM and MPF. Biomarker levels were no indicators of worse survival or asbestos exposure.

S07-3

Combination of serum mesothelin and plasmatic osteopontin in the diagnosis of malignant pleural mesothelioma

Rudy Foddis[1], Luciano Mutti[2], Silvia Simonini[1], Agnese Vivaldi[1], Giovanni Guglielmi[1], Alessandro Ferraro[1], Nicolino Ambrosino[3], Antonio Chella[3], Marco Lucchi[4], Pier Aldo Canessa[5]

[1]Division of Occupational and Preventive Medicine, University Hospital of Pisa, Italy, [2]Department of Medicine, Local HealthUnit11,Vercelli, Italy, [3]Pulmonary Unit, University Hospital of Pisa, Pisa, Italy, [4]Cardiothoracic Department, University of Pisa, [5]Pulmonary Unit, Hospital of Sarzana, La Spezia, Italy

Several authors have recently proposed the dosage of some proteins as markers of early diagnosis and/or risk factors of Malignant Pleural Mesothelioma (MPM). So far each single marker is characterized by rates of sensitivity and specificity not completely suitable for clinical or preventive applications. For this reason, we evaluated whether the combination of two markers (serum mesothelin and plasmatic osteopontin) could increase the sensitivity and specificity in diagnosis of epithelioid MPM.Serum and plasma samples were available from consecutive patients presenting at the University Hospital of Pisa. We measured serum mesothelin (SMRP) and plasmatic osteopontin (pOPN) levels in 93 healthy asbestos-exposed subjects and 25 patients with epithelioid MPM. SMRP and pOPN median values in epithelial MPM patients and healthy subjects were significantly different ($p<0.0001$). We compared sensitivity and specificity of SMRP and pOPN as well as the area under the ROC curve (AUC) with those derived from the combination of the two markers, obtained through a logistic regression analysis. The combination of the two markers resulted in an increase of both sensitivity and specificity as well as AUC value (AUC values: pOPN 0,819, SMRP 0,808, pOPN + SMRP 0,933).This study demonstrated that the combined use of SMRP and pOPN improves the diagnostic accuracy in diagnosis of epithelioid MPM compared to the single marker. Grant support: Noprofit Buzzi Unicem Foundation for Pleural Mesothelioma Research

S07-4

SMRP expression in a cohort of australian asbestos-exposed power industry workers

AD LaMontagne, AH Holloway, GA Lee

McCaughey Centre, Melbourne School of Population Health, University of Melbourne, Australia

A cohort study was undertaken in power industry workers in the Latrobe Valley power-generating region of Victoria (Australia), which suffers the state's highest rates of mesothelioma from extensive past use of asbestos. From an estimated 55,000 current and past workers in 2003, we aimed to recruit approximately 1,000 of these asbestos-exposed workers.The main aims of the study include (i) establishing a cohort of asbestos-exposed workers, (ii) developing a biospecimen bank, (iii) assessing the natural history of serum mesothelin-related protein (SMRP) levels in the cohort in relation to feasibility for use in the early detection of mesothelioma, and (iv) developing and evaluating a community based smoking cessation program. Blood samples are being collected in annual waves up to 36 months.A total of 708 subjects consented, the majority were born between 1948 and 1965. Consenting participants completed questionnaires on demographics, employment and exposure histories, and health. Ten mL blood samples were collected. For the initial wave of data collection, 594 questionnaires were completed and 617 blood samples collected.To date, mesothelin assays have been done on a total of 450 wave 1 samples. The majority of SMRP levels were <1.5 nM (n=425, 94%)-the manufacturer's recommended "high " threshold. Twenty-two of the remaining 25 samples were between 1.5nM and 2.5nM, and three had mesothelin >2.5nM (total of 6% of samples "high "). One of the highest SMRP levels (5.0 nM) was from a participant with pleural plaques and lung cancer (now deceased). Analysis of SMRP by sex, age, co-morbid conditions and exposure history will also be presented. The potential for future collaboration on emerging early detection methods for mesothelioma will be discussed.

S07-5

Low serum SMRP (soluble mesothelin-related peptide) identifies patients with extended survival for malignant pleural mesothelioma

Lindsay Almquist[1], Karl Kelsey[2], Jessica LaRocca[2], Silvia Plaza[2], Geralyn Lambert-Messerlian[2], David Sugarbaker[3], Raphael Bueno[3], Andres Houseman[2], John Godleski[4], Carmen Marsit[2], Brock Christensen[2], Heather Nelson[1]

[1]Masonic Cancer Center, University of Minnesota, USA, [2]Brown University, [3]Harvard Medical School, [4]Harvard School of Public Health

Elevated serum soluble mesothelin-related peptide (SMRP) is present in a high proportion of Malignant Pleural Mesothelioma (MPM) cases, likely tagging a phenotypically distinct subset of tumors. Using a case series of MPM patients obtained from the International Mesothelioma Program at the Brigham and Womens Hospital, we tested the clinical utility of serum SMRP in determining patient outcome. SMRP was measured using ELISA (MESOMARK, Fujirebio Diagnostics, Malvern PA) from blood samples obtained prior to treatment (n=179). Positivity was defined as ≥ 1.5nM (recommended manufacturers cut-off and near our population median). Females were significantly more likely to be SMRP positive than SMRP negative ($p<0.04$). Further, SMRP positive cases were significantly older than SMRP negative cases ($p<0.001$). There were no associations between SMRP and either asbestos exposure (quantitative asbestos body count or self-reported) or histology type. Evaluating SMRP as a clinical marker, we found that MPM patients positive for SMRP had significantly worse survival overall ($p=0.052$), and that this relationship was very strong after consideration of histology and sex (stratified log-rank test, $p=0.009$). In a Cox regression model adjusting for sex, age and histology, SMRP positive patients had a HR of 1.5 (95% CI 1.0, 2.1). This work demonstrates that serum SMRP is significantly associated with MPM patient outcome. Future work examining response-to-therapy based on SMRP status may lead to promising targeted therapies for this highly fatal disease.

S08

PET scan in MPM Imaging

S08-1

Functional MR tools for diagnosing malignant pleural mesothelioma

Johan Coolen[1], Philippe Nafteux[2], Frederik De Keyzer[1], Walter De Wever[1], Christophe Dooms[2], Ilse Roebben[1], Wim Van Hecke[1], Eric Verbeken[2], Paul De Leyn[2], Stefan Ghysels[1], Dirk Van Raemdonck[2], Kristiaan Nackaerts[2], Johny Verschakelen[1], Steven Dymarkowski[1]

[1]Department of Radiology, University Hospitals Leuven, Belgium, [2]Departments of Thoracic Surgery, Pneumology, and Histopathology, University Hospitals Leuven, Belgium

PURPOSE
Correct diagnosis and staging of malignant pleural mesothelioma (MPM) is primordial because of its infaust prognosis. Therefore, we prospectively evaluated the potential role of diffusion weighted imaging (DWI) sequence in this setting. Additionally, we examined if dynamic contrast-enhanced MRI (DCE-MRI) could further improve DWI diagnostic value.

MATERIAL AND METHODS
Thirty-one consecutive patients with pleural abnormalities suspect for malignant pleural disease underwent chest CT or whole-body PET-CT (PET/CT), MR with DWI and DCE-MRI and explorative thoracoscopy with histopathological confirmation. PET/CT staging was based on the clinical routine patient diagnosis, while DWI was evaluated first by calculating the apparent diffusion coefficient (ADC) of the suspect lesion and secondly by interpretation of the curves derived from DCE-MRI data. Both PET/CT and MR data were correlated with pathology.

RESULTS
In this cohort, there were 10 patients with MPM, 4 pleural metastases, 1 pleural fibroma and 16 benign pleural diseases. The sensitivity and specificity of PET/CT in differentiating benign from malignant pleural disease is 100% and 38%, respectively (accuracy 68%).
Pure ADC-based diagnosis showed an optimal threshold of 0.00152 mm^2/s to discriminate between benign and malignant lesions, with sensitivity and specificity of 66% and 100%, respectively (accuracy 84%). This could be improved to 87% and 94% (accuracy 90%) using the DCE-MRI-data in case of ADC between 0.00152 and 0.00200 mm^2/s. In total, 18 patients were staged correctly with PET/CT, 11 incorrectly and 2 undetermined. DWI staged 26 patients correctly and 5 incorrectly. The undetermined cases on PET/CT were correctly diagnosed on MRI.

CONCLUSION
DWI supplemented with DCE-MRI seems a promising tool for the differential diagnosis and staging of pleural diseases and particularly for MPM. ADC-based diagnosis can be used first to discriminate between benign and malignant lesions, while DCE-MRI provides additional information rectifying some false negative ADC findings.

S08-2

Role of PET/CT in staging and surgery for malignant pleural mesothelioma

Cosimo Lequaglie[1], Gabriella Giudice[1], Christian Rolfo Cervetto[2], Rita Marasco[1], Margherita Garramone[1]

[1]Thoracic Surgery, IRCCS Ospedale Oncologico Rionero in Vulture, Italy, [2]Medical Oncology Clinica Rotger Palma de Mallorca, Spain

Objectives: To show the PET/CT role in reducing the exploratory procedures with an early diagnosis, in a better staging of disease and in a postoperative follow-up in MPM.Materials/Methods: Sixty-seven potentially extrapleural pneumonectomy (EPP) submitted patients (range age of 31-79 years) were observed between 1999 and 2009. All patients underwent PET/TC scan: median preoperative SUV was 6.8 (range 3-20). The same surgeon team operated 45 patients (29 male - 16 female), 23 right-sided lesions and 22 left-sided. Forty patients underwent EPP, 1 pleural decortications, 3 exploratory thoracotomies for chest wall or inferior vena cava invasion, and 1 laparoscopy for peritoneal metastases. One patient were no surgical proposed for macroscopically evidence of extended diseases and received chemotherapy. Thirty-seven patients had epithelioid subtype, 3 sarcomatous and 6 biphasics. Eighteen tumors were pStage I-II, 24 pStage III and 4 pStage IV according IMIG staging system. Results: All T4 or M1 cases were detected by PET/CT preoperative scan. The follow-up study reported a PET/TC sensibility of 95% with a diagnostic accuracy of 92% regarding the local recurrences or distant metastases. The analysis correlation in patients with high and low SUV and epithelioid and non-epithelioid histotype showed a better prognosis in both low SUV and epithelioid tumor.The median follow-up for all surviving patients was 36 months. Disease free survival were 11 and 21 months for the high and low SUV groups, respectively. Median survivals were 16 and 29 months for the high and low SUV groups, respectively. In a multivariable analysis, high SUV tumors were associated with a 4.1 times greater risk of death than low SUV tumors ($p = 0.04$). Conclusions: PET/CT appears to give a good support in staging and prognosis in MPM. A systematic use of PET scan could be anticipate the recurrence of MPM in follow-up period in EPP submitted patients.

S08-3

^{18}F-Fluorothymidine positron emission tomography (FLT PET) imaging in malignant pleural mesothelioma (MPM)

Roslyn Francis[1,2,7], Anna Nowak[2,3,7], Tatiana Segard[1], Michael Philips[4], Peter Robins[1], Peter Swift[1], Agatha van der Schaaf[1], Elizabeth Thomas[1], Laurence Morandeau[5], Jan Boucek[1], William Musk[6,7], Michael Millward[2,3]

[1]Department of Nuclear Medicine/ WA PET Centre, Sir Charles Gairdner Hospital, Australia, [2]School of Medicine and Pharmacology, University of Western Australia, [3]Department of Medical Oncology, Sir Charles Gairdner Hospital, Western Australia, [4]Cancer Council Clinical Trials Centre, Western Australian Institute for Medical Research, Western Australia, [5]Medical Technology and Physics, Sir Charles Gairdner Hospital, Western Australia, [6]Department of Respiratory Medicine, Sir Charles Gairdner Hospital, Western Australia, [7]National Research Centre for Asbestos Related Diseases

18F-fluorodeoxyglucose (FDG) PET is emerging as a useful modality in prognostication and response assessment of MPM. 18F-Fluorothymidine (FLT) is a PET cellular proliferation tracer, which has been studied in response evaluation in solid tumours, but not in MPM.
Patients with confirmed MPM commencing first line chemotherapy were prospectively recruited. Patients had FLT and FDG PET scans at baseline and after 1 cycle of chemotherapy. PET scans were assessed visually and semi-quantitatively using a 3D region growing algorithm. Patients received routine clinical care, including CT scans for response assessment at baseline and after cycles 1, 4 and 6. Patients were followed for progression and survival.
33 patients have been recruited and visual analysis for PET stage and response performed. 32 of 33 (97%) patients demonstrated tumour FLT activity. There was a significant correlation between FLT and FDG PET T ($\kappa = 0.65$ $p<0.001$), N ($\kappa = 0.41$ $p=0.012$), M ($\kappa = 0.71$ $p<0.001$) and UICC TNM summary stage ($\kappa = 0.62$ $p=0.001$). FLT PET trended towards slightly higher N stage. 28 of 33 patients were assessable for response after 1 cycle of chemotherapy. Measured agreement between two independent readers was 94% for visual response assessment. There was however only 50% concordance between FLT and FDG consensus response after 1 cycle of chemotherapy, with 8 of 14 discordant responses (57%) from FDG partial response/ FLT stable disease category. FLT PET had fewer partial responses (18% FLT, 43% FDG partial response) and more progressive disease (25% FLT and 14% FDG progressive disease) compared with FDG PET.
Mesothelioma demonstrates proliferative activity. FLT PET tumour stage correlates closely with FDG stage. Concordance between FLT and FDG PET visual response assessment after 1 cycle of chemotherapy is poor, predominantly because FLT changes post therapy appear less pronounced than FDG changes. Outcome data is being compiled.

S08-4

Comparison of dynamic contrast enhanced MRI (DCE-MRI) parameters with integrated PET-CT (TGV and SUVmax) and serum mesothelin in the baseline assessment of malignant pleural mesothelioma (MPM)

Clare Hooper[1], David Hall[2], Paul Virgo[3], Paul White[4], Mike Darby[5], Toby Hall[6], Jeremy Braybrooke[7], Julie Searle[2], Iain Lyburn[2], Nick Maskell[1]

[1]University of Bristol, Pleural Clinical Trials Unit, Southmead Hospital, UK, [2]Cobalt Cheltenham Imaging Centre, Cheltenham, UK, [3]Department of Immunology, Southmead Hospital, Bristol, UK, [4]University of the West of England, Bristol, UK, [5]Department of Radiology, Southmead Hospital, Bristol, UK, [6]Department of Radiology, Royal United Hospital, Bath, UK, [7]Bristol Oncology Centre, UBHT, Bristol, UK

PET-CT and serum mesothelin have shown promise in predicting prognosis and evaluating treatment response in MPM but may be less reliable with sarcomatoid histology or prior talc pleurodesis. DCE-MRI with pharmacokinetic analysis, is a novel metabolic imaging modality providing a measure of tumour angiogenesis. We prospectively examined the relationship between pharmacokinetic parameters on DCE-MRI (Gadolinium washout rate) with PET-CT (SUVmax and TGV), serum mesothelin and histological sub-type in MPM patients at diagnosis.Method: Pre-treatment patients with histologically proven MPM underwent DCE-MRI and integrated PET-CT scans and serum mesothelin assay (MESOMARK™) at a single visit. TGV was calculated using MIM software version 4.2.2 (MIMvista corp.). Gadolinium washout rate (GWR) on DCE-MRI was measured via CAD software (ViewForum R6.3 V1L3, Philips Medical Systems). Results: 30 patients.70%(21/30) epithelioid and 30%(9/30) sarcomatoid histology. 43%(13/30) had undergone prior talc pleurodesis. Histological sub-type did not statistically significantly affect SUVmax, TGV or GWR. Mesothelin was significantly greater in the epithelioid group (3.2nM/L (2.0,6.3) vs 0.6nM/L (0.5,0.8) P<0.001). There was no significant difference in mesothelin, SUVmax, TGV or GWR between talc pleurodesed and non-pleurodesed patients in the whole group, but in the epithelioid sub-group there was a trend to significantly higher TGV with talc pleurodesis (talc: 2799 (1931,11257) no talc: 955.5(146.8,2354) P=0.053) that was not observed with GWR (P=0.4179). While SUVmax strongly correlated to TGV (r=0.725, P0<.001), there was no correlation between GWR and TGV (r= 0.203, P=0.282) or between mesothelin and any of the imaging values.Conclusion: Metabolic imaging provides a reliable baseline assessment of epithelioid and sarcomatoid MPM while Mesothelin is usually low in the latter. DCE-MRI with GWR may be less sensitive to talc pleurodesis than PET-CT parameters and is a cheaper, more readily available modality that involves shorter patient appointment times warranting further study in MPM prognostic evaluation and treatment response monitoring.

S08-5

Computerized measurement of malignant pleural mesothelioma

William Sensakovic[1], Samuel Armato III[1], Christopher Straus[4], Rachael Roberts[1], Philip Caligiuri[3], Adam Starkey[1], Hedy Kindler[2]

[1]Committee on Medical Physics, The University of Chicago, Chicago, USA, [2]Dept. of Medicine, The University of Chicago, Chicago, Illinois, USA, [3]Dept. of Radiology, University of Utah, Salt Lake City, Utah, USA, [4]Dept. of Radiology, The University of Chicago, Chicago, Illinois, USA

Background: Quantification of mesothelioma tumor extent is required to evaluate the efficacy of clinical trials. The manual acquisition of linear tumor thickness measurements on each of three sections on serial computed tomography (CT) scans is the current standard for tumor response assessment. Previous studies have determined that volume could be a more accurate indicator of response; however, the time necessary to manually delineate tumor volume makes implementation difficult. The purpose of this study was to create a computerized system to identify mesothelioma tumor in 3D and measure tumor volume. **Methods:** CT scans from 18 mesothelioma patients were collected, and a computerized system based on active surfaces identified mesothelioma in each scan. Three observers manually outlined tumor on a subset of sections for each scan. The similarity among observers and between the manual and computer-generated outlines was measured using the Dice coefficient (D), which equals 1 if outlines are identical and 0 if they do not overlap. Area for manual outlines and 2D cross-sections of the computer-defined volume were also compared.**Results:** The median D between pairs of observers were 0.620, 0.635, and 0.777. Median D between each observer and the computer-generated outlines were 0.550, 0.627, and 0.640. The bias between pairs of observers ranged from 367mm^2 to 910mm^2, and the corresponding 95% limits of agreement for area differences were (-2645.24,1911.32) and (-4476.43,2657.39). The bias between the mean observer area and the computer area was 593mm^2, and the 95% limits of agreement were (-4059.34,2872.96).**Conclusion:** A computerized system to delineate mesothelioma tumor in 3D and measure tumor volume was presented. The computer-generated outlines and volume were accurate representations of the tumor (as defined by observer outlines and 2D area) and indicate that the computer method could be applied in future studies to evaluate tumor progression and treatment efficacy.

S09

Thoracoscopy

S09-1

Keynote Speaker

Thoracoscopy in mesothelioma

Hans Hoffmann, Hendrik Dienemann

Department of Surgery, Thoraxklinik, University of Heidelberg, Germany

Thoracoscopy is the preferred **diagnostic procedure** in MPM because it allows complete visual examination of the pleura, multiple, deep and large biopsies (preferably including fat and/or muscle to assess tumor invasion) and provides a diagnosis
in 90% of cases (1). If thoracoscopy is performed, it is recommended to take biopsies of both normal and seemingly abnormal pleura (grade 1C) (1). In patients who are candidates for EPP, the incision(s) should be placed in line with the planned subsequent thoracotomy incision, so that the trocar incisions can be excised at the time of the EPP.
There is limited but emerging evidence that VATS **debulking pleurectomy/decortication can provide good symptom control and may have a beneficial effect on survival** (2). The objective of the operation is to relieve an entrapped lung by removing the visceral tumour cortex. Removal of the parietal tumor cortex may relieve a restrictive ventilatory deficit and reduce chest wall pain. At present there is an absence of randomised trials, but a national study is ongoing in the UK which is being supported by the National Cancer Research Institute comparing VATS debulking with chemical pleurodesis (MesoVATS) (1).
Thoracoscopy with **pleurodesis** is useful in preventing recurrent effusions. Sterile talc is preferred to other agents (grade 1A). Pleurodesis is most effective when performed early in the disease process (grade 1C) but it should not be performed before sufficient tissue for diagnosis has been obtained (grade 1A) (1).

1. Scherpereel et al., Guidelines of the ERS and ESTS for the management of malignant pleural mesothelioma, Eur Respir J 2010; 35:479-495
2. Srivastava et al., Does video-assisted thoracoscopic decortication in advanced malignant mesothelioma improve prognosis?, ICVTS 2009; 8:454-456

S09-2

A new electrocautery pleural biopsy technique using an insulated tip diathermic knife during semirigid pleuroscopy

Shinji Sasada[1], Kunimitsu Kawahara[2], Norio Okamoto[1], Masashi Kobayashi[1], Tomonori Hirashima[1], Hidekazu Suzuki[1], Motohiro Tamiya[1], Yuka Matsuura[1], Yashu Chang[3], Teruomi Miyazawa[4], Ichiro Kawase[1]

[1]Thoracic Malignancy, Osaka Prefectural Medical Center for Respiratory and Allergic Diseases, Japan, [2]Pathology, Osaka Prefectural Medical Center for Respiratory and Allergic Diseases, [3]Pulmonary Medicine, North Shore Hospital, [4]Pulmonology, St. Marianna University School of Medicine

Introduction: Biopsy size obtained with standard flexible forceps (SFF) during semirigid pleuroscopy is often insufficient pathologically. An insulated-tip diathermic knife (IT knife: IT-1, IT knife 2; IT-2) appears to be safe resection of a larger lesion in gastrointestinal endoscopy. We sought to validate an electrocautery pleural biopsy technique using the IT-1/IT-2 during semirigid pleuroscopy. Methods: Thirty-three patients with thickening pleura without obvious changes in bulge were eligible for biopsy using the electrocautery (IT-1 in 26 / IT-2 in 7) between 2006 and 2009. After subpleural injection of saline with lidokine and epinephrine, the pleural lesion was electorically incised in a circular shape with full-thickness using IT-1or IT-2. Pathological findings of the specimens obtained by IT-1/ IT-2 were compared to those by SFF, and we reviewed the pleuroscopic parameters such as complications, procedure time, and diameter of the specimens.Results: Diagnostic yields from specimens obtained with IT-1, IT-2, and SFF were 76.9% (20/26), 100 % (7/7), and 69.7 % (23/33), respectively. IT-1/IT-2 biopsy was superior to SFF in 10 of 33 patients (mesothelioma in 3, inflammation in 5, breast cancer in 1, and TB in 1). These pleural lesions revealed thickened, smooth abnormal appearances. The overall diagnostic yield for both IT-1/ IT-2 and SFF was 100%. The median time of the procedure was 21 min (10-92), and median diameter of specimens was 14 mm (6-26). There were no severe complications.Conclusions: Electrocautery biopsy using IT-1/IT-2 during semirigid pleuroscopy has great potential for diagnosing smooth abnormal pleura which are difficult to biopsy with SFF.

S09-3

Narrow band imaging: A new technology for the diagnosis of malignant pleural mesothelioma

Atsuko Ishida[1], Miho Nakamura[1], Yuka M Miyazu[1], Takahiro Kida[1], Hiroshi Handa[1], Hiroki Nishine[1], Seiichi Nobuyama[1], Takeo Inoue[1], Taeko Shirakawa[1], Masamichi Mineshita[1], Teruomi Miyazawa[1], Noriaki Kurimoto[2], Takashi Nishisaka[3], Philippe Astoul[4]

[1]Division of Respiratory and Infectious Diseases, Department of Internal Medicine, St Marianna University School of Medicine, Japan, [2]Division of Chest Surgery, Department of Surgery, St Marianna University School of Medicine, Kawasaki, Japan, [3]Department of Pathology, Hiroshima Prefectural Hospital, Hiroshima, Japan, [4]Division of Thoracic Oncology, Department of Thoracic and Cardiovascular Diseases, St Marguerite Hospital University of the Mediterranean, Marseille, France

Narrow band imaging (NBI) is an endoscopic image enhancement technology that highlights blood vessels. NBI is known to be useful in the early diagnosis of pharyngeal and esophageal cancer by detecting subtle changes of vascular patterns. We applied NBI to pleuroscopy to evaluate the efficacy of NBI in the diagnosis of malignant pleural mesothelioma. From May 2006 to January 2010, we performed pleuroscopy using a pleura-videoscope with white light (WL) and NBI under local anesthesia in 75 patients. Of these, malignant pleural mesothelioma was diagnosed in 15 patients (10 epithelial type, 3 biphasic type, and 2 sarcomatous type). Two patients were excluded from evaluation, as careful observation of the pleura using NBI was impossible due to multiple or bloody fibrinous adhesion. For the remaining 13 patients, 27 biopsy specimens were obtained from particular sites showing distinct vascular patterns seen by WL and/or NBI. All endoscopic images recorded by WL and NBI were classified visually by vascular patterns. All biopsy specimens collected for this study were confirmed pathologically as malignant mesothelioma. Of the 27 lesions, WL displayed blood vessels in 10 lesions, and NBI in 24 lesions (WL vs. NBI; $p = 0.0001$). Blood vessels seen on the lesions were classified mainly into 2 patterns, punctate vessels and blood vessels with irregular caliber. Punctate vessels were seen in 4 lesions under WL, and 10 in NBI. Blood vessels with irregular caliber were seen in 6 lesions under WL, and 14 in NBI. Our study demonstrated that NBI visualized blood vessels significantly better than WL for malignant pleural mesothelioma, and suggests that NBI applied to pleuroscopy may be useful in selecting optimal biopsy sites by highlighting punctate vessels or blood vessels with irregular caliber in the diagnosis of malignant pleural mesothelioma.

S09-4

Thoracoscopy combined with autofluorescence imaging and narrow band imaging for the diagnosis of malignant pleural mesothelioma

Akihiro Yasumitsu[1], Kazuya Fukuoka[1], Hitomi Kamiya[1], Miki Honda[1], Eriko Masachika[1], Hisaya Okuwa[1], Yoshitaka Nogi[1], Risa Maeda[1], Taiichiro Otsuki[1], Kouji Mikami[1], Noriko Hirayama[1], Takayuki Terada[1], Asuka Okada[1], Aki Murakami[1], Shusai Yamada[1], Kunihiro Tamura[1], Kozo Kuribayashi[1], Chiharu Tabata[1], Noriaki Tsubota[2], Takashi Nakano[1,2]

[1]Division of Respiratory Medicine, Department of Internal Medicine, Hyogo College of Medicine, Japan, [2]Department of Thoracic Oncology, Hyogo College of Medicine, Hyogo, Japan

Background and Objective. Thoracoscopy is an essential procedure for the definitive diagnosis of malignant pleural mesothelioma (MPM). However, in some cases of early-stage MPM, it is difficult to detect a lesion even through conventional thoracosopy. In the present study, to improve the diagnostic accuracy of thoracoscopy on MPM, autofluorescence imaging (AFI) and a narrow band imaging (NBI) system was assessed in combination with the conventional method.

Subjects and Methods. Thoracoscopy combined with AFI and NBI was performed on 34 patients with pleural fluid retention, suspected of MPM. Thoracoscopy under local anesthesia and video-assisted thoracoscopic surgery (VATS) were performed on 32 and 2 patients, respectively. Examination was carried out using a flexible bronchoscope (Olympus BF-F260) for AFI and a conventional white light thoracoscope (Olympus LTF-240) for NBI.

Results. In 31 of 34 patients, a diagnosis of MPM was made by pleural biopsy using thoracoscopy combined with AFI and NBI. Autofluorescence thoracoscopy showed that nodules suspicious for mesothelioma were clearly visualized as magenta fluorescence, while the intact pleura appeared green in color. In the cases of MPM, thoracoscopy with NBI demonstrated emphasized irregularity of the pleura was as compared with findings on conventional white light thoracoscopy, and abnormal pink/white nodules with increased vessel growth was easily detected.

Conclusion. Thoracoscopy combined with AFI and NBI is a novel tool for the diagnosis of MPM. There is a possibility that this procedure may make it possible to distinguish between pleural lesions due to MPM and the intact pleura more accurately.

S10

Second-line treatment

S10-7 Keynote Speaker

Beyond first-line systemic therapy for MPM

Christopher Lee

Medical Oncology, BC Cancer Agency-Fraser Valley Centre, Canada

In the last decade, first-line chemotherapy for malignant pleural mesothelioma (MPM) has become accepted as a standard of care. There is no longer therapeutic nihilism in management of the disease, and this has spurred increased interest in drug development. Assessment of novel agents in the second-line setting is now routine, and such studies are helping to advance a general understanding of the biology of MPM. At the same time, salvage chemotherapy at the time of progression and maintenance therapy are strategies that have major implications for clinical care and continue to undergo evaluation. Population-based data and follow up of patients enrolled on clinical trials indicate already significant utilization of second-line chemotherapy, and there is a need to provide appropriate evidence to support this practice. Also a priority is ongoing research to better define community standards of supportive and palliative care. Managing the symptoms associated with MPM warrants a tailored approach that should be achievable in more than just specialized centres. Beyond first-line systemic therapy for MPM are a host of challenges that require continued dedicated effort and collaboration amongst researchers.

S10-1

VANTAGE 014: Vorinostat in patients with advanced malignant pleural mesothelioma (MPM) previously treated with pemetrexed and either cisplatin or carboplatin: A phase III, randomized, double-blind, placebo-controlled trial

Lee Krug[1], Paul Baas[2], Hedy Kindler[3], Hilary Calvert[4], Maurizio Marangolo[5], Christian Manegold[6], Anne Tsao[7], Dean Fennell[8], Xing Sun[9], Gregory Lubiniecki[9]

[1]Memorial Sloan-Kettering Cancer Center, USA, [2]The Netherlands Cancer Institute, Amsterdam, Netherlands, [3]University of Chicago, Chicago, IL, USA, [4]University College London Cancer Institute, London, UK, [5]Azienda USL, Ravenna, Italy, [6]University Medical Center Mannheim, Heidelberg University, Heidelberg, Germany, [7]MD Anderson Cancer Center, Houston, TX, USA, [8]Queen' s University Belfast & Northern Ireland Cancer Centre, Belfast, Northern Ireland, [9]Merck Research Laboratories, Upper Gwynedd, PA, USA

Background: Vorinostat, a histone deacetylase inhibitor, alters gene expression and protein activity. In a phase I study of single–agent vorinostat, 5 of 13 previously treated patients with MPM experienced stable disease lasting 4–13 months. This global phase III study investigates the overall survival and tolerability of vorinostat plus best supportive care (BSC) vs placebo plus BSC in patients with MPM. Methods: Patients with pathologically confirmed MPM and disease progression following 1 or 2 prior systemic regimens are eligible. Patients receive oral vorinostat 300 mg (or matching placebo) twice daily for 3 days each week of a 3–week cycle. Primary endpoints are overall survival and safety/tolerability (NCI–CTCAE, version 3.0). Secondary endpoints are objective response rate, progression–free survival, pulmonary function, and patient–reported symptoms. Tumor samples are also being collected to analyze for markers that correlate with activity. Enrollment of 660 patients is planned, which will provide 90% power to detect a hazard difference of 25% (e.g. median survival from 6 to 8 months). Results: As of April 2010, 458 patients, most of whom are men (84%) and have had a single line of prior therapy (73%), have enrolled at 77 sites. Baseline patient characteristics are median age 64 years, epithelial histology (83%), and median Karnofsky performance status 80%. Of 427 patients, 95% have reported ≥1 adverse event (AE). The most common all-grade AEs (incidence of grade 3–5 events) are nausea 44% (3%), fatigue 38% (10%), dyspnea 31% (13%), and decreased appetite 29% (3%). The independent data monitoring committee met in December 2009 for the third protocol–specified interim analysis and advised continuation of enrollment. No safety concerns were identified at that analysis of 372 patients. Conclusions: This is the largest randomized study of patients with MPM. If successful, vorinostat would fill an unmet medical need for patients with previously treated MPM.

S10-2

Open label randomized phase III maintenance study of thalidomide (arm A) vs. observation (arm B) in patients with MPM after treatment with cisplatin and pemetrexed: interim report

Paul Baas[1], Wieneke Buikhuisen[1], Andrew Vincent[1], Rob van Klaveren[3], Frank Custers[4], Hugo Schouwink[5], Otilia Dalesion[1], Nick Pavlakis[2], Sjaak Burgers[1]

[1]The Netherlands Cancer Institute, Netherlands, [2]Sir Gardner Hospital Sydney, [3]Erasmus University Rotterdam, [4]Atrium medical Center, Heerlen, [5]Medical Spectrum twente, Enschede

Introduction: Since standard treatment with a platin and anti-folate does not lead to long survival the addition of an anti vascular agent seems reasonable. In patients who did not progress after chemotherapy, suppression of the neo-vasculature by adding thalidomide might lead to improved progression free survival. We here report on the toxicityM and M: All received a platin (carboplatin AUC 5 or cisplatin 75mg/m2), pemetrexed 500 mg/m2 (q3) or pemetrexed 500 mg/m2 alone (q3) for 4 courses. Patients without signs of progression and who gave informed consent, were randomized to receive thalidomide 200 mg/day orally (A) or no treatment (B). CT scanning of the thorax and physical examination was performed every two months or earlier when indicated. Progression free survival was calculated from start of randomization. A number of 190 events was required to show an improvement of 50% in PFS (5 to 7.5 months).Results: From 05/2004 until 12/2009 222 patients from 8 Dutch and 4 Australian centers were included in this study (111 in each arm). Median age: 64 yrs (range 41-82); 190 epithelial type; 216 WHO 0-1 and 6 WHO 2. Toxicity: At the time of this report 205 (103 arm A; 102 arm B) patients were evaluable for toxicity. There were few observed toxicities. Total % of grade 3 toxicity was 28% for arm A and 15% for arm B. Low platelet count was observed in 4 patients in arm A and 8 in arm B. Two patients in arm B experienced leukopenia. In arm A two patients experienced constipation and two neurosensory problems, while neither toxicity occurred in arm B. No cases of drug related cardiovascular toxicity were seen. Conclusions: This is currently the largest maintenance study in patients with MPM. During the meeting an update will be presented.

S10-3

Randomized, multicentre phase IIIb study of ranpirnase + doxorubicin (DOX) versus DOX in patients with unresectable malignant mesothelioma (MM)

Joachim von Pawel[1], M. Krzakowski[2], J. Jassem[3], C. Eschbach[4], J. Kozielski[5], J. Costanzi[6], U. Gatzemeier[8], K. Shogen[7], M. Reck[8]

[1]Asklepios Fachkliniken Muenchen-Gauting, Germany, [2]Institute of Oncology, Warsaw, Poland, [3]Medical University, Gdansk, Poland, [4]Asklepios Klinikum Harburg, Hamburg, Germany, [5]Medical University, Zabrze, Poland, [6]Lone Star Oncology Consultants, Austin, TX, USA, [7]Alfacell, Somerset, NJ, USA, [8]Krankenhaus Grosshansdorf, Grosshansdorf, Germany

Ranpirnase (Onconase®) is a novel ribonuclease with preclinical activity against various tumors. A median survival of 8.3 months was seen in a multicentre phase II trial (chemonaive and pretreated pts) who met the CALGB prognostic criteria group 1-4 (Mikulski,JCO 20,2001).
Methods: This multicenter controlled phase III trial compared efficacy and safety of Dox with or without ranpirnase. Primary endpoint was OS, secondary endpoints included RR, TTP, safety and disease related symptoms. Eligibility criteria: unresectable MM; CALGB group 1-4, ECOG PS 0-1. Stratification was by CALGB group and histology (epithelioid vs. non-epithelioid). One line of prior therapy was permitted. Between 08/01 and 09/07 413 eligible pts were randomized to DOX 60 mg/m2 3-weekly with or without ranpirnase 240 μg/m2 weekly (cycle1) and 480 μg/m2 if no severe toxicity had occurred. The study was designed to detect an increase of 4 ms (9vs.13) in median OS (MST) using a two-sided logrank test (α=5%) with 90% power.
Results: Both arms were well balanced (DOX+ranpirnase/DOX: 203/210pts): Mean age 62.2/61.8 yrs; males 157/156; PS 0 52/60; PS 1 151/150; prior chemo 65/65 pts (pemetrexed 35/35;other chemo 30/30). CALGB groups 14/14(1), 45/51(2), 117/115(3), 27/30(4). Investigator assessed tumor regression and stabilization rate was in favor of combination arm (tumor regression: 28%vs18%, tumor stabilization: 47%vs47%, p =0.04). In the ITT there was no significant difference in OS (MST: 11.1vs10.7ms; HR1.02, 95%CI 0.82-1.26) whilst in a preplanned analysis including 130 pretreated patients a significant advantage in survival in favor of DOX+ranpirnase was found (MST:10.5vs9ms; HR1.49, 95%CI1.02-2.17). The safety profile for both treatment arms was similar. Most frequent side effects reported for both treatment groups included nausea, fatigue and alopecia.
Conclusion: Combination of ranpirnase and DOX is a safe and feasible treatment in unresectable MM and showed a significant impact on survival of pretreated patients compared to DOX alone.

S10-4

Vinorelbine (V) in pemetrexed-pretreated patients (PTS) with malignant pleural mesothelioma (MPM)

Paolo Zucali[1], Elena Lorenzi[1], Giovanni Ceresoli[2], Fabio De Vincenzo[1], Letizia Gianoncelli[1], Matteo Simonelli[1], Cristina Ripa[2], Laura Giordano[1], Armando Santoro[1]

[1]Department of Medical Oncology, Istituto Clinico Humanitas IRCCS, Italy, [2]Department of Oncology, Istituto Humanitas Gavazzeni, Bergamo, Italy

BACKGROUND: The role of second-line therapy in MPM is currently undefined. V has shown activity in MPM. The aim of this study was to retrospectively evaluate the activity and toxicity of V in a series of pts previously treated with first-line pemetrexed-based chemotherapy. METHODS: V 25 mg/m2 was administered intravenously on days 1,8q21 for 6 cycles or until progression or unacceptable toxicity either as second-line (2L) or further-line (>2L) therapy. RESULTS: 43 pts were enrolled (2L:18pts;>2L:25pts). Median age: 67 (range 43-82). EORTC prognostic score: 19 pts good, 24 pts poor. Partial response was observed in 4 (9.3%) pts, stable disease in 12 (27.9%). Disease control rate (DCR) was 37.2%. Median progression free survival (PFS) was 2.1 months (mo) (0.6-10mo); median overall survival (OS) was 5.2mo (0.8-40.3mo). Pts with a DC achieved a longer PFS (5.2vs1.4mo;p<.001), and a longer OS (8.3vs3.2mo;p.017). Pts with a good EORTC score showed an higher DCR (66.7%vs28.6%;p.023), a longer PFS (3.2vs1.5mo;p.004), and a longer OS (12.5vs2.9mo;p<.001). The prognostic role of the EORTC score was confirmed in a multivariate analysis for OS (HR5.9;CI95%2.1-16.9;p<.001). No difference was observed in terms of DCR, PFS, and OS stratifying for the line of therapy and the response to first line. Grade 3/4 haematological toxicity: neutropenia in 5 (11.6%) pts, without febrile neutropenia. Non-haematological toxicity: grade 2 fatigue in 11 (25.5%) pts and constipation in 4 (9.3%).CONCLUSIONS: V was moderately active, with an acceptable toxicity profile, in pemetrexed-pretreated MPM pts, particularly with a good EORTC score. The role of second-line therapy in MPM remains to be evaluated in prospective trials on large series of pts.

S10-5

Platinum-based doublet chemotherapy in pretreated Malignant Pleural Mesothelioma (MPM) patients: a mono-institutional experience

Giulia Pasello[1], Samuele Nicotra[2], Giuseppe Marulli[2], Federico Rea[2], Laura Bonanno[1], Paolo Carli[1], Cristina Magro[1], Antonio Jirillo[1], Adolfo Favaretto[1]

[1]Medical Oncology Department of Istituto Oncologico Veneto, IRCCS, Italy, [2]Thoracic Surgery Department of University Hospital, Padua, Italy

BACKGROUND: The major clinical problems of MPM management are the short duration of response and the early relapse. Currently, after the first-line standard pemetrexed/platinum combination there is not a defined regimen for the second line treatment, and the clinical benefits in fit patients are uncertain. We analyzed the feasibility of gemcitabine/platinum chemotherapy in pretreated MPM patients.METHODS: Eligible patients should have relapsed after first-line chemotherapy with pemetrexed plus cisplatin (24%) or carboplatin (76%); 71% of the patients had received trimodality treatment, 29% were inoperable. Patients had to have PS=0-2, adequate organ function, measurable disease. Chemotherapy was gemcitabine 1000 mg/m^2 days 1,8 plus the alternative platinum compound respect to 1st line, i.e. cisplatin 75 mg/m^2 or carboplatin AUC5 day 1 every 3 weeks, for 3-6 cycles. Baseline staging and reassessment after cycles 3 and 6 were performed with CT-scan. RESULTS: Since 2006 17 relapsed MPM patients were referred to our centre. Patients were 12 males and 5 females; median age: 61 years (range 47-74); histology: 12 epithelial, 4 sarcomatoid and 1 biphasic. PS 1-2 (15:2).The combination of gemcitabine with carboplatin/cisplatin was administered as second line treatment in 13(76%) patients, as third line in 4(24%) patients. Two patients are ongoing, 2 were lost to follow-up without re-evaluation, therefore radiologic and clinical response was assessable in 13(76%) patients. We showed stable disease in 9(69%) patients and progressive disease in 4(31%) patients. Symptoms improved in 7(54%) cases. Median survival: 33.5 weeks, median time-to-treatment failure: 15.5 weeks.Toxicity profile showed 2(14%) grade-4 and grade-3 thrombocytopenia, 6(43%) grade-3 leucopenia, 3(21%) grade-3 anaemia and neutropenia. Grade-3 non haematological toxicities were nausea (7%) and asthenia (21%).CONCLUSION: Gemcitabine-platinum regimens are well-tolerated and able to control symptoms and disease progression. They can be considered valid therapeutic options for MPM relapsed patients after at least one line of chemotherapy

S10-6

Second-line chemotherapy in malignant pleural mesothelioma: Results of a retrospective multicenter study

Paolo Zucali[1], Matteo Simonelli[1], Giovanni Michetti[2], Giovanni Ceresoli[3], Marcello Tiseo[4], Elena Collova'[5], Alessandro Follador[6], Fabio De Vincenzo[1], Elena Lorenzi[1], Matteo Perrino[1], Letizia Gianoncelli[1], Laura Giordano[7], Armando Santoro[1], Marina Garassino[8]

[1]Department of Oncology, Istituto Clinico Humanitas IRCCS, Rozzano, Italy, [2]Medical Oncology Unit, Ospedali Riuniti, Bergamo, Italy, [3]Medical Oncology Unit, Humanitas Gavazzeni, Bergamo, Italy, [4]Medical Oncology Unit, University Hospital of Parma, Parma, Italy, [5]Medical Oncology Unit, Ospedale Civile, Legnano, Italy, [6]Medical Oncology Unit, Azienda Ospedaliera Universitaria, Udine, Italy, [7]Biostatistics Unit, Istituto Clinico Humanitas IRCCS, Rozzano (Milan), Italy, [8]Medical Oncology Unit, Ospedale Fatebenefratelli e Oftalmico, Milan, Italy

Background. Pemetrexed-cisplatin chemotherapy is the standard of care in the first-line (FL) treatment of unresectable malignant pleural mesothelioma (MPM). Second-line (SL) chemotherapy is considered for MPM patients, but the optimal treatment has not been defined yet. The aim of this study was to evaluate the clinical outcomes of 2L therapy in a series of MPM patients included in a large retrospective multicenter database.Materials and Methods. The clinical records of MPM-patients who received a SL treatment in 7 Italian centers from 1996 to 2008 were reviewed. The study endpoints were response-rate (RR), overall-survival (OS), and progression-free-survival (PFS) for SL, stratified for patient characteristics, FL outcomes, and type of SL. Only patients with full clinical information were included in the analysis.Results. Out of a group of 423, 181 patients with full clinical data were identified. Their characteristics were: median age 64 years (range:36-85); male gender 115 (53%); good EORTC-score 109 (60.2%); epithelial histology 135 (74.6%). After FL, 147 (81.2%) patients achieved a disease control (DC) with a time-to-progression (TTP) ≥12 months in 45 patients. After SL, 95 patients (52.5%) achieved a DC (21 partial-response; 74 stable-disease); median PFS and OS were 4.3 and 8.7 months, respectively. According to multivariate analysis, DC after 2L therapy was significantly related to pemetrexed-based re-treatment (OR:2.46;95%CI:1.18-5.14;p=0.017) and FL-TTP ≥12 months (OR:3.5;95%CI:1.4-8.6;p=0.006). PFS was related to younger age (<65years) (HR:1.43;95%CI:1.01-2.02;p=0.046) and to FL-TTP ≥12 (HR:0.45;95%CI:0.29-0.69;p<0.001). OS was significantly related to FL-TTP ≥12 (HR:0.54;95%CI:0.35-0.83;p=0.005).Conclusions. SL chemotherapy seems to be active in MPM patients, particularly in younger patients with a prolonged (≥12 months) TTP after FL chemotherapy. Re-challenge with pemetrexed-based regimens appears to be an interesting option for SL-setting.

S11

Peritoneal mesothelioma

S11-4 **Keynote Speaker**

Intraperitoneal pemetrexed combined with intravenous cisplatin: A well tolerated adjuvant treatment for diffuse malignant peritoneal mesothelioma

Paul Sugarbaker, Lana Bijelic, O. Anthony Stuart
Washington Cancer Institute, USA

Cytoreductive surgery and heated intraoperative intraperitoneal chemotherapy is used successfully to treat diffuse malignant peritoneal mesothelioma (DMPM) with median survival of approximately 50 months. We hypothesized that adjuvant intraperitoneal pemetrexed and intravenous cisplatin would have low morbidity and increased efficacy. From September 2007 until September 2009, 10 patients with DMPM were enrolled in a phase II, single-institution protocol of adjuvant intraperitoneal (IP) pemetrexed combined with intravenous (IV) cisplatin following best surgical cytoreduction with hyperthermic intraperitoneal chemotherapy. The treatments were given every 3 weeks for 6 cycles and consisted of 500 mg/m2 of IP pemetrexed and 75 mg/m2 of IV cisplatin. Pemetrexed was delivered through a peritoneal port placed at the time of cytoreduction. Toxicities were prospectively evaluated using the Common Toxicity Criteria, Version 2.0.Eight patients had epithelioid mesothelioma and 2 had biphasic. Nine of the 10 patients were able to complete all 6 cycles of therapy. One patient had a catheter infection requiring catheter removal and completed two cycles of intravenous therapy. No patients required dosing modifications. Five of the 9 patients (55%) experienced grade I nausea. Four patients (44%) experienced grade I and one patient (11%) grade II abdominal pain. There were no hematologic toxicities and no grade III or IV toxicities except the catheter infection. Pharmacologic analysis of pemetrexed peritoneal and plasma levels was performed showing a peritoneal fluid to plasma AUC ratio of 70. Adjuvant intraperitoneal pemetrexed combined with intravenous cisplatin can be administered with very low morbidity and has favorable short-term outcomes. The intraperitoneal administration of pemetrexed is associated with few adverse events.

S11-1

A novel tumor node metastasis (TNM) staging system of diffuse malignant peritoneal mesothelioma based on outcome analysis of a multi-institutional database

Tristan Yan[1], Marcello Deraco[2], Dominique Elias[3], Olivier Glehen[4], Edward Levine[5], Brendan Moran[5], David Morris[5], Pompiliu Piso[5], Terence Chua[5], Paul Sugarbaker[5]

[1]The Baird Institute for Applied Heart and Lung Surgical Research, Australia, [2]National Cancer Institute of Milan, Milan, Italy, [3]Institut Gustave Roussy, Villejuif, France, [4]Hospices Civils de Lyon and Unviversite Lyon, Centre Hospitalier Lyon Sud, Pierre Benite, France, [5]Peritoneal Oncology Group

Background: Currently, no tumor-node-metastasis (TNM) staging system exists for patients with diffuse malignant peritoneal mesothelioma (DMPM). The primary objective was to formulate a clinicopathological staging system through the identification of significant prognostic parameters. Methods: Eight international institutions with prospectively collected data on 405 patients who underwent cytoreductive surgery and hyperthermic intraperitoneal chemotherapy contributed to the registry. Two hundred and ninety-four patients had complete clinicopathological data and formed the basis of this staging project. Results: Peritoneal Cancer Index (PCI) was categorized into T1 (PCI 1-10); T2 (PCI 11- 20); T3 (PCI 21-30) and T4 (PCI 30-39). Twenty-two patients had positive lymph nodes (N1) and 12 patients had extra-abdominal metastases (M1). The survival for patients with T1 (PCI 1-10) N0 M0 was significantly superior to the rest of patients. This group of patients is therefore designated as Stage I. The survival of patients with T2 (PCI 11-20) and T3 (PCI 21-30), in absence of N1 or M1 disease was similar and therefore categorized as Stage II. The survival of patients with T4 (PCI 30-39), N1 and/or M1 was similarly poor and therefore categorized as Stage III. Three prognostic factors were independently associated with survival in the multivariate analysis: histological subtype, completeness of cytoreduction and the proposed TNM staging. The 5-year survival associated with Stage I, II and III disease was 87%, 53% and 29% respectively. Conclusions: The proposed TNM staging system for diffuse malignant peritoneal mesothelioma emphasizes the prognostic importance of Peritoneal Cancer Index, lymph node involvement and extra-abdominal metastasis. The staging system results in significant stratification of survival by stage when applied to the current multi-institutional registry data.

S11-2

Clinical utility of positron emission tomography with ^{18}F-FDG for malignant peritoneal mesothelioma

Anna Domenech[1], Ichise Masanori[1], Michael Rasiej[1], John Chabot[2], Elethea Hare[2], Mary Hesdorffer[2], Gabe Leinwand[2], Carolyn Visser[2], Robert Taub[2]

[1]Department of Radiology, Columbia College of Physicians and Surgeons, USA, [2]Columbia Mesothelioma Center, Department of Medicine, Columbia College of Physicians and Surgeons, NY NY, USA

Background:Diagnosis and management of malignant peritoneal mesothelioma (MPM) depends closely on imaging. 18F-FDG-PET has been shown to be effective in detecting MPM but its exact role has not yet been established. The purpose of the study was to evaluate its clinical utility.Methods: We analyzed retrospectively clinical and imaging data of 40 MPM patients (23 women and 17 men, mean age 51 y, range 22-80 y) who had multiple 18F-FDG-PET/CT or PET scans. Results:Six patients had their initial scans pretreatment and all 6 scans (100%) showed FDG-avid diffuse or linear lesions characteristic of MPM. 34 patients had their initial scans post-treatment. 18 (53%) scans showed recurrences, 15 (44%) scans showed no disease, and 1 (3%) scan, performed between cycles of chemotherapy, showed persistent disease (PD). All 40 patients had their final follow-up scans 38 ± 15 months post-treatment. 9/13p (69%) with no lesions were in clinical complete resolution (CCR). 3/13p had PD and 1/13p died. 17/23p (74%) with scan progression had either PD (3/23p), recurrence (2/23p) or died (12/23p) and 6/23p were in CCR. 1/2p with scan improvement had PD and 1/2p died. 2p with stable scans were in CCR. The overall final follow-up scan findings were consistent with clinical states in 27/40p (68%). Conclusion:18F-FDG PET appears to be a valuable imaging modality in detection and management of MPM patients.

S11-3

1st line treatment with platinum and vinorelbine in malignant peritoneal mesothelioma

Jens Sorensen, Sandra Wallerek

Dept. Oncology, Finsen Centre, National Universiyty Hospital, Denmark

Purpose: No standard chemotherapy regimen has been defined for advanced Malignant Peritoneal Mesothelioma (PeritMeso). Vinorelbine (VNB) and Platinum is among the most active combination chemotherapy regimens in 1st line treatment in Malignant Pleural Mesothelioma and hence this regimen was explored in PeritMeso. Methods: Inoperable PeritMeso patients (pts) with performance status (PS) 0-2 and normal organ function, with no major comorbidity, received either Cisplatin 100 mg/m2 (n=8 pts) or Carboplatin AUC 5 (n=3 pts) day 1 together with VNB 30 mg/m2 i.v. day 1 and 8 q. 3 weeks for 4-8 courses. CT-scans were done initially and for every 2-3 courses. Modified recist criteria were used for response assessment. The treatment was approved by the local Institutional Board and pts gave written informed consent.Results: Median age among 11 pts included was 65 years (range 46-70), there were 82% males, 55% had epithelial subtype, and PS 1 and 2 occurred in 27% and 18%, respectively. None had resectable disease. Median no. of courses were 4 (range 1-8) and median time on treatment was 110 days. Only CTC grade 4 toxicity encountered was for leucopenia (9%). There was one febrile leucopenia and no toxic deaths. Postponement of treatment occurred in 4 pts due to delayed haematological recovery. Partial remission occurred in one patient (9%). 9 pts have died, medians of Progression Free Survival was 280 days (range 49-644 days) and Overall Survival was 301 days (range 56-630 days). Fractions of pts alive after 1- and 2-years were 55% and 18%, respectively.Conclusions: This 1nd line treatment with Platinum, either Cisplatin or Carboplatin, together with VNB in PeritMeso was confined with moderate hematologic toxicity and poor activity in terms of response rate. The progression free survival and overall survival were relatively good compared to hitherto published results with other cytotoxic regimens.

S12

Radiation biology & Radiotherapy-I

S12-1

Keynote Speaker

IMRT provides excellent local control in pleural mesothelioma with and without surgery

Aaron Allen, J. Menhel, Y. Refaely, M. Kramer, O. Eldan, M. Saute

Department of radiation oncology, Dana-Farber Cancer Institute, Brigham and Women's Hospital, Harvard Medical School, USA

Purpose: To determine the effectiveness and safety of restricted field IMRT in the treatment of pleural mesothelioma with and without surgery
Methods: Patients with localized pleural mesothelioma were treated with IMRT at the Davidoff Center. Patients underwent extrapleural pneumonectomy if their KPS and disease permitted. Patients unable to undergo EPP were offered pleurectomy or no surgical therapy. IMRT was delivered using a nine field restricted technique. Inverse planning was done on the ECLIPSE planning software (Varian CA). Dose calculations were done with PB and AAA algorithms. Delivery was performed with dynamic sliding window treatment. Post-EPP the lung was limited to a mean lung dose (MLD) of 9 Gy or V20 of 10%. With both lungs MLD was limited to 16 Gy and V20 < 35%. All patients underwent initial FDG-PET scans for staging.
Results: From 10/07- 12/09 ten patients were treated with IMRT at the Davidoff Center. Seven patients received IMRT after EPP and three patients were treated without surgical resection. The median follow-up of both groups is 12 months (range 3-31 months). The median MLD of the post-EPP patients was 3.3 Gy (2.5-5.5 Gy) and V20 1% (0.3%-9%). No radiation pneumonitis was observed. The local control in these seven patients was 86 % with a single patient failing at the inferior edge of the field. Five patients experienced distant failure and have died and two patients remain alive and NED. The median survival 13 months (7-30 mos). Of the patients with unresected disease the MLD was 15.3 Gy (4.8-15.7 Gy). 2/3 patients received systemic therapy prior to IMRT and died of distant disease (median survival of 14 months). One patient was treated with RT alone and died of competing illness at 20 months following RT.
Conclusions: IMRT is safe and effective in preventing recurrence and progression in localized mesothelioma.

S12-2

Acute toxicities observed with neoadjuvant short accelerated hemithoracic radiotherapy (RT) followed by extra-pleural pneumonectomy (EPP) for malignant pleural mesothelioma (MPM): preliminary results

B.C. John Cho[1], Ronald Feld[2], Natasha Leighl[2], Marc de Perrot[3]

[1]Department of Radiation Oncology, Princess Margaret Hospital, University of Toronto, Canada, [2]Department of Medical Oncology, Princess Margaret Hospital, University of Toronto, [3]Division of Thoracic Surgery, Toronto General Hospital, University Health Network, University of Toronto

Purpose: To evaluate the acute treatment toxicities seen in MPM patients treated on study with short accelerated hemithoracic RT followed by EPP. Materials and Methods: We are conducting an research ethics board approved prospective study evaluating the feasibility of short accelerated neoadjuvant hemothoracic RT followed by EPP the next week for clinically resectable early stage MPM. The dose prescribed is 25 Gy/5 fx over 1 week to the entire ipsilateral hemithorax. Acute treatment related toxicities are presented (defined as any toxicity seen within 3 months of treatment completion). Toxicities are graded (G) according the CTCAE v3.0 criteria.Results: Five patients (3 male, 2 female) of an intended 12 have been accrued to this study. Mean age is 61 years (range: 46-69 years). All patients were ECOG 1 or better. Median follow-up is 4 months. All patients were clinically staged cT1-2 N0 M0 prior to neoadjuvant RT. After EPP, the pathological stages were: ypT4 N0 M0 (n=2); ypT4 N2 M0 (n=2); and ypT4 N3 M0 (n=1). No fatal (G5) or life-threatening toxicities (G4) were observed during treatment. Two severe (G3) toxicities were seen during the peri-operative period (thrombus of subclavian vein requiring anticoagulation; hemothorax requiring operative correction). Conclusions: These results are preliminary and, therefore, should be interpreted cautiously. Short accelerated neoadjuvant hemithoracic RT followed by EPP appears feasible. More mature follow-up is needed to evaluate outcomes and late toxicities.

S12-3

Liver toxicity following extrapleural pneumonectomy and adjuvant three-dimensional conformal radiation therapy for treatment of malignant pleural mesothelioma

Robert Lin[1], MoMo Tin[1], James Chen[1], Regina Tse[1], Brian McCaughan[2]

[1]Department of Radiation Oncology, Sydney Cancer Centre, Royal Prince Alfred Hospital, Australia, [2]Department of Cardiac Thoracic Surgery, Royal Prince Alfred Hospital, Sydney Australia

Purpose:To assess liver biochemical toxicities following extrapleural pneumonectomy and adjuvant three-dimensional conformal radiotherapy (3DCRT)Methods and Materials:Since 2004, 33 patients with malignant pleural mesothelioma (MPM) were treated with extrapleural pneumonectomy (EPP) and adjuvant 3DCRT to the hemithorax. 16 patients also received chemotherapy prior to surgery. Serial liver function tests (LFTs), mean liver dose and liver dose-volume histogram (DVH) were analysed in 17 patients with right-sided disease. Changes in LFTs were graded using CTCAE v4.02. Patients (n=12) with left-sided disease and patients (n=4) who failed to complete the intended treatment were excluded from the analysis due to low liver dose. The mean radiation dose was 45.7Gy (range 41.4Gy - 52.4Gy).Result:Total of 102 LFTs from 17 patients were analysed, from start of radiotherapy and followed for a median of 18 months (range 1 - 63) post-treatment. Grade two adverse events in Alkaline phosphatise (ALP) were observed in 5 patients (29%) with no grade three toxicity. Grade two or worse adverse events for Gamma-glutamyl transpeptidase (GGT) were observed in 10 patients (59%). The LFT abnormalities generally peaked at 1~3 months post-RT, at 5-14 times the upper level of normal. Alanine and Aspartate Aminotransferases levels were normal in all patients. All patients who had grade two toxicities or worse had V30 liver dose greater than 50% and mean liver dose of greater than 30Gy. Most patients (94%) did not have ongoing LFT abnormality. The most severe toxicity was observed in a patient who had abnormal LFTs prior to radiotherapy.Conclusion:Our results suggest a correlation between liver biochemical toxicity and mean liver dose and liver DVH measurements. ALP and GGT during and post-treatment are more sensitive than the transaminases in monitoring liver toxicity.

S12-4

Hypofractionated palliative radiotherapy for malignant mesothelioma using helical tomotherapy

Rufus Scrimger, Quincy Chu, Michael Smylie, Charles Butts, Anil Joy, Zsolt Gabos

Department of Oncology, University of Alberta, Canada

IntroductionRadiotherapy has traditionally played a limited role in the management of malignant mesothelioma. The technical challenges of irradiating the pleural surface of the lung have limited the dose of radiation that could be safely delivered. This study explored the toxicity and efficacy of helical tomotherapy for patients with symptomatic mesothelioma. Methods:Patients were eligible if they had pathologically confirmed mesothelioma of the pleural surface causing local symptoms. Only patients who progressed on or refused systemic chemotherapy were enrolled. Treatment involved radiotherapy using helical tomotherapy, treating all gross disease in the hemithorax to a dose of 40 Gy given in 15 daily fractions. The primary endpoint of symptom control was evaluated using a patient-assessed symptom questionnaire. Quality of life, radiotherapy dosimetry and toxicity, and radiological response rates were also evaluated. Results:13 patients were accrued over a period of approximately 36 months. Four patients were unable to complete all treatments due to discomfort or disease progression. The remaining 9 tolerated the treatment well with only mild toxicity (grade 1-2 fatigue and pneumonitis). Pulmonary function testing at one month post-treatment showed a mean change in DLCO of -7% [range: +30% to -21%], in spite of high ipsilateral lung doses. Although the ultimate disease course was not altered, with radiographic evidence of progression in all but one patient at 3 months, the short term response rates were encouraging, with 71% of patients achieving significant improvements in pain at the one month follow-up visit. Half of patients reporting good pain relief at 1 month had a durable response lasting 3+ months.Conclusions:With the caveat of small patient numbers, helical tomotherapy appears to be an effective modality to combat the pain associated with malignant mesothelioma. In spite of treatment, however, disease progression was observed in almost all patients by 3 months.

S13

Molecular target therapy

S13-1

Final results of a phase II study of sunitinib as second-line therapy in malignant pleural mesothelioma

Anna Nowak[1,4], Michael Millward[1,4], Roslyn Francis[1,2], Agatha van der Schaaf[2], Tatiana Seguard[2], Jenette Creaney[1], Arman Hasani[4], Arthur Musk[3], Michael Byrne[3,4]

[1]School of Medicine and Pharmacology, University of Western Australia, Australia, [2]WA PET Centre, Sir Charles Gairdner Hospital, [3]Department of Respiratory Medicine, Sir Charles Gairdner Hospital, [4]Department of Medical Oncology, Sir Charles Gairdner Hospital

Sunitinib is a multi-targeted tyrosine kinase inhibitor of rational targets in MPM including VEGFR and PDGFR. We tested sunitinib as second-line therapy in MPM.Eligible consenting patients had progressive MPM during/after first-line platinum/antimetabolite, ECOG PS 0-1, adequate organ function, and measurable disease. Treatment: sunitinib 50 mg/day x28d q6 weeks. Primary endpoint: objective response (OR) defined by either a). Modified RECIST Criteria (MRC) or b) metabolic response on FDG-PET in pts without prior talc pleurodesis. Imaging was performed at baseline, after cycles 1, 2, and every 2 cycles thereafter; most PET imaging was during treatment break. Simon 2 stage design: 2 responses in the first 23 pts and 5 responses in 51 assessable pts gave $\alpha = 0.05$, $\beta = 0.1$ assuming an OR of 20% of interest.From 05/06 to 12/09, 53 eligible patients were accrued: 51 assessable for CT response; 20 without pleurodesis assessable on FDG-PET. Demographics: M/F (44/9); median age 64 (range 45 - 81); histology epithelial/sarcomatoid/mixed/unknown (37:1:9:6). ECOG PS 0/1 (15:38). Best MRC response: PR 6 (12%); SD 33 (65%); PD 12 (23%). Metabolic response: 6/20 assessable (30%), 1 also with CT PR; 4 with CT minor response. Protocol-defined responses 11/51 (22%, 95% CI 13%-36%). Median OS was 7 months. Median TTP was 3.5 months. Adverse events (number patients experiencing;%): Grade 3/4: thrombocytopenia (3;6%), neutropenia (2;4%); Grade 3: fatigue (11;21%,). 15/53 required dose reduction. There was one possible treatment-related death from penumonitis and a second grade 4 pneumonitis. Four patients developed increasing pleural effusions or ascites without other radiologic evidence of PD. VEGF-A, VEGF-C, VEGF-R2 and VEGF-R3 did not correlate with outcomes. Sunitinib has modest activity in previously treated MPM; testing at a continuous lower dose may be warranted to ameliorate toxicity. Timing of FDG-PET is important in studies of targeted therapies.

S13-2

A phase II study of dasatinib (D) in patients (pts) with previously treated malignant mesothelioma(MM): CALGB 30601

Arkadiusz Dudek[1], Herbert Pang[2], Robert Kratzke[1], Gregory Otterson[3], Everett Vokes[4], Hedy Kindler[4]

[1]University of Minnesota, USA, [2]Duke University, Durham, NC, [3]The Ohio State University Medical Center and Arthur G. James Cancer Hospital and Solove Research Institute, Columbus, OH, [4]The University of Chicago, Chicago, IL, Cancer and Leukemia Group B, Chicago, IL, USA

Background: D is a potent inhibitor of SRC family kinases that are frequently over-expressed in MM. There are no approved therapies for MM pts who progress on pemetrexed. We therefore conducted a phase II trial of D in MM pts who had received 1 prior pemetrexed regimen. Methods: Single arm phase II. Eligible pts had unresectable MM, PS 0-1, measurable disease, and no symptomatic effusions. Primary endpoint: Progression-free survival (PFS) at 24 weeks (wks) > 34%. D 70 mg BID was given orally. CT scans were obtained every 8 weeks. Results: 46 pts enrolled (9/07-8/09), 43 are evaluable for PFS, 26 for response, and 46 for toxicity. Pt characteristics: Male 72%; median age 68 (range 35, 81); PS 0 41%; epithelial histology 72%, pleural 76%. Median cycles given 2 (range 1-8). The starting dose was reduced to 50 mg BID after the first 23 pts enrolled because 50% of the first 12 pts enrolled had AE > grade 2. Grade 3/4 toxicities (% pts): fatigue 11%, non-malignant pleural effusion 9%, hyponatremia 7%, dyspnea 6%, pericardial effusion 2%, pneumonitis 2%, anemia 2%. There were 3 grade 5 toxicities: 2 respiratory failures, 1 unknown. Efficacy: 24-week PFS rate 21% (95% CI 11.7%, 37.4%), median PFS 9 wks (7.7, 16.6), median overall survival 26 wks (18.6, 33.9), partial response 2%; stable disease 23%. Correlative science: pre- and post-treatment median plasma VEGF (393 pg/ml), PDGFβ (13 ng/ml), serum CSF-1 (1518 pg/ml), and mesothelin-related protein (2.51 nM) were analyzed. CSF-1 was associated with worse overall survival (HR 1.683). Blood mononuclear cells were evaluated for phosphorylation of Src before and after treatment. Tumors were evaluated for expression of EphA2 and PDGFRβ. Conclusions: Dasatinib is inactive in previously-treated MM; the 70 mg dose is poorly tolerated. Correlative markers will be evaluated for association with PFS and OS.

S13-3

Randomized phase II trial of pemetrexed/cisplatin with or without CBP501 in patients with advanced malignant pleural mesothelioma (MPM)

Lee Krug[1], Sunil Sharma[2], Antoinette Wozniak[5], Jose Luis Morero[4], Christina Rodriquez[8], Ernesto Wasserman[6], Cristian Fernandez[6], Takumi Kawabe[7], Marianna Koczywas[3]

[1]Memorial Sloan-Kettering Cancer Center, USA, [2]Huntsman Cancer Institute, Salt Lake City, Utah, USA, [3]City of Hope Cancer Center, Duarte, CA, USA, [4]Hospital Maria Ferrer, Buenos Aires, Argentina, [5]Karmanos Cancer Center, Detroit, MI, USA, [6]ZeeCRO, San Antonio, Texas, USA, [7]Canbas Co., Ltd., Numazu City, Shizuoka, Japan, [8]Cleveland Clinic Taussig Cancer Institute, Cleveland, OH, USA

Background: Malignant cells preferentially repair damaged DNA at the G2 checkpoint, rather than at G1, because of loss of the G1 checkpoint. CBP501 was identified by cell cycle phenotype based optimization of a rationally designed G2 checkpoint abrogator. It inhibits several kinases which phosporylate CDC25C. Although the mechanism of action is still under investigation, CBP501 enhances the cytotoxicity of cisplatin in cell lines and xenografts, including NCI-H226 human mesothelioma. Phase I trials combining CBP501 with cisplatin alone or with pemetrexed/cisplatin have shown acceptable safety profiles and encouraging activity. The most common toxicity of CBP501 is an infusion-related histamine release syndrome causing urticaria which is easily treated with diphenhydramine. **Methods:** Previously untreated patients with unresectable MPM are randomized 2:1 to treatment with pemetrexed/cisplatin plus CBP501 25 mg/m2 IV (Arm A) or pemetrexed/cisplatin alone at standard doses (Arm B). The primary endpoint is progression free survival (PFS). 42 patients are planned for enrollment in Arm A; if greater than 23 are free of progression more than 4 months, the combination will be deemed worthy of further study. 21 patients will be enrolled in Arm B. In addition to standard CT imaging to assess response and PFS, PET scans, pulmonary function tests, and mesothelin levels are also being performed.**Results:** As of March 2010, 28 patients have been registered among 17 participating institutions and 26 have been treated (16 in Arm A, 10 in Arm B). Overall demographics are median age 66, 86% male, 72% epithelioid histology. Grade 3/4 treatment-related toxicities have been uncommon, no different than expected from standard chemotherapy, and comparable in the two arms. One patient in each arm died during treatment due to disease progression. 73% of patients treated with CBP501 had grade 1-2 allergic reactions.**Conclusions:** Enrollment continues on this randomized trial. Updated results will be presented at the meeting.

S13-4

Tissue response in patients with MPM after treatment with cisplatin, pemetrexed and axitinib: First results of a feasibility study

Wieneke Buikhuisen[1], Arjan Griffioen[2], Koen Marijt[2], Marion Scharpfenecker[1], Suzanne Onderwater[1], Tiny Korse[1], Paul Baas[1]

[1]Netherlands Cancer Institute, The Netherlands, [2]Angiogenesis Laboratory, Dept. of Medical Oncology, Free University - Cancer Center Amsterdam

Introduction: The vasculature in MPM is considered an important target. Since standard treatment with a platinum combined with an anti-folate does not lead to long survival, the addition of an anti-VEGF-Receptor strategy seems logical. Using intermediate tissue markers, early signals of response would be necessary before embarking on larger phase II or III studies.M and M: We performed a feasibility study in patients who received cisplatin 75 mg/m2, pemetrexed 500 mg/m2 (q3) and daily 2x 5 mg axitinib orally. Before treatment and after 3 courses a thoracoscopy was performed to collect tissue and evaluate the intrathoracic response. Material was collected for assessment of angiogenesis parameters, microvessel density (CD31/34 staining); number of proliferating endothelial cells. Results: 6 male patients were included of which 4 had both 3 courses of chemotherapy and evaluable thoracoscopies. One patient progressed during treatment and one allowed only true cut biopsies after chemotherapy. Median age: yrs 57 (range 56-61); Pathology: 4 epithelial type, 1 sarcomatoid type, 1 mixed type; WHO 0-1: 5pts, WHO 2: 1pt. Hematological toxicity: neutropenia grade 3: 60%, grade 4: 20%, no febrile neutropenia/infection occurred. All patients experienced grade 2 hypertension, no other cardiovascular toxicity was seen. Assessment of angiogenesis parameters in the tumour tissues before and after therapy revealed a decrease of microvessel density by factor of 2.72. Moreover, the number of proliferating endothelial cells decreased by approximately 4-fold. Conclusions: In patients with MPM it is feasible to perform repeat thoracoscopies to obtain tissue samples for analysis of intermediate markers. The combination of cis/pem/axitinib was well tolerated. There was an apparent decrease in microvessel density and proliferating endothelial cells after treatment.

S14

Radiation biology & Radiotherapy-II

S14-5

Keynote Speaker

Major advances in locoregional control and palliation of mesothelioma using high dose radiotherapy with new technologies and ^{18}F-FDG PET scanning

Malcolm Feigen, C. Lawford, M. Lawlor, K. Churcher, C. Hamilton

Radiation Oncology Centre, Austin Health, Heidelberg West, Victoria, Australia

Purpose:Mesothelioma is a cancer with a very high mortality where standard treatments with surgery, chemotherapy and radiotherapy are generally insufficient to prevent locoregional disease progression. Most symptoms are produced by gross invasion of local tissues. We aimed to investigate the effect of new radiotherapy techniques using advanced technologies on mesothelioma patients with unresected disease, utilizing ^{18}F-FDG PET scans to precisely define target volumes and assess treatment response. Methods and Materials We developed techniques to deliver high dose radiotherapy to selected patients with mesothelioma confined to one hemithorax, following progression after surgery and chemotherapy. PET scans were performed prior to radiotherapy to assist in targeting metabolically active disease. Patients received high doses of radiation for palliation using various new technologies, including intensity-modulated radiotherapy (IMRT), PET-CT fusion software and image guidance equipment. Locoregional control was validated by total glycolytic volumes (TGV) measurements on serial PET scans. ResultsSince 2003 we have delivered radiation doses up to 60 Gy to 40 patients, the majority having residual disease after incomplete surgery. Planning target volumes were progressively increased from two to nine liters. Eleven patients received IMRT to the entire hemithorax, eight on the right and three on the left side. TGVs were assessed on 40 PET scans from 14 patients followed for up to five years post-irradiation, with an average TGV reduction of 67% and a locoregional control rate of 71%. Acute toxicities were moderate, with no grade 4 or 5 toxicities at 18 months median followup. ConclusionWe have demonstrated effective local control of pleural mesothelioma with high dose hemithoracic radiotherapy, resulting in durable palliation and modest toxicity. Multimodality programs should include radiotherapy using advanced technologies to prevent or delay locoregional disease following surgery. High radiation doses provide long-term palliation.A phase II trial using these techniques is currently under development.

S14-1

Feasibility of pleural intensity-modulated radiation therapy (IMRT) for malignant pleural mesothelioma (MPM)

Marjorie Zauderer[1], Lee Krug[2], Benjamin Laser[1], Ellen Yorke[1], Raja Flores[3], Valerie Rusch[3], Kenneth Rosenzweig[1]

[1]Department of Radiation Oncology, Memorial Sloan-Kettering Cancer Center, USA, [2]Department of Medicine, Thoracic Oncology Service, Memorial Sloan-Kettering Cancer Center, [3]Department of Surgery, Thoracic Service, Memorial Sloan-Kettering Cancer Center

Purpose: In patients with MPM who are unable to undergo pneumonectomy, it is difficult to deliver tumoricidal doses of radiation to the pleura without significant toxicity. We implemented a technique of using IMRT to treat these patients.
Methods: Between 2005 and 2010, 36 patients with MPM at Memorial Sloan-Kettering Cancer Center were treated with pleural IMRT to the hemithorax (median dose: 46.8 Gy, range: 41.4-50.4 Gy). Treatments were delivered with 6 MV photons utilizing the sliding window method with dynamic multi-leaf collimators and beam directions consisted of 6-8 angles.
Results: Patient characteristics were: right-sided 56%; male 81%; age median 67 (range 42-82); epithelioid 78%, sarcomatoid 6%, mixed 17%; stage I: 6%, II: 38%, III: 33%, IV: 33%. Thirty-two patients (89%) received induction chemotherapy. 44% had unresectable disease while the other 56% underwent pleurectomy/decortication. Of 35 patients evaluable for acute toxicity, 7 (20%) had grade 3 or worse pneumonitis (including one death) and 2 had grade 3 fatigue. In 23 patients assessable for late toxicity, 5 had continuing grade 3 pneumonitis. With a median follow-up of 13 months from the beginning of any treatment, the 1-year and 2-year overall survival rates were 73% and 40% respectively (77% and 59% in surgical patients; 69% and 13% in inoperable patients) with a median overall survival of 17.4 months. The median time to local failure from the end of radiation therapy was 15.7 months in surgical patients and 4.9 months in inoperable patients.
Conclusions: Pleural IMRT in patients with MPM and an intact lung is a safe and feasible treatment option with an acceptable rate of pneumonitis. We have initiated a phase II trial of induction chemotherapy with pemetrexed and cisplatin +/- pleurectomy/decortication followed by pleural IMRT. This approach seems to improve local control, particularly in patients who have undergone pleurectomy.

S14-2

Trimodality treatment for malignant pleural mesothelioma : Intensity-modulated radiotherapy after extrapleural pnemonectomy

Naoya Yamasaki[1], Tsutomu Tagawa[1], Tomoshi Tsuchiya[1], Takuro Miyazaki[1], Yoichi Nakamura[2], Tetsuya Ida[2], Katsumi Nakatomi[2], Nobuko Matsuo[6], Kazuto Ashizawa[3], Nobuyuki Hayashi[3], Tomayoshi Hayashi[4], Kuniko Abe[4], Naoe Kinoshita[4], Shiro Obata[5], Takeshi Nagayasu[1]

[1]Division of Surgical Oncology, Nagasaki University Graduate School of Biochemical Sciences, Japan, [2]Second Department of Internal Medicine, Nagasaki University Graduate School of Biochemical Sciences, Nagasaki, Japan, [3]Radiology and Radiation Biology, Nagasaki University Graduate School of Biochemical Sciences, Nagasaki, Japan, [4]Department of Pathology Nagasaki University Hospital, Nagasaki, Japan, [5]Department of Radiology, Nagasaki Prefectual Shimabara Hospital, Nagasaki, Japan, [6]Department of Respiratory Disease, Nagasaki Municipal Medical Center, Nagasaki, Japan

Purpose : To investigate combined modality treatment with neoadjuvant chemotherapy followed by extrapleural pneumonectomy(EPP) and adjuvant radiotherapy including Intensity Modulated Radiotherapy(IMRT) in patients with malignant pleural mesothelioma(MPM). Patients and Methods : Between July 1998 and November 2009, 15 patients with MPM were reviewed in this retrospective study. Before 2005, five of 15 patients underwent intaraoperative cisplatin immersion without neoadjuvant chemotherapy. Since 2004, neoadjuvant chemotherapy consisted of 3 cycles of a combination of cisplatin and gemcitabine(n=3) or cisplatin and pemetrexed(n=7). Inversed planned IMRT with a median target dose of 50 Gy was applied in 2 Gy fractions. Results : All 15 patients underwent complete EPP. The mean age was 54 years (range 51 to 71). Pathological types were epithelioid in 6, biphasic in 5, and sarcomatoid in 4. IMIG stage was II in 4, and III in 11. T status was T2 in 7, and T3 in 8. Node status was N0 in 9, N1 in 1, and N2 in 5. Eleven patients underwent tri-modality treatment. Five of 11 patients received IMRT. Four of remaining 6 patients received 3D planning radiotherapy, but two patients could not received adjuvant radiotherapy because of their poor condition. Ten patients have died of progressive mesothelioma from the day of EPP (range 9 to 45 months). Three patients die of other disease. Two patients remain alive without evidence of disease with a follow up of 4 and 9 months, respectively. Of five patients who received IMRT, two patients(40%) developed local recurrence in the pleural space or chest wall, but remaining three patients achieved good local control. On the other hand, eight of 10 patients(80%) without IMRT developed local recurrence. One patient developed fetal pneumonitis after completion of IMRT. Conclusions : Although trimodality therapy including IMRT have better local control, careful planning should be given to treat with IMRT after EPP.

S14-3

Feasibility and efficacy of trimodality treatment with intensity modulated radiation therapy for malignant pleural mesothelioma - preliminary assessment

Kenichi Okubo[3], Takuji Fujinaga[2], Makoto Sonobe[2], Tsuyoshi Shoji[2], Hiroaki Sakai[2], Ryo Miyahara[2], Toru Bando[2], Hiroshi Date[2], Keiko Shibuya[3], Masahiro Hiraoka[3]

[1]Thoracic Surgery, Tokyo Medical and Dental University, Tokyo, Japan, [2]Thoracic Surgery, Kyoto University, Kyoto, Japan, [3]Thoracic Surgery, Tokyo Medical and Dental University, Japan

Objective: Although trimodality treatment is applied for malignant pleural mesothelioma (MPM), the method of radiation therapy (RTx) has been controversial. We examine our results of intensity modulated radiation therapy (IMRT) for patients with MPM.

Patients and Methods: Since 1998, 19 patients with resectable MPM were intended to treat with trimodality therapy; an extrapleural pneumonectomy and multi-cycles of platinum-based chemotherapy, followed by external beam RTx for hemithorax. Fifteen patients eventually received RTx. During the period we changed RTx programs in 2006, from conventional hemithorax RTx (CRTx, n=10) to IMRT (n=5). Radiation field of IMRT included the initial site of parietal pleura especially in the lower thorax. Feasibility of IMRT was examined in these patients, and the efficacy of IMRT was investigated with comparing overall and disease-free survivals, and relapse patterns with those after CRTx.

Results: Subtype of 5 patients with IMRT was epithelioid in 4 and sarcomatoid in 1. IMIG staging was II in 2, III in 2, and IV in 1. Chemotherapy was administered with multiple cycles in 4, and 1 cycle in 1. All 5 patients completed irradiation with a total dose of 44-50.4 Gy without specific adverse events. Overall and disease-free survivals in IMRT-group were not significantly different from those in CRTx-group. In CRTx-group intrathoracic recurrences were seen in 5 patients (50%) and all were within 20 months after RTx. In IMRT-group no intrathoracic recurrences were seen at this time of the longest follow of 42 months (n.s.).

Conclusion: Inclusion of IMRT into the trimodality protocol for MPM was feasible. IMRT is promising for local control after extrapleural pneumonectomy. Longer follow-up duration is needed and larger number of patients would be examined.

S14-4

Hemithoracic irradiation in malignant mesothelioma patients (MPM); the Amsterdam experience and a review of the literature

Paul Baas, Rick Haas

The Netherlands Cancer Institute, the Netherlands

Radiotherapy is often used in patients with MPM as adjuvant treatment after radical surgery with curative intent, as palliation and as prevention for scar recurrences. This presentation will focus on these three different indications and presents an update of the available literature. For palliation of pain a total dose of 36 Gy (>4 Gy fractions) is considered to be effective in the majority of cases (de Graaf-Strukowska) and the addition of hyperthermia can be considered a good alternative (van der Zee). This is considered to be a standard procedure in the treatmen of patients with advanced MPM. The use of RT as prophylaxis for drain tracts remains a matter of debate. Three studies have been performed leading to contra dictionary results (Boutin, O Rourke, Bydder). It is doubtful whether energy and resources should be put into a large randomized study concerning this issue. Over the last few years the use of IMRT has been used with a range of good and even fatal reactions. The data from the literature (Ahamad, Miles and Allen) all present different levels of safety. We have summarized these data and added the results of 16 patients treated with post-operative IMRT in our institute. A clear correlation is observed between the incidence of pneumonitis and different levels of V20 and mean lung dose. During the meeting a proposal shall be made how to implement IMRT in this setting and which dose constraints are considered to be optimal.

S15

Screening

S15-1 **Keynote Speaker**

New light on early diagnosis and therapy of asbestos-induced mesothelioma

Okio Hino

Department of Pathology and Oncology, Juntendo University School of Medicine, Japan

The Eker (*Tsc 2* gene mutant) rat model of hereditary renal carcinoma (RC) is an example of Mendelian dominantly inherited predisposition to a specific cancer in an experimental animal. *Tsc2* is an 'initial gene' of carcinogenesis and the abnormal networks of gene expressions should be involved in tumor formation. To search for such alterations, we identified the highly expressed genes in Eker RC as the Erc (expressed in renal carcinoma) gene. After we determined the complete primary structure of rat *Erc* cDNA, it was showed that the putative rat Erc product has an identity with human megakaryocyte potentiating factor, MPF/mesothelin. Rat Erc and human mesothelin are functional orthologues and the authors shall refer to this protein as Erc/mesothelin. We have succeeded in establishing specific antibodies against Erc/mesothelin, and after validation by immunohistochemical studies on diseased tissue of mesothelioma patients, an enzyme-linked immunosorbent assay (ELISA) system has been developed. The usefulness of which has been assessed and demonstrated as a diagnostic tool. We have started a large-scale prospective study on building construction workers, who run the risk of asbestos exposure, using our ELISA system. Recently, we found unique functions of this gene. The specific mechanism should throw new light on a definitive therapy for mesothelioma.

S15-2

Low-dose computed tomography as a screening tool for malignant pleural mesothelioma and early lung cancer in asbestos exposed individuals: The Toronto Experience

Demetris Patsios[1], George Dong[3], Brenda O'Sullivan[3], Heidi Roberts[1], TaeBong Chung[1], Elsie Nguyen[1], Steve Herman[1], Gord Weisbrod[1], Narinder Paul[1], Marc de Perrot[2]

[1]Department of Medical Imaging, University Health Network, Mount Sinai and Women's College Hospital, Canada, [2]Department of Thoracic Surgery, University Health Network, Toronto, Ontario, Canada., [3]Research Department, Department of Medical Imaging, University Health Network, Toronto, Canada

Purpose: We establised a screening program for prior asbestos workers using Low-Dose Computed Tomography (LDCT) to detect early malignant pleural mesothelioma and lung cancer . We report on the first five years of the study.Methods: The study cohort consisted of individuals with a history of asbestos exposure over 20 years prior to trial entry and/or known pleural plaques on previous imaging. Following informed consent, LDCT was performed (40-60 mA, 120 kV, 1-1.25mm). Parenchymal nodules were followed according to lung cancer screening recommendations. The morphology and location of pleural plaques and any pleural thickening was noted. Blood was drawn for a separate analysis of biomarkers. Results: We enrolled 1026 individuals between 03/2005 and 03/2010, average age 60.7 years. 743 (72.4%) were current or former smokers. 719 (70.1%) participants have had an annual and 32.4% had a biennial repeat screening LDCT. 595 (58%) subjects had pleural plaques. 890 subjects (86.7%) had pulmonary nodules, 195 (19%) had at least one nodule greater than 5 mm; 12 new or growing nodules were found on the annual repeat CT. 47 subjects had suspicious for malignancy pleural or parenchumal nodules that were closely followed up. In addition, 14 subjects with pleural effusions had diagnostic thoracentesis with one positive for malignancy. These resulted in 8 confirmed lung cancers, 3 pleural mesotheliomas and 1 peritoneal mesothelioma; the overall malignancy rate is 1.12%. 3 pleural mesotheliomas, 3 peritoneal mesotheliomas and 1 lung cancer were diagnosed in the interval of screening studies or in 0.7% of participants. Conclusion:More lung cancers were detected than pleural mesotheliomas. As many malignant pleural mesotheliomas were detected within the study in asymptomatic individuals as in interval imaging because of symptoms. We therefore question the utility of LDCT as a screening tool for early malignant pleural mesothelioma.

S15-3

Japanese general screening study for asbestos-related diseases (JG SARD study): Preliminary results

Kazuto Ashizawa[1], Nobuhiko Seki[2], Kenji Eguchi[2], Takuhiro Yamaguchi[3], Masahiro Kaneko[4]

[1]Clinical Oncology Center, Nagasaki University Hospital, Japan, [2]Teikyo University School of Medicine, [3]Tohoku University Hospital, [4]National Cancer Center Hospital

BACKGROUND: The number of patients with pleural mesothelioma and lung cancer associated with asbestos exposure has recently been increasing in Japan. PURPOSE: To evaluate the preliminary results of screening for asbestos-related diseases in a group of Japanese general population. MATERIALS AND METHODS: This prospective study was approved by the institutional review board; informed consent was obtained. Between March in 2006 and December in 2008, 9810 people (5283 men and 4527 women; mean age, 57 years) underwent chest radiography and low-dose CT examinations in 26 institutions in Japan. Clinical information such as histories of smoking and asbestos exposure was reviewed. Chest radiographs and CT scans were interpreted independently by 15 experienced pulmonologists or chest radiologists. RESULTS: The history of asbestos exposure was definitely present in 1253 (12.8%) individuals, possibly present in 2058 (21.0%), and absent in 6499 (66.2%). On chest radiograph, pleural plaque and pleural thickening were seen in 61 (0.6%) and 65 (0.6%) individuals, respectively. On low-dose CT, pleural plaque and pleural thickening were identified in 264 (2.7%) and 245 (2.5%) individuals, respectively, and non-calcified pulmonary nodule/mass was seen in 1003 (10.2%). The history of asbestos exposure was not confirmed in 77 out of 264 individuals (29.2%) having pleural plaques on low-dose CT. Based on the logistic regression analysis, pleural plaque on low-dose CT was significantly correlated with male, age more than 60 years, a history of asbestos exposure and smoking.CONCLUSION: Our results indicate that the detectability of pleural lesions on low-dose CT is approximately 4 times higher than that on chest radiographs, and that about 30% of individuals with pleural plaques on low-dose CT are not aware of the asbestos exposure.

S15-4

Research in asbestos disease-affected communities - Can research process aid social healing?

AD LaMontagne[1], AH Holloway[1], CE Hunter[2]

[1]McCaughey Centre, Melbourne School of Population Health, University of Melbourne, Australia, [2]Centre for Health Program and Policy Evaluation, Melbourne School of Population Health, University of Melbourne

In communities hard-hit by asbestos-related disease (ARD), the process through which research is conducted merits careful consideration. We present a case study spanning several years of research in one such community. Attempting to balance research imperatives with community-expressed needs, we have conducted community-based social research as a prelude to epidemiological studies. We first conducted an in-depth interview study with community members on what they saw as the most important ARD problems, and what they thought could or should be done about them. The explicit goal here was to develop respectful and collaborative relationships, and to merge researcher-with community expertise in finding ways forward. Community and researcher insights were integrated to propose a comprehensive "social and public health response " to ARD. "Social " in this context expresses the need for healing at the social or community level (e.g., need for apologies, memorials). Community also expressed a need for documenting their particular history of asbestos use and consequences, which led us to conduct historical research tracing local responses to ARD over time. This work informed a substantial piece in a Melbourne newspaper outlining the need for an apology from state government for its past contribution to ARD as the responsible employer-the State Electricity Commission of Victoria, followed four months later by a public apology from the state's Premier. In another concrete enactment of community participation in finding ways forward, we edited a collection of articles on ARD in Australia in which community advocates, an exposed worker, and the Australian Council of Trade Unions contributed independently-authored papers. This foundation of mutual respect and trust led to a current community-based project exploring new early detection methods for mesothelioma. While continuing to pursue traditional "research outcomes, " we simultaneously seek to make the research process a positive "social healing " intervention in itself.

S15-5

Longitudinal stability and screening value of mesothelioma biomarkers in an asbestos-exposed population

Kevin Hollevoet[1], Joris Van Cleemput[2], Joel Thimpont[3], Paul De Vuyst[4], Lionel Bosquee[5], Paul Germonpre[6], Kristiaan Nackaerts[7], Yoshiro Kishi[8], Joris Delanghe[9], Jan van Meerbeeck[1]

[1]Department of Respiratory Medicine, Ghent University Hospital, Belgium, [2]Occupational Health Service, Eternit NV, Kapelle-op-den-Bos, Belgium., [3]Occupational Diseases Fund, Brussels, Belgium, [4]Department of Respiratory Medicine, Erasme Hospital ULB, Brussels, Belgium, [5]Department of Respiratory Medicine, CHU Sart Tilman, Liege, Belgium, [6]Department of Respiratory Medicine, Antwerp University Hospital, Antwerp, Belgium, [7]Department of Respiratory Medicine, University Hospital Gasthuisberg, Leuven, Belgium, [8]Department of Research and Development, Ina Institute, Medical & Biological Laboratories, Co. Ltd., Nagano, Japan, [9]Department of Clinical Chemistry, Ghent, University Hospital, Belgium

Background: Soluble mesothelin (SM) and megakaryocyte potentiating factor (MPF), two serum biomarkers of mesothelioma, are potentially useful to screen asbestos-exposed individuals. However, the longitudinal stability of SM and MPF and the impact of changes in covariates, like age and glomerular filtration rate (GFR), on serial biomarker measurements have not yet been prospectively assessed. **Methods:** Healthy asbestos-exposed individuals are followed for two years with annual blood sampling. Serum SM and MPF levels were assayed with ELISA kits. Critical thresholds of SM and MPF were arbitrary set at 2.10 nM and 13.00 ng/ml, respectively. **Results:** In total, 214 individuals were included at baseline, and 162 already provided a second sample. Baseline and follow-up biomarker levels significantly correlated ($P<0.001$, $r_{SM}=0.87$; $r_{MPF}=0.76$). A random intercept model showed that age and GFR had a significant, yet limited longitudinal effect. A one-unit increase in age and decrease in GFR resulted in an increase of respectively 0.02 nM and 0.01 nM for SM, and respectively 0.11 ng/mL and 0.03 ng/mL for MPF. Twenty-seven individuals had elevated levels at baseline or follow-up. In these individuals, GFR and age were significantly lower and higher, respectively, compared to those with normal biomarker levels ($P<0.001$). One individual with elevated biomarker levels, but also a severely declined GFR, presented with prostate cancer at follow-up. In two participants with normal biomarker levels, an epithelioid malignant pleural mesothelioma and a lung cancer, respectively, were reported. **Conclusions:** Serial measurements of SM and MPF displayed a long-term stability. In contrast to the substantial association at baseline, longitudinal changes in age and GFR had little effect on biomarker levels. Further follow-up is required to establish whether elevated biomarker levels reflect an insidiously developing malignancy or merely a false positive, e.g. due to a decreased renal function.

S15-6

Serum SMRP (soluble mesothelin-related peptide) is determined by tumor MSLN methylation status in malignant pleural mesothelioma: implications for screening studies

Heather Nelson[1], Lindsay Almquist[1], Jessica LaRocca[2], Silvia Plaza[2], Geralyn Lambert-Messerlian[2], David Sugarbaker[3], Raphael Bueno[3], John Godleski[4], Carmen Marsit[2], Brock Christensen[2], Karl Kelsey[2]

[1]Masonic Cancer Center, University of Minnesota, USA, [2]Brown University, [3]Harvard Medical School, [4]Harvard School of Public Health

Malignant pleural mesothelioma (MPM) remains a cancer of poor prognosis. It is hoped that implementation of effective screening biomarkers will lead to earlier diagnoses and improved outcomes. Serum-measured soluble mesothelin-related peptide (SMRP) has been demonstrated to have excellent specificity for MPM, but poor sensitivity precludes its use as a screening biomarker. Using a case series of MPM patients from the International Mesothelioma Program at the Brigham and Womens hospital, we sought to identify the molecular underpinnings of the poor sensitivity of SMRP. Specifically, we examined whether cases with low serum SMRP had somatic alterations in their tumors that explained this poor sensitivity. There was limited evidence that MSLN (the gene encoding mesothelin) was deleted, or that microRNA alterations in the tumor were associated with serum SMRP values. We identified three potential target regions for CpG methylation silencing in the MSLN promoter, one of which was amenable to bisulfite pyrosequencing. MSLN promoter methylation was significantly higher in normal pleura than tumor tissue ($p < 6x10-9$). Next, we compared cases according to serum SMRP status and observed that MSLN methylation was significantly higher among tumors from patients testing negative for SMRP (<1.5nM) versus those that were SMRP positive ($p<0.03$). These results demonstrate that MSLN is normally methylated in the pleura, and that methylation is lost in most tumors. However, in a subset of tumors methylation is retained, and this mechanism explains the poor sensitivity of the SMRP assay. These results may lead to additional biomarker targets that will resolve the poor sensitivity of the SMRP assay and allow implementation of screening among exposed populations.

S16

Animal models

S16-1 **Keynote Speaker**

Mechanisms of asbestos-induced carcinogenesis: Animal model studies

Shinya Toyokuni[1], Li Jiang[1], Qian Hu[1], Hiroki Ohara[1], Hirotaka Nagai[1], Takashi Takahashi[2], Yasumasa Okazaki[1], Shinya Akatsuka[1], Yoriko Yamashita[1]

[1]Department of Pathology and Biological Responses, Nagoya University Graduate School of Medicine, Japan, [2]Department of Tumor Biology, Nagoya University Graduate School of Medicine

Respiratory exposure to asbestos has been associated with malignant mesothelioma (MM) in humans. An increase in mortality from MM is expected in Asian countries including Japan. Here we studied the ability of chrysotile, crocidolite and amosite to induce oxidative DNA damage and their modulating factors. Electron spin resonance analyses showed that crocidolite and amosite containing high amounts of iron, but not chrysotile, catalyzed the generation of hydroxyl radicals in the presence of H2O2. These reactions occurred indeed in the presence of natural iron chelators, including citrate and ATP. Then, we used time-lapse videomicroscopy to evaluate whether cells take up asbestos fibers. Not only RAW264.7 cells but also MeT-5A and HeLa cells engulfed crocidolite fibers, which reached not only cytoplasm but also nucleus. Third, crocidolite and amosite fibers induced DNA double strand breaks in supercoiled plasmids preferentially at C:G and repeat sequences. Lastly, intraperitoneal administration of chrysotile, crocidolite and amosite to rats induced MM, with iron deposition in the surrounding tissue. Most of the tumors examined showed homozygous deletion of CDKN2A (p16INK4A), which frequently occurred in human MM as well as in iron-induced rat renal cell carcinoma. Thus, asbestos-induced carcinogenesis is oxidative stress-dependent. Whereas physicochemical characteristics of asbestos play a role in carcinogenesis, modulation of iron appears a promising strategy for the prevention of MM in people who have already been exposed to asbestos. Other animal mesothelioma models will also be discussed.References1. Toyokuni S. Role of iron in carcinogenesis. Cancer Sci 100: 9-16, 2009.2. Jiang L et al. Characteristics and modifying factors of asbestos-induced oxidative DNA damage. Cancer Sci 99: 2142-2151, 2008.3. Toyokuni S. Molecular mechanisms of oxidative stress-induced carcinogenesis: from epidemiology to oxygenomics. IUBMB Life 60: 441-447, 2008.4. Toyokuni S. Mechanisms of asbestos-induced carcinogenesis. Nagoya J Med Sci 71: 1-10, 2009.

S16-2 **Keynote Speaker**

Animal models of malignant mesothelioma: An overview

Richard Lake

National Centre for Asbestos Related Diseases (NCARD) The University of Western Australia, Australia

Animal models of mesothelioma provide the basis for clinical trials of novel therapies, providing important information on toxicity, dose, scheduling, and efficacy. They can provide pivotal information on the genetic cause of this cancer and inform studies that elucidate biological mechanisms, leading to the identification of druggable targets. There are three main types of model that are widely used in this regard: the induction of cancer using a carcinogen; the transplantation of a cancer cell line into syngeneic or immunocompromised mice; and transgenic mice with a genetic predisposition to develop mesothelioma. Variations on these strategies include the use of carcinogens in genetically modified animals to accelerate the course of disease or increase the penetrance of the disease. Each system has advantages and disadvantages so that ultimately the selection of model depends upon the question that is being asked. Subcutaneous transplantation has been extensively used to study mesothelioma, but despite predictable tumor development, rapid growth, and ease of access for measurement and interventions, it is not suitable for testing chemopreventative strategies following asbestos exposure.

S16-3

Role of protein kinase Câ and VEGF Receptor in malignant pleural mesothelioma: Therapeutic implications and the usefulness of *C. elegans* model organism

Rajani Kanteti, Essam El-Hashani, Margaret C. Nickels, Hedy L. Kindler, Ravi Salgia

Section of Hematology/Oncology, Department of Medicine, University of Chicago, USA

Malignant pleural mesothelioma (MPM) is a highly aggressive tumor with poor prognosis that may involve abnormalities in multiple signaling pathways. Protein kinase C ß (PKCß) signaling is associated with the vascular endothelial growth factor receptor (VEGFR), and is known to mediate cell proliferation, differentiation, and apoptosis. Herein, we show that PKCâI, PKCâII and VEGFR-2/KDR are overexpressed in 9 MPM cell lines (H2452, H2691, H2461, H513, H2596, H2373, H28, MSTO, H2052) compared to normal mesothelial cells (MeT-5A). We also observed similar dramatic increases in tumor tissues (42 tumor samples of malignant pleural mesothelioma (MPM) including 29 epithelioid (69%), 9 sarcomatoid (21%), 1 biphasic/mixed (2.5%), 1 desmoplastic (2.5%), and 2 well differentiated papillary mesotheliomas (5%)) as compared to the surrounding normal tissues. A PKCâ inhibitor, blocked angiogenesis and cell migration in H2461 cells. Alone or in combination with KRN633 (a selective inhibitor of VEGFR-2 tyrosine kinase) suppressed VEGF induced proliferation in 9 mesothelioma lines including H2461 cells but not in H2373 and MeT-5A cells and the combinatorial effect of the two drugs was additive. This was characterized by PCK-inhibition mediated reduction in VEGF induced tyrosine, serine and threonine phosphorylation in H2461 epithelioid mesothelioma cells. Also, phosphorylation of VEGR-2/KDR, p-PKCâII and p-AKT (S473) was decreased. In *C. elegans*, both KDR, the human ortholog of VEGFR and PKC β appear to be ubiquitously expressed and localized to similar compartments. As a proof of principle, we generated transgenic *C. elegans*, expressing the wild type PKCâ fused with a GFP reporter gene, and showed that treatment with PKC-inhibition resulted in significant inhibition of the expressed transgene demonstrating that *C. elegans* can be used for screening of drugs that target VEGFR/PKC β.

S16-4

Intrapleural application of polymeric films containing cisplatin and pemetrexed in a rat tumour model of malignant pleural mesothelioma

Luca Ampollini[1,2,3,4,5], Elisabetta Barocelli[2], Fabio Sonvico[3], Paolo Colombo[3], Rocco Bilancia[1], Andrea Cavazzoni[4], Pier Giorgio Petronini[4], Annamaria Cantoni[5], Michele Rusca[1], Paolo Carbognani[1]

[1]Thoracic Surgery, Department of Surgical Sciences, University Hospital of Parma, Italy, [2]Department of Pharmacological, Biological and Chemical Sciences, University of Parma, Parma, Italy, [3]Pharmaceutical Department, University of Parma, Parma, Italy, [4]Department of Molecular Pathology and Immunology, University Hospital of Parma, Parma, Italy, [5]Department of Veterinary General Pathology and Pathological Anatomy, University of Parma, Parma, Italy

Objective: To investigate the effect of intrapleural polymeric films containing cisplatin and pemetrexed on the local recurrence of malignant pleural mesothelioma in a rat tumour model. Materials and Methods: Polymeric films for the local delivery of anticancer drugs were constructed: hyaluronate and chitosan were loaded with cisplatin (100 mg/m2) and pemetrexed (10-100mg/kg). In vitro and pharmacokinetic studies were firstly performed. Then, an orthotopic model of malignant pleural mesothelioma was used. Mesothelioma cells were injected subpleurally in the anaesthetised rats. Six days later, a pleural tumour of 5mm was resected and left pneumonectomy and pleural abrasion were performed. Thereafter, the drug-loaded and unloaded films or cisplatin and pemetrexed solution were randomly intrapleurally applied. After 6 days, animals were euthanised and organs harvested for evaluations. The primary endpoint was the volume of tumour recurrence. The secondary endpoints were treatment-related toxicity; plasmatic and local drug concentration were also measured. ANOVA was used for statistical analysis.Results: Tumour volume was significantly reduced in the hyaluronate-cisplatin and hyaluronate-chitosan-cisplatin groups in comparison to control groups ($p = 0.001$ and $p < 0.0001$, respectively). Animals treated with hyaluronate-chitosan-cisplatin had a tumour recurrence significantly lesser than animals receiving cisplatin solution ($p = 0.003$) and hyaluronate-cisplatin ($p = 0.032$). The association of pemetrexed (10-100mg/kg) to polymeric films loaded with cisplatin did not result in a significant further reduction of tumour volume. No significant treatment-related toxicity was observed. On postoperative days 1 and 2, plasmatic concentration of cisplatin was significantly higher in the hyaluronate-cisplatin and hyaluronate-chitosan-cisplatin groups, in comparison to cisplatin solution, and was maintained over time. Pemetrexed was completely eliminated after 72h.Conclusions: Polymeric films loaded with chemotherapeutic drugs were significantly effective in reducing tumour recurrence compared with cisplatin/pemetrexed solution. Hyaluronate and hyaluronate-chitosan loaded with cisplatin assured significantly higher and more prolonged plasmatic drug concentrations than cisplatin solution without increasing toxicity.

S16-5

Mechanism of action of ADI-PEG20 in pleural malignant mesothelioma: *in vitro* and *in vivo* studies

Barbara Delage[1], Julie Foster[1], Claude Chelala[1], Alex Chacko[2], Dean Fennell[2,3], Stephen Mather[1], Nicholas Lemoine[1], Peter Szlosarek[1]

[1]Institute of Cancer, Queen Mary University of London, UK, [2]Centre for Cancer Research and Cell Biology, Queens University Belfast, Belfast, Northern Ireland, [3]Northern Ireland Cancer Centre, Belfast, Northern Ireland

Novel treatments are urgently required for malignant pleural mesothelioma (MPM), a chemorefractory disease with a median survival of less than 1 year that is increasing worldwide. We have explored the role of pegylated arginine deiminase (ADI-PEG20), an arginine catabolizing enzyme, in argininosuccinate synthetase (ASS1)-negative MPM cell lines. MPM tumors, lacking ASS1, are auxotrophic for arginine and therefore sensitive to arginine depletion driven by ADI-PEG20. First, we assessed the impact of ADI-PEG20 on global gene expression to identify novel interacting pathways in the ASS1-negative MPM cell line, JU77. Next, three ASS1-negative MPM cell lines (JU77, 2591 and MSTO) were treated with ADI-PEG20 and assessed for cellular viability by the MTS colorimetric assay. Apoptosis protein induction by ADI-PEG20 was measured by western-blot after mitochondrial and cytosolic fractionation. Lastly, ADI-PEG20 activity was modelled in vivo using the MSTO cell line combined with small animal 18F-FDG-PET/CT imaging. ADI-PEG20 modulated several thousand genes involved in cell cycle and DNA damage in the ASS1-negative JU77 MPM cell line by 24hrs of drug treatment. We validated several interacting pathways including ADI-PEG20-induced downmodulation of the mTOR/p70S6K signaling pathway and the ribonucleotide reductase subunits, RRM1 and RRM2, known to be involved in cell proliferation and drug sensitivity. In contrast, stable transfection of ASS1 cDNA in the JU77 cell line resulted in ADI-PEG20 resistance with minimal evidence of gene modulation. ADI-PEG20 triggered mitochondrial apoptosis as evidenced by SMAC release in ASS1-negative MPM cell lines. Finally, small animal PET-CT studies suggested that ADI-PEG20 suppressed metabolically active subcutaneously implanted MSTO cells and reduced RRM2 protein expression. In conclusion, ADI-PEG20 modulates numerous biological pathways of therapeutic importance in MPM cell lines and suggests that arginine depletion may have a role to play in the future management of MPM. A clinical trial of ADI-PEG20 in patients with advanced MPM is underway in the UK.

S16-6

Staphylococcal enterotoxin-C, a pleurodesing agent, delays tumour growth in a murine model of malignant mesothelioma

Sally Lansley[1], Shaohua Ren[2], Ai Ling Tan[1], YC Gary Lee[1,3]

[1]Lung Institute of Western Australia, Australia, [2]Lishui Central Hospital, Zhejiang, China, [3]School of Medicine & Pharmacology, University of Western Australia, SCGH

Malignant mesothelioma (MM) remains an incurable cancer and its global incidence is rising rapidly. Bacterial products have been trialled in an effort to enhance local immunity and have demonstrated tumoricidal activity. Staphylococcal enterotoxins (SE) are classic models of superantigens that have potent mitogenic activity on T cells and demonstrated anti-tumour effects in several cancer models. Intrapleural delivery of SE has been used in parts of China for many years as a pleurodesing agent. However, it is unknown whether SE actually kills cancer cells. In this study, we examined the efficacy of one type of SE, SE-C, in the treatment of MM *in vivo* and *in vitro*. Using a flank model of murine MM (using $5x10^5$ AB1-HA cells), SE-C was intratumorally administered (10 days post tumour inoculation) at 2ng daily for six days. Directly following the treatment period, MM tumours in the saline-treated controls were significantly larger in size ($9.92 \pm 4.13mm^2$) compared to SEC treated tumours which remained very small ($2.63 \pm 0.75mm^2$), $p<0.001$. These results were verified in a repeat experiment ($n=20$ in total). Upon cessation of SE-C treatment, MM tumours resumed growth. *In vitro*, SE-C (0-10ng/ml) induced dose-dependant cytotoxicity and reduced viability in all seven human and murine MM cell lines tested, using WST-1 assays and trypan blue exclusion methods. Annexin V staining and flow cytometry revealed increased apoptosis in SE-C treated MM cells compared to untreated controls. These actions were not cytokine mediated as, *in vitro*, no significant release of IL8, VEGF, MIP-2, MCP-1, IL-10 or TNFα were observed in SE-C treated MM cells over controls. These results suggest that SE-C, a drug already shown to be safe in humans, kills MM cells *in vitro* and suppresses MM growth *in vivo*.

S16-7

MexTAg mice exposed to asbestos develop cancer that faithfully replicates key features of the pathogenesis of human mesothelioma

Cleo Robinson, Richard Lake, Anna Nowak

National Centre for Asbestos Related Diseases, University of Western Australia, Australia

Animal models have an important role in helping us understand the aetiology and pathogenesis of cancer as well as providing a platform for investigating the efficacy of treatments. We have previously described MexTAg mice in which the mesothelin promoter directs expression of the SV40 large T antigen to mesothelial cells. Here, we show how this model replicates key features of human asbestos-induced mesothelioma. MexTAg mice that are exposed to asbestos develop mesothelioma more rapidly than wild type mice and with 100% incidence. The mice do not develop spontaneous mesotheliomas. Interestingly, we found that the TAg transgene did not affect the rate of progression of disease, suggesting that TAg expressing tumors are no more aggressive than wild type tumors and that TAg does not alter the course of disease once initiated. The instillation of an alternative inflammatory agent, thioglycollate, did not induce mesotheliomas; demonstrating inflammation per se is not sufficient for tumour development in MexTAg mice. We found that neither the mouse age at the time of exposure or gender were prognostic factors: two questions which have not been satisfactorily answered in human epidemiology. We have explored the functionality of the model in the context of putative carcinogens, therapy, and chemoprevention, and have demonstrated that MexTAg mice offer a robust system suitable for a range of translational experimental work. Briefly, mesotheliomas in MexTAg mice respond to cytotoxic chemotherapy in a comparable way to human disease, making them an ideal model in which to test the efficacy of novel therapies. We also investigated the efficacy of a COX-2 inhibitor as a cancer prevention strategy and whilst this particular drug was ineffective in this regard, the data demonstrate the suitability of the model for testing other preventative strategies.

S17

Multimodality treatment

S17-1 Keynote Speaker

Multimodality treatment paradigm for mesothelioma

David Sugarbaker

Division of Thoracic Surgery, Department of Surgery, Brigham and Women's Hospital, USA

Malignant pleural mesothelioma (MPM) is an aggressive disease with few effective treatments. Median survival in patients who do not receive treatment or palliation is between 6 and 9 months. Single modality approaches to treatment, that is, surgery, chemotherapy, or radiation, alone, have failed to effectively extend survival. An aggressive multimodality treatment strategy has been developed that combines the effects of cytoreductive surgery for macroscopic complete resection (MCR) with adjuvant modalities like chemotherapy, radiotherapy or biologics, to complete the cytoreductive process by targeting microscopic residual disease both locally and systemically. High local recurrence remains the chief barrier to long-term survival. Maximum cytoreductive surgery has evolved as the cornerstone of the multimodality therapeutic approach. Pleurectomy/decortication (P/D) and extrapleural pneumonectomy (EPP) are the two operations currently performed. P/D is better tolerated physiologically and has a lower morbidity and mortality, but MCR is less frequently attained. EPP is a maximally cytoreductive procedure. The concern for radiation-induced pneumonitis is obviated, and sterilization of surgical margins is permitted by radiation and other adjuvant modalities. P/D is more suited for superficial disease that spares the lung fissures, whereas EPP is more appropriate for locally aggressive disease. Combination chemotherapy with cisplatin and pemetrexed has shown superiority over single agents. EPP after neo-adjuvant chemotherapy has demonstrated good results in some centers. Intracavitary chemotherapy achieves lower toxicity levels in plasma and provides better local control. Hyperthermic intraoperative chemotherapy (HIOC) has been shown to be both feasible and safe. Radiation therapy to the hemithorax remains a challenge, with the potential for iatrogenic injury to vital structures. Treatment with intensity-modulated radiation therapy (IMRT) after surgery is highly effective for local control; however, it has been associated with lethal pulmonary toxicity. Other approaches to multimodality treatment include different means of radiation delivery, biological agents, virally mediated gene therapy, photodynamic therapy, and immunotherapy.

S17-2

Trimodality therapy for malignant pleural mesothelioma: Radical pleurectomy followed by chemotherapy with cisplatin/permetrexed and radiotherapy

Servet Bolukbas[1], Christian Manegold[2], Michael Eberlein[3,4], Thomas Bergmann[1], Annette Fisseler-Eckhoff[5], Joachim Schirren[1]

[1]Department of Thoracic Surgery, Dr.-Horst-Schmidt-Klinik, Germany, [2]University Medical Center Mannheim, Heidelberg University, Mannheim, Germany, [3]Critical Care Medicine Department, National Institutes of Health, Bethesda, MD, USA, [4]Division of Pulmonary and Critical Care Medicine, Johns Hopkins University School of Medicine, Baltimore, MD, USA, [5]Institutes for Pathology and Cytology, Dr.-Horst-Schmidt-Klinik, Wiesbaden, Germany

Objective: To analyze the efficacy and results of Radical Pleurectomy (RP) in a trimodality therapy concept.Methods: From November 2002 to October 2007, 35 out of 102 consecutive patients with histological diagnosis of Malignant Pleural Mesothelioma (MPM) underwent trimodality therapy, including lung - sparing surgery with RP followed by 4 cycles of chemotherapy with Cisplatin (75 mg/m2)/Pemetrexed (500 mg/m2) and radiotherapy 4–6 weeks after operation. Results:Median age was 65 years. Epithelial histology was diagnosed in 27 patients (77.1%). Nineteen patients were in advanced stages III and IV (54.3%). The surgical procedures had to be extended in 4 cases: partial resection and reconstruction of the chest wall (n=1), partial resection and reconstruction of the diaphragm (n=1), partial resection and reconstruction of the chest wall and diaphragm (n=1) and partial resection of the aortal adventitia (n=1), respectively. Macroscopic complete resection could be achieved in 18 patients (51.4%). Treatment related morbidity and mortality were 20.0% and 5.8%, respectively. 33 patients completed the trimodality therapy. Overall median survival was 30.0 months with a median follow–up of 21.7 months. One–, 2–, and 3–year–survival were 69%, 50% and 31%, respectively. Advanced stages III/IV (p=0.06), macroscopic incomplete resections (p=0.001), non–epithelial histology (p=0.55) and nodal metastases (p=0.19) were associated with poorer survival. In the subgroup analysis of 8 patients (22.9%), who were initially eligible for EPP at stage I, had epithelial histology and underwent macroscopic complete resection, the median survival was 56.4 months (95%CI 33.0–79.8).Conclusions:Promising results in terms of longterm survival, morbidity and mortality can be achieved by the trimodality therapy concept. In a multimodality therapy setting, less aggressive, lung sparing surgical treatment seems to be an alternative to more aggressive surgical approaches. This treatment approach warrants further prospective controlled multicentre studies.

S17-3

Hyperthermic intrathoracic chemoperfusion (HITHOC) in combination with pleurectomy / decortication (P/D) for treatment of malignant pleural mesothelioma (MPM)

K. Uwe Gruetzner[1], Michael Lindner[1], Thomas Duell[2], Rudolf Hatz[1]

[1]Munich Center for Thoracic Surgery, Asklepios Fachkliniken Muenchen-Gauting, Germany, [2]Department of Pneumological Oncology, Asklepios Fachkliniken Muenchen-Gauting, Germany

Objective:Our objective was to evaluate the feasibility and safety of P/D + HITHOC (Cis-Doxo) in elderly patients with potentially resectable MPM. Patients and Methods:From 11/2009 until 4/2010, a total of 12 patients were treated by open pleurectomy and complete decortication of the lung to remove all visible tumor mass. After closing the chest cavity a Performer HT (Rand, Medolla, Italy) was used for chemoperfusion with cisplatin (40mg/l) and doxorubicin (20 mg/l) in a total volume of 5000 ml normal saline for 90 minutes at 42 degrees Celsius.Results:Twelve patients (10 male / 2 female, age 65-79, mean 72.2 years were successfully treated. All visible tumour could be removed, all chemoperfusions were completed as planned. Time under anaesthesia was between 5:50 and 6:30 hrs, extubation was possible right after the operation in all cases.Postoperative treatment in the ICU was necessary for 1-6 days (mean 1.4), mean hospital stay was 13,3 days, no reoperation was indicated. No potential side effects of the chemoperfusion were observed, such as wound healing disorders, nausea, or significant rise in creatinine or drop in WBC. We observed cardiac arrythmias in 4 patients that were treated medically, 1 pneumonia and 1 secondary air leak with the necessity to place a chest tube.Within the short follow-up period no tumour relapse occurred.Conclusion: P/D + HITHOC (Cis-Doxo) proofed to be a feasible and safe method to treat MPM. Despite the long time under anesthetic and high dose local chemotherapy patients are doing remarkably well after surgery. A longer follow-up is needed to define if this multimodality approach results in additional benefit as compared to standard procedures.

S17-4

11 years single centre experiece with induction chemotherapy followed by extrapleural pneumonectomy for malignant pleural mesothelioma

Isabelle Opitz[1], Michaela Tutic[1], Peter Kestenholz[1], Rolf Stahel[2], Niklaus Schaefer[2], Walter Weder[1]

[1]Division of Thoracic Surgery, University Hospital Zurich, Switzerland, [2]Department of Oncology, Universitty Hospital Zurich, Zurich

Objective: To summarize our single centre 11 years experience with multimodality treatment Material and Methods: Eligible patients had MPM with clinical stage T1-3 N0-2 M0 disease considered to be completely resectable. Neoadjuvant chemotherapy consisted of a combination of cisplatin and gemcitabine (cis/gem) or cisplatin and pemetrexed (cis/pem) followed by extrapleural pneumonectomy (EPP). Postoperative radiotherapy was optional. Results: From May 1999 to January 2010, 167 patients were included in the multimodality treatment concept for MPM; 40% received the combination cis/gem and 60% cis/pem, 1% was lost to follow up. 116 patients underwent extrapleural pneumonectomy corresponding to a resectability rate of 69%. The median follow-up time of all patients was 15 months (2-124 months).The median overall survival (OAS) of the whole patient cohort was 19 months. The patients having fulfilled the whole concept survived significantly longer with 22 months (95% CI: 19; 23) in comparison to 10 months (95%CI: 9; 11) for the patients treated with chemotherapy alone (p=0.0001). Several prognostic factors for overall survival (OAS) and progression free survival were analyzed, out of which IMIG stage and EORTC score showed a significant influence on OAS.Conclusion: After 11 years of experience with induction chemotherapy plus EPP, a median OAS of approximately 2 years is confirmed.

S17-5

Feasibility study on multimodality therapy for MPM

Seiki Hasegawa[1], Fumihiro Tanaka[1], Takeharu Yamanaka[2], Morihito Okada[3], Toshinori Soejima[4], Norihiko Kamikonya[1], Kohei Yoki[5], Kazuya Fukuoka[1], Takashi Nakano[6]

[1]Department of Thoracic Surgery, Hyogo College of Medicine, Japan, [2]National Kyusyu Cancer Center, Fukuoka, Japan, [3]Hiroshima University, Hiroshima, Japan, [4]Hyogo Cancer Center, Akashi, Japan, [5]Nagoya University Hospital, Nagoya, Japan, [6]Hyogo College of Medicine, Nishinomiya, Japan, the Japan Mesothelioma Research Center (JMRC); Aichi Cancer Center, Nagoya, Japan; Osaka Medical Center for Cancer and Cardiovascular Diseases, Osaka, Japan; Okayama University Hospital, Okayama, Japan; Cancer Institute Hospital of JFCR, Tokyo, Japan; Kobe University Hospital, Kobe, Japan; National Cancer Center Hospital, Tokyo, Japan; Shikoku Cancer Center, Matsuyama, Japan; Shizuoka Cancer Center, Naga-izumi, Japan; Chiba University Hospital, Chiba, Japan; Tokyo Medical University, Tokyo, Japan; Tohoku University Hospital, Sendai, Japan; Nagasaki University Hospital, Nagasaki, Japan; Nagoya University Hospital, Nagoya, Japan; Hyogo College of Medicine Hospital, Nishinomiya, Japan; Hyogo Cancer Center, Akashi, Japan; Hiroshima University Hospital, Hiroshima, Japan; Fujita Health University Hospital, Toyoake, Japan

Backgrounds:
Recent Japan's nationwide investigation on malignant pleural mesothelioma (MPM) reported that, between 2002 and 2006, extrapleural pneumonectomy (EPP) had been completed in 171 patients with low morbidity/mortality rate, but that only 12 of them were performed in trimodality setting. Thus, feasibility and safety of trimodality treatment still remain unclear in Asian population, though limited information is currently available from a few Western studies. In this context, we conducted a feasibility study of multimodality treatment consisting of preoperative induction chemotherapy with pemetrexed plus cisplatin (PC), EPP and postoperative RT for potentially resectable MPM.
Methods:
With support by the Special Coordination Funds for Promoting Science and Technology from the Japanese Ministry of Education, Culture, Sports, Science, and Technology, the Japan Mesothelioma Research Center (JMRC) conducted a prospective study to assess the feasibility of trimodality treatment for potentially resectable clinical stage I-III MPM patients. Based on the results of a phase I/II study of PC for unresectable MPM conducted in Japan and a single-institutional pilot study of induction PC for resectable MPM, we adopted cisplatin at the dose of 60mg/m2 in combination with pemetrexed (500mg/m^2). After 3 cycles of PC, patients received EPP followed by RT (54Gy in 30 fractions of 1.8Gy per day). The primary endpoints are macroscopic complete resection rate and treatment-related mortality, and secondary endpoints included completion rate of trimodality treatment, adverse events, radiographic response rate of induction chemotherapy, and 2-year disease-free survival and overall survival. A total of 40 patients will be accrued over 3 years.
Results:
Thirty-two eligible patients have been enrolled as of July 15, 2010. We expect that patient registration will be completed until March 2011.
Conclusions:
This nationwide multi-institutional study will provide a variety of information on trimodality treatment in Japanese population, which contributes to planning future prospective studies for resectable MPM.

S18

Clinical trials

S18-1

Valproic acid plus doxorubicin: effective therapy for progressing mesothelioma. A ELCWP phase II study

Arnaud Scherpereel[1], Thierry Berghmans[2], Jean-Jacques Lafitte[1], B Colinet[3], M Richez[4], Y Bonduelle[5], Anne-Pascale Meert[2], Xavier Dhalluin[1], Nathalie Leclercq[2], Marianne Paesmans[6], Luc Willems[7], Jean-Paul Sculier[2]

[1]Pulmonary and Thoracic Oncology Department, Hospital of the University of Lille II, France, [2]Department of Intensive Care Unit and Thoracic Oncology, Institut Jules Bordet, Centre des Tumeurs, Universite Libre de Bruxelles (ULB), Brussels, Belgium,, [3]Department of Pneumology, Hopital Saint-Joseph, Gilly, Belgium, [4]Department of Pneumology, CHR St Joseph, Warquignies, Belgium, [5]Department of Pneumology, CH Peltzer-La Tourelle, Verviers, Belgium, [6]Data Centre, Institut Jules Bordet, Centre des Tumeurs, Universite Libre de Bruxelles ELCWP, Brussels, Belgium, [7]Biology Laboratory, FUSAGx (University of Agronomic Sciences of Gembloux), Belgium

Rationale: No treatment is recommended for patients with malignant mesothelioma (MM) failing after first line cisplatin-based chemotherapy (CT). In vitro data suggested that valproic acid (VA), a histone deacetylase inhibitor(HDACi), has pro-apoptotic effect and synergized with doxorubicin (D) to induce apoptosis in MM cells. Our primary endpoint was to determine response rate of combined VA and D in patients with unresectable MM failing after platinum-based chemotherapy.Methods: Treatment consisted of D (60 mg/m2) plus VA. An interim analysis for response rate (RR) was planned after the first 16 registered patients. All the cases were centrally reviewed.Results: From 07/2006 to 03/2009, 45 eligible patients with pleural MM were registered. The majority of the patients were male (73%), had a performance status equal or > 80 (76%) and epithelial histologic subtype (80%). There were 7 partial responses (RR 16%; 95% CI 3-25%), all in patients with PS 80-100. Best disease control rate was 36% (95% CI 22-51%). Two toxic deaths were observed (febrile neutropenia, sudden death), both in patients with poor PS (60-70).Conclusion: VA, an HDACi, plus doxorubicin appears an effective CT regimen in good PS (80-100) patients with refractory or recurrent MM, for which no standard therapy is available.

S18-2

A Phase II Trial of anti-TGFbeta Monoclonal Antibody (GC1008) in Relapsed Malignant Pleural Mesothelioma (MPM)

James Stevenson[1,2], Hedy Kindler[2], Daniel Schwed[1], Daniel Sterman[1], Corey Langer[1], Steven Albelda[1]

[1]Thoracic Oncology Program, University of Pennsylvania School of Medicine, USA, [2]University of Chicago

Transforming growth factor-beta (TGF-beta)is a cytokine made by tumors and white blood cells within tumors. Although its functions are varied and context dependent, in advanced cancers it clearly functions to enhance tumor growth by supporting tumor blood vessels, stimulating the supporting cells for the tumor, and by altering the immune system. Our preclinical work, as well as that of other investigators, has established that TGF-beta is produced in large amounts by malignant mesothelioma (MPM) and plays an important role in promoting tumor growth and progression via a variety of mechanisms. Blockade of TGF-beta markedly inhibits the growth of MPM in animal models, primarily through immunologic effects (Suzuki et al., Ca. Res, 67:2351). Genzyme, Inc. has developed a clinical grade monoclonal antibody directed against TGF-beta (GC1008) that has been tested in Phase 1 clinical trials in cancer with minimal toxicity. We have opened a Phase II trial of GC1008 (3 mg/kg) in patients with relapsed MPM as of June 2010. Patients will receive an infusion of the antibody every three weeks. In the absence of toxicity or disease progression, GC1008 will be continued for 6 cycles or more. Our target population will include patients with MPM with good performance status and evaluable disease by Modified RECIST criteria, whose disease has progressed following one or two previous systemic therapies, at least one of which contained pemetrexed. We aim to enter 40 patients over two years at two sites. Our primary objectives for the trial will be determination of progression-free survival at 3 months, as well as toxicity and safety of this antibody. Our secondary goals will include response assessment and overall survival. Additional objectives will include measurement of tumor biomarkers (such as mesothelin), identification of effects of TGF-beta blockade, and immune responses against the tumor.

S18-3

Phase II study of pegylated arginine deiminase in patients with ASS1-negative malignant pleural mesothelioma

Peter Szlosarek[1], Dean Fennell[2], Robin Rudd[1], Jeremy Steele[1], Li-Hui Chen[1], Hanna Nicholas[1], David Gilligan[3], Mike Lind[4], James Spicer[5], Paul Taylor[6], Allan Hackshaw[7], John Bomalaski[8], Nicholas Lemoine[1]

[1]Barts Cancer Research UK Center, UK, [2]CCRCB, Belfast, UK, [3]Addenbrooke's, Cambridge, UK, [4]Princess Royal Hospital, Hull, UK, [5]Guy's Hospital, London, UK, [6]Christie Hospital, Manchester, UK, [7]UCL Cancer Institute , London, UK, [8]Polaris Pharmaceuticals, San Diego, USA

BACKGROUNDArgininosuccinate synthetase (ASS1) deficient malignant pleural mesothelioma (MPM) appears to confer a worse prognosis but undergoes caspase-dependent apoptosis with the arginine-depriving agent, pegylated arginine deiminase (ADI-PEG20). Thus, we have initiated a multicenter phase II study of ADI-PEG20 in patients with advanced ASS1-negative MPM.PATIENTS AND METHODSThis is a 'window of opportunity' randomized phase II trial (2:1) of best supportive care with ADI-PEG20 versus best supportive care alone in patients with chemonaive ASS1-negative MPM. Patients must have non-resectable disease and a performance status of 0 or 1 with evaluable disease on CT at study entry. Patients will be stratified according to gender, histopathological subtype (epithelioid/mixed or sarcomatoid subtype) and cancer center.ENDPOINTSWe expect to screen 126 patients to enrol the required 63 patients with an ASS1-negative MPM from six cancer centres within the UK. The primary endpoint will be progression free survival (PFS), with secondary endpoints including response rate, overall survival and toxicity. We are looking for a 20% or greater improvement in the PFS in patients receiving ADI-PEG20 compared to patients receiving best supportive care alone. Patients will be offered systemic chemotherapy on progression with pemetrexed and platinum. Translational endpoints will include measurement of methylation status of the ASS1 promoter in primary tumoral samples, plasma mesothelin levels, metabolomics using LC/MS and 1H-NMR, plasma nitrite and nitrate levels, and the validation of pathways 'downstream' of ASS1 identified using MPM cell lines.CONCLUSIONTargeting arginine is a potential novel therapeutic strategy in patients with MPM tumors that are deficient in the enzyme ASS1. This Cancer Research UK sponsored randomized phase II study seeks to confirm the efficacy and safety of ADI-PEG20 in the management of patients with advanced ASS1-negative MPM.

S18-4

Retreatment with pemetrexed based chemotherapy (PBC) in patients with malignant pleural mesothelioma (MPM)

Giovanni Ceresoli[1], Paolo Zucali[2], Fabio De Vincenzo[2], Letizia Gianoncelli[2], Matteo Simonelli[2], Elena Lorenzi[2], Cristina Ripa[1], Laura Giordano[2], Armando Santoro[2]

[1]Oncology , Humanitas Gavazzeni, Italy, [2]Oncology, Istituto Clinico Humanitas , Milan, Italy

Background: The role of second-line therapy in patients with MPM progressing after first-line PBC is currently undefined. Recent case series have suggested a possible role of re-treatment with PBC. In this observational study, the activity and safety of this therapeutic option was assessed in a consecutive series of patients.**Methods**: Patients with complete response (CR), partial response (PR) or stable disease (SD) lasting for at least 3 months after first-line PBC were retreated with PBC, either as second-line (2L) or further-line (>2L) therapy. **Results**: Between October 2004 and July 2009, 32 patients (22 males, 10 females) received re-treatment with PBC as 2L (19 patients) or >2L therapy (13 patients). Median age was 65 years. Sixteen patients were re-treated with pemetrexed alone, 16 with a pemetrexed/platinum combination. An objective response was achieved in 6 patients (one CR,5 PRs), for a response rate of 19%. Ten patients (31%) had SD. Overall, the disease control rate (DCR) was 50%. Median PFS and overall survival (OS) were 3,9 months and 10,3 months. PFS and OS after re-treatment were correlated with PFS achieved after first-line PBC (FL-PFS). Patients with a FL-PFS > 12 months had a median PFS of 5.6 months, compared to 2.5 months in patients with a FL-PFS <12 months (p=0.002); no patients in this group was progression-free at 1 year. Toxicity was mild, with grade 3 or 4 hematological toxicity occurring in 9,3% of patients.**Conclusion**: Re-treatment with PBC should be considered as second-line therapy in MPM patients achieving a durable (> 12 months) disease control with first-line PBC.

S18-5

Polysaccharide films: a drug delivery platform for loco-regional therapy of malignant pleural mesothelioma

Fabio Sonvico[1], Luca Ampollini[2], Elisabetta Barocelli[3], Andrea Cavazzoni[4], Claudio Mucchino[5], Pier Giorgio Petronini[4], Paolo Carbognani[2], Paolo Colombo[1]

[1]Pharmaceutical Department, University of Parma, Italy, [2]Thoracic Surgery, Department of Surgical Sciences, University Hospital of Parma, Parma, Italy, [3]Department of Pharmacological, Biological and Chemical Sciences, University of Parma, Parma, Italy, [4]Department of Molecular Pathology and Immunology, University Hospital of Parma, Parma, Italy, [5]Department of General and Inorganic Chemistry, Clinical Chemistry, Physical Chemistry, University of Parma, Parma, Italy

Introduction: In current clinical practice, best survival data in the treatment of mesothelioma were observed after multimodality treatment including chemotherapy, radiotherapy and surgery. Nevertheless, local recurrence rate remains a major problem. Intrapleural therapy is an emerging treatment option for local tumour control and polymeric films can be a dosage form, able to provide the control on drug release and to guarantee a certain degree of adhesion to biological substrates.Materials and methods: Polysaccharide films based on hyaluronate or chitosan were prepared by layer deposition of a solution with film forming agents and plasticizers and oven drying. Cisplatin and pemetrexed, representing the currently reference drug combination in the chemotherapy schedules, were loaded by dissolving the drug with polymers and excipients and its final concentration was 0.5% and 2% w/w, respectively in the dry film. Films were characterized for physicochemical properties, mechanical properties and drug release. Results: Films manufactured were thin (around 100 μm) and flexible; chitosan films showed higher resistance. In vitro drug release for pemetrexed in an excess of PBS was prompt, the drug was released in 1h. The high aqueous solubility of the drug and the hydrophilicity of the film components did not allowed a control of the release over longer times. In the case of cisplatin, on the contrary, because of the interaction between the drug and the polysaccharides, the release of the drug was prolonged. After 96 hours, in the case of hyaluronate films, 95% of the loaded drug was approximately released, while cisplatin was released from chitosan films only adding lysozyme, an enzyme mimicking bioerosion; the 20% of the loaded drug was then released in 28 days.Conclusions: Polymeric films represent an administration approach that can be tailored for the delivery of a number of innovative anticancer compounds with a potential use in malignant mesothelioma.

S19

Prognostic factors

S19-1 **Keynote Speaker**

Prognostic factors in malignant mesothelioma

Jacobus Burgers

Department of Thoracic Oncology, The Netherlands Cancer Institute, The Netherlands

A prognostic factor should accurately predict the outcome of a disease. Many(potential) prognostic factors are known for mesothelioma, but still prediction of disease-related future remains bothersome. This presentation will present the most recent data on prognostic factor research in mesothelioma, and illustrate their limitations and potential use. During the 2008-Amsterdam IMIG meeting 11 out of the 229 abstracts had their main focus on prognostic factors. Ten were published. Nevertheless, they illustrate the limitations of prognostic factor research. All studies involved a different marker, 10 studies were exploratory. Most included a limited number of patients or concerned retrospective analyses. Although these imperfections limit its use as prognostic marker, the association between the marker and the prognosis is confirmed and launches the marker as potentially meaningful in mesothelioma. Only few markers have been studied for their potential to discriminate between high and low risk patients in mesothelioma (phase II studies). In addition, phase III confirmatory studies, using the marker prospectively to discriminate between patients with high or low risk of disease progression or death,are rare. Two studies from the last IMIG mentioned properties of the markerbeyond its prognostic potential. Nowak et al. confirmed the prognostic value of FDG-PET in mesothelioma and constructed a prognostic nomogram includingweight loss, pleurodesis and Total Glycolytic Volume to predict the survival of their patient population (Clin. Cancer Res. 2010). Richards et al. usedtheir database on resectable epithelial mesothelioma to compare the Brighamand AJCC/UICC staging system and proposed adjustments to this system (Cancer, 2010). This latter nicely fits with the IMIG plans to prospectively collect data to confirm and to improve the prognostic value of the current mesothelioma staging system, a phase III prognostic study that is wholeheartedly recommended.

S19-2

Identification of an estrogen-regulated gene associated with mesothelioma outcome

Melissa Coleman, Assunta De Rienzo, William Richards, David J. Sugarbaker, Raphael Bueno

Division of Thoracic Surgery and the International Mesothelioma Program, Brigham and Women's Hospital, and Harvard Medical School, USA

Malignant pleural mesothelioma (MPM) is a highly lethal pleural malignancy with a median survival of approximately 12 months from the time of diagnosis. Given the generally poor prognosis and limited therapeutic options, several studies have focused on MPM prognostic factors. Female gender has been identified as a positive prognostic factor for MPM, however there has been little study of estrogen receptors or estrogen-regulated genes in relation to outcome. Our laboratory has previously described a novel four-gene expression ratio-based algorithm for the prediction of MPM outcome. Beyond assigning predictions of MPM outcome, we now demonstrate that this test has the potential to be used as a tool in the discovery of biomarkers and/or pathways which may play a critical role in prognosis. The aim of this study was to utilize the four-gene ratio test to identify and investigate estrogen-regulated genes associated with MPM outcome. Therefore, we applied the ratio test to gene expression profiling data obtained by two different microarray platforms, Illumina (Agilent) and CodeLink (Applied Microarrays). Forty MPM specimens, with 11 samples in common, were analyzed in each microarray. Utilizing this predictive test the samples were divided into good and poor outcome groups. Genes regulated by estrogen were then identified by literature search. The expression of these genes was evaluated in each of the MPM samples, revealing an estrogen-regulated gene more highly expressed in good outcome than poor outcome samples in both microarray data sets ($p<0.0001$). These results have been further validated in a subset of the MPM samples by quantitative real-time PCR. In addition, preliminary analysis of the estrogen-regulated gene demonstrates its expression in three out of nine mesothelioma cell lines tested. Further studies of this gene are currently in progress to evaluate its role as a biomarker for MPM prognosis and as a possible therapeutic target.

S19-3

Circulating and tumor-infiltrating myeloid cells correlate with poor survival in non-epithelial malignant pleural mesothelioma

Bryan Burt, Scott Rodig, Tamara Tilleman, Raphael Bueno, David Sugarbaker

Division of Thoracic Surgery, The Brigham & Women's Hospital, USA

Introduction: Mesothelioma is a devastating cancer with limited therapeutic options. Mesothelioma tumor cells produce copious amounts of myeloid cell stimulating factors. We hypothesized that an increased number of circulating and tumor-infiltrating myeloid cells in mesothelioma patients would correlate with poor survival. Methods: Preoperative absolute blood monocyte counts were analyzed in 667 mesothelioma patients treated surgically from 1991-2009. Immunohistochemistry of the macrophage-defining antigen CD68 was performed on 60 resected tumors. Data were analyzed via t-tests and cox proportional-hazards determinations. Results: Thirty-five percent of patients had tumors of non-epithelial histology. This group had significantly worse survival than the epithelial group (9.3 vs. 16.6 months; $p<0.0001$) and higher numbers of circulating monocytes (580 ± 20 vs. 520 ± 10 cells/ml, $p=0.003$). In both groups, increasing monocyte count correlated with poor survival [Non-Epi: HR 15.1(5.3-39.8),$p=0.02$; Epi: HR 2.9(1.0-5.0),$p=0.003$], but only in the non-epithelial group was this independent of WBC (HR 9.8(2.2-37.3),$p=0.002$]. In mesothelioma tumor sections, macrophages comprised $27\pm9\%$ of all nucleated cells. By flow cytometry, these tumor macrophages demonstrated an immunosuppressive phenotype with high expression of CD163, CD206, and the IL-4Rα. The degree of macrophage infiltration negatively correlated with survival in non-epithelial [HR 1.12(1.02-1.18),$p=0.006$] but not epithelial ($p=0.5$) groups. Although a higher percentage of macrophages was found in advanced stage non-epithelial tumors ($p=0.006$), their negative correlation with survival was independent of stage [HR 1.12(1.029-1.209),$p=0.008$]. Furthermore, macrophages infiltrating the tumor cell island component of non-epithelial tumors predicted overall survival ($p=.007$), whereas those infiltrating the tumor stroma did not ($p=0.14$). Conclusions: Increasing numbers of circulating monocytes and tumor-infiltrating macrophages portend poor survival in human non-epithelial pleural mesothelioma and enable a novel target for immunotherapy.

S19-4

Validation of blood neutrophil-to-lymphocyte ratio as a prognostic factor in patients with malignant mesothelioma

Steven Kao[1,2], Nick Pavlakis[3], Rick Abraham[4], Janette Vardy[1], Michael Boyer[1], Nico van Zandwijk[2], Stephen Clarke[1,2]

[1]Department of Medical Oncology, Sydney Cancer Centre, Australia, [2]Asbestos Diseases Research Institute, Bernie Banton Centre, Sydney, Australia, [3]Department of Medical Oncology, Royal North Shore Hospital, Sydney, Australia, [4]Department of Medical Oncology, Prince Charles Hospital, Brisbane, Australia

Background
Prognosis in malignant mesothelioma (MM) patients remains poor. Prognostic factors are not routinely used in MM. Previously, we demonstrated that blood neutrophil-to-lymphocyte ratio (NLR), an index of systemic inflammation, predicted for survival in MM patients undergoing chemotherapy. We sought to validate the prognostic value of NLR in an independent cohort of MM patients taking part in prospective clinical trials.
Methods
MM patients recruited for clinical trials since 2000 at Sydney Cancer Centre and Royal North Shore Hospital were included in this validation study. Survival was determined by the Kaplan Meier method. Potential predictors of prognosis such as age, gender, Eastern Cooperative Oncology Group (ECOG) performance status, histological subtype, baseline symptoms of chest pain, fatigue, cough, dyspnoea and sweats, baseline white cell, haemoglobin, and platelet counts, as well as neutrophil-to-lymphocyte ratio (NLR) were analysed against overall survival from the commencement of protocol therapy. Multivariate analyses using Cox Regression model were performed with significant factors ($p<0.05$) to determine their independent effect.
Results
A cohort of 105 patients was identified: median age 61 years (range 36-79); 81% males; 82% ECOG 0-1; histology: 58% epithelioid, 34% non-epithelioid. At the time of report, 88% of patients were deceased. Median survival was 9.7 months (95% CI, 8.1-11.2). Female gender, epithelioid histological subtype, baseline white cell count $<8.3x10^9/l$, baseline platelet count $<400x10^9/l$, and NLR <5 were predictive of longer survival ($p=0.048$, 0.002, 0.007, 0.016, 0.001 respectively). After multivariate analysis, histological subtype (hazard ratio 2.3; 95% CI, 1.4-3.6; $p=0.001$) and NLR (hazard ratio 2.3; 95% CI, 1.5-3.7; $p<0.001$) remained independent predictors of survival.
Conclusions
We have validated NLR as an independent predictor of survival for patients with MM undergoing systemic therapy. Given its low cost, easy reproducibility and wide accessibility, NLR may become an important tool to predict outcome of MM patients undergoing systemic therapy.

S19-5

Extrapleural pneumonectomy for malignant pleural mesothelioma: Outcomes of treatment and prognostic factors

Tristan Yan, Brian McCaughan, Paul Bannon

Royal Prince Alfred Hospital, Sydney and The Baird Institute for Applied Heart and Lung Surgical Research, Australia

Objective: This study aimed to evaluate the perioperative and long-term outcomes associated with extrapleural pneumonectomy (EPP) for patients with malignant pleural mesothelioma (MPM). Methods: From October 1994 to April 2008, 70 patients were selected for EPP. Univariate analysis was performed using Kaplan-Meier method and compared using the log-rank test. Multivariate analysis was used. The prognostic factors included age, gender, side of disease, asbestos exposure, histology, PET, date of surgery, neoadjuvant chemotherapy, completeness of cytoreduction, lymph node involvement, perioperative morbidity, adjuvant radiotherapy and pemetrexed-based chemotherapy. Results: The mean age was 55 (S.D. = 10) years. Fifty-eight patients had epithelial tumors. Six patients received neoadjuvant chemotherapy; 28 patients received adjuvant radiotherapy and 16 patients received postoperative pemetrexed-based chemotherapy. Forty-four patients had no lymph node involvement. The perioperative morbidity and mortality were 37% and 5.7%, respectively. The complications included hemothorax (n = 7), atrial fibrillation (n = 6), empyema (n = 4), bronchopulmonary fistula (n = 3), right heart failure (n = 2), pneumonia (n = 1), constrictive pericarditis (n = 1), acute pulmonary edema (n = 1), small bowel herniation (n = 1) and disseminated intravascular coagulopathy (n = 1). The median survival was 20 months, with a 3-year survival of 30%. Asbestos exposure, negative lymph node involvement and receipt of adjuvant radiation or postoperative pemetrexed -based chemotherapy were associated with improved survival on both univariate and multivariate analysis. Conclusions: The present study supports the use of EPP-based multi-modal therapy in carefully selected MPM patients.

S20

TNM staging for MPM

S20-1

Keynote Speaker

The International Association for the Study of Lung Cancer (IASLC) International Staging Committee (ISC): from retrospective to prospective registration of data

Ramon Rami-Porta

Thoracic Surgery Service, Hospital Universitario Mutuade Terrassa, Spain

The staging project of the IASLC started in 1998 with the constitution of an ISC. The objective was to revise the 6th edition of the tumour, node, and metastasis (TNM) classification of lung cancer. The ISC collected retrospective data on patients with lung cancer world-wide from 1990 to 2000. More than 100,000 patients were registered in the IASLC database. After the required exclusions, 81,495 were included for analysis (68,463 with non-small cell carcinoma and 13,032 with small cell carcinoma). The analysis of the database allowed the revision of the following descriptors: T descriptors: tumour size, additional tumour nodules in the same lobe or in another ipsilateral lobe, and pleural dissemination; N descriptors: no modifications were made, but the concept of nodal zones was devised to assess the prognostic impact of nodal tumour burden; M descriptors: intrathoracic metastases were separated from extrathoracic metastases. The database allowed the testing of the TNM classification in small cell lung cancer and bronchopulmonary carcinoids. However, most descriptors could not be validated because many registries lacked the required information on TNM descriptors to analyse their prognostic impact. The limitations of the retrospective staging project prompted the prospective collection of data to revise future editions of the TNM classification. To achieve this objective the information on the specific descriptors of the TNM is fundamental. In 2009, the Board of Directors of the IASLC decided to expand the activities of the staging project to mesothelioma, thymic tumours, and oesophageal cancer. Now the activities of the ISC are divided into four domains: lung (including non-small cell lung cancer, small cell lung cancer, neuroendocrine tumours), mesothelioma, thymic tumours, and oesophageal cancer. The stage of development of each domain is different, but the leading common idea is to collect prospective data for the subsequent revisions of the classifications of these tumours.

S20-2

Applicability of proposed TNM modifications to biphasic mesothelioma

William Richards[1], Maria McIntire[2], Matthias Hofer[2], Lucian Chirieac[2], David Sugarbaker[1]

[1]Division of Thoracic Surgery, Brigham and Women's Hospital, Harvard Medical School, USA, [2]Department of Pathology, Brigham and Women's Hospital, Harvard Medical School, Boston, Massachusetts, USA

Background: We recently proposed modifications to TNM staging criteria for patients with epithelial malignant pleural mesothelioma (MPM) undergoing extrapleural pneumonectomy (EPP) based multimodality therapy (Cancer 2010;116:1510-7). We now explore the ability of these criteria to stratify the survival of similarly-treated patients with biphasic MPM. Method: Two hundred twenty-eight patients were identified who underwent EPP between 6/8/1988 and 10/30/2008 with a pathologic diagnosis of biphasic MPM. Vital status was confirmed as of 5/30/2010. To date, available slides were retrieved from 205 cases and evaluated. The predominant component (sarcomatoid or epithelioid) by light microscopy of each of the biphasic MPM specimens was recorded. Pathologic stage was derived using our published modifications of TNM criteria. Kaplan-Meier survival functions and Cox proportional hazard estimates were obtained for patients grouped by stage and stratified by predominant histology. Results: Among the 205 biphasic tumors, 116 were predominantly epithelioid and 89 were predominantly sarcomatoid. Stage distribution, associated hazard ratios and median survival for predominantly epithelioid tumors were stage I: N=4, HR=1.0, 22.2 months; stage II: N=46, HR=1.4, 12.7 months; stage III: N=46, HR=2.2, 10.4 months; stage IV: N=20, HR=2.8, 7.4 months. For predominantly sarcomatoid tumors, corresponding values were: stage I: N=6, HR=1.0, 15.9 months; stage II: N=38, HR=1.2, 12.6 months; stage III: N=27, HR=3.2, 6.4 months; stage IV: N=18, HR=3.7, 6.0 months.Conclusions: Proposed modifications to TNM staging criteria based on analysis of epithelial histology tumors provide good stage distribution and survival stratification for patients with biphasic MPM undergoing EPP, particularly those with predominant epithelioid histology. This study provides validation of our published proposed classification criteria in an independent cohort of MPM patients and suggests that modeling of individual T, N and grouping criteria would further improve staging accuracy of patients with biphasic tumors.

S20-3

Patterns of metastases to N2 lymph nodes from biphasic pleural malignant mesothelioma

Maria McIntire[1], William Richards[2], David Sugarbaker[2], Lucian Chirieac[1]

[1]Department of Pathology, Brigham and Women's Hospital, USA, [2]Department of Thoracic Surgery, Brigham and Women's Hospital, Boston, MA, USA

BACKGROUND: Pathologic classification of diffuse malignant mesothelioma (DMM) into epithelioid, sarcomatoid, and biphasic types, according to the current WHO criteria, is an important predictor of survival. Studies have shown that patients with extrapleural lymph node metastases have a poor prognosis following surgery. However, there are no studies examining which component is more likely to spread to N2 lymph nodes. The goal of this study was to characterize the histology of metastases to N2 lymph nodes from patients with biphasic DMM. DESIGN: We identified 231 consecutive patients with biphasic pleural DMM treated by extrapleural pneumonectomy (EPP) at Brigham and Women's Hospital between 1988 and 2009 and found 74 patients who also had a diagnosis of DMM metastatic to mediastinal lymph nodes. We evaluated 26 of these patients with biphasic DMM, N2 lymph node metastases, and available pathology material for the presence of epithelioid, sarcomatoid or both histologies in the positive N2 lymph nodes.RESULTS: All 26 patients (6 F/20 M; mean age 58.9; range 31-72) had a diagnosis of biphasic DMM metastatic to N2 lymph nodes. Nineteen patients (73%) with biphasic DMM had both epithelioid and sarcomatoid components in the N2 lymph nodes. Seven patients (27%) with biphasic DMM showed spread only of the epithelioid component in the N2 lymph nodes (p=0.058). No cases showed spread of the sarcomatoid component alone.CONCLUSION: Our data suggest that a diagnosis of biphasic DMM in patients with N2 lymph node metastases is highly predictive of the histology in the lymph node metastases. However, the findings of an epithelioid component alone in the mediastinal lymph nodes does not preclude from a diagnosis of biphasic DMM in the EPP specimen. The results of our study emphasize the importance of histologic classification and highlight the biologic complexity of tumor morphogenesis and progression in biphasic DMM.

S21

Chemotherapy

S21-1

In vitro chemoresistance to expanded drug panel in malignant pleural mesothelioma

Andrea Wolf, William_F. Powell, Jr., Tamara_R. Tilleman, Raphael Bueno, William_G. Richards, David_J. Sugarbaker

Division of Thoracic Surgery, Brigham and Women's Hospital, USA

OBJECTIVE: Malignant pleural mesothelioma (MPM) is an aggressive disease in which chemoresistance is high. An opportunity for more efficacious chemotherapy may be missed if a patient receives agents to which his tumor manifests resistance. We explored *in vitro* MPM resistance to an expanded profile of chemotherapeutics to provide more options for individualized therapy.METHODS: Between May 2008 and March 2010, 102 specimens were sent for Extreme Drug Resistance Assay (Oncotech) using methods previously described. Resistance patterns were assessed.RESULTS: Ninety-one specimens (89%) had sufficient growth for analysis. Extreme resistance to oxaliplatin was found in 5% and 4% epithelial and non-epithelial tumors, respectively; cisplatin 14% and 11%; gemcitabine 24% and 25%; vinorelbine 30% and 32%; cisplatin-gemcitabine 22% and 29%; doxorubicin 5% and 7%; cyclophosphamide 5% and 11%; taxotere 19% and 18%; etoposide 2% and 7%; irinotecan 5% and 11%; and capecitabine 5% and 4%. Intermediate resistance to oxaliplatin was found in 48% and 61% epithelial and non-epithelial tumors, respectively; cisplatin 27% and 32%; gemcitabine 30% and 32%; vinorelbine 48% and 39%; cisplatin-gemcitabine 25% and 21%; doxorubicin 13% and 14%; cyclophosphamide 30% and 11%; taxotere 38% and 32%, etoposide 13% and 11%; irinotecan 27% and 14%; and capecitabine 14% and 29%. Low resistance to oxaliplatin was found in 40% and 29% epithelial and non-epithelial tumors, respectively; cisplatin 49% and 50%; gemcitabine 43% and 43%; vinorelbine 19% and 29%; cisplatin-gemcitabine 41% and 39%; doxorubicin 79% and 71%; cyclophosphamide 56% and 68%; taxotere 33% and 43%; etoposide 81% and 75%; irinotecan 52% and 58%; and capecitabine 71% and 57%.CONCLUSIONS: *In vitro* assessment of MPM chemoresistance to an expanded panel of drugs is feasible. Low rates of extreme resistance to such agents as cyclophosphamide, doxobucin, and etoposide in both epithelial and non-epithelial tumors provide expanded treatment options based on resistance patterns for individual tumors.

S21-2

Drug sensitivity and cytogenetic changes in primary malignant mesothelioma cells

Adam Szulkin[1], Rita Otvos[2], Tunde Szatmari[1], Fang Zong[1], Anders Hjerpe[1], Eva Darai-Ramqvist[1], Laszlo Szekely[2], Katalin Dobra[1]

[1]Division of Pathology, Department of Laboratory Medicine, Karolinska Institutet and University Hospital Stockholm, Sweden, [2]Department of Microbiology Tumor and Cell Biology (MTC) Karolinska Institutet, Stockholm, Sweden

HYPOTHESIS: We hypothesize that the drug resistance pattern is individual for each mesothelioma patient and may correlate to cytogenetic changes in primary tumour cells. METHODS: Pleural effusions containing primary malignant mesothelioma cells were received from the diagnostic routine. Cells were seeded in a 384-well plate for a robotized ex vivo testing of drug sensitivity. In total, 30 different drugs were tested (2 topoisomerase inhibitors, 5 alkylating agents, 5 antimicrotubule agents, 1 proteasome inhibitor, 10 antimetabolites, 6 antitumor antibiotics and 1 corticosteroid). Each drug was tested at concentrations covering the clinically relevant span. Two new experimental drugs, J1 (a melphalan derivative) and RITA (a p53 reactivating agent) were also tested. To evaluate major cytogenetic changes, an array comparative genomic hybridization (Agilent oligonucleotide CGH array 4x244kb with ULS labeling kit) was performed with benign and malignant mesothelial cells.RESULTS: We have so far tested 14 samples. Among the tested established drugs, Actinomycin-D was the most effective, with cytotoxic effects in 13 out of 14 cultures. Among the different groups, the antimicrotubule agents (Docetaxel, Paclitaxel, Vinblastine, Vincristine and Vinorelbine) seemed to affect most cases.The sensitivity patterns varied greatly between cultures. Five of the isolates were resistant to most of the tested drugs and even the most sensitive cell culture was resistant to >50 % of the drugs. The two experimental drugs, J1 and RITA, showed promising results, with cytotoxic effects in all tested mesothelioma cell cultures.Array CGH analyses have been performed and results currently being evaluated. CONCLUSIONS: The robotized assay allows a simultaneous determination of chemosensitivity to 30 different drugs. The obtained drug sensitivity patterns vary greatly between different Malignant Mesothelioma isolates.

S21-3

Histoculture drug response assay for malignant mesothelioma of the pleura

Tatsuya Yoshimasu, Shoji Oura, Fuminori Ohta, Rie Nakamura, Yoshimitsu Hirai, Koma Naito, Megumi Kiyoi, Haruka Endo, Sayoko Hashimoto, Mitsumasa Kawago, Yoshitaka Okamura

Department of Thoracic and Cardiovascular Surgery, Wakayama Medical University, Japan

Background:Chemotherapy with combination of cisplatin and pemetrexed were used as the first line standard chemotherapy protocol for malignant mesothelioma. However, most patients do not cure after the first line chemotherapy. More numbers of effective chemotherapy agents should be provided for the second line chemotherapy. We investigated the chemosenitivities for surgically resected specimens of malignant pleural mesothelioma using histoculture drug response assay (HDRA).Patients and Methods:A total of 4 surgically resected specimens, that were obtained from the patients with malignant pleural mesothelioma, were used for this study. We examined chemosensitivities of these specimens to cisplatin (CDDP), doxorubicin (ADM), mitomycin C (MMC), 5-fluorouracil (FU), paclitaxel (PAC), docetaxel (DOC), etoposide (VP-16), irinotecan (CPT-11), gemcitabine (GEM), and vinorelbine (VNR), using HDRA technique. The HDRA technique was the same as we previously reported in lung cancer (Yoshimasu T, et al. J Thorac Cardiovasc Surg. 2007; 133: 303-8).Results:HDRA was evaluable in all patients. Chemosensitivities of CDDP, FU, ADM, MMC, PAC, DOC, VP-16, CPT-11, GEM, and VNR were measured in 4, 4, 4, 4, 3, 3, 3, 3, 2, and 1 patients, respectively. Chemosensitivities in each drug were judged positive in 3, 3, 3, 2, 2, 0, 0, 3, 2, and 1 patients, respectively. When the results were summarized in the aspect of individual patients, HDRA provided 7, 7, 3, and 2 drugs with positive chemosensitivity, in each patient.Conclusion:According to the HDRA results in this study, CDDP and GEM were also promising for malignant mesothelioma, as that was already well known. DOC and VP-16 did not seem to be useful. FU, ADM, MMC, PAC, CPT-11, GEM, and VNR might be considered as the candidates for the second line chemotherapy.

S21-4

A clinical study of 34 malignant pleural mesothelioma patients treated with pemetrexed from Nagasaki Thoracic Oncology Group

Takaya Ikeda[1], Minoru Fukuda[2], Masaaki Fukuda[3], Hiroshi Soda[4], Yoshifumi Soejima[5], Akitoshi Kinoshita[6], Hiroshi Gyoutoku[1], Nanae Tomonaga[1], Seiji Doi[1], Katsumi Nakatomi[1], Tetsuya Iida[1], Yoichi Nakamura[1], Shigeru Kohno[1]

[1]The Second Department of Internal Medicine, Nagasaki University Hospital, Japan, [2]Nagasaki Municipal hospital, [3]Japanese Red Cross Nagasaki Atomic Bomb Hospital, [4]Sasebo General Hospital, [5]NHO Ureshino Medical Center, [6]NHO Nagasaki Medical Center

Objective. We analyzed the clinical features and prognosis of malignant pleural mesothelioma patients treated with pemetrexed. Subjects. From 2007 to 2009, 34 patients were treated with pemetrexded in Nagasaki Thoracic Oncology Group. Results. Of the 34 patients, 28 were men and six were women with median age 68 years old (range 53 to 81). Twenty-six patients (76.5%) had exposed to asbestos. The histological subtype was epithelioid in 16 patients, sarcomatoid in 10 patients, biphasic in five patients, unknown in three patients. Twenty-four patients were stage III/IV disease. Twenty-six patients were chemo-naive patients and eight patients had received prior chemotherapy. Thirty patients had received pemetrexed and cisplatin chemotherapy. Three patients had received pemetrexed and carboplatin chemotherapy. One patient had received pemetrexed monotherapy. In pemetrexed and cisplatin group, the response rate was 13.3%, the median progression free survival was 3.4 months, the median survival was 8.9 months, the 1-year survival rate was 33.3%. In hematological toxicities, grade 3 or 4 neutropenia was recorded in six patients (20.0%), grade 3 or 4 anemia was recorded in one patient (3.3%). In non-hematological toxicities, grade 3 or 4 gastrointestinal toxicities were recorded in four patients (11.8%). Conclusion. Pemetrexed is useful in malignant pleural mesothelioma patients. The establishment of combined modality therapy including pemetrexed-containing chemotherapy is required for the improvement of survival in malignant pleural mesothelioma.

S21-5

Optimized intrapleural cisplatin chemotherapy with a fibrin carrier after extrapleural pneumonectomy for malignant pleural meosthelioma: a pre-clinical study

Isabelle Opitz[1], Barbara Erne[1], Seval Demirbas[1,2], Alexander Jetter[2], Burkhardt Seifert[3], Rolf Stahel[4], Walter Weder[1]

[1]Division of Thoracic Surgery, Universitiy Hospital Zurich, Switzerland, [2]Division of Clinical Pharmacology and Toxicology, University Hospital Zurich, Switzerland, [3]Biostatistics Unit, Institute of Social and Preventive Medicine, University of Zurich, Zurich, Switzerland, [4]Laboratories of Molecular Oncology, Clinic and Policlinic of Oncology, University Hospital Zurich, Switzerland

Objective: To evaluate whether platinum concentration in chest wall tissue and in serum are optimized by local application of cisplatin loaded to a fibrin carrier compared to cisplatin-solution in a randomized setting of a pig model. Methods: After left-sided pneumonectomy including parietal pleurectomy pigs were randomly assigned to receive either 90mg/m2 cisplatin (CDDP) intracavitary solution (n=6) or to receive 5mg CDDP-fibrin (n=5) applied on a predefined area of the chest wall. Platinum concentration in serum as well as in chest wall tissue was determined at several early time points until day 5 after treatment. Platinum levels were measured using mass spectrometric detection. Results: The dose- and surface-corrected (geometric) mean concentration of CDDP in chest wall tissue 2 h but also at day 5 after the application was doubled in animals treated with CDDP-fibrin compared to the animals treated with CDDP-solution. In serum, the dose- and surface-corrected exposure towards CDDP (AUC0-5d) was significantly lower with CDDP-fibrin than with CDDP-solution ($p<0.0005$). This is also reflected by significantly reduced serum-creatinine and -urea values in the CDDP-fibrin group ($p<0.0001$). Conclusion: After CDDP-fibrin treatment, CDDP tissue concentration was increased while systemic CDDP concentrations were significantly reduced in comparison to CDDP-solution treatment. This finding offers a clear advantage since rate and severity of systemic adverse events can be reduced while local cytotoxic concentrations are at least maintained.

S21-6

Pemetrexed/carboplatin(AC) or pemetrexed/cisplatin(AP) as first line treatment of malignant pleural mesothelioma(MPM): tolerability and response rate in operable patients

Giulia Pasello[1], Giuseppe Marulli[2], Samuele Nicotra[2], Federico Rea[2], Lucio Loreggian[3], Laura Bonanno[1], Paolo Carli[1], Cristina Magro[1], Antonio Jirillo[1], Adolfo Favaretto[1]

[1]Medical Oncology Department, Istituto Oncologico Veneto ,IRCCS, Italy, [2]Thoracic Surgery Department of University Hospital, Padua, Italy, [3]Radiotherapy Department of Istituto Oncologico Veneto, IRCCS, Padua, Italy

BACKGROUND: Trimodality treatment based on preoperative chemotherapy, surgery and adjuvant radiotherapy can be considered an effective therapeutic option for MPM selected patients. The objective of this study is to evaluate the tolerability and activity of AC or AP as neoadjuvant chemotherapy in MPM. METHODS: patients with histologically confirmed MPM, stage I-III, PS=0-1, received three cycles of pemetrexed 500mg/m^2 plus carboplatin AUC5 or cisplatin 75mg/m^2 day 1 every 21, with standard premedication. Baseline staging and preoperative restaging were assessed with CT-scan and PET-CT. RESULTS: since 2005, 54 patients were included in the study, 30 treated with AC, 24 with AP. Grade-3 haematological toxicities were neutropenia(24%), thrombocytopenia(3%) and anaemia(3%) in AC-treated patients and leucopenia(4%), neutropenia(13%), anaemia(8%) in the AP group. No grade-4 haematological toxicities were shown in the two arms. Grade-3 non-haematological toxicities were diarrhoea(3%) and asthenia(4%) in AC and AP cohorts respectively. Cumulative grade 2-3 asthenia at the last cycle of chemotherapy was commoner in the AP(21%) compared to the AC group(7%) and worsening of PS was shown in 29% and 17% of patients in the two groups respectively. 2 patients in AP arm had dose reduction because of hypercreatininemia and infection, 1 case of postoperative mortality was shown in this group. Response to AC and AP were: complete 3% vs 0%, partial 30% vs 17%, stable disease 64% vs 79%, progressive disease 3% vs 4%. Patients in AC and AP groups showed: resection rate 87% vs 83%; median survival 73 vs 65 weeks; progression free survival 52 vs 51 weeks. CONCLUSION: AC and AP are active and feasible neoadjuvant regimens without major toxicities. AC apparently gave higher response-rate, but resection rate, overall and progression free survival were similar. Cumulative non-haematological toxicities and PS worsening were commoner in AP-treated patients, and this could impair the clinical conditions of patients undergoing surgery.

S21-7

2nd line oral vinorelbine following 1st line platinum + pemetrexed in inoperable malignant pleural mesothelioma (MPM)

Jens Sorensen, Elin Aamdal

Dept. Oncology, Finsen Centre, National Universiyty Hospital, Denmark

Purpose: No standard chemotherapy regimen has been defined in 2nd line chemotherapy for advanced MPM. The activity of Vinorelbine (VNB) as 2nd line following progression after 1st line treatment with Platinum + Pemetrexed in inoperable MPM was explored.Methods: Inoperable MPM patients (pts) in performance status (PS) 0-2, with normal organ functions and no major comorbidity, who progressed after 1st line Platinum + Pemetrexed received oral VNB 80 mg/m2 day 1 and 8 q. 3 weeks for 4-6 courses. CT-scans were done initially and for every 2-3 courses. Modified recist criteria were used for response assessment. Treatment was approved by the local Institutional Board and pts gave written informed consent.Results: Median age among 15 pts included was 69 years (range 42-73), there were 80% males, 53% had epithelial subtype, and 67% had IMIG stage IV. PS 1 and 2 occurred in 47% and 33%, respectively. Median no. of treatment courses were 3 (range 1-6). Only grade 4 toxicity was leucopenia and thrombocytopenia (20% and 7% of pts, respectively) with 3 episodes of febrile leucopenia (with one death) and no bleeding episodes. Partial remission occurred in one patient (7%). 8 pts have died, medians of Progression Free Survival was 70 days (range 12-217) and Overall Survival was 77+ days (range 12-284+ days). Median overall survival from initial histological diagnosis, i.e. the combined effect of 1st and 2nd line treatment was 330 days (range 129 -550+ days) with 6 out of 15 pts (40%) surviving more than one year. Conclusions: This 2nd line treatment with VNB orally was confined with hematologic toxicity and poor activity in terms of response rate in this group of MPM patients with the majority having IMIG stage IV disease. The overall survival from time of histological diagnosis was relatively good compared to hitherto published results.

S21-8

Chemotherapy practice in patients with malignant pleural mesothelioma in the UK; the ChIMP project

Michael Peake[1], Liz A Darlison[1], Jill Lemon[1], Jeremy Steele[2]

[1]Mesothelioma UK, Glenfield Hospital, UK, [2]St Bartholomew's Hospital, London, UK

Background & aims; The incidence of Malignant Pleural Mesothelioma (MPM) has been increasing since the 1960s and currently over 2000 new cases are diagnosed in the UK pa. Evidence-based national guidelines are not available for MPM and during the process for the approval for the use of Pemetrexed in England by the National Institute for Health & Clinical Excellence (NICE), it was apparent that little was known about chemotherapy practice in the UK. We therefore carried out an audit of the use of chemotherapy in patients with MPM in the UK. MethodsChIMP (Chemotherapy In Mesothelioma Project) was an audit covering the period from 1st February 2008 to 31st January 2009. All cancer units in the UK were invited to enter sequential patients with MPM referred to an oncologist for consideration of chemotherapy. Results58 hospitals registered 736 patients, 686 with complete data (83% male; M:F ratio 4.9:1). Median age 72 yrs; 72.8% were of Performance Status 0/1. 368 (54%) of these received chemotherapy; 63% of PS 0 & 66.5% of PS1 patients. 27% of those who did not receive chemotherapy declined treatment. Pemetrexed was used in 91% of patients, 61% receiving it in combination with cisplatin and 29% with carboplatin. Over 50% received at least 3 cycles of treatment with a 28% overall response rate; 31% with Pem/Cis and 21% with Pem/Carbo. Treatment was well tolerated with a 3% febrile neutropenia rate. Conclusion This is probably the largest reported series of chemotherapy treatment in MPM in everyday clinical practice. Despite being referred to an oncologist, only 54% of patients actually received chemotherapy. There was indicative evidence that the combination of cisplatin with pemetrexed was more active than with carboplatin.

S21-9

Thymidylate synthase and excision repair cross-complementing group 1 as predictors of responsiveness in patients with malignant pleural mesothelioma treated with pemetrexed and carboplatin

Paolo Zucali[1], Anna Destro[2], Elisa Giovannetti[3], Giovanni Ceresoli[1], Manlio Mencoboni[4], Letizia Gianoncelli[1], Elena Lorenzi[1], Fabio De Vincenzo[1], Matteo Simonelli[1], Erik Thunnissen[3], Matteo Perrino[1], Laura Giordano[5], Godefridus Peters[3], Massimo Roncalli[2], Armando Santoro[1]

[1]Department of Oncology, Istituto Clinico Humanitas IRCCS, Rozzano, Italy, [2]Department of Human Pathology, Istituto Clinico Humanitas IRCCS, Rozzano (Milan), Italy, [3]Department of Medical Oncology, VU University Medical Center, Amsterdam; The Netherlands, [4]Department of Oncology, Ospedale Villa Scassi, Sampierdarena (Genova), Italy, [5]Biostatistics Unit, Istituto Clinico Humanitas IRCCS, Rozzano (Milan), Italy

Purpose: The combination of pemetrexed with a platinum agent represents the standard of care in the first-line treatment for malignant pleural mesothelioma (MPM). However, there are no established indicators of responsiveness that can be used to optimize treatment. This retrospective study aimed to assess the expression of excision repair cross-complementing group 1 (ERCC1) and thymidylate synthase (TS) in tumors, and correlate expression levels and several gene polymorphisms with the outcome of MPM patients treated with carboplatin/pemetrexed (CP) in a first-line setting.Patients and Methods: Analysis of TS mRNA and protein expression was performed by quantitative-PCR and immunohistochemistry (using the H-score) in tumor specimens from 99 MPM patients. TSER-2R/3R and ERCC1-C118T polymorphisms were also investigated in tumor specimens. Results: A significant correlation between low TS protein expression and disease control (DC) to CP (odd ratio [OR], 4.2, 95%CI 1.22-14.3; p=0.023), longer PFS (8vs6 months; hazard ratio [HR]:0.60, 95%CI, 0.39-0.93; p=0.023), or OS (18vs9 months; HR:0.59; 95%CI, 0.36-0.94; p=0.029) was found when patients were categorized according to median H-score. Similarly, patients with TS mRNA level below the median had significantly longer PFS (10vs6 months; HR:0.33, 95%CI, 0.15-0.59; p <0.001) and OS (22vs8 months; HR:0.17; 95%CI, 0.08-0.36; p < 0.001). The higher tertile of TS mRNA expression also correlated with higher risk of progressive disease (OR:2.5; p=0.044). Furthermore TS mRNA level and TS H-score confirmed their independent prognostic role for PFS and OS at multivariable analysis. In contrast, no correlation was detected between ERCC1 protein expression, TS and ERCC1 polymorphisms, and clinical outcome. Conclusion: In our series of treated MPM patients, low TS protein and mRNA levels resulted significantly associated to response, improved PFS and OS. Prospective trials for the validation of the prognostic/predictive role of TS in MPM patients treated with pemetrexed-based regimens are warranted.

S21-10

Personalized medicine in malignant mesothelioma: Can TS and ERCC1 predict response to chemotherapy?

Daniel Schwed[1], Charuas Deshpande[1], Leslie Litzky[1], Michael Feldman[1], Li-Ping Wang[1], Corey Langer[1], Simona Inghilleri[2], Liang-Chuan Wang[1], Anil Vachani[1], Steven Albelda[1], Giulia Stella[2]

[1]Division of Pulmonary, Allergy and Critical Care Medicine, Hospital of the University of Pennsylvania, USA, [2]Department of Respiratory Diseases, Fundazione IRCCS Policlinico San Matteo, 2700 Pavia, Italy

Background: ERCC1 over-expression has been associated with diminished benefit to cisplatin (cis) in advanced NSCLC; and over-expression of Thymidylate synthase (TS) has predicted lack of response to pemetrexed (pem). In contrast to NSCLC, relatively little work has been published in MPM on these molecular marker and their capacity to predict response to treatment. The purpose of this study was to evaluate the ability of intra-tumoral ERCC1 and TS expression levels to predict responses to cis/pem chemotherapy in patients with MPM.
Materials and Methods: We evaluated a cohort of 43 patients with MPM with complete clinical follow-up for semi-quantitative expression of TS and ERCC1 by immunohistochemistry using a modified Remmele ´ s score (range 0-12). Our primary outcome was the association between TS and ERCC1 expression and radiographic response to pemetrexed and cisplatin respectively.
Results: The mean age at diagnosis was 65 years. Mean overall survival was 13 months. Heterogeneity in the expression of both markers was noted, but overall expression was low. The mean immuonoreactive scores for TS and ERCC1 were 1.53 and 2.1 respectively. We found no significant association between TS or ERCC1 expression and response to chemotherapy.
Conclusions: In contrast to one published report, TS and ERRC1 expression levels failed to predict responsiveness to pem or cis. We are currently expanding the size of this cohort, and based on preclinical data, will evaluate two additional markers - p48 and pSTAT1 - to determine if disruption of the interferon pathway is associated with response to chemotherapy.

S21-11

Enhanced expression of multidrug resistance (MDR) protein in malignant pleural mesothelioma (MPM) patients is possibly achieved via osteoponin, CD44 variants and p-AKT expression

Abeer Bahnassy, Abdel-Rahman Zekri, Amany Abdel- Hamid, Fatma El-Kasem, Abdel-Rahman Abdel-Rahman, Ghada Sherif, Moustafa Mahmoud, Rabab Gaafar

Department of Pathology, National Cancer Institute, Cairo University, Egypt

Malignant pleural mesothelioma (MPM) is an aggressive disease, which is discovered late. It is resistant to chemotherapy and thus shows a dismal prognosis. Osteoponin (OPN) has recently been shown to be involved in the development of MPM via induction of multidrug resistance through unidentified mechanism(s).
We assessed the expression level (protein and RNA) of OPN, MDR, p-AKT and CD44 isoforms in 50 cases of MPM from Egypt. Twenty normal pleural samples were used as a control. Cases included 28 males and 22 females, 86% of patients gave a history of asbestos exposure Twenty four cases were epithelioid, 18 sarcomatoid and 8 mixed. Sixteen patients were stage I, 13 stage II, 12 stage III and 9 stage IV. The expression level of the studied markers was assessed by immunohistochemistry (IHC) and RT-PCR.
Overexpression of OPN, CD44, MDR and p-AKT proteins was detected in 32, 26, 32 and 25 cases; respectively. Increased RNA expression was reported in 31, 24, 30, 24 cases; respectively. There was a significant correlation between OPN expression and other markers, at the protein and RNA levels ($p < 0.05$). The concordance between OPN and MDR expression was the highest (98%). Control samples were negative for the studied markers except for 4 cases that showed faint focal expression of p-AKT and CD44. OS was significantly associated with performance status, increased expression of OPN, MDR and CD44 in univariate and multivariate analysis ($p<0.01$). A borderline significance was reported between poor response to treatment and increased expression of all studied markers either singly ($p=0.063$) or combined ($p=0.04$)
We conclude that OPN, CD44, and p-AKT could be used as poor prognostic markers in MPM being associated with reduced OS rates and poor response to treatment. This could be achieved via enhanced MDR expressionin which confers resistance to chemotherapeutic agents.

S22

Surgery-II

S22-1

Keynote Speaker

A technique for accessing the fused pleural space for gene therapy or other intrapleural treatments for mesothelioma

Joseph Friedberg, Andrew Haas, Stephen Albelda, Adri Recio, Colin Gillespie, Anil Vachani, Melissa Culligan, Daniel Sterman

University of Pennsylvania, USA

Background: Gene therapy for mesothelioma is an active area of research. Some of these protocols require that the gene therapy agents are placed into direct contact with tumor. This is not an issue in the setting of a free flowing malignant effusion, but many patients will have a fused pleural space as the result of previous therapeutic or palliative interventions. A fused pleural space presents a challenge for delivering gene therapy agents that, unless overcome, could preclude a patients enrollment in a gene therapy trial.Purpose: The purpose of this work was to develop a minimally invasive technique for delivering gene therapy agents to mesothelioma patients who presented with fused pleural spaces. Methods: Three patients with fused pleural spaces (2 talc pleurodeses, 1 radical pleurectomy) were referred for placement of a Pleurx catheter for gene therapy agent instillation as part of a gene therapy trial at the University of Pennsylvania. Through a 1 cm incision a video-tunneling device, designed for minimally invasive saphenous vein harvest, was introduced. A tunnel was created between the lung and the chest wall through which a 5 mm video thoracoscope could then be introduced. Under direct vision biopsies were performed to confirm the presence of tumor. The tunnel was then enlarged into a 60 cc cavity to accommodate the gene therapy instillation and a Pleurx catheter was tunneled into the space. Results: Each case was performed on an outpatient basis and resulted in successful placement of the catheter and gene therapy delivery. There were no injuries to the lung or bleeding complications. Conclusion: A safe and reproducible minimally invasive technique for accessing a fused pleural space has been developed. Using this minimally invasive technique, a fused pleural space does not need to be an exclusion criterion for any treatment that requires pleural access.

S22-2

Predictors of resectability in malignant pleural mesothelioma: Additive effects of volumetric analysis and preoperative pathophysiological variables. 5 year follow up and an update to IMIG 2008 report

Tamara Tilleman[1,2], Shin Matsuoka[2,3], Ritu Gill[2,3], William Richards[2,3], William Powell Jr.[1,2], Leonard Friedman[1,2], Raphael Bueno[1,2], Hiroto Hatabu[2,3], David Sugarbaker[1,2]

[1]Division of Thoracic Surgery, Department of Surgery, Brigham and Womens Hospital, USA, [2]International Mesothelioma Program, Boston, MA, [3]Department of Radiology, Brigham and Womens Hospital, Boston, MA, USA

Objective: At IMIG 2008, we reported preliminary data showing that lung volume predicts resectability in mesothelioma patients. Here we present the completed 5 year study.Methods: We retrospectively reviewed 302 MPM patients who underwent extrapleural pneumonectomy(EPP) at our center between 2005 and 2009 (IRB approved), recording demographics, clinical symptoms and volumetric analyses. For tumor and lung volumetric analysis, we used DICOM format CT images and ImageJ(public domain). The ipsilateral lung volume was standardized using an expected .45/.55 left-to-right ratio. Categorical and continuous variables were tested using Fisher' s exact and Wilcoxon rank sum, respectively. Independent predictors of resectability were analyzed by logistic regression.Results: Median age was 62.7yrs. (27-81), 255 patients were male(84%), 187 had epithelial histology(62%), 227 were resectable(75%). 148 patients with formatted CT images underwent volumetric analysis (112 resectable,76%). Median standardized lung volume was smaller in unresectable patients (30.5%;0-90.9vs51.3%;0-122.0,p=0.0001). Median tumor volume was larger in unresectable patients (703.6cc vs 415.5cc,p=0.0025). Patients taking pain medication were more often unresectable (OR=2.3,p=0.0353). Unresectable patients often had higher platelet counts (>450,000/μl,OR=2.5,p=0.0180). Resectability was not influenced by cell type. Overall survival was 11.9mo. Resectable patients survived longer than unresectable ones (12.7vs7.8 mo.;p=0.0053). Independent predictors of resectability found by univariate analysis (Tumor&lung volume, pain medication and age;p<0.05) were entered into a logistic regression model. Neoadjuvant chemotherapy was a confounder of resectability. Lung volume (>50%) predicted a resectability rate of 100% among those who had neoadjuvant chemotheraphy treatment, and tumor volume (<650) predicted a rate of 87% among those with no prior treatment. Conclusions: -Applying criteria specifying locally invasive disease may improve mesothelioma resectability-The novel method of volumetric analysis described herein provides a practical clinical tool. Tumor volume is the best predictor in chemotheraphy naïve patients whereas lung volume predicts resectability post-neoadjuvant treatment.-These models are currently being validated in a prospective study.

S22-3

The efficacy of argon beam ablation: Potential role in pleurectomy for mesothelioma

Andrea Wolf[1], William Richards[1], Scott Swanson[1], Aneil Mujoomdar[1], Michael Jaklitsch[1], Lucian Chirieac[2], David Sugarbaker[1]

[1]Division of Thoracic Surgery, Brigham and Women's Hospital, USA, [2]Department of Pathology, Brigham and Women's Hospital, Boston, MA, USA

OBJECTIVE: Pleurectomy for early mesothelioma (with gross sparing of the visceral pleura) requires resection of healthy-appearing visceral pleura (thickness = .025-.085mm). A less morbid approach than decortication would decrease the risk of air leak and bleeding. We evaluated the efficacy of argon beam pleural ablation and the microscopic changes associated with this technique.METHODS: Lobectomy patients without pleural-based malignancy or infection were eligible for enrollment in an Institutional Review Board-approved protocol. Argon beam ablation at 80W and 120W was used. Each surgical pathology specimen was serially sectioned at 0.5cm intervals and microscopic changes examined. Lung parenchyma with attached visceral pleura were sampled for histologic evaluation in an average of 10 H&E-stained slides (range 6-14), and assessed for both the depth of subpleural coagulative necrosis and subjacent cellular changes. RESULTS: Five patients were enrolled, including three thoracoscopic and two open resections. Argon beam ablation was not associated with bleeding, air leak, or other complications. One specimen was excluded due to coding error. Using 80W, median depth of penetration was 0.249mm (range 0.217-0.289) for total coagulative necrosis and 1.56mm (range 1.16-1.88) for subjacent cellular changes. At 120W, depths were 0.249mm (range 0.156-0.300) and 1.35mm (range 1.14-1.52) for total necrosis and any cellular changes, respectively.CONCLUSIONS: In this prospective study, we found that the argon beam (at 80W or 120W) effectively and completely ablated visceral pleura in both open and thoracoscopic approaches. Coagulative necrosis was observed to a depth nearly ten times the thickness of the visceral pleura. These pilot data suggest that argon beam ablative visceral pleurectomy may represent a safe alternative to decortication, with potential for reduced blood loss and air leak. Argon beam ablation of the visceral pleura may have a role as an adjunct to parietal pleurectomy in the treatment of pleural mesothelioma.

S22-4

Extrapleural pneumonectomy for malignant pleural mesothelioma results correlated to selection criteria

Cosimo Lequaglie, Gabriella Giudice, Rita Marasco, Margherita Garramone

Thoracic Surgery, IRCCS Ospedale Oncologico Basilicata, Italy

Background: To show the role of selection criteria in extrapleural pneumonectomy (EPP) for malignant mesothelioma (MPM).Materials and Methods: Sixty-five potentially EPP submitted patients were selected between 1999 and 2009: PS 0-1, cStages I-II, epithelioid histology, predictive postoperative Fev1 more than 1.0 L and resectable lung perfusion less 50%, PaO2 more 65 mmHg, PaCO2 less 45 mmHg, ejection fraction more 40%. CT and/or MR with PET/CT scan were employed. All had videothoracoscopy for diagnosis and pleurodesis.The same team cured 44 patients (28 females and 16 males, range age of 31-79 years). Twenty-three right MPMs and 21 left ones were observed. Forty patients underwent EPP, 3 exploratory thoracotomies for chest wall or inferior vena cava invasion, and 1 laparoscopy for peritoneal metastases. Thirty-five were epithelioid subtype, 3 sarcomatous and 6 biphasics. Seventeen tumors were pStage I-II, 23 pStage III and 4 pStage IV (no radically resected) according to IMIG. Four re-thoracotomies were for hemostasis, 2 bronchial stump fistulae, 1 superior vena cava thrombosis, 1 gastric rupture, 1 recurrent nerve paralysis, 1 late empyema without fistulae, 1 chilothorax and 2 renal failures. The post-operative mortality is limited to 1 patient for ARDS (2.2 %).Twenty-three patients completed the trimodality treatment. Five had intraoperative chemohypertermic therapy, 1 simply chemotherapy, but 7 patients no therapy for poor PS. Results: Nine deaths for disease were at the end of follow-up, 11 deaths for other causes (6 vascular diseases, 3 respiratory causes, 2 chemotoxicity), 5 alives with recurrence, 15 alives without cancer (longer survival = 105 months). The 3-yr stage I survival was 100% and fall down to 25% in stage III. Conclusions: Strict selection, prevention of complications, attention and standardization of operative procedure reduce hospitalizations and improve quality of life. The ideal treatment for MPM does not exist. Histotype, stage and trimodality therapy may prolong survival.

S22-5

Integrating immunotherapies with debulking surgery to target residual and metastatic mesothelioma

Amanda Cleaver, Renae Weller, Steve Broomfield, Kasia Kania, Malte Wellhaussen, Richard Lake, Bruce Robinson, Andrew Currie

National Centre for Asbestos Related Diseases, School of Medicine & Pharmacology, University of Western Australia, Australia

Most malignant mesothelioma patients present with aggressive and invasive tumours, which are very difficult to completely resect using surgical methods. In general, surgical debulking is palliative but not curative, often delaying tumour growth by only a matter of months. Immunotherapies have been trialed previously in mesothelioma patients with limited responses, but few studies have looked to see how immunotherapy can best be integrated with debulking surgery. We used an established mouse model of mesothelioma to explore the anti-tumour and immunological responses invoked by different immunotherapy strategies when used in combination with debulking surgery. Debulking surgery (75%) in combination with the locally delivered TLR7 adjuvant molecule imiquimod (IMQ) induced a systemic, tumour-specific CD8 response and led to a significant survival benefit above treatment groups that had surgery or IMQ treatment alone. Immunological memory was also induced, as surviving animals resisted tumour rechallenge. However, despite the induction of a systemic CD8 response, IMQ and surgery combination did not effectively target "metastatic " tumour, tested using a dual tumour model. The addition of agonistic anti-CD40 (a potent DC activator), systemically to the IMQ/surgery combination led to a systemic response and a further survival benefit in residual and metastatic tumour models. These findings suggest that a multimodality approach, combining different immunotherapies with conventional debulking, may be beneficial in the treatment of normally unresponsive tumours.

Difficult Cases01

Difficult cases: ask the expert

DC01-1

A calcified pleural tumour in a patient with asbestos exposure: Osteosarcoma or osteogenic sarcomatoid mesothelioma?

Clare Hooper[1], Mike Darby[2], Nick Maskell[1]

[1]Pleural Clinical Trials Unit, North Bristol Lung Centre, Southmead Hospital, UK, [2]Department of Radiology, Southmead Hospital, Bristol BS10 5NB UK

Histological differentiation of primary pleural osteosarcoma (or osteosarcoma with pleural metastases) versus pleural sarcomatoid mesothelioma with prominent osteosarcomatous differentiation is extremely challenging, not least because expression of immunohistochemical markers of mesothelioma is often absent in the latter.

We illustrate this with the case of a 62 year old man, with no significant past medical history, who presented with breathlessness. A CT thorax demonstrated a large right pleural effusion and a soft tissue mass, with extensive patchy ossification, involving the anterior end of the first rib. A calcified pleural plaque was also noted in the left hemithorax. The patient had had moderate asbestos exposure, 40 years ago, whilst working in a power station.

VATS pleural biopsies demonstrated tissue dominated by osteoid closely related to a large number of atypical osteoblasts with intervening dilated vascular channels. On the surface there were atypical pleomorphic spindle cells with no epithelial component. Immunohistochemistry was negative for CK 5/ 6, Calretinin , WT1 and SMA. There was strong osteoblast expression of CD99. Appearances were consistent with a malignant spindle cell tumour with abundant malignant osteoid and bone formation.

Initial multidisciplinary review resulted in diagnosis of osteogenic sarcomatoid mesothelioma and chemotherapy with cisplatin and pemetrexed was initiated. The patient deteriorated following the first cycle. A repeat CT showed gross disease progression, including concentric pleural thickening with extensive ossification, liver metastases and contralateral lung metastases. He died 3 months after his initial presentation.

Several expert histological opinions were sought, including within the regional mesothelioma MDT meeting with both primary osteosarcoma (favoured by imaging and and the striking density of malignant osteoid and bone formation within pleural biopsy specimens) and osteogenic sarcomatoid mesothelioma (favoured by the rarity of pleural osteosarcoma and the asbestos exposure history) proposed.

DC01-2

Localized epithelial malignant mesothelioma

Gunnar Hillerdal

Lung Department, Karolinska Hospital, Sweden

An 80-year-old man, former building worker with asbestos exposure, was referred because of a large apical tumor of the right lung. His main camplaint was increasing pain in the shoulder since more tha a year. He was otherwise in a n excellent condition for his age. A fine0needle blopsy showed malignant epithellal cells, and the patient was referred to thoracic surgery. An upper lobe resection was performed. An epitheliod tumor with intravasal infIltration, invading the pleura but without any lymph node metastases, was found. In the immediate surroundings were pleural plaques, with some atypical sarcomatoid proliferation. Immunohistochemistry: MNF116+, CK5-+, CK7+-, CK14-, CK18+, CK19+, CK20-, EMA+, p63+, CEA-/+, BerEp4+/-, CD138-/+, S-100-, SMMS1-, Actin-, Factor VII-, CD31-, CD34-, E-Cadherin-, TTF-1-, NapsinA-, PSA-, PSAP-, D2-40-+, Thrombomodulin+-, Calretinin+, Mesotelin-, HBM1-+, Vimentin+, Calponin-, CD30-, Chromogranin-, Desmin-, Smoothelin-, CD99-, Synaptosin-, CD45-.

Our pathologist's final diagnosis: malignant epitheloid tumor, most likely a low differentiated malignant mesothelioma.

The patient is doing well, so far no sign of recurring disease, so we have not started Pemetrexed treatment as yet especially since we have not received any definite diagnosis.

DC01-3

Successfully treated stage III myxomatous epithelial mesothelioma presenting with a recurrent three liter mucinous pleural effusion

Joseph Friedberg, Cengel K, Culligan M, Stevenson J

Penn Mesothelioma and Pleural Disease Program Coordinator Thoracic Surgery Clinical Practice and Research Coordinator, USA

41yo otherwise healthy female presented to her primary care physician with complaints of increasing shortness of breath and lower back pain. She ultimately underwent a chest x-ray, which revealed a large right-sided pleural effusion. Thoracentesis yielded three liters of mucinous fluid, cytology suspicious for malignancy – "*hypercellular and consists of large neoplastic cells with abundant cytoplasm and monomorphic appearing nuclei with small nucleoli*." The fluid rapidly reaccumulated, was repeatedly drained but defied definitive diagnosis. RVATS/Pleural Biopsy yielded a diagnosis of "*diffuse nodular malignant mesothelioma, epithelial type with myxoid stroma*." Her extent of disease evaluation raised question of transgression of tumor below her diaphragm. She underwent laparoscopic inspection and biopsy of multiple abnormal appearing areas within her peritoneal cavity, all of which revealed no evidence of metastatic disease. She then underwent Right Extrapleural Pneumonectomy and Intraoperative Photodynamic Therapy. The chest revealed a bizarre appearing polypoid mucinous mesothelioma variant and the tumor was found to superficially invade the adjacent underlying lung parenchyma and involved multiple lymph node levels including: intercostal, periaortic, periesophageal, pericaval, periphrenic and posterior recess. The right hemithorax also contained subpleural collections of tumor that were separate from the pleural tumor itself. Post-operatively she was treated with hemithoracic radiation followed by Alimta based chemotherapy. She has recently developed contralateral lung nodules and malignant ascites but continues to lead an active lifestyle, nearly three years from the time of her diagnosis.

DC01-4

20 year-old female with inhibin-positive epithelioid mesothelioma

Elad Sharon

Medical Oncology Fellow, National Cancer Institute, USA

The patient is a 20-year-old female who originally presented to her primary physician with 2-month history of fatigue, weight loss, fevers, and night sweats in August 2009. Upper and lower GI endoscopies normal, and a computed tomogram of the abdomen and pelvis demonstrated ascites and omental masses with peritoneal implants. The patient was originally thought to be likely due to primary peritoneal carcinoma, as her CA-125 at this time was measured to be 2300. An extensive debulking of tumor was performed, which was suboptimal due to extensive diaphragmatic disease. Pathology specimens were obtained locally and sent to the NIH for review. Immunohistochemical stains revealed tumor cells positive for inhibin, WT1 (strong diffuse staining) and EMA. There was patchy staining for calretinin. Stains were negative for synaptophysin,chromogranin, cytokeratin 5/6, CEA (monoclonal) and desmin. The immunohistochemical staining profile was not typical of malignant mesothelioma, but the histologic features were suggestive of epithelioid malignant mesothelioma. Electron microscopy was performed, which confirmed the diagnosis. The diagnosis was confirmed at two other expert centers. She was diagnosed with peritoneal mesothelioma. The patient continued to have daily fevers, night sweats, anorexia, and fatigue. She subsequently had an extensive debulking of her tumor in November 2009. Pathology from her second surgery confirmed the earlier diagnosis with similar staining and morphological features.

Difficult Cases02

Pathology-Ⅱ: Difficult cases: ask the expert

DC02-1

A rare case of papillary well-differentiated peritoneal mesothelioma with transition into diffuse malignant mesothelioma.

Marcello Deraco[1], Dario Baratti[1], Antonello Cabras[2], Elvira Mingrone1[1], Shigeki Kusamura[1], Rossella Bertulli[3], Massimiliano Solazzo[4], Francesco Puccio[4]

[1]Departments of Surgery-Peritoneal Malignancy Unit, Fondazione IRCCS Istituto Nazionale dei Tumori, Italy, [2]Departments of Pathology, [3]Departments of Medical Oncology-Rare Tumors Fondazione IRCCS Istituto Nazionale dei Tumori, Milano, Italy, [4]Dpt of Surgery Manerbio Hospital Brescia Italy

Papillary well-differentiated peritoneal mesothelioma (PWDPM) is a rare subtype of diffuse malignant peritoneal mesothelioma (DMPM) with an enigmatic natural history and no standard therapy. Cytoreductive surgery (CRS) with hyperthermic intraperitoneal chemotherapy (HIPEC) has been proposed as the therapy of choice for DMPM, but it is not widely accepted for PWDPM, since the disease often shows an indolent behaviour. We present the clinical history of a patient whose initial diagnosis of PWDPM was eventually changed to DMPM only after he underwent CRS and HIPEC.
CASE REPORT A 65-year old woman sought medical attention for severe ascites, abdominal pain and moderate weight loss. CT-scan showed diffuse thickening of the omentum, pelvic, diaphragmatic and parietal peritoneum with involvement of the ileocecal region. Laparoscopic biopsies revealed PWDPM and the patient was referred to our center to undergo CRS and HIPEC. Since the disease did not demonstrate clinical benign behaviour, due to the diffuse intra-abdominal spread and the severity of the clinical picture that was discordant with the pathological type, we decided to anticipate the patient treatment. Macroscopically complete cytoreduction was obtained with total anterior, bilateral diaphragmatic and pelvic peritonectomy with greater and lesser omentectomy, splenectomy, sigmoidectomy, appendectomy and cholecistectomy. Peritoneal disease involvement was scored as a peritoneal cancer index of 20/39. The microscopic residual tumor was treated by closed-abdomen HIPEC with cisplatin and doxorubicin. Postoperative course was uneventful. Pathological examination of the surgical specimens showed coexistence of typical WDPPM and epithelial DMPM, with tubulo-papillary differentiation and deep tissue invasion. Immunohistochemical studies were positive for calretinin, cytokeratin 5/&, and WT-1, and negative for pCEA and BerEp4.
CONCLUSION Differential diagnosis between PWDPM and DMPM may be difficult pre-operatively. Transition of PWDPM into a malignant process or coexistence of both components may be missed, unless extensive tumour sampling from many different anatomic sites is provided by CRS. In this case, comprehensive treatment made possible accurate diagnosis and adequate clinical management.

DC02-2

Experiences of cytology evidence for early recognizing occupational malignant mesothelioma in Taiwan

Lukas Lee,

Division of Environmental Health and Occupational Medicine, National Health Research Institutes, Taiwan

Background: In Taiwan, there has been an increasing trend of malignant mesothelioma (MM), as found through analysis of the Taiwan Cancer Registry database, although under-diagnosis is likely. The unique findings of specific groups with long-term survivors and of MM' s incidence difference between males and females [male to female= 3:2] need further investigation.**Purpose:** To increase early diagnosis of malignant pleural effusion caused by MM, and to identify the histopathological pattern for early-stage pleural MM caused by low-dose and brief exposure to asbestos, we retrospectively analyzed the cytology specimens of pleural effusion of all available MM cases diagnosed at the National Taiwan University (NTU) Hospital in the past two decades.
Method: Based on the computerized disease/cancer registry databases at NTU Hospital available from 1977, we collected MM cases of ICD-9 code 163 [pleural cancer] and 158 [peritoneal cancer]. Medical records and proxy interviews were conducted to collect the patients' histories of asbestos exposure, and the cases were classified as probable, possible, or no exposure. Cytology specimens of pleural effusion of pleural MM will be scrutinized by two senior cytologists who are blind to the exposure level. Immunocytochemical staining of the previous cytology slides will be performed. Difficult cases will be presented in front of experts in the IMIG 2010 conference.
Results: A total of 39 cases of clinically diagnosed MM were found during 1977-April 2010, including pleural [28], peritoneal [5], and solitary [6] cancers. Cytology specimens after 2000 were reviewed by clinical pathologists in order to identify difficult cases in morphological patterns. An independent anatomical pathologist will be involved in the special staining. Among the low-dose exposure group, we may recognize the early morphological changes in pleural MM. The most difficult cases in early diagnosis by cytology will be presented in the conference.

DC02-3

KG case abstract for IMIG 2010 Difficult Cases: Ask the Experts

Andrea Wolf

Division of Thoracic Surgery, Brigham and Women's Hospital, USA

A 29-year old woman without known asbestos exposure presented with pneumonia in December 2003. Chest CT revealed large left pleural and small pericardial effusions with pneumothorax, left upper lobe consolidation, and mediastinal adenopathy. She underwent left thoracotomy, blebectomy, and mechanical pleurodesis at an outside hospital. Biopsy revealed left epithelial diffuse malignant mesothelioma (dMPM; PanK, calretinin, WT-1, and EMA positive). Further workup revealed a right effusion with pleural PET avidity. Our team performed right pleuroscopy with biopsies confirming right epithelial dMPM (AE1/AE3, calretinin, and WT-1 positive; LeuM1 and CEA negative). Her FEV1 was 1.38 L (46% predicted) with 74% perfusion to the right lung and no ventricular dysfunction or pulmonary hypertension. After multidisciplinary consultation, a treatment plan was formulated to include staged pleurectomies with heated intracavitary chemotherapy and adjuvant chemotherapy.
In February 2004, the patient underwent left radical pleurectomy with diaphragmatic resection and splenectomy, bicavitary intraoperative heated cisplatin and diaphragmatic reconstruction. Pathology confirmed left epithelial dMPM, involving parietal and visceral pleurae without diaphragmatic muscle or lung parenchymal invasion. FEV1 improved postoperatively (1.54L; 52%). In May 2004, the patient underwent right radical pleurectomy with heated intrapleural cisplatin. Pathology again confirmed right epithelial dMPM, involving parietal and visceral pleurae, without lung or lymphovascular invasion. Postoperatively, she developed ventilator dependence, requiring tracheostomy and feeding tube (both eventually removed). She completed 4 cycles of adjuvant cisplatin-pemetrexed by December 2004. The patient did well until she developed clinical depression with anorexia and impaired nutrition, progressing to respiratory failure requiring reoperative tracheostomy in February 2008.
Since removal of her feeding tube in January 2009, she has continued to maintain nutrition orally. Her tracheostomy is capped during the day and she rests on the ventilator overnight. Now six years from surgery without evidence of recurrent disease, she is active in physical fitness (even playing basketball) and patient advocacy.

P01

Epidemiology- I

P01-1

Environmental asbestos related diseases: a South African experience

Jill Murray[1,2], Ntombizodwa Ndlovu[1], Jim teWater Naude[3]

[1]National Institute for Occupational Health, National Health Laboratory Service, South Africa, [2]School of Public Health, University of the Witwatersrand, Johannesburg, South Africa, [3]Asbestos Relief Trust, South Africa

Introduction; The Asbestos Relief Trust (ART) and Kgalagadi Relief Trust (KRT) were created as a result of litigation. The Trusts compensate claimants who developed asbestos-related diseases (ARDs) as a result of working or living near a qualifying operation. This preliminary study describes asbestos exposure and ARDs in environmental cases that were submitted to the ART and KRT from 2004 to 2010.MethodsThe asbestos exposure histories and medical findings of all claims registered with the Trusts are entered onto the ARTmis (Asbestos Relief Trust Management Information System). The frequencies of disease were determined. The paper-based files of the environmental cases were also reviewed to obtain comprehensive exposure information. ResultsThere were 15 463 claimants on the database, of which 1% were confirmed environmental cases. Of these, 52% were diagnosed with malignant mesothelioma. DiscussionThe Trusts have received fewer environmental claims than anticipated. Unlike occupational claims, there is no active case finding for environmental claims. In addition, the onus lies with the claimant or his/her dependent to provide the diagnosis of an ARD as well as evidence that the claimant lived near a qualifying operation or was domestically exposed to asbestos.

P01-2

Mesothelioma incidence and survival in UK cancer networks and regions

Michael Peake[1], Sharma P Riaz[2], Henrik Moller[2]

[1]National Cancer Intelligence Network, UK, [2]Thames Cancer Registry, London, UK

Objective; To describe mesothelioma incidence and survival in cancer networks and regions in England MethodWe extracted data on patients diagnosed with mesothelioma in England from the National Cancer Information Service. We extracted age-standardised rates (per 100,000 European standard population (ASR (E)) and 1-year relative survival (%) in cancer networks, by sex. Results North England, Essex, Kent and Medway, NE London, and Central South Coast (CSC) all had high incidence rates. Men had a higher incidence than women; the highest rate was among men living in CSC (7.7 (ASR (E)). Between 1987 to 2006 we observed a steeper increase in incidence in men than in women. Women had a higher relative survival than men. There was low relative survival in Southern England. The highest 1-year survival in women occurred in the Humber and Yorkshire Coast (69.6%) and the lowest in Avon, Somerset and Wiltshire (ASW) (23.5%). The highest relative survival in men was in Pan Birmingham (39.2%) and the lowest was in, ASW (26.6%). From 1987 to 2006 we observed an increase in relative survival with the % of males being alive at 1 year increasing from 24.9% in 1987- 1991, to 27.2% in 1992-1996, 29.8% in 1997-2001 and 33.7% in 2002-6. Conclusion The incidence of mesothelioma is increasing and will continue to rise due to exposure of asbestos in the 1960s and 1970s. There is significant variation in survival rates across England, but there has been an improvement in survival rate over the study period. This is most likely to be a result of earlier diagnosis.

P01-3

Genoa and Trieste, Italy: malignant mesothelioma in two coastal areas

Claudio Bianchi, Tommaso Bianchi

Center for the Study of Environmental Cancer - Italian League against Cancer, Italy

The coasts facing the Gulf of Genoa, northwestern Italy, and the Gulf of Trieste, northeastern Italy, are the sites of many industries. Studies on mesothelioma started in these areas some 50 years ago. Collections of cases published in the 1960s firstly draw attention on mesothelioma phenomenon in these districts. In such phase asbestos was not identified as the cause. However, studies conducted since the early 1970s indicated asbestos as the responsible agent. The Provinces located along the two gulfs emerged as the areas with the highest mortality rates for pleural cancer among men in Italy. Data on mesothelioma incidence became available during the last two decades. Besides the high incidence, mesothelioma in the two areas shows various common features, including a large prevalence of men (80-90%), a prevalence of shipyard workers, and long latency periods elapsed between first exposure to asbestos and diagnosis of the tumor. Moreover, in both areas numerous mesothelioma cases have been observed in seafarers, and dock workers. In addition, studies in Genoa Gulf area revealed a high incidence of pleural mesothelioma among petrochemical workers. In the Trieste area, a series of necropsy-based investigations gave information about the patterns of asbestos exposure in the different occupational categories. Although asbestos use has been reduced in both areas in the late 1970s, and stopped since 1992, mesothelioma epidemic does not show signs of abatement. In 2004-2005, age standardized incidence rates on European population among men were 10.6/100,000 in the Trieste Province, and 9.9/100,000 in Gorizia Province. In Liguria (the Region of Genoa Gulf) the rate was 10.5 in 2005. Ancient work in shipbuilding remains the principal cause of mesothelioma in Genoa and Trieste. Rising mesothelioma incidence has recently been reported from other shipyard areas of the Mediterranean region, such Split and Rijeka (Croatia).

P01-4

Malignant mesothelioma in nonagenarian people

Claudio Bianchi[1], Tommaso Bianchi[1], Sergio Bucconi[2]

[1]Center for the Study of Environmental Cancer - Italian League against Cancer, Italy, [2]Institute of Pathological Anatomy - University of Trieste - Italy

Malignant mesothelioma developing at very old ages, 90 years or more, is a rare event, with about 1% of cases in large series. Diagnosis is generally more difficult at these ages, because invasive procedures are not employed. Serious difficulties may also be encountered in determining the etiology of the tumor, since remote settings, occupational and environmental, have to be reconstructed. From the pathogenetic point of view, the reasons of this unusual late development have to be clarified. Different explanations are plausible such as very mild exposure to asbestos, individual resistance to the asbestos effects, unusually late exposure to asbestos, etc.. In the present study, eight cases of malignant pleural mesothelioma, diagnosed in the Trieste-Monfalcone area, northeastern Italy, in nonagenarian persons, were reviewed. The group included seven men and one woman, aged between 90 and 93 years. The diagnosis was confirmed by necropsy in all the cases. All people had histories of occupational exposure to asbestos. Six patients had worked in the shipyards, one in merchant marine, and one had been trader in marine setting. The latency periods elapsed between first exposure to asbestos and diagnosis of mesothelioma, calculated in six cases, ranged between 64 and 74 years. Asbestos bodies were found on routine lung sections in six cases. Isolation of asbestos bodies after chemical digestion of the lung tissue, performed in two cases, showed 72,000 bodies/gram of dry tissue in a 90-year-old man, who had worked in the shipyards for 34 years, and 150 bodies/gram in a 93-year-old woman, who had worked in the shipyards for 23 years. Mild or late exposures to asbestos do not seem to explain the late development of mesothelioma in this group of cases. Individual resistance or other factors could have played a role.

P01-5

Estimation of the effects of temporal patterns of occupational asbestos exposure on the risk of pleural mesothelioma: results from a French pooled case-control study

Aude Lacourt[1], Karen K. Leffondre[2], Celine C. Gramond[1], Stephane S. Ducamp[3], Anabelle A. Gilg Soit Ilg[4,5], Ellen E. Imbernon[3,5], Joelle J. Fevotte[6], Marcel M. Goldberg[5], Yuriko Y. Iwatsubo[5], Louis-Rachid LR. Salmi[7], Patrick P. Brochard[1]

[1]Laboratoire Sante Travail Environnement EA 3672, Equipe Associee en Sante Travail, ISPED, Universite Victor Segalen Bordeaux 2, France, [2]ISPED, Universite Victor Segalen Bordeaux 2, Bordeaux, France, [3]Departement Sante Travail, Equipe Associee en Sante Travail, Institut de Veille Sanitaire, Bordeaux, France, [4]Registre Multicentrique a Vocation Nationale des Mesotheliomes Pleuraux (MESONAT), Caen, France, [5]Departement Sante Travail, Institut de Veille Sanitaire, Saint Maurice, France, [6]Unite mixte de recherche epidemiologique et de surveillance en transport, travail et environnement Umrestte (UCB Lyon 1 / InVS/ Inrets), Lyon, France, [7]Centre de recherche INSERM U897 Epidemiologie et Biostatistique, Universite Victor Segalen Bordeaux 2, Bordeaux, France

Case-control studies do not clearly demonstrate how the risk of pleural mesothelioma is affected by various temporal aspects of asbestos exposure in the general population. The main objective of this study was to explore the quantitative relationships between several temporal patterns of asbestos exposure including total duration of exposure, age at first exposure and time since last exposure. Cases were pooled from a previous French case-control study and from pleural mesothelioma registered by the French National Mesothelioma Surveillance Program until 2006 in order to increase the power of the study. Controls with a full job history were selected from a random sample representative of the French population and frequency matched by sex and year of birth. Occupational asbestos exposure was obtained by linking job histories with a job-exposure matrix. The effect of temporal pattern was studied using a restricted cubic-spline function in a non-conditional logistic regression model. Among exposed men, an exposure-response relationship was confirmed with the average intensity and total duration of exposure. However, the intensity of the effect according to the total duration of exposure decreased with time since last exposure. The risk of pleural mesothelioma increased until 30 years after cessation of exposure (OR_{30}=2.4 [1.2-4.6]) and then decreased (OR_{50}=1.7 [0.7-3.9]). Due to its long latency, the risk of pleural mesothelioma increased even after cessation of exposure and the decrease of the risk 30 years after cessation of exposure may reflect a possible clearance of asbestos fibres from the lungs. Finally, the younger the subjects when exposed, the greater was their risk of developing pleural mesothelioma (OR_{15vs7}=0.37 [0.07-2.04]) and OR_{30vs7}=0.08 [0.01-0.56]). These findings document the relation between asbestos and pleural mesothelioma according to temporal patterns, an issue still largely unexplored in case-control studies.

P01-6

Epidemiology of malignant pleural mesothelioma in Yekaterinburg

Sergey Kashanskiy, Aleksandr P. Shaburov

Yekaterinburg Medical Research Center, Russia

Object: Study of epidemiology of malignant pleural mesothelioma (MPM) in Yekaterinburg - one of the largest old industrial cities of Russia with the population about 1,5 millions of people, at the territory of which there are situated about 450 large enterprises of heavy and medium engineering, where 450 thousands of people work. The city is divided into 7 administrative districts.Methods: retrospectively the epidemiology of MPM in Yekaterinburg was studied from 1981 until 2004.Results: For the period it was diagnosed 60 cases of MPM in Yekaterinburg, and 27 (45%) from them were diagnosed in the first three years of the third millennium. The age of patients was from 12 to 78 years, at the average - 59.3. The proportion of men and women is 1.5:1.Among the patients 21 people were born and spend their lifetime in Yekaterinburg, and 39 patients were born in other places of the country. The period of habitation at the same address varied in men from 12 to 38 years, at average 22.6, and in women - from 20 to 45 years, at average 32.0.Occupational asbestos exposure was shown only in two patients.At the beginning of XXI century the morbidity of MPM grew fourfold, from 1.46 for the million of people in the year at the last decades of XX century to 5.94 cases at the beginning of the third millennium ($p<0.01$). Incidence rate was noted as in male, so in female population. In different districts of the city morbidity varied from 3.52 to 12.11. Maximum morbidity 7.58 and 12.11 was registered in two the most environmentally neglected districts, where enterprises of metallurgic and machine-building industry are situated. Conclusion: In the last years in Yekaterinburg the statistically significant fourfold incidence rate of MPM is registered, especially in the most environmentally neglected districts.

P02

Epidemiology-Ⅱ

P02-1

Magnitude of misclassification of current address which is used as environmental asbestos exposure surrogate

Dongmug Kang[1], Daeho Kim[2], Kyounghye Lee[1,2], Seonhee Kim[1]

[1]Pusan National University, Research Center for Asbestos Related Diseases, Korea, [2]Pusan National University, Department of Preventive & Occupational Medicine, [3]Pusan National University Yangsan Hospital, Department of Occupational & Environmental Medicine, Korea

Objectives¦ To described the magnitude of misclassification occurred when current address is used as past environmental exposure indicator for epidemiology of asbestos.**Methods**¦ We investigated past and current addresses of people who live presently within 500 meter distance from a former asbestos textile factory site. Current residents were divided into two groups, asbestos exposure group is current residents who had lived in a study area during 1969-1992 which is a running period of the factory and others considered as a non-exposure group.We surveyed the past residence records of the study population by resident registry data and respiratory symptoms of asbestos exposure group, respiratory diseases and others using a structural environmental asbestos exposure questionnaire.**Results**¦ The number of asbestos exposure group is 4,928 (21.0%) and that of non-exposure group is 18,530 (79.0%) which is magnitude of misclassification. Death ratio of exposure group is higher than non-exposure group with statistical significancy ($p<0.001$). **Conclusions**¦ Because asbestos related diseases need long latent period, using current address as a surrogate indicator of environmental exposure might yield misclassification which could weaken asbestos effects.Researchers who use address as a environmental exposure indicator needs to be careful for these kind of biases.

P02-2

The first nationwide survival analysis of Japanese mesothelioma patients from "Vital Statistics of Japan"

Keisuke Aoe[1], Akio Hiraki[2], Nobukazu Fujimoto[3], Kenichi Gemba[3], Takumi Kishimoto[3]

[1]Department of Medical Oncology and Clinical Research, National Hospital Organization Yamaguchi-Ube Medical Center, Japan, [2]Health Care Center, Okayama University, Okayama, Japan, [3]Asbestos-related Disease Center, Okayama Rosai Hospital, Okayama, Japan

The incidence of mesothelioma is increasing in Japan. Mesothelioma has become an independent category from thoracic malignancy since 1995 in "Vital Statistics of Japan". Whilst the death cases caused by mesothelioma were 500 cases in 1995, the number increased to 1170 in 2008. However, there has been no report for nationwide survival analysis of Japanese mesothelioma patients until now. Among the 6030 mesothelioma death cases extracted from the document "Vital Statistics of Japan" for 2003-2008, we used 5100 cases for survival analysis. Survival rate was calculated by Kaplan-Meier method. We studied 3998 men (median age, 70 years; range 6 to 100 years) and 1102 women (median age, 75 years; range 18 to 104 years). The numbers of pleural, peritoneal, pericardial, origin-unknown mesothelioma cases are 3598, 464, 38, and 1000, respectively. Overall median survival time (MST) of all mesothelioma patients was10 months. Survival rates for one, two, and three year are 35.7%, 14.3%, and 6.8%, respectively. Although there was no significant difference in survival curves among each year, MST was found to be increased gradually from 8 to 11 months if the data in 2003-2004 and 2005-2008 were combined separately. The one year survival rates of each year were 30.3%, 33.3%, 36.3%, 36.5%, 35.8%, and 40.1% in 2003, 2004, 2005, 2006, 2007, and 2008 death patients, respectively. There was no significant difference in survival curves among each origin, pleura, peritoneum, and pericardia or no significant difference in survival curves by sex for all the five-year patients whilst the longer survival for women was observed in patients only in 2003. Elderly patients (over 80 years) with mesothelioma showed shorter survival. Thus, these data indicated the trends of the survival of Japanese mesothelioma patients. We need to develop common system for the survey of mesothelioma in Japan.

P02-3

Years of potential life lost due to malignant mesothelioma: a global assessment

Giang Le[1], Ken Takahashi[1], Vanya Delgermaa[1], Lukas Lee[2], Eun-Kee Park[1]

[1]Department of Environmental Epidemiology, University of Occupational and Environmental Health, Japan, [2]Department of Environmental and Occupational Medicine, National Taiwan University Hospital, Taipei, Taiwan

Background: Mesothelioma is gaining recognition as a major public health issue on a global scale. A range of countries has been reporting mortality data to the WHO for some time but such information has been rarely investigated. The objective is to apply the relatively underutilized indicators of years of potential life lost (YPLL) and average years of potential life lost (AYPLL) to characterize the global burden of this important disease.Methods: We analyzed YPLL to life expectancy by sex, type, national income. Countries with available data for mesothelioma mortality (C45, ICD10) in the WHO mortality database during 1994-2008 and life expectancy were included in the study. YPLL was calculated as the summed product of the number of deaths and life expectancy across 5-year age categories with a unit of person-years (p-y). AYPLL was calculated by dividing the YPLL by the total number of deaths with a unit of years (yr).Results: 69 countries that satisfied the selection criteria recorded 90,885 deaths during 1994-2008 with an overall average age of death at 69.6 yr. The YPLL was calculated to be 1,572,170 p-y and AYPLL to be 17.30 yr. The majority of deaths were male (71,025 [78.1%]), high income (80,911 [89%]), account for 1,107,008 p-y (75.1%) and 1,376,817 p-y (87.6%), respectively. In contrast, higher AYPLL values were recorded mostly by female (18.5 yr), middle income countries (19.6 yr), reflecting deaths at lower ages in comparison to the life expectancy.Conclusion: The application of YPLL and AYPLL shed light on previously unreported aspects of the global burden of mesothelioma. The fact that high AYPLL is a feature of developing countries may be related to a predilection of developing countries to diagnose mesothelioma at younger ages but underdiagnose mesothelioma at older ages.

P02-4

Cancelled

P02-5

Difficulties experienced by mesothelioma patients in Japan

Yasuko Nagamatsu

St Luke's College of Nursing, Japan

Objective: This qualitative study was set up to explore difficulties experienced by patients with mesothelioma.Method: Semi-structured interviews were conducted with 18 mesothelioma patients (14 males and 3 females). Participants were interviewed one to two times and each interview was from one to three hours long. The data was analyzed by content analysis. Ethical approval was granted by the Research Ethics Committee of St. Luke ´ s College of Nursing.Result: The result of the analysis indicated five main categories: I.Incurability 1) Torment of wait for death idly 2) Fear of Death 3) Endless Pain 4) Anxiety because they cannot imagine their futureII.Rareness 1) Limited information 2) Good Medical care is not available (Hospitals that can provide diagnosis and treatment are limited. Doctors are short of knowledge and experience) 3) People ´ s misunderstanding and lack of compassion 4) No opportunity to sympathize with patients that share the same problemIII. VictimhoodIV.Ability deprivation 1) Not being able to do things 2) Loss of jobV.Painful treatmentDiscussion: Mesothelioma patients were under strain of fear of death and neglect. It is advisable to remind the medical staff on hand to show these patients support and compassion, so they do not feel forsaken. Giving mesothelioma patients more information about their condition, concrete advice on how to live a productively, and making counselors available may increase their quality of life.

P02-6

Official acknowledgement method of mesothelioma patients by the Asbestos-Related Health Damage Relief Law in Japan

Hirotaro Miura[1], Mitsunori Sakatani[2], Takumi Kishimoto[3], Kouki Inai[4], Yuichi Ishikawa[5], Teruaki Oka[6], Kenzo Hiroshima[7], Masanori Akira[8], Fumikazu sakai[9], Nobuhiko Kohyama[10]

[1]The Department of Respiratory Medicine, Yokosuka General Hospital, Japan, [2]National Health Insurance Seika-town Hospital, [3]The Department of Internal Medicine, Okayama Rosai Hospital, [4]The Department of Pathology, Hiroshima University, [5]The Department of Pathology, The Central Institute of Japan Foundation for Cancer Research, [6]The Department of Pathology, Kanto Central Hospital of the Mutual Aid Association of Public School Teachers, [7]The Departmrnt of Pathology, Tokyo Women's University Yachiyo Medical Center, [8]The Department of Radiology, National Hospital Organization Kinki-Chuo Chest Medical Center, [9]The Department od Radiology, Saitama Medical University International Medical Center, [10]Toyo University, Japan

In Japan, the Asbestos-Related Health Damage Relief Law was enforced on 27 March 2006. This law aims at relief of the patients of mesothelioma and asbestos-related lung cancer. It covers victims of these two malignancies, who are not compensated by workers insurance or the other official schemes. Neighbors around asbestos factories, families of asbestos workers, and self employed workers are included in the candidate. All types of mesothelioma are contained with or without clear exposure history to asbestos. By contrast, in the case of lung cancer it is required that a clear view of asbestos exposure to raise the risk of lung cancer twice. The diagnostic criteria differ before or after the enforcement of the law, especially in mesothelioma the difference is big. If the patient had died before it, only the statement of mesothelioma in the death certificate is required, although the diagnostic accuracy is about 80% on another research. On the other hand after it, the probability of diagnosis of mesothelioma is required. At first, on the application cases are deliberated at a Sub-committee which consists of 5-6 persons in the members of 10 pathologists, 9 clinicians, 10 radiologists and 2 asbestos fiber analysts. The pathologist group includes specialists of ovarian/ peritoneal cancer, sarcoma, and serosal cytology. Finally they are determined in the Acknowledgement Committee consisting of 4 pathologists, 3 clinician, 2 radiologists and a fiber analyst. From April 2006 to March 2010, a total of 6,578 sufferers from mesothelioma applied for the acknowledgment, and 5,189 patients were recognized by this Law and 371 were dismissed because the diagnosis was incorrect. As for lung cancer 1,883 sufferers applied, but only 703 patients were recognized, and 717 were dismissed according to the criteria for asbestos-related lung cancer.

P02-7

Estimation of lifetime direct medical cost of pleural mesothelioma in Taiwan

Lukas Lee[1,2], Ching-Chun Huang[2,3], Saou-Hsing Liou[1], Po-Ching Chu[2], Yu-Yin Chang[1], Jung-Der Wang[2,3,4]

[1]Division of Environmental Health and Occupational Medicine, National Health Research Institutes, Taiwan, [2]Department of Environmental and Occupational Medicine, National Taiwan University Hospital, Taipei, Taiwan, [3]Institute of Occupational Medicine and Industrial Hygiene, College of Public Health, National Taiwan University, Taipei, Taiwan, [4]Department of Internal Medicine, National Taiwan University Hospital, Taipei, Taiwan

Background and Objective: Malignant pleural mesothelioma (MPM) is primarily caused by asbestos exposure. Little data is available concerning the lifetime medical cost of treating MPM. To estimate the lifetime direct medical costs of treating MPM, we analyzed the reimbursement database of National Health Insurance (NHI) and the survival data of National Cancer Registry in Taiwan. Methods: A semi-parametric method with Monte Carlo simulation was applied to extrapolate the survival to 50 years. The Taiwan NHI reimbursement database for each patient diagnosed as MPM (ICD-9 code 163) during 1997-2008 was used to estimate the direct medical costs. Lifetime direct medical costs were estimated by cumulative sum of the product of monthly mean medical costs since diagnosis and the corresponding survival probability. Sensitivity analyses considering the effect of annual discount rate and disease duration were performed. Results: There were 284 cases of histopathologically verified MPM during 1979-2005. The estimated lifetime maximum survival for MPM since diagnosis was 8.82 [95% Confidence Interval 7.10-10.53] years. A total of 12 patients with MPM were identified in the representative sample of 1 million people of the NHI reimbursement database during 1997-2008. The average cost per hospital care was USD 3,732 [95%CI 2,968-4,495]. The consummate cost of lifetime medical care for the patients with MPM paid by the NHI was in average USD 26,903 [95%CI 10,048-43,757] after adjusting for a 3% annual discount rate. Conclusions: We demonstrated a practical approach for estimating lifetime direct medical costs for asbestos-related cancer using the NHI reimbursement database and cancer registry. The substantial burden on the society may be prevented by strict regulatory policies on asbestos.

P03

Oncogenesis- I

P03-1

MET receptor tyrosine kinase as a potential therapeutic target in malignant mesothelioma

Nicholas Campbell, Rajani Kanteti, Rifat Hasina, Hedy L. Kindler, Ravi Salgia

Chicago University, USA

MET receptor tyrosine kinase (RTK) is involved in a number of biological activities in cancer—such as proliferation, invasion, metastasis, and angiogenesis. We have been studying the role of MET RTK and its ligand hepatocyte growth factor (HGF) in malignant pleural mesothelioma (MPM). We have identified that MET was overexpressed in both epitheloid and sarcomatoid MPM, alongwith HGF. As well, in immunohistochemical (IHC) analysis, phosphorylated MET was overexpressed in MPM. Interestingly, downstream target of MET, protein kinase C (PKC) was also overexpressed in MPM. In mutational analysis of the MET gene, we identified several interesting mutations of MET in the semaphorin (sema, ligand binding site) and juxta-membrane domain. In particular, N375S mutation of MET was present in MPM, and is localized with the sema domain. As compared to wild-type MET, N375S mutation has differential ligand binding. Interestingly, the binding to HGF is less in N375S, and also there was difference in angiogenesis (as reflected through transfection into endothelial cells). In response to small molecule MET inhibitor, the N375S mutation responds less to inhibition as compared to wild-type MET. Aside from sema domain mutations, we have identified several mutations of the juxtamembrane (JM) domain—such as R988C, and T1010I. The response to MET inhibitor is enhanced in the T1010I MET mutation as compared to wild-type MET. Aside from mutations, there are several MPM samples that have increased gene copy number for MET. At this time, we are also testing the role of MET in C. elegans modeling system. We have identified "gain-of-function" of the JM domain mutations in C. elegans for MET. Since MET is an important molecule in MPM, we are currently designing potential clinical trials for first line and second line therapies for MPM. We believe that there are also important prognostic and predictive biomarkers as related to MET therapeutics.

P03-2

Involvement of adaptor protein Crk in malignant features of human mesothelioma

Mishie Tanino[1], Lei Wang[1], Taichi Kimura[1], Hiroshi Nshihara[2], Masumi Tsuda[3], Yoshihiro Matsuno[4], Kenzou Okamoto[5], Seiji Yano[6], Shinya Tanaka[1,2]

[1]Laboratory of Cancer Research, Department of Pathology, Hokkaido University Graduate School of Medicine, Japan, [2]Laboratory of Translational Pathology, Department of Pathology, Hokkaido University Guraduate School of Medicine, Sapporo, Japan, [3]Laboratory of Pathophysiology and Signal transduction, Department of Pathology, Hokkaido University Guraduate School of Medicine, Sapporo, Japan, [4]Department of Surgical Pathology, Hokkaido University Hospital, Sapporo, Japan, [5]Department of Surgical Pathology, Hokkaido-ChuoRosai Hospital, Japan Labour Health and Welfare Organization, Iwamizawa, Japan, [6]Division of Medical Oncology, Cancer Research Institute, Kanazawa University, Ishikawa, Japan

Rationale: Crk is a signaling adaptor protein which is mostly composed of SH2 and SH3 domains, and has been demonstrated to play a pivotal role in cell proliferation, differentiation, and migration. It has been demonstrated that Crk is involved in the pathogenesis of human cancers including brain tumors and ovarian tumors, however the role of Crk in malignant mesothelioma has not clarified.Method: To investigate the role of Crk in malignant mesothelioma, we first performed immunohistochemical staining with an anti-Crk antibody on surgically resected specimens of malignant mesothelioma. SDS-PAGE and immunoblotting were carried out in six malignant mesothelioma cell lines such as MESO1, MESO4, H2452, H2052, H28 and 211H. Next, we examined cell motility by wound healing assay, adhesion on collagen I-coated culture dishes, anchorage-dependent and independent growth, Rac activity by pull down assay and FRET based time-lapse analysis in Crk-knockdown cell line. Results: Crk was expressed in cytoplasm and/or nucleus in malignant mesothelioma cells of surgical specimens and also in all six cell lines. Furthermore, knockdown of Crk reduced cell motility, adhesion on collagen I, anchorage-dependent and independent growth, and Rac activity in malignant mesothelioma cell lines. Conclusion: These results suggest that Crk is involved in cell motility, adhesion to collagen I, and growth via activation of Rac in malignant mesothelioma cells.

P03-3

Oncogenic effect of Cul4A in mesothelioma development

Ming-Szu Hung[1], Liang You[2], Zhidong Xu[2], Jian-Hua Mao[3], Yu-Ching Lin[1], Cheng-Ta Yang[4], David Jablons[2]

[1]Department of Pulmonary and Critical Care Medicine, Chang Gung Memorial Hospital, Taiwan, [2]Thoracic Oncology Laboratory, Department of Surgery, Comprehensive Cancer Center, University of California, San Francisco, CA , USA, [3]Life Sciences Division, Lawrence Berkeley National Laboratory, University of California, Berkeley, CA, USA, [4]Department of Pulmonary and Critical Care Medicine, Chang Gung Memorial Hospital, Linkou, Taiwan

Cul4A, which is a member of evolutionally conserved cullin proteins family, plays important roles in cell survival, development, growth, and cell cycle through ubiquitin-mediated proteolysis of key regulatory proteins. However the role of Cul4A in cancer development has not been addressed. In this study, we first identified the amplification of Cul4A gene in four out of five mesothelioma cell lines by fluorescence in situ hybridization (FISH) analysis. Consistent with Cul4A copy number change, overexpression of Cul4A protein was found in these mesothelioma cells. Additionally, overexpression of Cul4A was also found in 64% primary malignant pleural mesothelioma (MPM) tumors. Knockdown of Cul4A with shRNA resulted in up-regulation of p21 and p27 tumor suppressor proteins in two p14ARF-null mesothelioma cell lines (H290 and H28) and in a p53-null colon cancer cell line HCT-116, suggesting its regulation of CDK inhibitors p21 and p27 proteins is p53-independent. Furthermore, down-regulation of Cul4A induced significantly G0/G1 cell cycle arrest in the H290 cell line, and reduced numbers of colony formation of mesothelioma cell lines H290, H28 and MS-1, which is the first time to show that Cul4A knockdown suppresses cancer cell growth. Moreover, G0/G1 cell cycle arrest is reversed by siRNA down-regulation of p21 and/or p27 levels in Cul4A shRNA transfected cells. Importantly, p21 appears to have a major effect on G0/G1 arrest in Cul4A knockdown cancer cells. Taken together, we proposed that Cul4A plays an essential role in the pathogenesis of mesothelioma, and may be a potential prognostic marker and therapeutic target for mesothelioma.

P03-4

Analysis of lipoxygenase pathways in malignant pleural mesothelioma

Vijay Agarwal[1,2,3], Dulani Ranatunge[1,2,3], Anne Campbell[1,4], Michael Lind[1,2,3], Lynn Cawkwell[1,2]

[1]Cancer Biology Proteomics Group, Postgraduate Medical Institute, University of Hull, UK, [2]Hull York Medical School, Hull, UK., [3]Queens Centre for Oncology and Haematology, Castle Hill Hospital, Hull and East Yorkshire NHS Trust, Hull, UK., [4]Histopathology Department, Hull Royal Infirmary, Hull and East Yorkshire NHS Trust, Hull, UK.

Introduction: Despite recent advances in chemotherapy, advanced malignant pleural mesothelioma (MPM) is associated with poor prognosis. Arachadonic acid is metabolised by the cyclooxygenase (COX) and lipoxygenase (LOX) pathways. This results in the production of prostanoids, leukotrienes, hydroxyeicosatetraenoic acids and hydroperoxyeicosatetraenoic acids, which have been implicated in carcinogenesis. The expression of 5-LOX and 12-LOX has been demonstrated to be associated with carcinogenesis in various solid tumours, however little is known about their clinical relevance in MPM. We aimed to assess the expression of 5-LOX and 12-LOX in a large series of MPM tissue samples.Methodology: Immunohistochemical analysis was performed in 93 archival MPM tissue samples (48 epithelial, 27 biphasic, 18 sarcomatoid) to determine 5-LOX and 12-LOX expression. Univariate and multivariate analyses were used to determine the presence of any prognostic factors.Results: Positive 5-LOX expression was seen in 73% (65/88) of MPM samples and this was associated with improved survival (median overall survival 13.3 months versus 7.3 months; p=0.006). However, when histological subtype was taken into consideration, multivariate Cox regression analysis demonstrated that 5-LOX expression was not an independent prognostic variable (p=0.074). Positive 12-LOX expression was seen in 83% (69/83) of MPM samples, but this was not associated with survival (p=0.455). Correlating this LOX expression data with our previously published COX2 expression results in the same cohort (Eur J Cancer 41:1645-8; 2005) revealed significant correlations. Positive 5-LOX expression correlated with positive COX2 expression (p=0.002). Positive 12-LOX expression correlated with 5-LOX co-expression (p=0.006).Conclusion: We have demonstrated that 5-LOX and 12-LOX are expressed in a significant number of MPM samples and therefore may provide novel therapeutic targets.

P03-5

YAP induces malignant mesothelioma cell proliferation via induction of CCDN1 expression

Tetsuya Mizuno[1,2], Hideki Murakami[1], Makiko Fujii[1], Futoshi Ishiguro[1,2], Yutaka Kondo[1], Shinya Akatsuka[3], Shinya Toyokuni[3], Yuichi Ueda[2], Kohei Yokoi[2], Hirotaka Osada[1,4], Yoshitaka Sekido[1,4]

[1]Division of Molecular Oncology, Aichi Cancer Center Research Institute, Japan, [2]Department of Cardio-Thoracic Surgery, Nagoya University Graduate School of Medicine, Nagoya, Japan, [3]Department of Pathology and Biological Responses, Nagoya University Graduate School of Medicine, Nagoya, Japan, [4]Department of Cancer Genetics, Program in Function Construction Medicine, Nagoya University Graduate School of Medicine, Nagoya, Japan

Malignant mesothelioma (MM) is one of the most aggressive neoplasms. Genetic alterations except for $p16^{INK4a}$/$p14^{ARF}$ and neurofibromatosis type2 (NF2) genes are not well known in connection with this neoplasm. Recent studies have shown that the Hippo signaling pathway, a possible downstream cascade of Merlin (a product of NF2), plays a key role in organ size control by regulating cell proliferation and apoptosis. We previously reported gene amplification of YAP transcription coactivator, a downstream effector of the Hippo pathway, in a subset of MM specimens. Although these findings suggest a potential involvement of the inactivation Hippo pathway and consequent activation of YAP for MM cell proliferation, the detailed functions of YAP on MM cell growth/survival are still unclear. Here we generated two YAP-knockdown MM cell lines and detected 716 genes which were potentially regulated by YAP with microarray analysis. Among them, cyclin D1 (CCND1), which is known to be overexpressed in most MM cells, showed 0.1-0.5-fold decrease of expression by YAP knockdown. Since cyclin-dependent kinase (CDK) inhibitors, p21cip1 and p27kip1, were expressed and the increment in their expression according to higher cell density in tissue culture was also observed, the sustained overexpression of CCND1 suggested that, on cell contact at high cell density, the regulation of CCND1 expression is impaired at the transcription level and that this may be induced by YAP activation. To confirm this, we constructed a YAP expression construct and a luciferase reporter construct with the CCDN1 promoter region. We found that YAP transduction strongly elevated CCND1 promoter activity. Moreover, chromatin immunoprecipitation assay revealed the direct binding of YAP and CCND1 promoter region. These results indicate that CCND1 is one of the direct transcriptional target genes of YAP and their interaction may play an important role in MM cell progression.

P03-6

Expression and functional analysis of Hairy Enhancer of Split 1 (HES1) in human malignant mesothelioma cell lines

Hideki Murakami[1], Shigehisa Kawata[1], Tetsuo Taniguchi[2], Koji Kawaguchi[2], Testuya Miuzuno[2], Futoshi Ishiguro[2], Makiko Fujii[1], Yutaka Kondo[1], Hirotaka Osada[1], Yoshitaka Sekido[1]

[1]Division of Molecular Oncology, Aichi Cancer Center Research Institute, Japan, [2]Department of Cardio-Thoracic Surgery, Nagoya University Graduate School of Medicine, Japan

Malignant pleural mesothelioma (MPM) is a highly aggressive neoplasm. Survival of patients with MPM is very poor because of inherent resistance to chemotherapy. Cellular and molecular aspects of MPM remain to be elucidated. To reveal genes relevant for MPM, we carried out oligo-nucleotide expression array experiments on 21 MPM cell lines. Whole Human Oligo Microarray including about 41,000 probes was used for the array experiments. Our study revealed several over-expressed and under expressed genes that play a role in the regulation of cell cycle, cell growth and motility to be common to 10 or more MPM cell lines. Among them, hairy enhancer of split 1 (HES1) which encodes a transcription factor was down-regulated in most of mesothelioma cell lines compared to normal mesothelial cells. Down-regulation of HES1 was confirmed with quantitative reverse-transcriptase PCR and Western blot analyses. We also introduced a HES1 construct into NCI-H290 mesothelioma cell line, and found that exogenous HES1 inhibited cell proliferation and induced cell cycle arrest in G1/G0 phase. Exogenous HES1 expression induced up-regulation of E-cadherin and down-regulation of MET and CCND1, which have been previously reported to play an important role for progression of mesothelioma cells. These results suggest that HES1 is potentially one of the key molecules in mesothelioma development.

P04

Oncogenesis-Ⅱ

P04-1

RON/MST1R, a receptor tyrosine kinase expressed in malignant pleural mesothelioma

Steven Gray[1], David Easty[1], Isabelle Opitz[2], Daisuke Nonaka[3], Harvey I. Pass[3], Luciano Mutti[4], Alex Soltermann[2], Kenneth J. O'Byrne[1,5]

[1]Department of Clinical Medicine, Trinity College Dublin, Ireland, [2]University Hospital Zurich, Zurich, Switzerland, [3]New York University School of Medicine, New York, NY, [4]Department of Medicine, Local Health Unit 11, Borgosesia, Italy, [5]HOPE Directorate, St. James's Hospital, Dublin, Ireland

Background: Receptor tyrosine kinases (RTK) represent novel therapeutic targets for the treatment of malignancy. Using a phospho-RTK array strategy we identified macrophage stimulating 1 receptor (MST1R/RON) as an RTK frequently expressed in malignant pleural mesothelioma (MPM). **Methods:** Expression and function of RON was studied in 4 MPM cell lines, 1 transformed mesothelial cell line, and 16 MPM and 5 benign mesothelial surgically resected specimens. Western Blot (WB) analysis in fresh frozen samples, immunohistochemistry (IHC) on fixed formalin paraffin embedded (FFPE) specimens, and cellular migration assays in cell lines were performed. **Results:** RON (mRNA & protein) is present in fresh frozen tumour and benign pleural specimens and the 4 MPM cell lines but not in the SV-40 transformed normal mesothelial MET-5A cells. MPM expressed different isoforms of RON compared to benign pleural plaques: both benign pleural plaques and MPM expressed the shortform of RON (sf-RON) whereas the larger RON variants, delta160 and delta165, were seen in MPM samples only. IHC was performed on a TMA array of FFPE samples resected from 352 patients. 94% showed expression as follows - weak (26%), moderate (37%), strong (31%). No correlations were observed for age, histology or gender with Global Ron Score per patient. Cox regression analysis of Global Ron Score identified a statistically significant correlation [HR 0.8 (95%CI: 0.6;0.9) p=0.014] between RON positivity and survival. Using a pre-clinical anti-RON monoclonal antibody, migration assays demonstrate that targeting MST1R/RON prevents cellular migration. **Conclusions:** Based on the phospho-RTK assays and protein expression studies MST1R/RON is frequently expressed in MPM. The migration inhibition assays suggest that RON may be a novel target for therapy in mesothelioma. Previously, MST1R/RON was shown to mediate epithelial mesenchymal transition (EMT). Therefore RON may play a role in the epithelioid to sarcomatoid spectrum of disease seen with mesothelioma.

P04-2

All-*trans*-retinoic acid inhibits tumor growth of malignant pleural mesothelioma in mice

Chiharu Tabata[1], Rie Tabata[2], Noriko Hirayama[1], Akihiro Yasumitsu[1], Shusai Yamada[1], Aki Murakami[1], Kunihiro Tamura[1], Takayuki Terada[1], Kozo Kuribayashi[1], Kazuya Fukuoka[1], Takashi Nakano[1]

[1]Division of Respiratory Medicine, Department of Internal Medicine, Hyogo College of Medicine, Japan, [2]Department of Internal Medicine, Hyogo Prefectural Tsukaguchi Hospital, Hyogo, Japan

Malignant pleural mesothelioma (MPM) is an aggressive malignant tumor of mesothelial origin associated with asbestos exposure. Because MPM has limited response to conventional chemotherapy and radiotherapy, the prognosis is very poor. Several researchers have reported that cytokines such as interleukin-6 (IL-6) play an important role in the growth of MPM. Previously we reported that all-*trans*-retinoic acid (ATRA) inhibited the production and function of interleukin-6 (IL-6) and transforming growth factor (TGF)-β_1 in the experiments using lung fibroblasts. We investigated whether ATRA had an inhibitory effect on the cell growth of MPM, the origin of which was mesenchymal cells similar to lung fibroblasts, using a subcutaneous xenograft mouse model. We estimated the tumor growth and performed quantitative measurements of IL-6, TGF-β_1 and platelet-derived growth factor (PDGF) receptor beta (PDGFR-β) mRNA levels both of cultured MPM cells and grown cells in mice with or without the administration of ATRA. ATRA significantly inhibited MPM tumor growth. In vitro studies disclosed that the administration of ATRA reduced 1) mRNA levels of TGF-β_1, TGF-β_1 receptors, and PDGFR-β, and 2) TGF-β_1-dependent proliferation and PDGF-BB-dependent migration of MPM cells.
These data may provide a rationale to explore the clinical use of ATRA for the treatment for MPM.

P04-3

Novel mechanism implicated in asbestos-induced malignant mesothelioma

Li Jiang, Shinya Akatsuka, Shinya Toyokuni

Department of Pathology and Biological Responses, Nagoya University, Japan

Exposure to asbestos fibers is considered a major factor contributing to the development of most malignant mesotheliomas (MM). We highlighted the role of asbestos in MM and summarized cytogenetic and molecular genetic findings in this malignancy. A rat model of malignant mesothelioma was built using injection of three types of asbestos fibers (UICC) including chrysotile, crocidolite and amosite with or without nitrilotriacetate (NTA). We found NTA enhanced the carcinogenicity of asbestos-induced mesothelioma especially chrysotile. Array-based comparative genomic hybridization analyses (CGH) results showed p16/CDKN2A homozygous deletion (Chrysotile, 89%; Crocidolit, 89%; Amosite, 100%) mapping to 5q32 and many high-copy amplified genes locating wide region of chromosome 7 in MM. Gene expression microarray suggested the expression of ctgf gene is significantly different between EM (epithelioid mesothelioma) and SM (sarcomatoid mesothelioma). And mRNA levels were increased 2~8-fold in EM but increased 16~180-fold in SM. As we have known that ctgf is associated with TGF-β signal pathway, we try to find the important role of ctgf in asbestos-induced malignant mesothelioma.

P04-4

Epigenetic inactivation of tumour suppressor genes by DNA methylation in malignant mesothelioma

Yuen Yee Cheng[1], Hong Chuan Jin[2], Francis Chan[3], Lyn Schedlich[1], Michaela Kirschner[1], Nico van Zandwijk[1], Glen Reid[1]

[1]Asbestos Diseases Research Institute, University of Sydney, Australia, [2]Biomedical Research Center Sir Runrun Shaw Hospital Zhejiang University, China, [3]Department of Medicine and Therapeutics, Prince of Wales Hospital, The Chinese University of Hong Kong, China

Epigenetic inactivation of tumour suppressor genes plays a crucial role in the progression of cancers including malignant mesothelioma (MM). Inappropriate silencing of critical genes can result in the inactivation of tumour suppressed genes (TSGs). Reactivation of affected TSGs has therapeutic potential in mesothelioma. DNA hypermethylation is the most well recognized epigenetic change in regulating gene expression. Recent cumulative studies of aberrant DNA methylation in human cancer showed high rates of aberrant promoter methylation in a subset of cancers, termed the CpG island methylator phenotype, which may also contribute to MM formation. However, there is currently limited information available regarding the DNA methylation status in MM. In this study, we aimed to investigate the relationship between gene silencing and DNA methylation in MM. We first looked at the changes in gene expression of five know TSGs (SFRP2, FBP1, Zic1, SLC19A3 and PCDH10) in established and primary MM cell lines upon treatment with the demethylating agent 5'Azacitidine. The mRNA expression level of SFRP2, FBP1, Zic1 and SLC19A3 were studied using conventional RT-PCR, and DNA methylation analysis was examined using COBRA, methylation specific PCR (MSP) and bisulfite sequencing. Our preliminary results indicated that these genes are reactivated by DNA demethylation, suggesting that epigenetic inactivation of TSGs could be a common event in MM which may play a pivotal role on MM development.

P04-5

Genomic profile of human malignant pleural mesothelioma: A CGH-array comparison between primary tumors and cells in culture

Didier Jean[1], Maria Paciencia[2,3], Emilie Thomas[4], Marie Brevet[2,3], Annie Renier[1], Martin Figeac[5], Aurélien de Reynies[4], Véronique Abonnet[2,3], Françoise Le Pimpec-Barthes[1,6], Nolwenn Le Stang[3], Jessica Zucman-Rossi[1], Marie-Claude Jaurand[1], Françoise Galateau-Sallé[2]

[1]INSERM UMR 674, "Functional Genomics of Solid Tumors", IUH, Université Paris Descartes, France, [2]MESOPATH Center, Centre Hospitalier Universitaire, Caen, France, [3]MESONAT Registry, Centre Hospitalier Universitaire, Caen, France, [4]Ligue Nationale Contre le Cancer, Paris, France, [5]Platform of Genomic and Sequencing, IFR114, Institute of Cancer Research, Lille, France, [6]Service de Chirurgie Thoracique, Hôpital Européen Georges-Pompidou, Assistance Publique-Hôpitaux de Paris, Université Paris Descartes, Paris, France

Malignant pleural mesothelioma (MPM) cells in culture are routinely used to study mechanisms of oncogenesis and to identify molecular targets for diagnosis, prognosis and treatment. However, this procedure could select subpopulations from the primary tumor, and genetic evolutions may be observed when tumor cells are maintained in culture. Large-scale genomic studies are needed to evaluate the relevance to use MPM cultured cells.
In order to compare genomic regions and genes altered between MPM primary tumors and cultured cells, we performed comparative genomic hybridization (CGH) array. Analyses were carried out using genomic DNA samples extracted from 42 MPM primary tumors and 35 MPM cells established in our laboratory and cultured for less than 10 passages.
Our results confirmed that MPMs are characterized by a complex pattern of genetic changes where genomic regions copy number losses are more frequent than gains. The most frequent loss in both MPM cultured cells and primary tumors involved the 9p21 region encoding *CDKN2A* and *CDKN2B* genes. Other common genomic deletions included major portions of chromosome 22 and regions surrounding 3p21 and 14q11.2. Higher frequency of alterations was observed with MPM cultured cells than primary tumors probably due to contamination of tumor specimens with normal tissue.
Some chromosomal allelic imbalances were solely observed in MPM cultured cells while others solely in primary tumors, as alterations in the 6p22 region. However, review of literature studies using CGH, CGH array or representational oligonucleotide microarray (ROMA) revealed that the main recurrent regions of chromosomal alterations we identified in MPM cultured cells were previously described in primary tumors. It seems that differences observed between primary tumors and cultured cells are more likely due to the high heterogeneity of MPM than to a culture artifact.

P05

Oncogenesis-Ⅲ

P05-1

Frequent deletions in 3p21.1 region in malignant mesothelioma cell lines established from Japanese patients

Yoshie Yoshikawa[1], Takashi Nakano[2], Ayuko Sato[3], Kazuya Fukuoka[2], Aki Murakami[2], Shusai Yamada[2], Kozo Kuribayashi[2], Chiharu Tabata[2], Yoshihiro Fujimori[4], Tomonori Morinaga[1], Tohru Tsujimura[3], Tomoko Hashimoto-Tamaoki[1]

[1]Department of Genetics, Hyogo College of Medicine, Japan, [2]Division of Respiratory Medicine, Department of Internal Medicine, Hyogo College of Medicine, Hyogo, Japan, [3]Division of Molecular Pathology, Department of Pathology, Hyogo College of Medicine, Hyogo, Japan, [4]Cancer Center, Hyogo College of Medicine, Hyogo, Japan

Frequent deletions in 9p21 and 22q regions have been reported in malignant mesothelioma (MM). The 3p21 region has also been reported one of the hot spots in homozygous or heterozygous deletions associated with MM, but the genes involved have not been identified. We established 15 MM cell lines from Japanese patients living in Kansai area, and performed array CGH. All cell lines showed homozygous deletions in the 9p21 region. The minimum common region of the deletion was 36 kb, which carries the CDKN2A/p16 and CDKN2B/p15 genes. Homozygous or heterozygous deletions in the 22q12.2 region carrying NF2 gene were also detected in 80% of cell lines. We also found deletions in 3p21.1 in half of them. Among several genes located in this region, we focused on the BAP1 (BRCA1-associated protein 1) gene which functions as a tumor suppressor gene interacting with the RING finger domain of BRCA1. Then we performed copy number analysis of this gene by real-time PCR with 20 MM cell lines. We detected homozygous or heterozygous deletions of this gene in 10 lines; three of them showed homodeletions and seven showed heterozygous deletions. Homodeletions of BAP1, as well as CDKN2A/p16, were also detected in primary tissue specimens and MM cells collected from malignant pleural effusion. The functional significance of deletions in the BAP1 gene in tumor progression is not clear at present. However, deletions in the BAP1 gene, in addition to those in the CDKN2A/p16 and NF2 genes, might be useful for MM diagnosis.

P05-2

Knockdown of ZEB1, a master epithelial mesenchymal transition (EMT) inducing gene, suppresses growth of pleural mesothelioma cell lines

Mihoko Horio[1], Mitsuo Sato[2], Yoshihiro Takeyama[2], Tetsunari Hase[2], Kenya Yoshida[2], Yoshitaka Sekido[3,4], Masashi Kondo[2], Yoshinori Hasegawa[2]

[1]Aichi Cardiovascular and Respiratory Center, Japan, [2]Departments of Respiratory Medicine , Nagoya Graduate University of Medicine, [3]Departments of Cancer Genetics, Nagoya Graduate University of Medicine, [4]Division of Molecular Oncology, Aichi Cancer Center Research Institute, Japan

Background: Epithelial to mesenchymal transition (EMT) causes human epithelial cancers to invade surrounding tissues and ultimately metastasize to distant sites. Very little is known about whether EMT also contributes to the pathogenesis of non-epithelial tumors including malignant pleural mesothelioma (MPM). Thus, we studied the role of ZEB1, one of the major regulators of EMT in epithelial cancers, in MPM. To this end, we examined the effect of ZEB1 knockdown on malignant phenotypes of MPM cell lines.Methods: 18 human MPM cell lines and one non-tumorigenic mesothelial cell line were used. Quantitative real-time RT-PCR and western blot of ZEB1, E-cadherin, and Vimentin were done. Transient ZEB1 knockdown was done by using three non-overlapping short interfering RNA oligos targeting ZEB1. Stable ZEB1 knockdown was done by using a retroviral vector expressing ZEB1 shRNA. Cell proliferation was measured by WST-1 and clonogenic growth was measured by liquid and soft agar colony formation assays. Fluorescence-activated cell sorting with propidium iodide staining was done to examine apoptosis and cell cycle. Results: The majority of MPM cells expressed higher levels of ZEB1 than a non-tumorigenic mesothelial cell. We performed ZEB1 knockdown experiments in two MPM cells, ACC-MESO-1(MESO-1) and H2052, which express high and moderate levels of ZEB1, respectively. Transient knockdown of ZEB1 resulted in increased expression of E-cadherin protein in MESO-1 cells but not in H2052 cells. Transient ZEB1 knockdown suppressed cell proliferation and liquid colony formation in the two MPM cell lines. Importantly, the ZEB1 knockdown dramatically suppressed soft agar colony formation in the two MPM lines. We did not see apoptosis or cell cycle arrest in either of the two lines. Stable knockdown of ZEB1 induced morphologic changes suggestive of mesenchymal-to-epithelial transition in MESO-1 cells but not in H2052 cells. Conclusion: These results suggest that ZEB1 serves as an attractive therapeutic target for MPM.

P05-3

Association of asbestos exposure and cigarette smoking with gene abnormalities in lung adenocarcinomas in Japan

Hironori Ninomiya[1], Ayako Tanaka[2], Norihiko Kohyama[3], Yuichi Ishikawa[1]

[1]Division of Pathology, The Cancer Institute, Japanese Foundation for Cancer Reseach, Japan, [2]St. Marianna University School of Medicine, [3]School of Economics, Toyo University, Japan

Asbestos, as well as tobacco, is known as a lung carcinogen and also a potent occupational and environmental toxin. Epidemiological studies have revealed asbestos and tobacco exerted a synergistic effect on causing lung cancer when both were combined together. However, the independent and cooperative biological effects of the two agents on lung remain uncertain. MATERIAL AND METHOD: To assess the cooperative effects of asbestos burden and smoking exposure on human lung adenocarcinoma, we examined LOH frequency presented by FAL (fractional allelic loss) values and p53 mutation status in 142 lung adenocarcinomas. Asbestos burden (AB, asbestos body number per unit 1 g dry lung tissue) was obtained using paraffin blocks of normal lung tissue by microscopic counting asbestos bodies after low-temperature incineration. All cases were classified into 9 groups according to smoking index (SI, a product of number of cigarettes a day and duration in years) (zero, from 1 to 499 and 500 or more) and asbestos burden (AB) (zero, from 1 to 999 and 1000 or more).RESULS (1) The p53 mutation ratio increased along with the elevation of AB and/or SI, and it was the lowest (21%) in the AB=SI=0 group. (2) LOH frequency was related to smoking rather than asbestos exposure. (3) hotspots mutations of p53 were observed among smokers, whereas no-specific mutations were detected in the groups exposed solely to asbestos.CONCLUSIONS Combined effects of smoking and asbestos exposure were confirmed by LOH and p53 mutation analyses. (1) The incidence of p53 mutation increased in relation of both AB and smoking. (2) Asbestos exposure did not increase LOH frequency by itself but did non-specific p53 mutations. To sum up, asbestos enhances genomic changes only together with smoking.

P05-4

Activated leukocyte cell adhesion molecule is involved in motility and invasion of malignant pleural mesothelioma

Futoshi Ishiguro[1], Hideki Murakami[1], Tetsuya Mizuno[1], Makiko Fujii[1], Yutaka Kondo[1], Noriyasu Usami[2], Kohei Yokoi[2], Hirotaka Osada[1], Yoshitaka Sekido[1]

[1]Division of Molecular Oncology, Aichi Cancer Center Reserch Institute, Japan, [2]Department of Cardiothoracic Surgery, Nagoya University Graduate School of Medicine

Activated leukocyte cell adhesion molecule (ALCAM) is a member of the immunoglobulin superfamily with five extracellular immunoglobulin-like molecules (D1-D5). ALCAM promotes homophilic (ALCAM-ALCAM) interactions, and its aberrant expression has been reported in various types of cancers including melanoma and breast cancer. However, the role of ALCAM in malignant pleural mesothelioma (MPM) remains unclear. We examined the expression status of ALCAM in 20 mesothelioma cell lines. Using quantitative real-time reverse transcriptase-PCR, all 20 cell lines were shown to express more than 4.5-fold ALCAM mRNA when the expression level of MeT-5A was arbitrarily set as 1.0. Elevated ALCAM protein was confirmed in the 20 cell lines with western blot analysis. We also detected positive staining of ALCAM in 16/22 (72.7%) MPM cases with immunohistochemistry.Since ALCAM has been reported to be involved in the promotion of tumor cell motility, we investigated whether or not inhibition of ALCAM suppresses any malignant phenotypes of MPM cells. ALCAM knockdown with RNA interference suppressed cell migration and invasion of all three mesothelioma cell lines of H290, Y-MESO-14 and Y-MESO-27 tested in vitro. These ALCAM knockdown cells also showed impaired anchorage-independent cell growth. A soluble isoform of ALCAM (sALCAM) consisting only of the membrane-distal domain D1 was recently isolated as an alternative, shortened transcript. This secreted splicing variant was shown to have an inhibitory effect on ALCAM-ALCAM homophilic interaction, and its impaired migratory capacity of normal endothelial cells or melanoma has been described recently. To determine whether this inhibitory effect can also be observed in MPM cells, we constructed expression constructs of the secreted variants and are currently investigating their effects on mesothelioma cells. Our study suggests that heightened expression of ALCAM contributes to tumor progression in MPM and that ALCAM might be a potential target of anti-cancer therapy.

P05-5

Identification of tumor initiating cells in malignant pleural mesothelioma

Claudia Frei[1], Isabelle Opitz[2], Bruno Fischer[1], Ubiratan Moura[1], Alex Soltermann[3], Walter Weder[2], Rolf Stahel[1], Emanuela Felley-Bosco[1]

[1]Laboratory of Molecular Oncology, Zurich University Hospital, Switzerland, [2]Thoracic Surgery, Zurich University Hospital, [3]Institute for Clinical Pathology, Zurich University Hospital

We hypothesize that a tumor initiating cell (TIC) population is present in malignant pleural mesothelioma (MPM). The aim of our study was to investigate whether the side population (SP) phenotype was associated with an increased tumorigenic potential and a higher chemoresistance. The SP phenotype is due to drug efflux by ABC transporters activity. By FACS analysis we identified the ABCG2 transporter on about 5 % of MPM ZL55 cells and on a human MPM xenograft cell culture. The SP assay resulted in the identification of SP cells with self-renewal capacity in both models. SP cells had an increased expression of the drug transporter ABCG2 which is apparently responsible for the SP phenotype since the ABCG2 specific inhibitor FTC could abolish this phenotype. Furthermore ZL55 SP cells had a decreased expression of differentiation markers mesothelin and N-cadherin and an increased expression of the stem cell maintenance gene Sox2. In the absence of known cell surface markers of mesothelium progenitor cells we tested mesenchymal stem cell (MSC) markers in both models and found that SP cells were $CD105^{low}$.
After implantation of sorted cells under the renal capsula of NOD/SCID mice "bona fide " mesothelin and N-cadherin expressing MPM tumors grew from both ZL55 SP and non-side population (NSP) cells. However, we identified a 5% sarcomatoid histotype in the SP-T xenografts compared to a 100% epithelioid histotype in ZL55 NSP-Ts. The sarcomatoid histotype of ZL55 SP-Ts was accompanied by an increased expression of stem cell Sox2 and coelomic mesoderm podoplanin markers, and by an important accumulation of HLA negative stromal cells compared to NSP-Ts. In addition, ZL55 SP-T cells were more chemoresistant and were able to regenerate tumors compared to ZL55 NSP-T cells. Similar results were observed in the human MPM xenograft culture.
Taken together these results indicate that TICs are present in MPM.

P06

Asbestos

P06-1

Analysis of early lesions in rats after intraperitoneal administration of nanofibers

Shuichi Adachi[1], Miho Mizoi[1], Sei-ichi Omura[2], Takahiro Kobayashi[2], Okio Hino[3]

[1]Dept. Pub. Health, Grad.Sch.Nutr.Sci., Sagami Women's Univ., Japan, [2]Integrated Research Inst., Tokyo Inst. Tech., Kanagawa, Japan, [3]Dept. Mol. Pathogenesis, Grad.Sch.Med., Juntendo Univ., Tokyo, Japan

Nanomaterials are the most important new materials in various field of usage. Regarding the necessity of hazard identifications for nanomaterials, we have examined the lesions as an early sign of carcinogenic process in experimental animal after i.p. administration of nanomaterials. Multi-wall carbon nanotube (MWCNT), three different dimensional features of TiO2 (P:spheric particle, F100:short fiber, F400:long fiber), chrysotile asbestos (Chr), two types of crystalline whiskers (potassium titanate and silicon carbide) and vehicle (1% Tween 80 in saline) as control (V). At 1, 3, 5 days, 1, 2, 4, 10, 20 weeks after i.p. administration, histological lesions and plasma N-ERC level were examined. No significant change was observed in P and V groups without the coagulations of TiO2 particle on the surface of liver tissue. In contrast, 3 fibrous materials (F100, F400 and Chr) induced obvious inflammatory lesions between liver and diaphragm at 1 day and progressed to adhesion at 5days point. These lesions were the severest in Chr group and comparatively moderate in F400 and F100 groups. Plasma N-ERC levels in F400 were continuously high in Chr and whiskers, however, it gradually decreased to control level in fibrous TiO2 and MWCNT. These results suggest that plasma N-ERC is a possible indicator to evaluate the potency of mesothelioma inducible activity for fibrous nanomaterials.

P06-2

Asbestos body analysis in patients with malignant pleural mesothelioma who underwent extrapleural pneumonectomy

Kazunori Okabe, Eisuke Matsuda, Hiroyuki Tao, Hiromasa Yamamoto, Seiki Kobayashi, Kazuro Sugi

Division of Thoracic Surgery, National Hospital Organization, Yamaguchi Ube Medical Center, Japan

[Background] Malignant pleural mesothelioma (MPM) has been recognized as related to asbestos inhalation. However, the mechanism by which asbestos causes MPM is still unclear. The aim of this study was to analyze asbestos bodies (AB) in the lungs of patients with MPM who underwent extrapleural pneumonectomy (EPP).
[Methods] Twenty consecutive MPM patients who underwent EPP from June 2006 to February 2010 were reviewed. AB quantification involved the digestion of 1-4 grams of lung tissue in bleach employing a modified Smith and Naylor method[1)]. Scanning electron microscopic analysis was reported by us previously[2)].
[Results] The median AB number was 6,168/g dry lung (lower than the detection limit ~ 443,571). An insulator factory worker and a plumber had very high AB numbers (443,571 and 319,989). The proportion of AB in the insulator factory worker was 95% amosite and 5% crocidolite. The AB numbers in four of the twenty patients were equal to those in the general population. A school teacher is classified as a job which involves a risk of asbestos exposure. However, the AB numbers of three school teachers were low (2,030, 711, and lower than the detection limit). Two MPM cases were judged to have been caused by environmental asbestos exposure in Amagasaki City. Their AB numbers were 5,127 and 4,027. The proportion of AB in the latter patient was 85% crocidolite, 12% amosite, and 2% others. Two railroad car makers in the same factory had similar AB numbers: 19,916 and 16,556. The proportion of AB in the latter patient was 90% crocidolite and 10% amosite.
[Conclusions] The analysis of AB in the lungs of patients with MPM is useful to elucidate the carcinogenetic nature of asbestos.
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P06-3

Trends in asbestos and nonasbestos fiber concentrations in the lung tissues of Japanese patients with mesothelioma

Kiyoshi Sakai[1], Naomi Hisanaga[2], Eiji Shibata[3], Michihiro Kamijima[4], Gaku Ichihara[5], Yasuhiro Takeuchi[5], Tamie Nakajima[5]

[1]Nagoya City Public Health Research Institute, Japan, [2] the Center for Health Care, Aichi University of Education, Kariya, Japan, [3]the Department of Health and Psychosocial Medicine, Aichi Medical University School of Medicine, Nagakute, Japan, [4]the Department of Occupational and Environmental Health, Nagoya City University Graduate School of Medical Sciences, Nagoya, Japan, [5]the Department of Occupational and Environmental Health, Nagoya University Graduate School of Medicine, Nagoya, Japan

Objective: The change in both asbestos and nonasbestos fiber concentrations in the lungs that occur over time and the cause of these changes were investigated in Japanese mesothelioma patients.Methods: Lung tissues were obtained from 52 mesothelioma patients who had undergone surgery or an autopsy between 1971 and 2009. The patients had a history of occupational asbestos exposure and were classified by decade, from the 1970s to the 2000s. The asbestos and nonasbestos fiber concentrations were determined by transmission electron microscopy with energy-dispersive X-ray analysis using a low-temperature ashing procedure.Results: The geometric mean of asbestos concentration markedly decreased from the 1970s (73.2 million fibers/g dry lung) to the 2000s (1.23 million fibers/g dry lung). The mean duration of asbestos exposure decreased from the 1970s (33.7 years) to the 2000s (17.1 years), and the mean of the duration elapsed since the last asbestos exposure increased from the 1970s (0.3 years) to the 2000s (20.9 years). Although there was no significant correlation between the asbestos concentrations in the lungs and the duration of asbestos exposure, the asbestos concentration in the lungs was significantly inversely correlated with the duration elapsed since the last asbestos exposure ($p<0.01$). These relations were also found for nonasbestos fibers. Conclusion: The asbestos and nonasbestos fiber concentrations in lung tissues of the mesothelioma patients decreased from the 1970s to the 2000s. This was supposed to be resulted from a reduction in asbestos exposure in both occupational and living environments and increased elimination or disappearance of asbestos and nonasbestos fibers from the lung.

P06-4

Asbestos fiber concentration in lung tissues resected for malignant pleural mesothelioma

Noriyasu Usami[1], Kiyoshi Sakai[2], Koichi Fukumoto[1], Haruko Suzuki[1], Takehiko Okagawa[1], Koji Kawaguchi[1], Toshiki Okasaka[1], Kohei Yokoi[1]

[1]Division of Thoracic Surgery, Nagoya University Graduate School of Medicine, Japan, [2]Nagoya City Public Health Research Institute, Japan

Background; Although the association between asbestos exposure and the development of malignant pleural mesothelioma (MPM) is well recognized, the relationship between asbestos fiber concentrations by fiber types and the development for MPM remains unclear. **Methods;** Asbestos fiber concentrations in the lung tissues of 8 patients who underwent a surgical resection for MPM were analyzed by transmission electron microscopy with energy-dispersive X-ray analysis using a low-temperature ashing procedure. The geometric mean concentrations of crysotile and amphibole asbestos in the control subjects without asbestos exposure were used as reference, which were 0.5 and 1.0 (x 10^6 fibers / g dry lung), respectively. (Reference: Sakai K et al. Asbestos Concentration and Fiber Size in Lungs of the Urban Residents. Japanese Journal of Public Health, 1991; 38: 762-770) **Results;** Patients consisted of 6 men and 2 women with a median age of 58 years. All male patients had a history of asbestos exposure, but 2 female patients had none. All patients underwent an extrapleural pneumonectomy. Histological subtypes were epithelioid in 5 patients and biphasic in 3. Ratios of the MPM cases to the control of crysotile concentration ranged from 0.6 to 12.4 in all men and 0.1 to 0.4 in two women. Those of amphibole asbestos ranged from 0.28 to 19.9 in all men and 0.02 to 0.17 in two women. **Conclusions;** Two women who had no history of asbestos exposure showed lower concentrations of crysotile and amphibole asbestos than the control subject. On the other hand, most patients with a history of asbestos exposure had higher concentrations of crysotile and amphibole asbestos than the control subject, but even in some patients with a history of asbestos exposure the low concentrations were observed. To elucidate the relation between the pulmonary asbestos fiber concentrations by fiber types and development of MPM, further investigations are necessary.

P07

Immunology

P07-1

Human MT-2 cell line displays enhanced suppressive function by chronic exposure to asbestos

Megumi Maeda[1], Yasumitsu Nishimura[1], Naoko Kumagai[1], Hiroaki Hayashi[1], Yoshie Miura[2], Takemi Otsuki[1]

[1]Department of Hygiene, Kawasaki Medical School, Japan, [2]Department of Molecular Genetics, Medical Institute of Bioregulation, Kyushu University, Fukuoka, Japan

Chronic exposure to asbestos results in malignant mesothelioma. As this disease has a long incubation period, $CD4^+CD25^+$ regulatory T (Treg) cells-mediated anti-tumor immune response may be impaired. To reveal whether Treg cells exposed to asbestos attenuate an anti-tumor immunity in patients with malignant mesothelioma, six chrysotile-induced apoptosis-resistant sublines (MT-2Rst; CA1, CA2, CA3, CB1, CB2, CB3) were established by long-term (more than 8 months) and low-level (10 μ g/ml) exposure to chrysotile-A (CA) or -B (CB) from the HTLV-1-immortalized human T-cell line MT-2 (MT-2Org) having a Treg-like suppressive function. The results of flow cytometry showed that MT-2Org and MT-2Rsts cells expressed high levels of two markers for Treg, Foxp3 and GITR. The regulatory function of these cells was determined in an allogeneic MLR co-culture using [^{3}H]-Thymidine incorporation. Five MT-2Rst cell lines suppressed proliferation of autologous $CD4^+CD25^-$ responder T-cells (Tresp) upon stimulation with irradiated allogeneic peripheral blood mononuclear cells (PBMCs) more strongly than MT-2Org. The mechanisms of the suppressive function were clarified in MT-2Rst-CB1 using a CFSE assay. When Tresp were stimulated not with coated anti-CD3 antibody and soluble anti-CD28 antibody, but with coated anti-CD3 antibody and autologous induced-dendritic cells, the proliferation was suppressed by MT-2Rst-CB1 more strongly than MT-2Org. Furthermore, shRNA knockdown showed that the immunosuppressive cytokines IL-10 and TGF-β1 produced from MT-2Rst-CB1 partially mediated suppression in the proliferation of Tresp using Transwell. In conclusion, MT-2Rst-CB1 enhanced the suppressive function on effector T-cell proliferation through cell contact-dependent mechanisms and soluble factors. These findings suggested that chronic exposure to asbestos enhance suppressive activity of Treg cells and lead to a decrease in anti-tumor immune function.

P07-2

The effects of continuous long-term exposure to asbestos, chrysotile and crocidolite on HTLV-1 immortalized human T cell line, MT-2

Takemi Otsuki[1], Megumi Maeda[1], Yoshie Miura[2], Naoko Kumagai[1], Hiroaki Hayashi[1,3], Shoko Yamamoto[1], Yasumitsu Nishimura[1]

[1]Department of Hygiene, Kawasaki Medical School, Japan, [2]Department of Molecular Genetics, Medical Institute of Bioregulation, Kyushu University, Fukuoka, Japan, [3]Department of Dermatology, Kawasaki Medical School, Kurashiki, Japan

As we know silica influence human immune system and dysregulate autoimmunity, Asbestos, which is the mineral silicate, may influence the biocellular characteristics of human immune-competent cells. Whether or not continuous long-term exposure to asbestos, chrysotile and crocidolite, on HTLV-1 immortalized human T cell line, MT-2, alter its cellular characteristics was investigated. Transient and high-dose exposure to chrysotile or crocidolite induced apoptosis of MT-2 cells through the phosphorylation of proapoptotic p38 and JNK signaling molecules, activation of mitochondrial apoptotic pathway, and production of ROS. Then, continuous and low-dose exposure to chrysotile or crocidolite was started. Chrysotile is less carcinogenic although crocidolite is well-known as strong carcinogen because of its higher contents of iron to induce DNA damage. Eight months later, continuous and low-dose exposed sub-lines to chrysotile and crocidolite had revealed asbestos-induced apoptosis resistance and designated as MT-2CB (exposed to chrysotile) and MT-2CR (to crocidolite). To compare the effects of chrysotile and crocidolite, on human immunocompetent cells may be important, since less carcinogenicity and more usage of chrysotile compared with less usage and more carcinogenicity of crocidolite. The molecular mechanisms of acquisition of resistance o asbestos-induced apoptosis were similar. There are the activation of STAT3 caused by higher secretion and autocrine usage of IL-10 caused by activation of Src-family kinase and upregulation of anti-apoptotic Bcl-2 protein located down-stream f STAT3. Although cDNA array analysis showed differences of altered genes between two sublines, array CGH revealed similar pattern of loss and gain of chromosome. The alteration of cellular features of immunocompetent cells caused by continuous exposure to asbestos may influence the reduction of tumor immunity.

P07-3

Suppressive effect of asbestos-exposure on the differentiation of human cytotoxic T lymphocytes, accompanied with decreases in IFN-γ and TNF-α

Naoko Kumagai[1], Yasumitsu Nishimura[1], Megumi Maeda[1], Hiroaki Hayashi[1], Takumi Kishimoto[2], Takemi Otsuki[1]

[1]Department of Hygiene, Kawasaki Medical School, Japan, [2]Okayama Rosai Hospital, Japan

[Background and Purpose] Asbestos fibers have tumorigenicity, which is thought to cause mesothelioma. However, in contrast, its effect on anti-tumor immunity remains unclear. Therefore, the present study investigated effect of asbestos on differentiation from näive $CD8^+$ T cells into CTL and functional properties of $CD8^+$ cells from asbestos-exposed people with pleural plaque (PL).
[Materials and Methods] CTL were induced by allogenic mixed lymphocyte reactions (MLR). PBMCs from healthy volunteer (HV) were cultured with irradiated allogenic PBMCs with chrysotile B (CB) or crocidolite (CR) asbestos at 5μg/ml. After 7 days, cellularity, proliferation, apoptosis, cytotoxicity, intracellular levels of granzyme B (GB), perforin and IFN-γ of $CD8^+$ T cells and productions of cytokines in supernatants were assayed by flow cytometry. PBMCs from HV and PL were assayed for some of the parameters mentioned above, before or after stimulation with PMA and ionomycin.
[Results] CB suppressed the increase in cell-number of $CD8^+$ T cells during MLR, where allogenic cytotoxicity decreased, in contrast to no effect of CR. They showed decreases in GB^+ cells, IFN-γ^+ cells, $CD45RO^+$ effector/memory cells and $CD25^+$ activated cells in $CD8^+$ cells and an increase in $CD45RA^+$ cells, where the proliferated $CD8^+$ cells were few, but apoptotic those did not increase. The productions of IL-10, IFN-γ and TNF-α but not IL-2, decreased upon CB-exposure. $CD8^+$ T cells in PL-group unexpectedly showed higher levels of GB and perforin after stimulation compared with HV, although the percentage of IFN-γ^+ cells was normal.
[Discussion] These results indicate that CB has a potential to suppress induction of CTL with decreases in IFN-γ and TNF-α, but not related with suppressive effect of IL-10 or toxicity of CB for $CD8^+$ T cells. The results from specimens paradoxically suggest the possibility that PL-positive people might be protected from tumor by increased cytotoxic potential of $CD8^+$ T cells.

P07-4

Pleural mesothelioma instigates tumor associated fibroblasts to promote progression via malignant cytokine network

Qi Li[1], Wei Wang[1], Tadaaki Yamada[1], Yasuhiko Nishioka[2], Saburo Sone[2], Seiji Yano[1]

[1]Division of Medical Oncology, Cancer Research Institute, Kanazawa University. Japan, [2]Department of Respiratory Medicine and Rheumatology, Institute of Health Biosciences, University of Tokushima Graduate School, Tokushima, Tokushima, Japan

Background: Regardless of the recent advance of chemotherapy combining cisplatin and pemetrexed, the prognosis of malignant pleural mesothelioma (MPM) is extremely poor with median survival varying between 8 and 14 months. Tumor microenvironments are thought to play crucial roles in progression and chemosensitivity of various malignant diseases. We recently developed orthotopic implantation model of MPM in SCID mice, and observed that α-SMA positive fibroblast-like cells intensively accumulated into the tumors produced by human MPM cells, MSTO-211H. We have therefore assessed the role of interaction between MPM cells and microenvironments focusing on tumor-associated fibroblasts (TAF). Methods: We measured various cytokine productions in MSTO-211H cells and fibroblast cell line, MRC-5. The interaction between MPM and fibroblasts was analyzed in co-culture system in vitro and orthotopic SCID mouse model in vivo. Immunohistochemistry of various cytokines were performed in tumor specimens from fifty-one MPM patients to assess clinical relevance.Results: MSTO-211H cells highly produced FGF-2 and PDGF-AA that enhanced proliferation, migration, and HGF production of MRC-5 cells. HGF derived from MRC-5 cells stimulated proliferation and migration of MSTO-211H cells. MRC-5 cells promoted progression of MSTO-211H cells, which can be inhibited by TSU-68 (inhibitor of FGFR, VEGFR, and PDGFR) or NK4 (HGF antagonist). In vivo, TSU-68, NK4 or Imatinib (PDGFR inhibitor) effectively inhibited the growth of thoracic tumors of MSTO-211H cells, associated suppression of fibroblast infiltration into the tumors. Co-existence of HGF with FGF-2 and/or PDGF-AA was frequently detected in tumor tissues from MPM patients. Conclusion: These findings indicate that MPM instigates TAF to promote progression via malignant cytokine network, and that regulation of this cytokine network may be therapeutically useful for controlling progression of MPM.

P07-5

Decrease in NKp46 on NK cells upon exposure to asbestos, a possible marker to monitor anti-tumor immunity

Yasumitsu Nishimura[1], Naoko Kumagai[1], Megumi Maeda[1], Hiroaki Hayashi[1], Takumi Kishimoto[2], Takemi Otsuki[1]

[1]Department of Hygiene, Kawasaki Medical School, Japan, [2]Okayama Rosai Hospital, Okayama, Japan

Recently, we reported that peripheral blood NK cells in patients with malignant mesothelioma (MM) showed low cytotoxicity with decrease in NKp46, one of receptors utilized to recognize targets and transduce activation signal, which also decreased in NK cells included in PBMCs cultured with chrysotile B (CB) asbestos. Therefore, we explored the mechanism of decrease in NKp46 on NK cells upon CB-exposure, and examined NK cells in people positive for pleural plaque (PL) as well as MM-patients, compared with healthy volunteers (HV). NK cells isolated from PBMCs also showed a decrease in NKp46 when cultured with CB, where the decrease was smaller than that NK cells in PBMCs cultured with CB showed. The NK cells showed an increase in NKp46 when co-cultured with PBMCs without cell-cell contact using culture insert, and they showed a decrease in NKp46 when CB was added into the culture area of PBMCs. The analysis for cytokines showed low productions of TNF-α, IFN-γ and IL-12p70 in the culture of PBMCs with CB. These results indicate that the decrease in NKp46 on NK cells was caused indirectly by altered cytokine profiles in immune cells upon exposure to asbestos as well as by the direct effect of asbestos-exposure. Unlike MM-patients, NK cells of PL-positive people showed widely distributed expression of NKp46. However, the half of PL-positive people with low expression of NKp46 showed lower cytotoxicity than the other half. The statistical analysis of these whole data showed a significant correlation between cytotoxicity and expression level of NKp46. Based on these, the scores of 1, 2 and 3 were assigned to NKp46-high PL-, NKp46-low PL-groups and MM-group, respectively. This score was inversely correlated with cytotoxicity of NK cells. These results suggest the possibility of NKp46 as a marker to monitor anti-tumor immunity in people exposed to asbestos.

P08

Animal models

P08-1

Mesothelioma xenografts developed in the immune deficient mice and the clinical relevance

Ming-Sound Tsao[1], Licun Wu[1,2], Ming Li[1], Masaki Anraku[2], Tetsuzo Tagawa[2], Zhihong Yun[2], Katrina Rey-McIntyre Rey-McIntyre[2], Michael Johnston[2,3], Marc de Perrot[2]

[1]Ontario Cancer Institute/Princess Margaret Hospital, University Health Network, University of Toronto, Canada, [2]Latner Thoracic Surgery Research Laboratories and Division of Thoracic Surgery, Toronto General Hospital, Toronto, [3]Beatrice Hunter Cancer Research Institute, Dalhousie University, Halifax, Nova Scotia, Canada

We hypothesize that primary xenografts established directly from biopsies or resected malignant pleural mesothelioma (MPM) may provide novel models to study malignant mesothelioma, including the identification of novel genomic markers of this disease and as preclinical models of novel therapeutic and their predictive biomarkers identification. Methods: Fresh tumor tissues harvested from 42 MPM patients treated by extrapleural pneumonectomy (EPP) at the University Health Network were implanted into subcutaneous tissue of non-obese diabetic severe-combined-immunodeficient (NOD/SCID) mice. Xenografts were passaged up to five generation when the tumors have reached 15 mm diameter. At each passage tumor tissues were cryo-preserved, snap-frozen for banking in liquid nitrogen and fixed in formalin for histological examination. Response of models to cisplatin was evaluated. Results: 40.5% (17/42) of patients " tumors developed xenografts and six models have been passaged for five generations. The first generation models required 180±21 (70-344) days to reach 15mm diameter, while growth times for second to fifth generations were 98±14, 94±11, 67±5, and 64 ±6 days, respectively. Despite lower rate of engraftment for epithelioid MPM (12/32 or 34.4%) compared to other MPM types (6/10 or 60%), the difference was not statistically significant ($p=0.26$; Fishers Exact Test). For 41 patients with clinical follow-up information, patients whose MPM formed xenografts had statistically non-significant worse survival [Hazard ratio (HR) 1.65, 95% confidence interval 0.6-4.59, $p=0.33$] than patients whose tumors did not form xenografts. A similar result (HR 1.59, $p=0.45$) was obtained for epithelioid MPM patients only. There was no association between pre-operative chemotherapy and the ability of implanted tumors to establish xenografts (6/14 for >=3 cycles vs 11/28 for <3 cycles chemotherapy). Preliminary evaluation of the models to cisplatin treatment demonstrated growth inhibition activity in 5/7 of xenograft. Conclusion: Primary MPM xenograft model may be a good model to study the biology of MPM.

P08-2

Location matters: biological significance of pleural microenvironment in mesothelioma murine models

Elliot Servais, Christos Colovos, Luis Rodriguez, Marissa C. Velez, Valerie W. Rusch, Michel Sadelain, Prasad S. Adusumilli

Memorial Sloan-Kettering Cancer Center, USA

Introduction: This study aims to determine the importance of the pleural microenvironment in choosing a mesothelioma murine model that best recapitulates human malignant pleural mesothelioma (MPM) and facilitates translational investigations.
Methods: Mice were inoculated with equal number of human MPM cells, stably transduced to express GFP-Luciferase, either via intrapleural (orthotopic), intraperitoneal (pseudoorthotopic), subcutaneous (flank), or intravenous (systemic) route. Mesothelioma tumor pathobiology and locoregional progression were investigated by histology, immunohistochemistry (IHC), and lymphangiogenesis by CD34 and LYVE1 immunofluorescence. In control and treated mice, we assessed the ability of these models to allow noninvasive, accurate tumor progression monitoring by serial quantitative MRI, bioluminescence imaging (BLI), serum biomarker (soluble mesothelin-related peptide - SMRP) level, and survival.
Results: In contrast to intraperitoneal, flank, and systemic murine MPM models, the orthotopic pleural model accurately recapitulated human pathobiology demonstrating chest wall and diaphragmatic invasion and mediastinal lymph node (LN) metastases; retained strong, sustained expression of WT-1, Calretinin and Mesothelin even at late stages of disease; showed extensive lymphangiogenesis characteristic of MPM; facilitated accurate monitoring of tumor burden and progression by BLI, volumetric MRI, and SMRP ($r=0.9$, $p<0.0001$); permitted investigation of isolated thoracic radiation; and experienced worse survival ($p<0.001$) with terminal events mimicking human MPM.
Intraperitoneal mesothelioma showed carcinomatosis with minimal serosal involvement, few identifiable LNs, non-quantifiable tumor burden by MRI or BLI, prohibitive GI toxicity with radiation, and large-volume ascites requiring sacrifice. Flank tumor model showed minimal lymphangiogenesis, no LN metastases, absent SMRP even with large flank tumors, and required sacrificed for tumor bulk without evidence of systemic symptoms. The systemic mesothelioma model was not comparable by any of the above characteristics.
Conclusions: The orthotopic murine model accurately represents human MPM and allows quantitative tumor progression monitoring by noninvasive imaging and serum biomarkers. Our findings highlight the importance of the pleural microenvironment in investigating MPM.

P08-3

Antioxidants are ineffective for prevention of mesothelioma in the MexTAg asbestos-induced mouse mesothelioma model

Cleo Robinson, Anna Nowak, Richard Lake

National Centre for Asbestos Related Diseases, University of Western Australia, Australia

MexTAg transgenic mice universally develop mesothelioma following asbestos instillation. The latency period between exposure and development of disease is proportional to that observed in human mesothelioma in terms of species lifespan. Thus, MexTAg mice are an ideal system in which to investigate cancer prevention strategies. Any effective preventative agent that is identified using MexTAg mice could be readily translated to a clinical trial. An efficacious strategy would have profound implications for the millions of people who have been inadvertently exposed to asbestos either through their occupations or otherwise. Epidemiological studies have indicated that some dietary factors, mainly vitamins and minerals are associated with a lower cancer incidence. Due to the potential pathogenic role of reactive oxygen and nitrogen species that are induced by asbestos fibres we set out to investigate whether dietary supplementation with the antioxidants vitamins A, E and selenium could alter the survival rate of asbestos-induced mesothelioma in MexTAg mice. We found no evidence that these antioxidants would be useful in the prevention of asbestos induced mesothelioma.

P08-4

Microenvironment-dependent mesenchymal-to-epithelial transition in a xenograft model of malignant mesothelioma

Eva Ramqvist[1], Gustav Nilsonne[1], Stefan Imreh[2], Tunde Szatmari[1], Anders Hjerpe[1], Katalin Dobra[1]

[1]Department of Clinical Pathology/Cytology, Karolinska University Hospital, Sweden, [2]Department of Microbiology, Tumor and Cell Biology, Karolinska Institutet, Stockholm, Sweden

In malignant mesothelioma, much as in its benign counterpart, the cells may assume either an epithelioid or a fibroblast-like morphology. In certain systems, it has been shown that mesothelioma cells retain the capacity to transdifferentiate between these two phenotype. Mesothelioma cells of the two different phenotypes exhibit certain striking biological dissimilarities. Clinically, the presence of sarcomatoid cells is a marker for poor prognosis and increased therapy resistance. The acquisition of new malignant traits such as drug resistance and invasivity in cancer cells is linked to the genomic instability of the cells, facilitating new genetic changes. We have previously shown that specific break-points on chromosome 3 correlate to the degree of genomic instability in cancer cells. In this study, we aimed to investigate the role of the microenvironment in mesothelioma differentiation and the underlying genetic changes in a mesothelioma model system in SCID mice, using cell lines derived from the same tumor and induced to differentiate toward epithelioid and sarcomatoid phenotypes.Sarcomatoid cells gave rise to faster-growing tumors in SCID mice and required a shorter latency period before xenograft tumors became detectable.Array comparative genomic hybridization of chromosome 3 showed that the xenografts had a third genotype, similar to each other, resembling the sarcomatoid cell line. Copy number gain of 3q21-22 characterized the sarcomatoid cells and it was found in all xenografts. Immunohistochemistry and electron microscopy results strengthen the same finding. The morphology and protein profile of epitheloid and sarcomatoid cell lines converge in the xenografts, though certain line characteristics were maintained.In conclusion, our results suggest that the morphologic differences between epithelioid and sarcomatoid mesothelioma cells were due mainly to the microenvironment-driven clonal expansion, converging towards a similar genotype when grown in vivo.

P08-5

A conditional mouse model for spontaneous malignant pleural mesothelioma, and its detection by bioluminescence

Françoise Le Pimpec-Barthes[1,2], Annie Renier[1], Michiko Kawakita[1], Fabrice Chareyre[2], Jocelyne Fleury-Feith[3], Audrey Saint-Albin[1], Florence Gillaizeau[5,6,7], Julien Daubriac[1], Didier Jean[1], Marco Giovannini[3], Jessica Zucman-Rossi[1], Marie-Claude Jaurand[1]

[1]INSERM UMR 674, "Functional Genomics of Solid Tumors" , IUH, Université Paris Descartes, France, [2]Service de Chirurgie Thoracique, Hôpital Européen Georges-Pompidou, Assistance Publique-Hôpitaux de Paris, Université Paris Descartes, Paris, France, [3]House Ear Institute, Center for Neural Tumor Research, Los Angeles, CA, USA, [4]Laboratoire d'Histologie et de Biologie Tumorale, Hôpital Tenon, Paris, France, [5]Département d'Informatique Hospitalière, Hôpital Européen Georges-Pompidou, Assistance Publique-Hôpitaux de Paris, Université Paris Descartes, Paris, France, [6]INSERM UMR S 872, Equipe 20, Centre de Recherche des Cordeliers, Université Paris Descartes, Paris, France., [7]INSERM, Centre D'investigation Epidémiologique 4, Paris, France.

The objective of our study was to develop a working murine model of spontaneous malignant pleural mesothelioma (MPM), useful to study pathogenesis and to test new therapies and targeting agents, by conditional inactivation of genes already implicated in MPM, i.e. *NF2* and *CDKN2A*, and to follow disease progression by non invasive imaging.
Two groups of *Nf2* and Luciferase Reporter conditional mutant mice were used. First group (Gp1) had wild type *Cdkn2a* gene (LucRepNf2$^{Floxp16-/-}$ mice, n=45), and second (Gp2) constitutive inactivation of this gene (Gp2: LucRepNf2$^{Floxp16-/-}$ mice, n=47). *Nf2* inactivation was produced by a recombinant recombinase adenovirus vector (Ad*Cre*) inoculated into the pleural cavity. Control mice received empty adenovirus (n=12), physiological salt solution injection, or no injection (n=9). MPM was detected using clinical criteria and bioluminescence by Xenogen analysis after intraperitoneal injection of luciferin in living animals. Photo emission was measured in thoracic and abdominal regions. Anatomo-pathological studies were carried out using conventional staining, and immunological detection of cytokeratins, calretinin and vimentin.
Median survival times were not significantly different between groups Gp1 and Gp2, and according to treatment. A total of 24 tumours was observed, 22 following Ad*Cre* injection: 5 (23%) in Gp1 and 17 (77%) in Gp2 (p=0.006). MPM were identified 8.5 to 17 months after injection in 7 mice, all in Gp2. Bioluminescence levels were not significantly different between groups with and without MPM while different levels were found in thoracic areas between mice without or with MPM (means 6.71E+05 and 2.93E+05 respectively p= 0.046).
Murine MPM can be generated by inactivation of *Nf2*/*Cdkn2a* in mice. Bioluminescence analysis in thoracic area suggested MPM occurrence. The long delay for MPM occurrence and the low MPM incidence limit usefulness of this model, but the bioluminescence analyses appeared useful to follow MPM development.

P08-6

The therapeutic efficacy of anti-vascular endothelial growth factor antibody, bevacizumab, and pemetrexed against orthotopically implanted human pleural mesothelioma cells in severe combined immunodeficient mice

Wei Wang[1], Qi Li[1], Seiji Yano[1], Yasuhiko Nishioka[2], Saburo Sone[2]

[1]Division of Medical Oncology, Cancer Research Institute, Kanazawa University. Japan, [2]Department of Respiratory Medicine and Rheumatology, Institute of Health Biosciences, University of Tokushima Graduate School, Tokushima, Tokushima, Japan

Purpose: Malignant pleuralmesothelioma (MPM) is an aggressive malignancy, which has a poor prognosis with a median survival of less than 1 year. The vascular endothelial growth factor (VEGF) has been reported to be an ideal therapeutic target, and a multitargeted antifolate, pemetrexed, has been clinically used for the treatment of MPM. Experimental Design:We examined the therapeutic efficacy of the anti-human VEGF neutralizing antibody, bevacizumab, in combination with pemetrexed against two different human MPM cells, EHMES-10 and MSTO-211H, orthotopically inoculated into severe combined immunodeficient mice.Results: Bevacizumab inhibited a VEGF-induced proliferation of the human endothelial cells in a dose-dependent manner, but it had no effect on the proliferation of the two MPM cell lines in vitro. The orthotopically inoculated EHMES-10 cells (VEGF high expressing) produced thoracic tumors and a large volume of bloody pleural effusion, whereas the MSTO-211H cells (VEGF low expressing) produced thoracic tumors and a small volume of bloody effusions. Treatment with bevacizumab effectively inhibited the production of thoracic tumors and dramatically prevented the production of pleural effusion by the EHMES-10 cells but not the MSTO-211H cells. Treatment with bevacizumab reduced the number of enlarged tumor-associated vessels and proliferating tumor cells. Moreover, treatment with bevacizumab in combination with pemetrexed more effectively suppressed the formation of the pleural effusion and prolonged the survival compared with the control and monotherapy in the EHMES-10 cell-bearing severe combined immunodeficient mice.Conclusions: These results suggest that the combined use of bevacizumab and pemetrexed may therefore be promising for controlling the progression of MPM highly expressing VEGF.

P08-7

Establishment of *in vivo* fluorescence imaging in mouse models of malignant mesothelioma

Shuji Kubo[1], Norie Yamaoka[1], Yoshiko Kawasaki[1], Yunfeng Xu[1], Hideyuki Yamamoto[1], Nobuyuki Terada[2], Haruki Okamura[1]

[1]Laboratory of Host Defenses, Institute for Advanced Medical Sciences, Hyogo College of Medicine, Japan, [2]Department of Pathology, Hyogo College of Medicine, Nishinomiya, Japan

Malignant mesothelioma is a highly aggressive tumor with poor prognosis, and new treatment paradigms are urgently needed. For testing preclinical efficacy of new therapeutic agents, establishment of ideal animal models is crucial.
Here, we developed in vivo fluorescence imaging models for human malignant mesothelioma in mice using tumor cells engineered to express fluorescent proteins (EGFP, mRFP, mCherry, and mPlum) by lentiviral vectors. Among these fluorescent proteins, the expression of mCherry protein in the transduced tumor cells was shown to be robust and stable both in vitro and in vivo. In both, peritoneal disseminated and orthotopic pleural mesothelioma models, mCherry-positive tumors could be sensitively detected and tumor growth was successfully monitored.
This represents the first study to achieve sensitive tumor detection and tracking of tumor growth and development in the malignant mesothelioma mouse models by non-invasive in vivo fluorescence imaging. These imaging models can be versatile and powerful tools to explore new treatment paradigms for malignant mesothelioma.

P09

Pathology- I

P09-1

Expression of survivin and effects of survivin siRNA transfection on mesothelioma cell lines

Looniva Shrestha, Vishwa Jeet Amatya, Yukio Takeshima, Kei Kushitani, Kouki Inai

Department of Pathology, Hiroshima University, Japan

Aim: Survivin, a member of the inhibitors of apoptosis (IAP) protein family is overexpressed in many tumors including mesothelioma. The survivin protein functions to inhibit caspase activation leading to inhibition of apoptosis. Survivin also plays a role in cell proliferation and promotes angiogenesis. Our preliminary data showed expression of survivin in mesothelioma cells. Here, our aim is to study the effect of surivivin siRNA transfection on mesothelioma cells.Methods: Expression of survivin was analyzed in 2 mesotholioma cell lines (ACC-Meso-1, and ACC-Meso-4) by immunocytochemistry and western blot procedure. Survivin mRNA expression was analyzed by using RT-PCR before and after survivin siRNA transfection. Apoptosis assay and cell cycle assay were carried out by flowcytometry using NEXIN-reagent and cell cycle reagent respectively. Results: Expression of survivin protein and survivin mRNA were found in both the cell lines. More than 75% of survivin mRNA downregulation was found in both of cell lines by RT-PCR. Survivin protein downregulation was found by immunocytochemistry 2 to 4 days after siRNA transfection in control cells by flowcytometry. The analyses of apoptosis assay and cell cycle assays after survivin siRNA transfection and the possibility for mesothelioma therapy will be discussed.

P09-2

How to diagnose cytokeratin-negative anaplastic mesotheliomas?

Noriko Kimura[1], Fumiaki Yoshida[2], Osamu Wakabayashi[2], Hiromitsu Domen[3], Nozomu Iwashiro[3]

[1]Department of Clinical Research Pathology Section, National Hospital Organization Hakodate Hospital, Japan, [2]Deparment of Respiratory Medicine, National Hospital Organization Hakodate Hospital, Hakodate, Japan, [3]Department of Surgery, National Hospital Organization Hakodate Hospital, Hakodate, Japan

Immunohistochemical positivity of cytokeratin (CK) such as AE1/AE3, CAM5.2, CK 5/6 is usually requested to confirm sarcomatoid mesothelioma. However, how to diagnose CK–negative anaplastic mesothelioma remains to be solved. Here, we present two cases of sarcomatoid mesothelioma with special reference to differential diagnosis by immunohistochemical study. Patient 1 is an autopsy case of 72–year–old male. The whitish gelatinous tumor continuously encased entire right lung, pericardium and diaphragm, and focally infiltrated into the right lung. Histologically, the tumor shows two components: one was fascicular proliferation of various sized anaplastic cells with abundant collagen, and the other was smaller spindle–shaped cells with abundant mucinous component. Tumor cells in both components were immunohistochemically negative for CK, but strongly positive for vimentin. Calretinin, D2–40, thromobomodulin were focally and weakly stained. The tumor was diagnosed sarcomatoid mesothelioma mainly based on gross findings. Patient 2 is an operated case of 68–year–old male. He had a huge tumor localized in the left thoracic cavity. No other tumor was observed by whole body scanning by PET examination. The tumor entirely covered the left lung and was operated en block .On cut surface, a large amount of blood flew from the tumor. Histologically the tumor cells were extremely pleomorphic and mimic to so–called malignant fibrous histiocytoma with multinucleatd larger cells. Immunohistochemically, only vimentin was strongly positive, and D2–40 and thrombomodulin were focally positive. All other antibodies of immunohistochemical panel for mesothelioma and for other sarcomas including CD31, CD34, desmin, S100 were negative. Interestingly, these two tumors shared common immunohistochemical staining: positive for CD10 and cytoplasmic, but not nuclear staining of WT–1. In conclusion, D2–40, CD10, and cytoplasmic staining of WT–1 immunoreactivity may be characteristic for CK–negative anaplastic sarcomatoid mesothelioma. Further studies of many CK–negative sarcomatoid mesothelioms are necessary.

P09-3

Genomic gains and losses in malignant mesothelioma demonstrated by FISH analysis of paraffin-embedded tissues

Maiko Takeda[1], Takahiko Kasai[1], Yasunori Enomoto[1], Masato Takano[1], Kouhei Morita[1], Reina Inoue[1], Takahiro Yagyuu[1], Eiji Kadota[2], Hiroshi Maruyama[3], Akitaka Nonomura[1]

[1]Department of Diagnostic Pathology, Nara Medical University School of Medicine, Japan, [2]Department of Laboratory Medicine and Pathology, Kishiwada City Hospital, Osaka, Japan, [3]Department of Pathology, Hoshigaoka Koseinenkin Hospital, Hirakata, Japan

Purpose: Malignant mesothelioma (MM) results from the accumulation of a number of acquired genetic events at the onset. In MM, the most frequent changes were losses in 9p21, 1p36 and 22q12, and gains in 5p, 7p and 8q24 by CGH analysis. Although the diagnostic utility of 9p21 homozygous deletion by fluorescence in situ hybridization (FISH) analysis in MM has been reported only recently, other genes have not been well examined. This study analyzed the frequency of various genomic gains and losses in MM using FISH analysis. Materials and Methods: We performed FISH analysis using paraffin-embedded tissues from 42 cases of MM. Results: Chromosomal losses in MM were found at 9p21(83%), 1p36(43%), and 12q22(38%), whereas gains were found at 5p15(48%), 7p12(38%), and 8q24(45%). The frequency of each genomic gain or loss was similar to the findings on CGH in a previous study. There were no cases of adenomatoid tumor, benign mesothelial multicystic tumor, reactive mesothelial hyperplasia or pleuritis showing any gains or losses. At least one genomic abnormality was identified in all cases of MM. Among various histological subtypes, the chromosomal abnormality tended to be more common in cases showing sarcomatous elements (biphasic or pure sarcomatoid) than in cases showing an epithelioid histology. Conclusions: Our study suggests that genomic evaluation by FISH analysis might be helpful to distinguish MM from benign mesothelial proliferation.

P09-4

Molecular pathology of lung carcinoma in asbestos-exposed workers compared to mesothelioma

Pascal Andujar[1,2,3], Alexis Descatha[4], Claire Danel[5], Françoise Galateau-Sallé[6], Issam Abd-Alsamad[1], Pierre Validire[7], Marie-Annick Billon-Galland[8], Bénédicte Clin[6], Françoise Le Pimpec-Barthes[5], Denis Debrosse[7], Jean-François Régnard[9], Annie Renier[10], Jessica Zucman-Rossi[10], Jean-Claude Pairon[1,2,3], Marie-Claude Jaurand[10]

[1]Hôpital Intercommunal de Créteil, France, [2]INSERM, U955, Equipe 4, Créteil, France, [3]Université Paris-Est Créteil Val de Marne, Faculté de Médecine, Créteil, France, [4]INSERM, U687, Villejuif, France, [5]AP-HP, GHU Ouest, Hôpital Européen Georges Pompidou, Paris, France, [6]CHU Caen, Caen, France, [7]Institut Mutualiste Montsouris, Paris, France, [8]Laboratoire d¸Etude des Particules Inhalées, Paris, France, [9]Centre Chirurgical Marie Lannelongue, Le Plessis-Robinson, France, [10]INSERM, U674, IUH, Université Paris-Descartes, Paris, France

While cigarette smoking constitutes 80% of the attributable risk in lung cancer, asbestos fibres can also contribute to the development of lung cancer. So far, the nature of genetic alterations attributable to tobacco smoke and asbestos fibres respectively are not well defined. Asbestos specific biomarkers identification is important to better understand role asbestos in lung carcinogenesis. In a previous study, we showed that *P16*/*CDKN2A* gene inactivation in asbestos-exposed non-small-cell lung cancer (NSCLC) human cases mainly occurs via deletion after adjustment for age and cumulative tobacco consumption. This alteration was also found in human malignant mesothelioma (HMM), a cancer independent of tobacco smoking but associated with asbestos exposure, suggesting a possible relationship with an effect of asbestos fibres. The purpose of the present study was to characterize molecular alterations in NSCLC in asbestos-exposed workers compared to HMM alterations and to define asbestos exposure biomarkers in NSCLC.
We investigated *Ki-RAS*, *EGFr*, *NF2* and *TP53* genes known for high mutations frequencies in human NSLCL and/or in HMM. We analysed by genomic DNA sequencing 100 human frozen NSCLC tissues (50 asbestos-exposed and 50 asbestos-unexposed cases matched on age, gender, histologic type and smoking habits) obtained from 358 recruited NSCLC patients with well-defined smoking habits, and detailed assessment of asbestos exposure. In parallel, 34 primary human cell cultures obtained from confirmed HMM cases were studied.
No difference was found in gene mutations between asbestos-exposed and unexposed NSCLC cases. In contrast, some mutations were specific of the type of tumour, i.e *NF2* mutations were solely found in HMM, while *Ki-RAS* and *EGFr* mutations were only present in NSCLC.
These results demonstrated different transformation pathways between lung and pleural cells in a context of asbestos exposure. Nevertheless, the mechanism of gene inactivation may be specific of carcinogenic factor.

P09-5

Utility of immunohistochemistry in distinguishing between benign and malignant mesothelial proliferations

Kei Kushitani[1], Yukio Takeshima[1], Vashwa Jeet Amatya[1], Longnan Jin[1], Hidekazu Kohno[2], Hiromi Tsukiji[3], Kouki Inai[1]

[1]Department of Pathology, Graduate School of Biomedical Sciences, Hiroshima University, Japan, [2]Department of Respiratory Medicine, Hiroshima University Hospital, Hiroshima, Japan, [3]Hiroshima City Institute of Public Health, Hiroshima, Japan

[Aim] Differential diagnosis between benign and malignant mesothelial proliferations by light microscopy is problematic. The aim of this study is to investigate the utility of immunohistochemistry for the differential diagnosis between benign and malignant mesothelial proliferations. [Materials and Methods] 1. Forty-five cases of epithelioid mesotheliomas and 60 cases of non-neoplastic mesothelial cells were immunohistochemically analyzed using antibodies to desmin, alpha-smooth muscle actin (α-SMA), muscle specific actin, CD10, h-caldesmon, calponin, epithelial membrane antigen (EMA), mucin 1 (MUC1), p53, Ki-67, glucose transporter 1 (GLUT-1) and insulin-like growth factor II mRNA binding protein 3 (IMP3). 2. Nine cases of desmoplastic mesotheliomas and 9 cases of fibrous pleuritis were immunohistochemically analyzed using antibodies to desmin, cytokeratin (AE1/AE3 and CAM5.2), mesothelial markers (calretinin, D2-40 and Wilms' tumor 1) and α-SMA. [Results and Coclusion] 1. It was suggested that desmin and EMA were useful markers for differential diagnosis between epithelioid mesothelioma and non-neoplastic mesothelial cells, and the Utilities of MUC1, p53, Ki-67, GLUT-1 and IMP3 were limited, because the sensitivities or specificities of these markers was much inferior to those of desmin and EMA. 2. In the present study, it was suggested that desmin was useful for differential diagnosis between desmoplastic mesothelioma and fibrous pleuritis. However, further research and discovery of novel useful markers are necessary.

P10

Pathology-Ⅱ

P10-1

The trial of differentiation grading of epithelioid mesothelioma with reference to its clinicopathological significance

Yukio Takeshima[1], Kouki Inai[1], Yuichi Ishikawa[3], Teruaki Oka[3], Kenzo Hiroshima[4]

[1]Department of Pathology, Graduate School of Biomedical Sciences, Hiroshima University, Japan, [2]Department of Pathology, Cancer Institute, Japanese Foundation for Cancer Research (JFCR),Tokyo, Japan, [3]Department of Pathology and Laboratory Medicine, Kanto Central Hospital of the Mutual Aid Association of Public School Teachers, Tokyo, Japan, [4]Department of Pathology, Tokyo Women's Medical University Yachiyo Medical Center, Chiba, Japan

[Introduction] Epithelioid mesotheliomas (EMs) often show various morphological features, including papillotubular, solid, microcystic, cord-like, signet-ring cell like, deciduoid and so on. However, the significance of these morphological features is still not well established. Therefore, in this study, we tried to divide EMs into three differentiation grades based on morphology, and elucidated its clinicopathological significance. [Materials and Method] Fifty-three EMs were collected from Japanese medical institutes, and divided into three differentiation categories (i.e. well, moderate and poor) by focusing on "ppapillotubular" morphology. We also evaluated morphological features, including nuclear atypia, mitotic counts, necrosis and desmoplasia. Immunohistochemical analyses including calretinin, D2-40, CAM5.2, MIB-1, and p16 were also conducted.[Results & Discussion] The cases were divided into 11 cases (well), 38 cases (moderate), and 15 cases (poor), respectively, based on this grading system. The less-differentiated cases tended to be higher nuclear atypia, higher mitotic counts, and more massive necrotic foci and higher MIB-1 labeling index. It appeared that the expression of calretinin and CAM5.2 was higher in more differentiated cases. The prognosis of differentiated (well and moderate) EMs was significantly better than that of less-differentiated (poor) EMs. These results suggest that EM differentiation grading by morphological observation may be useful for the prediction of prognosis of EM patients.

P10-2

Morphologically-based grading of epithelial malignant pleural mesothelioma

Gina Cunto-Amesty[1], William Richards[2], David Sugarbaker[2], Joseph Corson[1], John Godleski[1]

[1]Department of Pathology, Brigham and Women's Hospital, USA, [2]Division of Thoracic Surgery, Brigham and Women's Hospital, Boston, MA, USA

Objective: To develop a morphologically-based grading system for epithelial malignant mesothelioma.Method: We conducted a pilot study in 20 cases that were selected from among 151 patients with epithelial MPM who were treated with extrapleural pneumonectomy, had complete pathologic staging, and were classified to stage II based on adjusted TNM criteria (Cancer 2010;116:1510-7). We evaluated cellular and architectural morpholgy, necrosis, and mitotic rates on semiquantitative and quantitative scales by microscopic examination of hematoxylin and eosin-stained sections from the clinical blocks (median 11 slides/case, range 2-17) with at least 5% viable tumor.Results: Using a preliminary multivariable algorithm, 12 tumors were classified as "well differentiated", 5 as "moderately differentiated", and 3 as "poorly differentiated". In this limited cohort, patients with well differentiated tumors trended toward longer overall survival (32 months median with 37% 5-yr survival) than those with moderately-poorly differentiated tumors (18 months median, 0% 5-yr survival; logrank p=0.2154) using this algorithm. Increased nucleolar size and solid architecture were prominent among the factors correlated with shorter survival.Conclusion: Epithelial MPM exhibits a range of morphologic features that may support a clinically applicable tumor grading system. This pilot demonstrates that such features may be modeled to derive prognostic information that is independent of tumor stage.

P10-3

The accuracy of pretreatment biopsy of pleural malignant mesothelioma in predicting histopathologic type in the extrapleural pneumonectomy specimen

Maria McIntire[1], Wai Foo[1], Mathias Hofer[1], Joseph Corson[1], David Sugarbaker[2], Lucian Chirieac[1]

[1]Department of Pathology, Brigham and Women's Hospital, USA, [2]Department of Thoracic Surgery, Brigham and Women's Hospital, Boston, MA, USA

Background: Pathologic classification of diffuse malignant mesothelioma (DMM) into epithelioid, sarcomatoid, and biphasic types is an important predictor of survival. The diagnosis of DMM is usually based on histopathologic examination of an adequate thorascopic or open biopsy. Since DMM is often heterogeneous, a biopsy may not be representative of the entire tumor. The goal of this study was to determine the accuracy of pretreatment biopsy in establishing the histopathologic type of DMM. Design: We examined 151 consecutive patients with pleural DMM treated from 1988 to 1997 at Brigham and Women s Hospital by extrapleural pneumonectomy (EPP) followed by heated chemotherapy all of whom had a pretreatment biopsy available for review. We characterized the presence of epithelioid and sarcomatoid histology in the resection and pretreatment biopsy specimens. Associations between the histology in pre- and post-treatment specimens were investigated.Results: The histology type of DMM in pretreatment biopsies were epithelioid in 120 patients (79%), mixed in 21 patients (14%), sarcomatoid in 8 patients (5%), and indeterminate in two patients (1%). The histology type of DMM in resection specimens was epithelioid in 93 patients (62%), mixed in 51 patients (34%), and sarcomatoid in 7 patients (4%). Biopsy findings were concordant with resection findings in 116 patients (Spearman r=0.64, p<0.0001). Conclusions: Our data suggests that a diagnosis of mixed or sarcomatoid DMM in the pretreatment biopsy is highly predictive of the histology in the resection specimen. A diagnosis of epithelioid DMM in the pretreatment biopsy is less accurate, and it changed to a less favorable one in a significant proportion of the cases. The results of our study emphasize the importance of thorough biopsy sampling in patients with malignant mesothelioma and the value of resection specimens for accurate diagnosis.

P10-4

Re-evaluation of malignant mesothelioma: similar results in a Norwegian and a Japanese study despite of different approaches

Helmut Sandeck[1], Oluf Roe[2], Kristina Kjaerheim[3], Helena Willen[4], Erik Larsson[5,6]

[1]Department of Pathology and Medical Genetics, St. Olav University Hospital, Norway, [2]Department of Oncology, St. Olav University Hospital, Trondheim, Norway, [3]Cancer Registry of Norway, Oslo, Norway, [4]Clinical Pathology and Cytology, Uppsala University Hospital, Sweden, [5]Department of Genetics and Pathology, Uppsala University, Sweden, [6]Department of Laboratory Medicine, Children's and Women's Health, Norwegian University of Science and Technology, Trondheim, Norway

Background: In connection with serological studies on malignant mesothelioma (MM), we re-evaluated biopsies and autopsy material with an earlier diagnosis of MM from 61 Norwegian patients from the period of 1980-2002. Methods: Immunoreactivity (IR) against basic positive markers Calretinin, EMA, Podoplanin and Mesothelin and negative markers CEA and Ber-Ep4, and, if needed, further markers was determined. Diagnoses were established by three pathologists, and compared with the earlier diagnoses and the published results of the Japanese study of Takeshima et al. (2009), the only other MM re-evaluation study known to us. Results: 49 cases (80 %) were considered being MM by a high degree of likelihood, five more cases possible MM. Of the remaining seven cases, three were diagnosed as adenocarcinoma, three as pleomorphic lung carcinoma, in one peritoneal case a clear entity diagnosis could not be given. In the cases where the initial MM diagnoses were confirmed, there had been applied either no IHC or, partially depending on available markers, between one and 18 markers. The 12 cases not confirmed by us had either lacked IHC (n=4), non-specific markers were used (n=4), IR was different (n=1), or specific markers had not shown positive IR in the right part of the MM cells (n=3). In some cases key positive markers had not yet been available. Otherwise choice of markers and IR interpretation were thus main concerns. The interdisciplinary re-evaluation study of Takeshima et al. that is based on a nearly 8-fold larger material shows 17 % of diagnoses classified as "definitively not/unlikely" and 71.5 % as "probable/definite" MM, while the corresponding numbers in our material are 16 % (8/49) and 80 %. Conclusions: Similar ranges of results in both studies may reflect valid approaches. Re-evaluation of earlier MM diagnoses is necessary before using tissue material for referential purposes. Keywords: re-evaluation, immunohistochemistry, classification, diagnosis, accuracy.

P10-5

Extent of the sarcomatoid component is an independent predictor of survival in malignant mesothelioma

Mathias Hofer[1], Maria McIntire[1], Wai Foo[1], Joseph Corson[1], Gavin Gordon[1], Raphael Bueno[2], David Sugarbaker[2], Lucian Chirieac[1]

[1]Department of Pathology, Brigham and Women's Hospital, USA, [2]Department of Thoracic Surgery, Brigham and Women's Hospital, Boston, MA, USA

Background: Diffuse malignant mesothelioma (DMM) is classified into epithelioid, sarcomatoid and biphasic types. The predictive value of each component in the biphasic type has not been well established. We investigated the clinical significance of the percentage of sarcomatoid and epithelioid components in biphasic DMM.Design: We evaluated 153 consecutive patients with epithelioid (n=106), biphasic (n=40), and sarcomatoid (n=7) DMM treated with extrapleural pneumonectomy from 1988 to 1997. The percentage of sarcomatoid component by light microscopy of each of the biphasic DMM specimens (average of 25.3 tumor-containing slides, range 11-79) was recorded by two pathologists. The average of the two readings and the pathologic stage (TNM, Sugarbaker and Bouchard) were correlated with overall survival.Results: The mean follow-up period after surgery was 25.4 months. Biphasic DMM had a bimodal distribution: predominantly sarcomatoid (more than 50% sarcomatoid component, n=19) or predominantly epithelioid (less than 50% sarcomatoid component, n=21). Patients with predominantly sarcomatoid biphasic DMM had similar survival as patients with monophasic sarcomatoid DMM (10.4 and 9.1 months). Patients with predominantly epithelioid biphasic MM had a better survival (17.5 months). The extent of sarcomatoid component was significantly associated with worse overall survival (p$lt;0.0001). The patients with monophasic epithelioid DMM performed best; median survival of 30.5 months, (p<0.0001). In multivariate analysis, including sex, age, and pathologic stage, sarcomatoid differentiation and age were independent prognostic indicators of survival, (p<0.0001) and (p=0.001), respectively.Conclusion: Our results indicate that the extent of sarcomatoid component predicts overall survival in patients with biphasic MM. When the sarcomatoid component is greater than 50%, the survival is similar to patients with monophasic sarcomatoid MM. In contrast, patients with predominantly epithelioid biphasic MM have an intermediate survival between monophasic epithelioid MM and biphasic sarcomatoid MM. Our data emphasize the importance of accurate histopathologic assessment and reporting of DMM.

P10-6

TLE1 expression in malignant mesothelioma: A potential pitfall in the immunohistochemical evaluation of pleuropulmonary sarcomatoid tumors

Joyce Ou, Paul Zhang, Leslie Litzky

Department of Pathology and Laboratory Medicine, University of Pennsylvania Medical Center, USA

Background: TLEs (transducin-like enhancer of split proteins) are mammalian homologues to the Drosophila protein Groucho. TLEs function as transcriptional corepressors that inhibit Wnt signaling pathways and play a role in repressing differentiation. Gene expression profiling identified TLE1 as a promising discriminator of synovial sarcoma. Subsequent tissue microarray studies of mesenchymal tumors established TLE1 as a sensitive immunohistochemical marker for synovial sarcoma, although other studies focusing on soft tissue sarcomas have challenged its specificity. Malignant mesothelioma is a tumor with variable histology that can be difficult to distinguish from other diffuse or localized pleuropulmonary tumors. Positive TLE1 expression in malignant mesothelioma has not been observed in the very limited number of tumors previously reported. This series is the first to evaluate TLE1 expression in malignant mesothelioma, using 32 clinically and pathologically well-characterized pleural-based tumors.
Methods: We examined TLE1 immunoreactivity in paraffin sections from 32 specimens (21 biopsies, 2 extrapleural pneumonectomies, and 9 radical pleurectomies). Twenty-two diffuse malignant mesotheliomas were characterized as epithelioid type, 7 as biphasic, and 3 as sarcomatoid. Santa Cruz Biotechnology monoclonal antibody TLE1 (M-101) was used with Vision Bio Bond-maX autostainer. TLE1 nuclear immunoreactivity was evaluated within the tumor cells.
Results: Positive TLE1 nuclear staining (defined as >10% of tumor cells) was seen in 16 of 32 malignant mesotheliomas (50.0%). Of the cases with positive staining, 56.2% were of the epithelioid type, 37.5% were biphasic, and 6.3% were sarcomatoid.
Conclusions: TLE1 nuclear staining is seen in a significant percentage of malignant mesotheliomas and should not be relied upon as a discriminating immunohistochemical marker, particularly when synovial sarcoma is in the differential diagnosis. The high percentage of malignant mesotheliomas with positive immunohistochemical staining for TLE1 may have implications for understanding the tumor biology of these neoplasms and suggests a potential target for future therapies.

P11

Pathology-Ⅲ

P11-1

Patterns of lymph node spread to N2 nodes predicts survival in patients with biphasic pleural malignant mesothelioma (MM)

Maria McIntire, William Richards, David Sugarbaker, Lucian Chirieac

Department of Pathology, Brigham and Women's Hospital, USA

BACKGROUND: In patients with diffuse MM, metastases to extrapleural N2 lymph nodes are a poor prognosis characteristic. Studies from our group have shown that metastases to N2 lymph nodes from biphasic MM have either both epithelioid and sarcomatoid histologies or only the epithelioid histology, but the clinical significance of this observation is unknown. In this study we investigated the clinical significance of the component metastatic to N2 lymph nodes from patients with biphasic MM.DESIGN: We identified 231 consecutive patients with biphasic MM treated by surgery at Brigham and Women's Hospital between 1988 and 2009 and found 74 with metastases to mediastinal N2 lymph nodes. We evaluated the N2 lymph node metastases of 41 of these patients with biphasic MM and available pathology material and correlated the findings with overall survival.RESULTS: All 41 patients (8 F/33 M; mean age 62; range 31-88) had a diagnosis of biphasic MM metastatic to N2 lymph nodes. Twenty-four patients (59%) with biphasic MM had both epithelioid and sarcomatoid components in the N2 lymph nodes and seventeen patients (41%) showed spread only of the epithelioid component to the N2 lymph nodes. The mean follow-up period after surgery was 11.2 months. The median survival of patients with mixed histology in the N2 lymph nodes was 8.9 months versus 11.9 months for those with an epithelioid component (p=0.059).CONCLUSION: Our data indicate that the presence of a mixed component in the N2 lymph nodes may predict a worse overall survival in patients with biphasic MM. The results of our study emphasize the importance of histologic classification of not only the surgical specimen but also the lymph node metastases and highlight the biologic complexity of disease progression in biphasic MM.

P11-2

Malignant pleural mesothelioma with tongue metastasis: more common than one would think?

Astero Klabatsa[1], Krishna Suchak[2], Jeremy P Steele[1], Robin M Rudd[1], Maria Calaminici[2], Kim Piper[2], Michael T Sheaff[2]

[1]Barts Mesothelioma Research, Department of Medical Oncology, St Bartholomews Hospital, Barts and The London Queen Marys Medical School, UK, [2]Department of Histopathology, Division of Cellular Pathology, Royal London Hospital, London, UK

We report a case of a previously diagnosed mesothelioma presenting with a two-month history of diffuse hard infiltration of the tongue. An incisional biopsy was taken from the left tongue and the suspected clinical diagnosis was amyloidosis. Histological examination showed mucosa covered by parakeratinising stratified squamous epithelium. Within the underlying corium, strands and cords of an infiltrative neoplasm were seen dissecting through the skeletal muscle fibres. The neoplastic cells were histomorphologically monotonous and epithelioid in character. There was absence of mitotic activity and necrosis. A background population of small lymphocytes was seen intimately admixed with the tumour cells. The tumour was present at the deep margin of the biopsy.The neoplastic cells were positive for immunohistochemical markers CK5/6, calretinin, MNF-116, WT1, D2-40 and EMA (membranous). There was no demonstrable mucin and all carcinoma and lymphoma markers were negative. The overall morphological and immunohistochemical features were those of metastatic malignant mesothelioma of the epithelioid type. We considered this to be a very unusual finding and wondered if it was unique. However, there are at least two previous case reports that mesothelioma can rarely metastasize to the tongue. It is interesting to speculate why the tongue should be a preferential site for metastasis of mesothelioma.

P11-3

Clinicopathologic characteristics of malignant mesotheliomas arising in patients with a history of radiation for Hodgkin lymphoma

Justine Barletta[1], John Godleski[1], Lucian Chirieac[1], David Sugarbaker[2]

[1]Department of Pathology, Brigham and Women's Hospital, USA, [2]Department of Thoracic Surgery, Brigham and Women's Hospital, Boston, MA, USA

Background: Recent studies have reported an association between malignant mesothelioma and chest radiation for Hodgkin lymphoma (HL). The clinicopathologic characteristics of malignant mesotheliomas arising in these patients have not been established. Design: We studied the clinicopathologic characteristics of nine malignant mesotheliomas from patients with a history of radiation to the chest for HL and no reported asbestos exposure (case group) with 12 random malignant mesotheliomas from patients with a history of asbestos exposure (control group). Clinical features ascertained were sex, age at mesothelioma diagnosis, asbestos exposure, dates of radiation for HL, and death. Tumors were classified as epithelioid, sarcomatoid, or mixed types according to WHO criteria. We reviewed an average of 14 H&E slides (range 2-25) from each tumor and recorded the presence of rhabdoid, clear cell, signet-ring cell, and myxoid morphology, pleomorphism, necrosis, mitoses, cytogenetic and molecular alterations. Result: Median time from treatment for HL to mesothelioma was 24.4 years (range 13-36). Eight of the cases (89%) of mesothelioma following HL were epithelioid and one was mixed. Two cases had anaplastic/pleomorphic histology; one had a myxoid morphology; one had clear cells, and three had signet ring cells. The cytogenetic and molecular alterations were numerous losses/deletions, including deletion of 22q, and deletion of the p16 gene. Patients with mesothelioma after radiation for HL were younger than the patients in the control group (median age 41 vs. 65, $p<0.0001$) and had a significantly longer median overall survival (31.5 vs. 11.2 months, $p=0.046$). Conclusion: Patients with mesothelioma after HL are significantly younger and have a longer overall survival compared to patients in the control group. Continued studies are needed to further define the clinicopathologic and molecular characteristics of patients with malignant mesothelioma and history of radiation for HL.

P11-4

Desmoplastic malignant mesothelioma: a clinical review of five pathologically diagnosed cases

Ryo Okuda, Takeshi Shinohara, Terufumi Kato, Akihiro Tagawa, Yoko Hashiba, Hiroshi Takahashi, Takashi Ogura

Department of Respiratory Medicine, Kanagawa Cardiovascular and Respiratory Center, Japan

AIMS:
Desmoplastic malignant mesothelioma (DMM) is rare, seldom curable disease. To determine its incidence and prognosis, the clinical features of DMM were analyzed.
METHODS:
We reviewed clinical course and pathological features of DMM patients who had diagnosed by histopathology in one institute.
RESULTS:
During January 2000 and October 2008, 36 patients were diagnosed as malignant mesothelioma, and 5 of them were confirmed as DMM. In 5 DMM patients, median age was 74 years (49 - 78), and 4 of the patients were male. Three patients were 0/1 of ECOG performance status and 2 patients were 2/3. One patient was in clinical stage I/II, other 4 patients were in stage III/IV. Symptoms at diagnosis were dyspnea in 1 patient, chest pain in 2 patients, and pleural effusion was found in 4 patients. Video-assisted thoracoscopic biopsy was necessary to diagnose in 3 patients, CT assisted lung biopsy in 1 patient, autopsy in 1 patient. Fluid cytology or closed pleural biopsy did not provide enough specimens to diagnose DMM in this study. Three of the patients were treated with combination chemotherapy including pemetrexed and cisplatin, resulted no responses.
CONCULUSIONS:
DMM is a rare variant form of malignant mesothelioma. Difficulty in diagnosis of DMM was compounded by small size of the specimens. Surgical biopsy was usually required to make an accurate diagnosis. Pemetrexed contained chemotherapy did not show enough outcomes in these patients.

P11-5

Cancelled

P11-6

Importance of clinical manifestations for differential diagnosis between sarcomatoid carcinoma and malignant mesothelioma

Per Fredrik Ekholdt

The Department of Internal Medicine,Sykehuset Ostfold Helsefortak Fredrikstad, Norway

A case report of a 62 year old woman will be presented.She is a fitness teacher in very good general health and never smoker.She had a major car crash i 1985 and was admitted to Chest CT because of familiar Alfa -1 Antitrypsin Deficency . CT scan showed an apical mass on the left side,a tumor adherent to the Aorta,minor pleural thickening on the left side,and a nodule in the basal part of the right lung.Coaxial biopsy from the tumor adherent to the Aorta was taken outside the lung as shown by the needle on CT scan.Histology by our own Pathology Department showed most probably a Sarcomatoid Carcinoma.As this is a rare entity the sample was sent to Oslo University Hospital for review.The slides were viewed by both the Sarcoma group and Mesothelioma group and a substantial number of markers were used.The conclusion was that it was a lung carcinoma but Malignant Mesothelioma could not be ruled out. Based on clinical data and the fact that she was a never smoker in very good health it was speculated that she may have a variant of Malignant Mesothelioma.After four courses of Carboplatin and Alimta most of the tumors disappeared.Updated result will be presented showing that in rare cases like this the clinical and radiological manifestation must be correlated to the immunohistochemical analysis when treatment shall be given.A brief review of the literature concerning differential diagnosis between Sarcomatoid Carcinoma and Malignant Mesothelioma will be given.

P11-7

Long-term survival of stage IV pleural mesothelioma presenting with retroperitoneal mass following multimodality treatment

Joseph Friedberg, Cengel K, Culligan M, Stevenson J

Penn Mesothelioma and Pleural Disease Program Coordinator, Thoracic Surgery Clinical Practice and Research Coordinator, USA

53yo otherwise healthy male presented with increasing shortness of breath, was found to have a right pleural effusion and underwent thoracentesis for > two liters of thick yellow fluid, suspicious but non-diagnostic for malignancy. He underwent RVATS for pleural biopsy and talc pleurodesis. Mixed immunohistochemistry confirmed neoplastic process, mesothelioma vs metastatic adenocarcinoma. Second and third pathologic opinions confirmed malignant mesothelioma, epithelial type with abundant myxoid stroma. Extent of disease work-up raised question of transgression of disease below his diaphragm as evidenced by a 1.2cm soft tissue density in the region of the gastrohepatic ligament and a 2.5cm mass adjacent to his right kidney. Both lesions were PET negative and interpreted as paraganglioma on CT and MRI of the abdomen (Figure 1). Laparoscopic excision of both the retroperitoneal mass and the gastrohepatic lymphadenopathy were both consistent with malignant mesothelioma, myxoid variant, morphologically resembling his pleural tissue specimen. He was subsequently treated with 4 cycles of Alimta-based chemotherapy, which he tolerated well. His pleural disease remained radiographically stable and his re-staging laparoscopy revealed no evidence of retroperitoneal disease. He then underwent Right Modified Extrapleural Pneumonectomy and Intraoperative Photodynamic Therapy followed by hemithoracic radiation and two additional cycles of Alimta-based chemotherapy. He currently has radiographic evidence of recurrent disease within his remaining lung which has thus far proved to be indolent in nature over the past year. He continues to live an active lifestyle, nearly 4 years from the time of his initial diagnosis of stage IV malignant mesothelioma.

P12

Pathology-Ⅳ

P12-1

Expression and localization of matrix metalloproteinase 9 (MMP-9) in mesothelioma cells and reactive mesothelial cells

Hironori Katayama[1], Masataka Tanno[1], Masaru Hosone[1], Zenya Naito[2]

[1]Department of Pathology, Nippon Medical school, Tama-Nagayama Hospital, Japan, [2]Department of Pathology, Integrative Oncological Pathology, Nippon Medical School, Tokyo, Japan

Objectives: Mesotheliomas occur in various forms in body cavity fluid. Immunohistochemical staining with a panel of antibodies is used to diagnose mesothelioma when the condition is strongly suggested by cytological findings. We performed a differential diagnosis of mesothelioma from reactive mesothelial cells using a matrix metalloproteinase 9 (MMP-9) antibody. **Materials and Methods:** Study subjects were 7 mesothelioma cases (pleural mesothelioma: 5 cases, peritoneal mesothelioma: 1 case, pericardial mesothelioma: 1 case) and 9 non-cancerous cases of reactive mesothelial cells (pleural effusion: 7 cases, peritoneal effusion: 2 cases) evaluated at our department. Immunostaining was performed in cell block sections prepared from body fluid and also performed in cell transfer sections prepared from Papanicolaou-stained specimens. Three mesothelioma cases were also examined for expression in tissue. Immunostaining was performed with a Dako Autostainer and EnVision visualization system. **Results:**1) Positive results were obtained in 6 cases of mesothelioma (86%), expressed in the cytoplasm and cytomembrane.2) Negative results were obtained in 7 cases of reactive mesothelial cells (76%), and weakly positive results in small cell numbers in the remaining 2 cases. **Conclusion:** Using the matrix metalloproteinase 9 (MMP-9) antibody is effective in differentiating mesothelioma from reactive mesothelial cells.

P12-2

Immunocytochemistry of CD146 is useful for discrimination between malignant pleural mesothelioma and reactive mesothelium in effusion cytology

Ayuko Sato[1], Ikuko Torii[1], Yoshihiro Okamura[1], Tadashi Yamamoto[1], Takashi Nishigami[1], Tatsuki Kataoka[1], Seiki Hasegawa[2], Takashi Nakano[3], Toshiaki Kamei[4], Tohru Tsujimura[1]

[1]Department of Pathology, Hyogo College of Medicine, Japan, [2]Department of General Thoracic Surgery, Hyogo College of Medicine, Hyogo, Japan, [3]Division of Respiratory Medicine, Department of Internal Medicine, Hyogo College of Medicine, Hyogo, Japan, [4]Division of Pathology, Yamaguchi Grand Medical Center, Yamaguchi, Japan

Malignant pleural mesothelioma (MPM) is a refractory tumor with poor prognosis associated with asbestos exposure. Pleural effusion is frequently observed in patients with MPM, and cytological analysis is effective to detect MPM. However, cytological discrimination between MPM and reactive mesothelium (RM) is often difficult because of the similarity of morphology and the lack of reliable discriminating markers. Increased expression of CD146, a cell adhesion molecule, has been reported to be closely related to an advanced stage of malignant melanoma, prostate cancer, and ovarian cancer. In this study, we evaluated the diagnostic utility of CD146 for discrimination between MPM and RM by examining immunocytochemically CD146 expression in MPM and RM using two clones of CD146 antibody, OJ79 and EPR3208, on smear specimens of effusion fluids. Immunocytochemical stains were semiquantitatively scored based on immunostaining intensity (0-3). CD146 expression was detected in 15 of 16 MPM with median immunostaining score 3 by OJ79 and 19 of 21 MPM with median immunostaining score 2 by EPR3208. OJ79 and EPR3208 were found to be complementary in diagnosis of three MPM cases, showing that all 23 MPM cases were positive for CD146 by either clone. Strong immunoreactivity of CD146 was observed at the apposing surfaces of cell-cell interactions on the plasma membrane of MPM cells. On the other hand, CD146 expression was undetectable in all 28 RM cases by both clones. The sensitivity of OJ79 and EPR3208 was 94% and 90%, respectively, and the specificity was 100% for both clones. We propose that CD146 is a sensitive and specific immunocytochemical marker enabling differential diagnosis of MPM from RM.

P12-3

Molded mesothelioma cells with hump-like cytoplasmic process in effusion cytology

Shinji Hamakawa[1], Kenji Kurashina[1], Youichi Kondo[1], Mie Kosaka[1], Natsumi Adachi[1], Tsutomu Sakurai[1], Yoshimi Kashiwasaki[1], Takao Oba[1], Seiichiro Shimizu[2], Toshiaki Kamei[3], Hirotoshi Akita[4], Sakae Hata[5], Kazuki Nabeshima[6]

[1]Department of Central Laboratory, Showa General Hospital, Japan, [2]Department of Diagnostic Pathology, Showa General Hospital, [3]Division of Pathlology, Yamaguchi Grand Medical Center, [4]Department of Medical Oncology, Hokkaido University Graduate School of Medicine, [5]Devision of Pathology, Kawasaki Medical School, [6]Department of Pathology, Fukuoka University Hospital and Faculty of Medicine, Japan

Objective: We observed characteristic hump-like cytoplasmic process in malignant mesothelioma in body cavity fluid cytology specimens.Study design: Using effusion material from 14 patients with epithelial malignant mesothelioma and 10 patients with adenocarcinoma, we conducted usual cytological examination and electron microscopic examination. Result: Malignant mesothelioma cells showed the following morphology, in a conceivably on going process; single cells, cell to cell apposition, molded cells with hump-like cytoplasmic processes, so-called pair cells, and molded or mutual inclusion cell clusters. In these cells immunocytochemistry was positive for calretinin, D2-40, WT1 and EMA, and electron microscopy revealed long thin microvilli and aggregates of intermediate filaments around nuclei.Conclusion: Typical molded cells with hump-like cytoplasmic processes, a diagnostic clue in effusion cytology for malignant mesothelioma, appear to be a stage in a conceivably on going process in which single mesothelioma cells mutually adhered, finally forming a small cell cluster.

P12-4

Collagenous stroma in body fluid cytology -Characteristic features and clinicopathological significance-

Sakae Hata[1], Naoki Kanomata[1], Toshiaki Kamei[2], Takuya Moriya[1]

[1]Department of Pathology, Kawasaki Medical School Hospital, Japan, [2]Division of Pathology, Yamaguchi Grand Medical Center

Objective: The aim of this study was to analyze characteristic features of collagenous stroma (CS) in body fluid cytology, and to find out its significance for cytological diagnosis.Study design: Cytological specimen from 36 cases of malignant mesothelioma (MM) and 4306 cases of benign reactive disease (BRD) were reviewed. CS was detected in 148 cases (23 of MM, 125 of BRD). CS were subdivided into three patterns according to different cell structures. Cytochemistry, immunocytochemistry and electron microscopy were additionally performed.Results: Type I clusters were seen in 118 cases (2.7%) of BRD, but not in MM. Type I clusters were significantly more frequent in peritoneal washing (11.4%) than aspiration cytology (0.09%) specimen ($p<0.001$). The detection rates for both type II and III clusters were significantly higher in MM cases (type II; 23case, 63.9%, Type III; 7 cases, 19.4%) than BRD (6 cases of each, 0.14%), respectively ($p<0.001$ and $p<0.001$). CS was always PAS positive, diastase resistant, and metachromatic in Giemsa staining. Immunocytochemistry, some of the type II CS showed expression for laminin or type IV collagen. Conclusions: Type I cluster was specific for BRD, and characteristically seen in peritoneal washing specimen. Both type II and III clusters were more frequent in MM cases than BRD. These results indicated that the CS in the body fluid cytology specimens might be of diagnostic use to evaluate malignant potential of mesothelial cells.

P12-5

Analysis of orangeophilic cells in effusion cytology

Toshiaki Kamei[1], Nobuo Sakuma[1], Hidemi Shibuta[1], Kaori Yasunaga[1], Hiroshi Okamura[1], Kenzo Hiroshima[2], Teruaki Oka[3], Toru Tsujimura[4], Takashi Hirano[5]

[1]Division of Pathology, Yamaguchi Grand Medical Center, Japan, [2]Department of Pathology, Tokyo Women's Medical University Yachiyo Medical, [3]Division of Pathology, Kanto Central Hospital, [4]Department of Pathology, Hyogo College of Medicine, [5]Department of Thoracic Surgery, Todachuo General Hospital, Japan

Objective: Cells with orange-colored cytoplasm (orangeophilic cells) are sometimes found in Papanicolaou-stained smears of serous effusions. We studied the frequency of their occurrence in effusions of various origins.Study Design: We retrospectively studied cytocentrifuged cell smears stained by Papanicolaou, i.e., 24 malignant mesotheliomas (MM), 53 reactive mesothelia (RM), 25 pulmonary adenocarcinomas (PAC), and 13 ovarian serous papillary adenocarcinomas (OSPAC). The numbers of orangeophilic cells were counted in each specimen. Results: Orangeophilic cells were observed in 18 (75.0%) MM, 2 (3.8%) RM, 2 (8.0%) PAC, and 3 (23.1%) OSPAC. Twelve (50.0%) MM, 1 (1.9%) RM, 1 (4.0%) PAC, and 1 (7.7%) OSPAC contained more than 5 orangeophilic cells in each specimen. The frequency in MM was statistically higher than in RM, PAC, or OPSAC ($p<0.001$) .Conclusion: Orangeophilic cells are very often found in effusions of MM, and are useful findings to differ MM from others. When we find orangeophilic cells in a specimen, we must carefully examine for MM. The presence of orangeophilic cells, especially more than 5 or more, is thus useful for diagnosing MM in serous effusions.

P12-6

Cytodiagnosis of malignant mesothelioma in effusion cytology - cell characteristic features and immunocytochemistry-

Toshiaki Kamei[1], Hidemi Shibuta[1], Hiroshi Okamura[1], Kaori Yasunaga[1], Kunimitsu Kawahara[2], Toshiaki Kawai[3], Yoshihiro Matsuno[4], Katsuji Marukawa[4], Shotaro Maeda[5], Chiho Ohbayashi[6], Toshiyuki Habara[7], Hiroshi Sonobe[7], Tetsuro Kodama[8], Yukio Takeshima[9]

[1]Division of Pathology, Yamaguchi Grand Medical Center, Japan, [2]Department of Pathology, Department of Pathology, Osaka Prefectural Medical Center for Respiratory and Allergic Diseases, [3]Department of Pathology & Laboratory Medicine, National Defense Medical College, [4]Department of Pathology, Graduate School of Medicine, Hokkaido University, [5]Pathology and Cytology Center, Mitsubishi Chemical Medience, [6]Department of Pathology, Hyogo Prefectural Cancer Center, [7]Department of Laboratory Medicine, Chugoku Central Hospital, [8]Respiratory Medicine, Tochigi Prefectural Cancer Center, [9]Department of Pathology, Graduate School of Medicine, Hiroshima University, Japan

Recently mesothelioma cases have been rapidly increased in number in Japan. For definitive diagnosis of mesothelioma (especially epithelioid and/or biphasic type), cytodiagnosis should be essential in pleural effusions and ascites especially in early stage. Characteristic cell features of mesothelioma are included in effusion cytology, such as (1)cell cluster (ball-like structure, papillary structure, window-formation, cell to cell engulfment, and type 2 collagenous stroma), (2)round or oval nuclei with one or two prominent nucleoli, (3) multinucleated cell, (4)thick basophilic cytoplasmas, (5)blurring of cell contour, (6)hump-like cellular processes. These cytological features would be useful in diagnosing mesothelioma. Moreover, we should performed trial of cell differentiation by immunohistochelial methods with antibody-panels (mesothelioma markers: two or three selection among calretinin, D2-40, WT1, CK5/6 and mesothelin, adenocarcinoma markers; two or three selection among in CEA, BerEP4, MOC31, TTF-1, and Napsin A. In our experiences, calretinin, D2-40, and WT1 are more recommended as mesothelioma markers, while CEA is one of essential adenocarcinoma markers especially in denying mesothelioma. EMA and/or desmin are usuful in differentiation of mesothelioma from reactive mesothelia. We wolud like to accentuate cytodiagnosis of mesothelioma in effusion cytology especially in clinically early stage.

P12-7

Diagnosis of malignant pleural mesothelioma: Comparison between pleural effusion cytology and pleural biopsy

Seiji Matsumoto[1], Kazue Yoneda[1], Masaki Hashimoto[1], Teruhisa Takuwa[1], Yoshitomo Okumura[1], Kazu Shiomi[1], Nobuyuki Kondo[1], Fumihiro Tanaka[1], Ikuko Torii[2], Torhu Tsujimura[2], Noriaki Tsubota[3], Kozo Kuribayashi[4], Kazuya Fukuoka[4], Takashi Nakano[4], Seiki Hasegawa[1]

[1]Department of Thoracic Surgery, Hyogo College of Medicine, Japan, [2]Division of Molecular Pathology, Department of Pathology, Hyogo College of Medicine, [3]Department of Thoracic Oncology, Hyogo College of Medicine, [4]Division of Respiratory Medicine, Dept. of Internal Medicine, Hyogo College of Medicine, Japan

Backgrounds: Pleural effusion cytology is sometimes insufficient to diagnose Malignant Pleural Mesothelioma (MPM) and pleural biopsy is essential for definitively diagnosis.
In this study, we compared pleural effusion cytology and pleural biopsy.
Patients and method: Patients suspected of MPM who underwent pleural biopsy under general anesthesia between February 2004 and January 2010 were included in this study. Pleural biopsy was performed by one skin incision on the supposed skin incision line of extrapleural pneumonectomy (EPP). A stamp-sized (5×4 cm) specimen including all layers of the parietal pleura was collected. It contains not only nodule but normal pleura.
Results: One hundred twenty one patients underwent pleural biopsy. Seventy-two patients were diagnosed as malignant tumors. 66 MPM (45 epithelioid, 6sarcoma, 6 desmoplastic, 5 biphasic, 1 anaplastic, 3 unknown), 2 lung cancer, 1 lymphoma, 1 invasive thymoma, 1 synovial sarcoma and 1 malignant pleural tumor. Thirty-two cases were inflammatory. Among 121 patients, 66 cases also underwent pleural effusion cytology. Three cases were pleural effusion cytology positive / pleura biopsy negative. Thirteen cases were pleural effusion cytology negative / pleura biopsy positive. Twenty-seven cases were pleural effusion cytology positive / pleura biopsy positive. Twenty-three cases were pleural effusion cytology negative / pleura biopsy negative.
Conclusions: Sampling through all layers and enough size of parietal pleura is essential to achieve a definitive diagnosis of MPM. The results of pleural effusion cytology and pleural biopsy are sometime discrepant.

P13

Biomarkers- Ⅰ

P13-1

Secretion of intelectin-1 from malignant pleural mesothelioma into pleural effusion

Shoutaro Tsuji[1], Yukio Tsuura[3], Takao Morohoshi[4], Tsutomu Shinohara[5], Fumihiro Oshita[6], Kouzo Yamada[6], Yoichi Kameda[7], Takashi Ohtsu[1], Yoshiyasu Nakamura[2], Yohei Miyagi[2]

[1]Division of Cancer Therapy, Kanagawa Cancer Center Research Institute, Japan, [2]Division of Molecular Pathology and Genetics, Kanagawa Cancer Center Research Institute, Yokohama, Japan, [3]Division of Pathology, Yokosuka-Kyosai Hospital, Yokosuka, Japan, [4]Division of General Thoracic Surgery, Yokosuka-Kyosai Hospital, Yokosuka, Japan, [5]Department of Clinical Research, Kochi National Hospital, Kochi, Japan, [6]Department of Thoracic Oncology, Kanagawa Cancer Center, Yokohama, Japan, [7]Department of Pathology, Kanagawa Cancer Center, Yokohama, Japan

Malignant pleural mesothelioma (MPM) is a rare but fatal tumor. Although most MPM patients show pleural effusion at even the early stage, it is hard to diagnose as MPM at the early stage because a sensitive and reliable diagnostic marker for MPM has not been found in plasma or pleural effusion. In the present study, we demonstrated that MPM cells secreted intelectin-1 specifically and that pleural effusion of MPM patient contained a large amount of intelectin-1. MPM cell lines, but not lung adenocarcinoma cell lines, secreted intelectin-1. In immunohistochemistry, epithelioid-type MPMs, but neither pleura-invading lung adenocarcinomas nor reactive mesothelial cells near the lung adenocarcinomas, were stained with anti-intelectin antibodies. Pleural effusion of MPM patients contained a higher concentration of intelectin-1 than that of lung cancer patients. In the pleural effusion, there was no correlation between intelectin-1 and hyaluronic acid, a high concentration of which is a reliable but not sensitive diagnostic marker for MPM. These results suggest that detection of intelectin-1 may be useful for a differential diagnosis of epithelioid-type MPM in immunohistochemistry and that a high concentration of intelectin-1 in pleural effusion can be utilized as another marker for clinical diagnosis of MPM.

P13-2

Diagnostic markers for malignant pleural mesothelioma: Serum antibody against antigens recognized by antibodies produced from tumor infiltrating B cells

Yoshiki Shigematsu, Koji Kuroda, Tetsuro Baba, Yoshika Nagata, Hidehiko Shimokawa, Yoshinobu Ichiki, Kenji Ono, Manabu Yasuda, Hidetaka Uramoto, Tomoko So, Mitsuhiro Takenoyama, Takeshi Hanagiri, Kosei Yasumoto

2nd Department of Surgery, University of Occupational and Environmental Health, Japan

Purpose: Malignant pleural mesothelioma (MPM) is difficult to be diagnosed at an early stage. This study attempted to obtain a tumor specific antibody against MPM derived from tumor infiltrating B lymphocytes (TIB) in MPM by using a xenotransplanted SCID mice model, and to identify the antigens recognized by the antibodies.
Methods: Tumor tissue specimens from 2 patients with MPM were engrafted subcutaneously in SCID mice and blood samples were obtained. A cDNA library was constructed from the mRNA of MPM. Immunoscreening of the libraries was performed by the serological identification of antigens by recombinant expression cloning method (SEREX). The titer of antibody against the antigens were measured in patients with MPM, lung cancer (with/without asbestos exposure), and breast cancer by ELISA. The function of the antigens was analyzed by inhibition with siRNA.
Results: 4 antigens were identified as MPM associated antigens. ELISA systems constructed by using 20 mer antigenic peptides correlated with the phage plaque assay to detect antibody titers against Gene-X and THBS-2. Antibody against Gene-X was detected in 46% of MPM patients and THBS-2 was detected in 84% of MPM patients. Among 88 control sera, including 25 normal healthy persons, 47 lung cancer patients and 16 breast cancer patients, only 2 were marginally positive against THBS-2, but not at all against Gene-X. Furthermore, the serum antibody titers decreased after surgical treatment of MPM and increased after recurrence of the disease. Gene-X was associated with an apoptosis-related genes and THBS-2 was associated with cell cycle related genes.
Summary: The titers of the antibodies against Gene-X and THBS-2 could be used as tumor markers for the diagnosis and follow-up of patients with MPM. Since Gene-X and THBS-2 are associated with cell proliferation, the regulation of these genes has a possibility to develop a new molecular target treatment.

P13-3

Circulating tumor cells (CTCs) in the diagnosis of malignant pleural mesothelioma (MPM)

Kazue Yoneda[1], Fumihiro Tanaka[1], Nobuyuki Kondo[1], Masaki Hashimoto[1], Teruhisa Takuwa[1], Seiji Matsumoto[1], Yoshitomo Okumura[1], Kazu Shiomi[1], Ayuko Sato[2], Tohru Tsujimura[2], Noriaki Tsubota[3], Kozo Kuribayashi[4], Kazuya Fukuoka[4], Takashi Nakano[4], Seiki Hasegawa[1]

[1]Department of Thoracic Surgery, Hyogo College of Medicine, Japan, [2]Division of Molecular Pathology, Department of Pathology, Hyogo College of Medicine, [3]Department of Thoracic Oncology, Hyogo College of Medicine, [4]Division of Respiratory Medicine, Department of Internal Medicine, Hyogo College of Medicine

Backgrounds: Circulating tumor cell (CTC), a surrogate of distant metastasis, is potentially useful in the diagnosis of malignant tumors, but its clinical significance in MPM remains unknown. The "CellCearch " system is a semi-automated detection system designed for capturing CTCs of epithelial origin with an antibody against epithelial cell adhesion molecule (EpCAM). As we had revealed that EpCAM expression was positive in around 50% of MPM tissues in a previous study (Yoneda K, et al. ASCO 2010), we prospectively examined the diagnostic capability of CTC.Methods: Patients who presented with suspicion or diagnosis of MPM were eligible. CTCs in 7.5mL of peripheral blood were quantitatively evaluated with the "CellSearch " system.Results: Among 114 eligible cases (mean age, 65 years; 27 females and 87 males), 92 were finally diagnosed as MPM and 22 as non-malignant diseases (NM). CTC was positive (CTC-count, one or more) in 38% (35/92) of MPM cases, and was also positive in 14% (3/22) in NM cases. CTC-count was significantly higher in MPM (range, 0 to 27) than in NM (range, 0 to 1; $p0<.05$), but a receiver operating characteristic (ROC) curve analysis failed to show a significant diagnostic performance of the CTC-test in discrimination between MPM and NM, with the area under curve (AUC) of 0.556 (95% confidence interval, 0.415 to 0.666). The sensitivity and specificity of the CTC-test were 38% and 86%, respectively. There was no significant correlation between CTC-count and tumor progression (clinical stage). Conclusions: CTC can be a useful tool for the diagnosis of MPM. However, the current CTC-test using anti-EpCAM antibody provides a low sensitivity for the diagnosis of MPM, which suggest a need for more sensitive CTC-detection system. Acknowledgement: This study supported by "The Special Coordination Funds for Promoting Science and Technology from the Japanese Ministry of Education, Culture, Sports, Science, and Technology"

P13-4

Clinical significance of serum VEGF in malignant pleural mesothelioma

Chiharu Tabata[1], Rie Tabata[2], Noriko Hirayama[1], Akihiro Yasumitsu[1], Shusai Yamada[1], Aki Murakami[1], Kunihiro Tamura[1], Takayuki Terada[1], Kozo Kuribayashi[1], Kazuya Fukuoka[1], Takashi Nakano[1]

[1]Division of Respiratory Medicine, Department of Internal Medicine, Hyogo College of Medicine, Japan, [2]Department of Internal Medicine, Hyogo Prefectural Tsukaguchi Hospital, Hyogo, Japan

Introduction: Malignant pleural mesothelioma (MPM) is an aggressive malignant tumor of mesothelial origin associated with asbestos exposure. MPM has a limited response to conventional chemotherapy and radiotherapy so diagnosing MPM early is very important. Vascular endothelial growth factor (VEGF) is an autocrine growth factor for MPM. Here, we investigated the serum levels of VEGF in patients with MPM in comparison to a population that had been exposed to asbestos without developing MPM.
Methods: Serum concentrations of VEGF were measured in 51 MPM patients and 42 individuals with benign asbestos-related diseases (asbestosis or pleural plaques) or who were healthy despite asbestos-exposure.
Results: We demonstrated that patients with MPM had significantly higher serum levels of VEGF than a population who had been exposed to asbestos but had not developed MPM, and the patients with advanced stage MPM showed higher levels of VEGF than the early stage MPM patients. The difference in overall survival between the groups with VEGF serum levels lower and higher than the assumed cutoff of 460 pg/ml was significant.
Conclusions: Our data suggest that the VEGF serum concentration could be a useful marker for screening MPM among asbestos-exposed individuals and as a prognostic factor.

P13-5

CD9 expression in mesothelioma: A correlation with clinicopathological factors and survival of patients

Vishwa Jeet Amatya[1], Yukio Takeshima[1], Taketo Yamada[2], Kei Kushitani[1], Chikao Morimoto[3], Kouki Inai[1]

[1]Department of Pathology, Hiroshima University Graduate School of Biomedical Sciences, Japan, [2]Department of Pathology, Keio University, Tokyo, Japan, [3]Division of Clinical Immunology, Advanced Clinical Research Center, Institute of Medical Sciences, University of Tokyo, Tokyo, Japan

CD9, a 24kD cell membrane glycoprotein, is a member of tetraspanins related to the suppression of tumor cell growth and motility. In cancer, decreased expression of CD9 protein has been implicated in progression of breast, lung, and colon cancers. Moreover, it was reported that low CD9 expression was correlated with poor prognosis in colon, lung, breast, and ovarian carcinomas.
We immunohistochemically examined the expression of CD9 in 8 mesothelioma cell lines and 174 human mesothelioma tissues (107 epithelioid, 42 sarcomatoid, 25 biphasic) using anti-CD9 antibody. CD9 expression was found in 4 out of 8 mesothelioma cell lines with prominent expression in epithelioid type. In mesothelioma tissue, 120 (69%) cases showed CD9 expression (41 (23.5%) cases with more than 50%, 41 (23.5%) cases, score 10-50%, 38 (21.8%) cases upto 10% of tumor cells). Ninety-three cases (87%) of epithelioid mesotheliomas with more frequent expression in differentiated type (95%) than less differentiated type (75%) showed CD9 expression. Only 6 (14%) cases of sarcomatoid mesothelioma showed CD9 expression. CD9 expression was statistically correlated with histological types (epithelioid type versus sarcomatoid type), IMIG staging (stage I, II versus III, IV), and differentiation degree (differentiated versus less differentiated epithelioid mesothelioma). The mesothelioma with CD9 expression showed higher median survival duration (18 months) compared to those without CD9 expression (10 months).
In conclusion, CD9 expression is an indicator of differentiated epithelioid mesothelioma and may also be a better prognostic factor of human mesothelioma.

P14

Biomarkers-Ⅱ

P14-1

Novel clinical role of angiopoietin-1 in malignant pleural mesothelioma

Noriko Hirayama[1], Chiharu Tabata[1], Rie Tabata[2], Akihiro Yasumitsu[1], Shusai Yamada[1], Aki Murakami[1], Kunihiro Tamura[1], Takayuki Terada[1], Kozo Kuribayashi[1], Kazuya Fukuoka[1], Takashi Nakano[1]

[1]Division of Respiratory Medicine, Department of Internal Medicine, Hyogo College of Medicine, Japan, [2]Department of Internal Medicine, Hyogo Prefectural Tsukaguchi Hospital, Hyogo, Japan

Malignant pleural mesothelioma (MPM) is an aggressive malignant tumor associated with asbestos exposure with limited response to conventional therapy, so diagnosing MPM early is very important. We have previously reported that angiopoietin (Ang)-1 was correlated with bleomycin-induced pulmonary fibrosis. Here, we investigated the association of Ang-1 with the development of MPM which originate from mesenchymal cells similar to lung fibroblasts, and demonstrated that Ang-1 stimulated the cell growth and migration of MPM cells in *in vitro* studies. We also demonstrated that patients with MPM had significantly higher serum levels of Ang-1 in comparison to a population who had been exposed to asbestos but had not developed MPM. The patients with advanced stage MPM showed higher levels of Ang-1 than the early stage MPM patients and the Kaplan-Meier method revealed a significant correlation between serum Ang-1 levels and survival. We propose the possibility that Ang-1 plays an important role in MPM tumor growth and our data suggest that the serum concentration of Ang-1 could be useful as prognostic factor.

P14-2

Platelet-derived growth factor (PDGF) in pleural effusion of malignant pleural mesothelioma and cancerous pleurisy due to lung cancer

Kunihiro Tamura[1], Kazuya Fukuoka[1], Hitomi Kamiya[1], Miki Honda[1], Eriko Masachika[1], Hisaya Okuwa[1], Yoshitaka Nogi[1], Risa Maeda[1], Taiichiro Otsuki[1], Kouji Mikami[1], Noriko Hirayama[1], Takayuki Terada[1], Asuka Okada[1], Aki Murakami[1], Shusai Yamada[1], Kozo Kuribayashi[1], Chiharu Tabata[1], Noriaki Tsubota[2], Takashi Nakano[1,2]

[1]Division of Respiratory Medicine, Department of Internal Medicine, Hyogo College of Medicine, Japan, [2]Department of Thoracic Oncology, Hyogo College of Medicine, Hyogo, Japan

Introduction: Platelet-derived growth factor (PDGF) has been implicated in the pathogenesis of malignant pleural mesothelioma (MPM).Serum PDGF-AB is considered to be a useful biomarker for the diagnosis of MPM.However,to date,there are few reports to evaluate fluid PDGF levels in MPM.In this study,we investigate whether PDGF in pleural effusion can be a useful biomarker of discrimination between MPM and cancerous pleurisy due to lung cancer (LC).
Materials and Methods: Pleural fluid was collected from 56 patients with MPM and 33 with cancerous pleurisy due to LC. PDGF-AA, BB and AB levels in effusion were determined by enzyme-linked immunosorbent assays (ELISA).
Results: The mean concentration of PDGF-AA was significantly higher in MPM patients than in LC patients (p=0.011).The mean concentration of PDGF-BB was significantly higher in MPM patients than in LC patients (p=0.041).The mean concentration of PDGF-AB was significantly higher in MPM patients than in LC patients (p=0.004).These results suggested that PDGF levels in pleural effusions may contribute to differentiate MPM from cancerous pleurisy due to LC.
Conclusion: PDGF in pleural effusion can be a useful biomarker of discrimination between MPM and cancerous pleurisy due to LC.

P14-3

A battery of biomarkers from effusions improve sensitivity for the diagnosis of malignant mesothelioma

Filip Mundt[1], Gustav Nilsonne[1], Sertac Arslan[2], Karola Csuros[1], Mervi Nurminen[1], Gunnar Hillerdal[3], Okio Hino[4], Huseyin Yildirim[2], Muzaffer Metintas[2], Katalin Dobra[1], Anders Hjerpe[1]

[1]Department of Laboratory Medicine, Karolinska University Hospital, Sweden, [2]Eskisehir Osmangazi University, Chest Diseases Department, Eskisehir, Turkey, [3]Lung Medicin, Karolinska University Hospital, Stockholm, Sweden, [4]Departments of Pathology and Oncology, Juntendo University School of Medicine, Tokyo, Japan

AIM: The aim of this study was to optimize a battery of markers found in pleural effusions to facilitate the diagnosis of malignant mesothelioma. MATERIAL AND METHODS: We used ELISA based techniques to screen for: mesothelin, hyaluronan, osteopontin, syndecan-2, syndecan-1 and thioredoxin in 190 effusions from patients diagnosed with malignant mesothelioma (n=46), other malignancy (n=50) or benign condition (n=94). All ELISA assays were commercially available except for syndecan-2, which was prepared in lab as described earlier. Determination of mesothelin was performed with two different ELISA kits: MESOMARK, which predominantly recognizes the C-ERC/Mesothelin fragment as well as SMRP, and the N-ERC/Mesothelin (7-16) ELISA kit (IBL, Japan) which is directed towards the N-ERC/Mesothelin fragment. All elevated hyaluronan values were further confirmed using HPLC. Statistical analysis is ongoing. Both logistic regression and Bayesian statistics are used to optimize a battery based on these markers. RESULTS: Univariate odds ratios show that hyaluronan and mesothelin are the most diagnostically useful, with some additional value of osteopontin and syndecan-2. N-ERC/Mesothelin performed better than C-ERC/Mesothelin. A logistic model with all factors included will correctly classify (MM or not) in >94% of cases. The ROC plot AUCs are similar with the two models, slightly better values being obtained with Bayesian statistics. Preliminarily, two parameters seem to be sufficient in an optimized battery analyzing effusions: hyaluronan and N-ERC/Mesothelin. Internal validation will be performed by a boot strap procedure. For further validation we also analyze a large external material (Eskisehir University, Turkey) consisting of pleural effusions from 53 mesothelioma patients, 166 other cancers and 165 benign effusions. The final battery and its validation will be presented.

P14-4

Soluble mesothelin for diagnosing malignant pleural mesothelioma: an individual patient data meta-analysis

Kevin Hollevoet[1], Hans Reitsma[2], Jan van Meerbeeck[1], Paul Baas[3], Jenette Creaney[4], Alfonso Cristaudo[5], Francesca Di Serio[6], Bogdan Grigoriu[7], Thomas Muley[8], Harvey Pass[9], Alex Rai[10], Bruce Robinson[11], Rodríguez-PortalJosé A[12], Arnaud Scherpereel[13], Joachim Schneider[14], Marco Tomasetti[15]

[1]Department of Respiratory Medicine, Ghent University Hospital, Belgium, [2]Department of Clinical Epidemiology, Biostatistics and Bioinformatics, Academic Medical Center, Amsterdam, The Netherlands, [3]Department of Thoracic Oncology, The Netherlands Cancer Institute, Amsterdam, The Netherlands, [4]National Center for Asbestos Related Disease, Western Australian Institute of Medical Research, University of Western Australia and Sir Charles Gairdner Hospital, Perth, WA, Australia, [5]Department of Endocrinology and Metabolism, Orthopaedics and Traumatology, Occupational Medicine, University of Pisa, Pisa, Italy, [6]Department of Clinical Pathology, University Hospital, Bari, Italy, [7]University of Medicine and Pharmacy, Iasi, Romania, [8]Translational Research Unit, Thoraxklinik am Universitätsklinikum Heidelberg, Heidelberg, Germany, [9]Department of Thoracic Surgery, New York University, Langone Medical Center, New York, USA, [10]Department of Pathology and Cell Biology, Center for Advanced Laboratory Medicine, Columbia University Medical Center, New York, USA, [11]National Center for Asbestos Related Disease, Western Australian Institute of Medical Research, University of Western Australia and Sir Charles Gairdner Hospital, Perth, WA, Australia, [12]Medical Surgery Unit for Respiratory Diseases, Virgen del Rocio University Hospital, Seville, Spain, [13]Pulmonary and Thoracic Oncology, University Hospital (CHRU) of Lille II, France, [14]Institute and Policlinic for Occupational and Social Medicine, Justus-Liebig Universität, Giessen, Germany, [15]Department of Molecular Pathology and Innovative Therapies, Polytechnic University of Marche, Ancona, Italy

Background: Soluble mesothelin is a serum biomarker of malignant mesothelioma. Its extensive diagnostic validation revealed differences in reported diagnostic performance, hampering the interpretation of its actual diagnostic value. To address this issue and indentify sources of heterogeneity, we performed an individual patient data (IPD) meta-analysis of soluble mesothelin. **Methods:** A literature search was conducted to identify studies which assessed the diagnostic performance of soluble mesothelin, measured with the Mesomark™ kit. Corresponding authors of eligible papers were invited to join the Soluble Mesothelin Collaboration, and provide IPD. Logistic regression models were used to examine the accuracy of soluble mesothelin, using random intercepts and slopes to account for the hierarchical nature of the data. Covariates were added to assess their impact on the diagnostic performance. Performance was expressed using odds ratios, receiver operating characteristics curves and areas under the curve (AUC). **Results:** The literature search identified 17 studies, and all corresponding authors provided IPD. Individual studies often included multiple control groups, which were classified as 1) healthy controls (n=909); 2) healthy asbestos-exposed (n=775); 3) individuals with a benign asbestos-related disease (n=736); 4) a benign respiratory disease (n=267) and 5) lung cancer (n=778). The number of patients with malignant pleural mesothelioma (cases) was 1026, resulting in a total of 4491 individuals. The ability of soluble mesothelin to discriminate between cases and controls was negatively influenced by higher age and depended on the type of control group. For example, after correcting for age, diagnostic performance differed significantly ($P<0.01$) in healthy controls (AUC=0.898) and lung cancer patients (AUC=0.797). **Conclusions:** This IPD meta-analysis demonstrates the impact of age and type of control group on the diagnostic performance of soluble mesothelin. Additional research will focus on case-specific covariates. Correcting for all these covariates allows us to interpret and discuss the diagnostic potential of soluble mesothelin more accurately.

P14-5

Serum mesothelin levels in asbestos exposed populations

Manlio Mencoboni[1], Fabio Spigno[2], Luigi Michelazzi[2], Virgina Mortara[3], Barbara Bobbio[4], Andrea Bruzzone[1], Fabrizio Rao[5], Bergaglio Marina[1], Omar Racchi[1], Rosangela Filiberti[4]

[1]Oncology Unit, Villa Scassi Hospital, Italy, [2]Occupational Medicine Unit, University of Genova, Genova, Italy, [3]INAIL Genova, Italy;, [4]National Cancer Institute, Genova, Italy, [5]Pneumology Unit, Villa Scassi Hospital, Genova, Italy

Background: The risk to develop asbestos-related tumors is proportional to the intensity of asbestos exposure in different categories of workers. Soluble mesothelin-related protein (SMRP) is raised in pleural mesothelioma and it has been shown that the concentration of the marker significantly increases with asbestos exposure. Aim of the study was to verify the association between serum SMRP and asbestos exposure in a large cohort of workers with occupationally exposure to different concentrations of asbestos fibres. Methods: A total of 1660 subjects, median age 60.9 years, were studied. Serum SMRP was measured by a specific ELISA assay. Subjects enrolled underwent clinical examination and were administered a detailed questionnaire on pathological anamnesis, individual habits and occupational exposure (duration of exposure, occupational task). A cumulative dose of inhaled asbestos fibres per year (fibres/cc/year) was estimated on the basis of the occupational risk. Results: Mean (±SD) SMRP was 0.55 (±0.39) nM/L. Mean fibres concentration was 22.4 (±24.7) ff/cc/year. On the basis of estimated fibres, three groups were distinguished: low (less than 12 ff/cc/year), intermediate (12-25 ff/cc/year) and high (more than 25 ff/cc/year). Lightly higher SMRP levels were found in subjects with higher exposure than in the remaining subjects (0.58±0.4 vs 0.53±0.4 ff/cc/year; p=0.06). The threshold for an abnormal SMRP result was set at 0.46 nM/L, corresponding to the median value of the marker in the all series. A relationship between higher SMRP levels and asbestos fibres was observed also after adjusting for age and presence of tumors in clinical anamnesis (OR= 1.21; 95% C.I.: 1.02-1.44 for high compared to low exposure).Conclusions: This study found a positive correlation between the serum SMRP levels and a high concentration of inhaled asbestos fibres in a large cohort of subjects with past occupational exposure. SMRP could be a promising marker of asbestos exposure.

P15

Biomarkers-Ⅲ

P15-1

Serum N-ERC as a useful biomarker for mesothelioma treatment

Takanori Mori[1], Ken Tajima[1], Michihiro Hirama[1,3], Naoko Aoki[4], Yoshiaki Hagiwara[4], Masahiro Maeda[4], Masaaki Abe[2], Okio Hino[2], Kazuhisa Takahashi[1]

[1]Department of Respiratory Medicine, Juntendo University, School of Medicine, Japan, [2]Department of Pathology and Oncology, Juntendo University, School of Medicine, Tokyo, Japan, [3]Department of Respiratory Medicine, Juntendo University Nerima Hospital, Tokyo, Japan, [4]Immuno-Biological Laboratories Co., Ltd., Gunma, Japan

Background: Recently, N-ERC/mesothelin (N-ERC) and Osteopontin (OPN) attract attention with malignant pleural mesothelioma (MPM) as diagnostic and treatment monitoring biomarkers. The aim of this study is to investigate whether serum N-ERC and plasma OPN levels correlate to therapeutic effect in patients with MPM. Methods: We recruited 24 patients between June 2005 and April 2010 at our hospital. We measured serum N-ERC and plasma OPN of patients before and after following chemotherapy. Results: The median age was 63.6 years old (range: 51-78); male/female, 19/5; pathological type, epithelial/sarcomatous, 20/4; stagel/II/III/IV, 1/5/7/11; chemotherapy regimen, cisplatin+pemetrexed/ cisplatin+gemcitabine/ carboplatin+gemcitabine/ pemetrexed, 16/5/2/1, respectively. The overall response rate was 20.8% with 5 partial response (PR), 9 stable disease (SD) and 10 progressive disease (PD). Average N-ERC ratio (ratios dividing C-ERC levels after chemotherapy by those before chemotherapy) of PR and SD+PD were 0.76 and 1.51, respectively (p-value <0.05). Average OPN ratio (ratios dividing OPN levels after chemotherapy by those before chemotherapy) of PR and SD+PD were 0.61 and 1.42, respectively (p-value <0.05). Conclusions: These data indicate that serum N-ERC and plasma OPN levels correlate with therapeutic effect of chemotherapy. We speculate that both N-ERC and OPN could be useful monitoring biomarker for MPM treatment. Another analysis regarding relationship between these markers and overall survival is ongoing, and could be reported soon.

P15-2

Megakaryocyte potentiating factor is effective for the differential diagnosis of malignant pleural mesothelioma

Kazuki Shimada[1], Yoshiro Kishi[2]

[1]National Institute of Biomedical Innovation, Laboratory for Immune Signal, Japan, [2]Ina Institute Medical & Biological Laboratories Co. Ltd., Department of Research and Development, Ina, Japan

Background: The early diagnosis of malignant pleural mesothelioma (MPM) is difficult. Megakaryocyte potentiating factor (MPF) and mesothelin variants (MSLN) which have been reported to represent candidate serum markers of MPM, have not yet been established which one (MPF or MSLN) is the most effective marker for the differential diagnosis of MPM. Therefore we have designed novel enzyme-linked immunosorbent assay (ELISA) systems to compare the diagnostic efficacy of MPF and MSLN as serum markers of MPM.Material and Methods: Serum samples were collected from 27 consecutive patients with non-resectable MPM (13 with epithelial type MPM, three with sarcomatoid type, five with mixed type and six with unclassified type). For controls, we used 47 patients with lung cancer, 35 with other cancers (18 ovarian, eight stomach and nine colon cancers), nine asbestos-exposed asymptomatic subjects and 38 healthy adults without a history of asbestos exposure.Results: Serum MPF and MSLN protein were elevated in MPM patients in comparison with every control group. While the area under the receiver operating characteristic curve (AUC) for serum MPF was 0.879, cutoff = 19.1 ng/ml (sensitivity = 74.1%, specificity = 90.4%), the AUC for serum MSLN was 0.713, cut-off = 93.5 ng/ml (sensitivity = 59.3%, specificity = 86.2%). A comparison between AUC for MPF and MSLN values showed that MPF is superior to MSLN ($p = 0.025$). Conclusions: Our analysis by ELISA for the diagnostic efficacy of MPF and MSLN as serum markers of MPM, revealed that MPF has superior sensitivity and specificity compared with MSLN.

P15-3

Role of SMRP, osteopontin and CA-125 for early diagnosis of malignant pleural mesothelioma

Katrina Rey-McIntyre, Masaki Anraku, Tetsuzo Tagawa, Licun Wu, Brenda O' Sullivan, Demetris Patsios, Geoffrey Liu, Zhihong Yun, Marc de Perrot

University of Toronto, Canada

PURPOSE: Plasma concentrations of soluble mesothelin-related protein (SMRP), osteopontin and cancer antigen-125 (CA-125) in MPM patients were compared to controls matched for gender, age, and smoking history, and a test group of asbestos exposed (AE) individuals undergoing prospective screening CT scan of the chest to determine the role of these markers in MPM detection.
METHODS: Quantification for each marker was conducted on untreated MPM patients (n=26), AE matched controls (n=44) and AE screened group (SG) (n=120).
RESULTS: Median biomarker levels were higher in MPM patients compared to AE controls in SMRP ($p< 0.0001$), osteopontin ($p= 0.0009$) and CA-125 ($p = 0.02$). In receiver operating curve (ROC) analysis, the sensitivity and specificity of SMRP in differentiating the controls from MPM patients were 69% and 73% (cut-off, 2.7nM), those of osteopontin were 61.5% and 91% (cut off, 425 ng/ml), and CA-125 were 61.5% and 77% (cut-off, 13.2 U/ml). The sensitivity and specificity of MPM patients to controls when all three makers were above their cut-off were 35% and 99%. None of the 120 SG individuals had all three markers above cut-off (specificity, 100%). However, 11 SG individuals had one value above the cut-off for SMRP (n=2), osteopontin (n=7) or CA-125 (n=2).
CONCLUSIONS: The three biomarker cut-off levels in the development group have established a potential indicator to be used in screening high-risk AE populations for MPM. The SG will increase and continue to be monitored to determine sensitivity, and if specificity will remain at 100%. New MPM patients with matched AE controls will also be used to determine if values remain constant.

P15-4

Gene expression ratio-based diagnostic and predictive tests using fine needle aspiration biopsies in malignant pleural mesothelioma

Assunta De Rienzo[1], Lingsheng Dong[1], Melissa Coleman[1], Beow Yeap[2], Roderick Jensen[3], William Richards[1], Gavin Gordon[1], David Sugarbaker[1], Raphael Bueno[1]

[1]Division of Thoracic Surgery and the International Mesothelioma Program, Brigham and Women's Hospital, and Harvard Medical School, USA, [2]Department of Medicine, Massachusetts General Hospital and Harvard Medical School, Boston, MA, USA, [3]Department of Biological Sciences, Virginia Tech, Blacksburg, VA 24060, USA

Malignant pleural mesothelioma (MPM) is a rare disease usually associated with previous asbestos exposure with an annual incidence in the US of 2,000 to 3,000 cases. The correct histological diagnosis is sometimes challenging and requires structural evaluation and complex immunohistochemical panels. To address these limitations, we combine gene expression ratio-based diagnostic and predictive tests with a minimally invasive pre-operative approach. One to 5 ex-vivo fine needle aspiration (FNA) biopsies of each tumor were taken immediately after tumor removal from 63 MPM patients and 92 lung cancer patients for a total of 276 ex-vivo MPM and 303 ex-vivo lung cancer samples. RNA was immediately isolated and all the FNA biopsies were analyzed using real time RT-PCR for the diagnostic (MPM vs. lung Adenocarcinoma) and predictive (MPM outcome prognosis) tests previously generated and validated by our group. We found that the sensitivity of the diagnostic test for MPM was 100% (95% CI: 95-100%), and the specificity in primary lung adenocarcinoma was 90% (95% CI: 81-95%). In addition, the FNA-based predictive classifications were concordant among 76% (95% CI: 65-87%) of patients with the risk assignments in a set of the matched surgical specimens previously analyzed by the predictive test. Furthermore, we extended our analysis to 155 ultrasound-guided (in vivo) FNAs from 54 MPM patients. When a majority rule was applied to the diagnostic test performed on multiple samples from the same patient, preliminary results indicated that 49 of 54 (91%) MPM patients were correctly classified as MPM. This study provides evidence that the analysis of gene expression from RNA obtained using image-guided FNA biopsies may represent a powerful and useful tool to diagnose, predict outcome and drive treatment decisions in MPM patients.

P15-5

Optimization of mesothelioma gene ratio tests for paraffin-embedded tissue

Melissa Coleman[1], Assunta De Rienzo[1], Beow Yeap[2], David Sugarbaker[1], Raphael Bueno[1]

[1]Division of Thoracic Surgery and the International Mesothelioma Program, Brigham and Women's Hospital, and Harvard Medical School, USA, [2]Department of Medicine, Massachusetts General Hospital and Harvard Medical School, Boston, MA, USA

Malignant pleural mesothelioma (MPM) is a highly lethal cancer with >90% 5-year mortality. A comprehensive pre-treatment staging strategy permitting effective treatment stratification is needed. Using frozen tissue, our laboratory previously described a novel gene expression ratio-based algorithm for MPM diagnosis and prediction of outcome. The diagnostic test has been show to be 99% accurate in differentiating MPM from lung adenocarcinoma, while the predictive test demonstrates statistically significant survival stratification ($p = 0.001$). However in clinical practice, most tissue specimens are formalin-fixed and paraffin-embedded. Therefore extension of gene ratio tests to such fixed specimens would allow rapid clinical application of this algorithm. Gene ratio tests are based on relative quantitative real-time PCR (qRT-PCR) gene expression. New qRT-PCR primers were designed to target smaller regions, in order to address the RNA fragmentation which occurs secondary to the fixation process. Predictive and diagnostic gene expression tests were performed with new and original primers comparing paraffin-embedded with matched frozen tissue. A 20 sample pilot analysis demonstrated $\geq$ 80% concordance comparing predictive and diagnostic gene ratio tests with paraffin-embedded and frozen tissue. A further large specimen cohort analysis is currently being performed to refine and validate these tests. In this pilot study we have successfully designed primers optimized for use with paraffin-embedded MPM tissue. Moreover we demonstrate high overall concordance in the results of the diagnostic and predictive test when analyzing matched paraffin-embedded and frozen specimens. Results of gene ratio tests comparing fixed and matched frozen tissue suggest the feasibility of extending the use of these tests to paraffin-embedded tissue in a pre-treatment staging algorithm.

P16

Imaging- I

P16-1

Computed tomographic assessment of apical involvement in clinical evaluation of malignant pleural mesothelioma

William Richards[1], Ritu Gill[2], Shin Matsuoka[2], Hiroto Hatabu[2], David Sugarbaker[1]

[1]Division of Thoracic Surgery, Brigham and Women's Hospital, Harvard Medical School, USA, [2]Department of Radiology, Brigham and Women's Hospital, Harvard Medical School, Boston, MA, USA

Background: We have demonstrated that the pathologic finding of disease involvement at the apical surgical margin after extrapleural pneumonectomy is related to decreased survival among patients with epithelial malignant pleural mesothelioma (MPM; Cancer 2010;116:1510-7). To determine if this prognostic factor could be assessed preoperatively, we investigated the relationship of apical pleural tumor involvement by imaging on computed tomography (CT) to survival among patients undergoing EPP.Method: With IRB approval, DICOM files of preoperative thoracic CT images were analyzed for evidence of apical involvement. Kaplan-Meier survival estimates were compared between groups of patients with and without CT-determined apical involvement using the logrank test. Epithelial and non-epithelial cases were analyzed separately due to distinct differences in expected survival duration. Results: 161 patients who had undergone extrapleural pneumonectomy between August 2001 and December 2007 had preopearative CT scans. Among 102 epithelial cases, survival was significantly longer for the 54 cases without apex involvement (25.9 months median) than for the 48 with apex involvement (13.1 months; $p=0.0005$). Among 59 non-epithelial cases, there was no survival difference between the 22 cases without apex involvement (8.6 months median) and the 37 with apex involvement (8.0 months; $p=0.3342$). Median CT-estimated tumor volumes differ significantly between cases with apex involvement (median 589 cc, range 103-3416 cc) and those without apex involvement (230 cc, 1-2717 cc; $p<.0001$ Mann-Whitney U test) for all cases. This contrast was relatively stronger for epithelial [746 (103-3416) cc vs 184 (1-2717) cc $p<.0001$] than for non-epithelial [477 (203-2830) vs 344 (34-2200) cc $p=0.0075$] cases with and without apex involvement, respectively. Conclusions: CT-assessed tumor involvement of the apical pleural margin correlates with prognosis in epithelial, but not non-epithelial MPM. Positive apical assessment is associated with higher tumor volume, and may represent a useful addition to the preoperative evaluation of epithelial MPM patients.

P16-2

Virtual surgical planning for pleural mesothelioma. Interactive volume visualization and automated quantification of pleural tumors on a 3D stereoscopic graphics cluster

Nigel Parsad[1], Jonathan Silverstein[1,2], Samuel Armato III[3], William Sensakovic[3], Ravi Salgia[4], Hedy Kindler[4], Wickii Vigneswaran[2]

[1]Computation Institute, University of Chicago, USA, [2]Departmant of Surgery, University of Chicago, Chicago, IL, USA, [3]Department of Radiology, University of Chicago, Chicago, IL, USA, [4]Department of Medicine, University of Chicago, Chicago, IL, USA

Surgical treatment of Pleurectomy Decortication (PD) and Extra Pleural Pneumonectomy (EPP) for Malignant Pleural Mesothelioma (MPM) appear to provide equivalent palliation. The choice of operation is surgeon dependent and an imaging technique that provides better visualization of the tumor may help to decide appropriate operative procedure. When tumor is infiltrating into the lung parenchyma, EPP may be a more suitable operation than PD if all other factors are equal. We explored the potential of immersive volume visualization environment in the calculation of pleural mesothelioma tumor volume utilizing both dynamic range of the data and the surgeon 3D input.

We have designed a parallel-processing, open-source, Linux based visualization software running on a nine machine computing cluster. CT datasets are loaded without pre processing. Volume rendering duties are distributed among eight "slave " nodes. Four slave nodes exclusively reconstruct each eye perspective producing 3D images. The difference between the two perspective reconstructions is an interoccular virtual camera offset that simulates binocular stereovision. The remaining "master " node assembles both camera perspective renderings. The virtual environment GUI shares features common to clinical radiology workstations but adds volumetric computation tools relying on tissue density based discrimination and voxel summation of user defined anatomical regions in 3D space.

Initial observations suggest that the 3D stereoscopic reconstruction and interactive manipulation of CT data sets improves surgical planning by providing superior visualization of tumor regions as compared to the traditional analysis using 2D imaging. Combined with our novel perceptual colorization algorithm, volumetric analysis potentially allows us to accurately determine the extent of pleural mesothelioma with efficiencies difficult to duplicate using grayscale, multiplanar CT images. The 3D stereoscopic volume rendering is both feasible and desirable and provides a powerful new tool for surgical planning. This technology has the potential to improve pre and postsurgical evaluation to assess response to treatment.

P16-3

Prognostic value of ^{18}F-FDG PET/CT in malignant pleural mesothelioma

Cecilia Bech[1], Jens Soerensen[1], Annika Loft[2]

[1]Department of Oncology, Finsen Centre/National University Hospital, Denmark, [2]Dept of Clinical Physiology, University of Copenhagen, Copenhagen, Denmark

BACKGROUND AND PURPOSE: Malignant pleural mesothelioma (MPM) is a rare disease with a poor prognosis, low response rates, and relapse is frequent. Better methods for choosing the right treatment regimen for the right patient are needed, and we need specific prognostic and predictive markers. 18F-fluoro-2-deoxy-D-glucose (FDG) PET/CT-scan is frequently used for staging in MPM and has proved superior to other imaging modalities in correct staging. The purpose of this study was to investigate prognostic value of 18F-FDG-PET/CT in patients with MPM and to correlate the standardized uptake value (SUVmax) with histopathological subtype, stage, response to treatment, and overall survival.SUV has been suggested as a prognostic and predictive marker in other cancer types, e.g. NSCLC, but not thoroughly investigated in MPM.MATERIALS AND METHODS: Retrospective review of pretreatment 18F-FDG PET/CT scans in inoperable MPM patients referred for palliative chemotherapy with cisplatin and vinorelbine. Measurement of SUVmax values of the metabolically most active tumor site were assessed and compared to clinical outcome.RESULTS:During 27 months in 2004-2006 12 patients with histopathologically biopsy proven MPM had pretreatment PET/CT scan (11 male, 1 female, median age 62 years). For patients with a SUVmax in the lower quartile survival was sigificantly better as 3 out of 3 patients were alive after 2 years compared to 0 out of 9 in the high-SUV-group ($p<0.01$).Two out of 3 patients with low SUV achieved a partial response to chemotherapy vs only 1 out of 9 in the high-SUV-group ($p=0.07$).CONCLUSIONS:A low SUVmax on pretreatment PET/CT is a predictor for long term survival in patients with malignant pleural mesothelioma and may also be predictive for treatment response.

P16-4

A fusion image of PET and pleural plaque 3D-CT for the early detection of malignant pleural mesothelioma in asbestos disease

Tadaaki Miyamoto[1], Toru Matsumoto[1], Kyosan Yoshikawa[4], Shinji Kato[2], Gen Satou[3], Hajime Kato[2]

[1]Chiba Prefecture Medical Association for Workers, Japan, [2]Funabashi Futawa Hospital, [3]Chiba Kensei Hospital, [4] Research Center Hospital for Charged Particle Therapy, National

PET has been reported to be a useful diagnostic method for malignant pleural mesothelioma (MPM). And CT has been shown to be useful to detect pleural plagues caused by asbestos exposure. Recently, we could develop a 3D display of pleural plague by reconstructing MDCT data. This volumetric CT technique was found to improve the accuracy of plague detection, and visualized the extent of plagues with their form and size on chest wall and diaphragm. In the present study, we challenge to create a fusion image of PET and 3D-CT of pleural plague for the early detection of MPM. A case with MPM and two cases with benign pleural plagues were tried to perform PET/CT scan using. ^{11}C-methionine. With use of AZE workstation (WS), a 3D image of the pleural plagues was reconstructed by MDCT, and a new fusion image of PET and pleural 3D-CT could be made successfully for all cases. In the MPM case, a positive PET area was shown on the pleural plague at the right anterior upper mediastinum. On the other hand, the two benign cases never founded a positive PET area on their pleural plagues. These fusion images will be presented. The usefulness of this method is awaited for further clinical trials, however.

P16-5

CT findings of benign asbestos pleural effusion

Katsuya Kato[1], Kenichi Genba[2], Takumi Kishimoto[2], Shinichi Fujimoto[2], Susumu Kanazawa[1]

[1]The Department of Radiology, Okayama University Hospital, Japan, [2]Okayama Rosai Hospital

PURPOSE; To present an adequate number of CT findings of benign asbestos pleural effusion. METHOD AND MATERIALS; The subjects were 36 patients with benign asbestos pleural effusion. In all patients thoracoscopic biopsy was conducted to exclude the possibility of malignant diseases including mesothelioma. All of the patients were male, aged 54 to 85 years (74 years on average). An occupational asbestos-exposure was found in 34 patients. RESULTS; Asbestosis was evident in 17% (6/36), pleural plaque in 92% (33/36), rounded atelectasis in 44% (16/36), and diffuse pleural thickening in 25% (9/36). Maximum thickness of the pleura was less than 5 mm in 64% (23/36), 5 mm and more in 36% (13/36), and more than 10 mm only in one case. No pleural irregularity was found in 22% (8/36), mild irregularity in 72%(26/36), and severe irregularity in 6% (2/36), and no mass formation was observed. Regarding the site of pleural irregularity (which overlapped), it was found on the mediastinal side in 30% (8/36), and in the lung base in 91%(32/36), and no irregularity was found in the interlobar region. An irregularity disappeared on CT in 7 of 8 patients who had exhibited irregularity on the mediastinal side during the 6month observation period and no change was found in only one patient. CONCLUSION; The occurrence rate of asbestos-related pulmonary or pleural lesions was higher in mesothelioma cases. About 6% of the patients showed highly irregular findings, and pleural thickening on the mediastinum, which is considered to be a characteristic of mesothelioma, were observed in 30% of the patients. However, these findings often disappeared during observation, and no patients with interlobar pleural irregularity were found, which can be used for discrimination from mesothelioma.

P17

Imaging-Ⅱ

P17-1

Physiologic and computed tomographic predictors of outcome following extrapleural pneumonectomy for mesothelioma

Saba Dhiya[1], Gretchen Deluke[4], Jonathan M. Barry[4], Christia P. Panizales[4], Aya Mitani[1,4], Hiroto Hatabu[5], David Sugarbaker[3], Gyorgy Frendl[1,4], George Washko[2]

[1]Department of Anesthesiology, Brigham&Women's Hospital, USA, [2]Department of Medicine, Division of Pulmonary Medicine, Brigham&Women's Hospital, Boston, MA, USA, [3]Department of Thoracic Surgery, Brigham&Women's Hospital, Boston, MA, USA, [4]STAR (Surgical Critical Care Unit Translational Research) Center, Brigham&Women's Hospital, Boston, MA, USA, [5]Department of Radiology, Brigham&Women's Hospital, Boston, MA, USA

Introduction: Post-operative complications of extrapleural pneumonectomy (EPP) include pulmonary hypertension, right ventricular (RV) dysfunction, and respiratory failure. While the exact pathogenesis of these events is not clear, there are no reliable peri-operative techniques to predict their occurrence.Objective: We primarily sought to evaluate the relationship between pulmonary vasculature cross-sectional area of small pulmonary vessels (CSA) on pre-operative high resolution chest computed tomography (HRCT) with the degree of pulmonary hypertension and length of ICU stay (ICU-LOS). We further examined the relationship of immediate post-operative invasive measures of pulmonary vascular disease (prior to any evidence of cardiac decompensation) to ICU-LOS. Methods: We performed a retrospective review of 39 consecutive patients from our comprehensive database that had pre-operative HRCTs and underwent EPP for malignant pleural mesothelioma in our institution between November 2008 and February 2010. In the pre-operative HRCT, the CSA<5mm2 was measured on the non-operative side. These measurements were completed prior to chart review in order to remain blinded to the clinical data. Invasive measures of mean pulmonary artery pressure (mean PAP) and pulmonary vascular resistance (PVR) were collected and in addition, ICU-LOS. Results: We found a tendency for CSA to be inversely related to post-operative PA pressures and PVR (R=-0.51, p=0.06 and R=-0.45, p=0.13 respectively). There was no relationship between CSA and ICU-LOS. Immediate post-operative measures of both MPAP and PVR were predictive of ICU-LOS (R=0.51, P=0.04 and R=0.58, P=0.02 respectively). Conclusions: In our pilot study, we demonstrate a tendency for association between CSA and physiological indicators of pulmonary hypertension. Further work is in progress in a larger cohort of patients to explore the predictive value of CSA for clinically important measures of outcome following EPP.

P17-2

Assessment of therapeutic response using FDG PET in patients with malignant pleural mesothelioma

Bumpei Yamaguchi[1], Hidenori Takizawa[1], Isano Hase[1], Toshiyuki Saginoya[2], Yoshiki Ishii[3]

[1]Department of Pulmonary Medicine, Southern Tohoku General Hospital, Japan, [2]Department of Radiology, Southern Tohoku General Hospital, Fukushima, Japan, [3]Department of Pulmonary Medicine and Clinical Immunology, Dokkyo Medical University School of Medicine, Tochigi, Japan

PURPOSE: We evaluated the ability of 2-deoxy-2-[18F]fluoro-D-glucose (FDG) positron emission tomography (PET) in the assessment of therapeutic response in patients with malignant pleural mesothelioma (MPM). PROCEDURES: FDG PET studies were performed before and during chemotherapy (every 3 courses) in 4 patients with MPM. The standardized uptake value (SUVmax) was measured. Tumor response after chemotherapy (combination of platinum and pemetrexed) was evaluated according to the modified Response Evaluation Criteria in Solid Tumors (RECIST). RESULTS: Median age was 56.2 (range 46-72). Male / female: 3 / 1. Epithelial / sarcomatoid: 3 / 1. Stage III /stage IV: 3 / 1. CDDP + PEM / CBDCA + PEM: 3 / 1. PR / SD: 3 / 1. Mean percent change of SUVmax in three PR patients was 0.21. Percent change of SUVmax in SD patient was 0.54. Mean percent change of thickness in pleural lesions measured for evaluating modified RECIST in the PR patients was 0.40. Conclusions: These findings suggest that assessment of therapeutic response using the percent change of SUVmax is more sensitive than that using thickness of the pleural lesions in patients with MPM.

P17-3

A simple scoring system to measure the volume of disease in malignant pleural mesothelioma

Aparna Deshpande, Sumit Gupta, Vimal Raj, Liz Darlison, James Entwisle

Gelnfield Hospital, University Hospitals of Leicester, UK

Purpose: Our aim in this pilot study is to propose a scoring system where the burden of mesothelioma can be easily established. Volume of disease is thought to be an important prognostic indicator.Currently,there is no universally agreed grading system whereby mesothelioma can be scored in a consistent and reproducible manner. Methods: Pleural thickness was recorded at the level of the aortic arch, right pulmonary artery and at the level where the IVC enters the right atrium. The affected lung was divided into 4 quadrants as follows: anteromedial, posteromedial, anterolateral and posterolateral. Pleural thickness in each quadrant was measured as follows. A grade of 0 for no pleural thickness, 1 for thickness less than 5mm, 2 for thickness 5 to10mm, 3 for over 10mm thickness and 4 for focal masses. Scores at each level were added up to give a final score. Scans of 17 consecutive patients diagnosed with mesothelioma in the year 2009 were studied by a Consultant thoracic radiologist and a trainee radiologist. Results: There were 4 quadrants at each of the three levels in 17 patients, giving a total of 204 quadrants. Agreement between the two observers using a weighted Kappa with qaudrantic weights, was calculated at 0.7174(95%CI, 0.5224 to 0.9124),which is substantial (range 0.61 to 0.8). Conclusion: Scans performed in arterial phase were thought to be more difficult to interpret in view of difficulty in differentiation of fluid and thickening. Agreement obtained shows this to be a promising technique. We intend to repeat the process with scans performed in the modified portal venous phase. This has real potential as a prognostic indicator and assessing response in clinical trials.

P17-4

In vitro and *in vivo* photodynamic diagnosis using 5-aminolevulinic acid in malignant mesothelioma

Abdellah Hamed Khalil[1], Hiromitsu Takizawa[2], Kazuya Kondo[1], Hisashi Matsuoka[2], Hiroaki Toba[2], Yasushi Nakagawa[2], Koichiro Kenzaki[2], Shoji Sakiyama[2], Soji Kakiuchi[3], Saburo Sone[3], Akira Tangoku[2]

[1]Department of Oncological Medical Services, Institute of Health Biosciences,The University of Tokushima Graduate School, Japan, [2]Department of Thoracic, Endocrine Surgery and Oncology. Institute of Health Biosciences,The University of Tokushima Graduate School, Japan, [3]Department of Respiratory Medicine and Rheumatology, Institute of Health Biosciences,The University of Tokushima Graduate School, Japan

Background: It is known that endogenously synthesized protoporphyrin IX (PpIX) following the administration of 5-aminolevulinic acid (5-ALA) is an effective photosensitizer for photodynamic diagnosis (PDD). Aim of the study: Was to test the in vivo and in vitro susceptibility of human mesothelioma cells to photodynamic diagnosis using 5-aminolevulinic acid as a photosensitizer.Materials and Methods: Three human mesothelioma cell lines (MSTO-211H, Y-MESO14 and NCI-H290) were incubated with 0.03% 5-ALA in serum free RPMI-1640 for 4 hours. PpIX fluorescence was detected using a fluorescence microscope. The intensity of fluorescence in the images was measured using Metamorph software. Pleural carcinosis was induced in 8 - 9 severe combined immunodeficiency disease (SCID) mice for each cell line to test the efficacy of PDD in vivo. Photosensitization was achieved by oral administration of 400 mg/kg 5-ALA solution. First we used conventional white light and subsequently blue light (380 - 449 nm) to excite PpIX-induced fluorescence. Tumor samples were surgically removed en bloc with surrounding tissue following in vivo imaging for histopathological examination.Results: In vitro experiment showed clear red fluorescence in the tumor cells. The mean fluorescence intensity of the three cell lines was 92.31 ± .69 for Y-MESO14, 165.16 ± 12.91 for NCI-H290 and 142.51 ± 26.85 for MSTO-211H. Conventional white light compared with fluorescence light showed (8.7 ± 5.8 vs 14.3 ± 8.8; P = 0.005) intrathoracic tumor foci in the Y-MESO14 group, (18.4 ± 6.3 vs 20.0 ± 5.7; P = 0.154) foci in the NCI-H290 group and (11.8 ± 2.1 vs 14.4 ± 3.5; P = 0.085) foci in the MSTO-211H group, which proved histopathologically. Conclusion: Human mesothelioma cells demonstrate marked and specific fluorescence after the application of 5-ALA, making PDD possible.

P18

Treatment- I

P18-1

Determination of resectability aided by ventilation and perfusion imaging in patients undergoing extrapleural pneumonectomy (EPP) for malignant pleural mesothelioma

Tamara Tilleman[1,2], Ritu Gill[2,3], William Richards[1,2], William Powell Jr.[1,2], Leonard Friedman[1,2], Raphael Bueno[1,2], Hiroto Hatabu[2,3], David Sugarbaker[1,2]

[1]Division of Thoracic Surgery, Department of Surgery, Brigham and Women's Hospital, USA, [2]International Mesothelioma Program, Boston, MA, [3]Department of Radiology, Brigham and Womens Hospital, Boston, MA, USA

Objective: Current methods (CT, MRI, echo, cMED) overestimate resectability of malignant pleural mesothelioma. We hypothesize that complementary information from ventilation and perfusion quantification (V/Q) may improve accuracy. Materials and Methods: In an IRB-approved retrospective study, we reviewed 208 consecutive patients who underwent EPP and had V/Q imaging. Demographic variables included histological subtype and surgical results. Imaging variables included V/Q and CT-derived tumor and lung volumes. 95/208 patients with V/Q data (45.7%) also had lung and tumor volume data. Chi square test was used for categorical variables; Wilcoxon rank sum for continuous variables; Spearman method for correlations.Results: Median age was 63.0 years (30.5-81.7), 174 were male (83.7%), 124 had epithelial histology (60%), 152 were resectable (73%). Resectability did not vary with cell type. Resectable patients had significantly higher ipsilateral ventilation (resectable: median=26.5%; range=0.0-95.5 vs. unresectable: median=17.5%; range=3.6-41.3, p=0.0005), and significantly higher ipsilateral perfusion (resectable: median=28.35 %; range=0.9-98.3 vs. unresectable median=21.35%; range=3.0-43.0, p=0.0004). Differences were not significant between sex, age or laterality. There was no difference in median ipsilateral ventilation or perfusion by cell type. Median contralateral ventilation and perfusion differed between resectable and unresectable patients (n=56) (contralateral-ventilation: 72.75 vs 82.5, p=0.0005; contralateral-perfusion: 71.56 vs 78.65, p=0.0004). Ipsilateral ventilation-to-perfusion ratio was not statistically different (p=0.4118).Ipsilateral ventilation ≤ 25% of predicted was 2.5 times more likely to be unresectable (p=0.0055, se=70%, sp=52%). Ipsilateral perfusion ≤25% of predicted was 3.1 times more likely to be unresectable (p=0.0005, se=66%, sp=61%).Ipsilateral V/Q values correlated significantly with lung and tumor volume (ventilation-tumor volume: -0.35, p=0.0006; ventilation-lung volume: 0.53, p<0.0001; perfusion-tumor volume: -0.44, p<0.0001; perfusion-lung volume: 0.50, p<0.0001).Conclusions: Reduced ventilation and perfusion are indicative of unresectability in mesothelioma. V/Q data are inversed to CT-derived tumor volume. A prospective study is required to validate whether preoperative V/Q can aid assessment of resectability prior to surgery.

P18-2

Surgical treatment for malignant pleural mesothelioma

Satoru Moriyama[1,2], Hiroshi Niwa[2], Masayuki Tanahashi[2], Haruhiro Yukiue[2], Eriko Suzuki[2], Hiroshi Haneda[2], Naoko Yoshii[2]

[1]Oncology, Immunology and Surgery, Nagoya City University Medical School, Japan, [2]Division of Thoracic Surgery, Respiratory Disease Center, Seirei Mikatahara General Hospital, Shizuoka, Japan

Objective. Early diagnosis and complete resection are needed for the improvement of survival in patients with malignant pleural mesothelioma. We investigated the outcome of surgical treatment for malignant pleural mesothelioma. Subjects. Between 1998 and 2009, 26 patients with malignant pleural mesothelioma were evaluated in our hospital. A retrospective review was performed in 12 patients received surgical treatment for malignant pleural mesothelioma. Extrapleural pneumonectomy was performed for patients with histologically confirmed malignant pleural mesothelioma, who were considered to have potentially resectable disease by diagnostic imaging and physiologic screening. Results. Of the 12 patients, eight were male and four were female, with a mean age of 58.9 years old. A past history of exposure to asbestos was identified in three patients (25%). Thoracoscopic pleural biopsy was the most effective method for diagnosis in 10 patients. It required 5.9 months (median) from the first visit to establish the diagnosis. Extrapleural pneumonectomy was performed in 10 patients and pleurectomy/decortication was in two patients. Complete resection was performed in seven patients (58.3%). There were no severe postoperative complications and operative deaths. Preoperative chemotherapy was given in one patient. Hyperthermo-chemotherapy during surgery was added in five patients. Postoperative chemotherapy was given in four patients, and radiotherapy in two patients. Histologically, nine patients were epithelial type and three were biphasic type. According to the IMIG pathological stage, three patients were Stage I, two were Stage II, six were Stage III, and one was Stage IV. The postoperative 5-year survival rates among all patients were 33.3%. The median survival time was 26.4 months. The postoperative 5-year survival rates for patients received complete resection were 57.1%, and that for Stage I, II were 100%. Conclusion. Extrapleural pneumonectomy promises good survival for Stage I, II malignant pleural mesothelioma. Thoracoscopic pleural biopsy is essential to detect early stage malignant pleural mesothelioma.

P18-3

Role of pleural pressure control following extra-pleural pneumonectomy during early postoperative days

Kenji Okita[1,2], Junya Kawazoe[2], Mitsuru Niwa[2], Koji Chihara[2]

[1]Thoracic Surgery Department, Kyoto Katsura Hospital, Japan, [2]Division of Thoracic Surgery, Shizuoka City Shizuoka Hospital, Shizuoka,Japan

A 56-year-old man who had malignant pleural mesothelioma diagnosed incidentally by biopsy of multiple nodules of the parietal pleura during the operation for the right spontaneous pneumothorax was treated with 4-course chemotherapy of cisplatin and pemetrexed. Subsequently, we performed a right extrapleural pneumonectomy (EPP). The amount of hemorrhage during the operation was 2423ml, and postoperative hemorrhage from the chest tube on POD1 was 1095ml. He suffered from severe coughing, atrial fibrillation (Af) by 170-180/min,and hypotension on POD1. A chest X-ray showed mediastinal shift to the left, however, no air-leakage from the tube was found. A chest CT confirmed a safe stump of the right main bronchus covered with an intercostal muscle flap. We tried to treat Af with defibrillator cardioversion, landiolol, and other medicines, but none of them was effective. To open the drainage tube clamped was an only way to solve the complication. The pleural pressure measured was as high as +15 cmH2O. After some amount of hemorrhage was drainaged, the tube was clamped. The amount of hemorrhage on POD1, 2, 3 were 1095, 535, 375ml, respectively, and the tube was withdrawal on POD4. We succeed repetitive thoracentesis to aspirate small amount of pleural fluid and air on happenings of Af next two weeks. The right pleural pressure was managed by it from more positive pressure to zero to -5 cmH2O, and it reached 0 cmH2O on POD29. He was no longer suffered from Af or tachycardia. It is very important to manage pleural pressure following EPP as physiological values, because the transmural pressure through the right atrium affects hemodinamics greatly.

P18-4

Discrepancy between clinical and pathological stages in patients undergoing extrapleural pneumonectomy for malignant pleural mesothelioma

Masaki Hashimoto[1], Teruhisa Takuwa[1], Yoshitomo Okumura[1], Nobuyuki Kondo[1], Seiji Matsumoto[1], Hayato Orui[1], Kazu Shiomi[1], Fumihiro Tanaka[1], Ikuko Torii[2], Torhu Tsujimura[2], Noriaki Tsubota[3], Kazuya Fukuoka[4], Takashi Nakano[4], Seiki Hasegawa[1]

[1]Department of Thoracic Surgery, Hyogo College of Medicine, Japan, [2]Division of Molecular Pathology, Department of Pathology, Hyogo College of Medicine, Hyogo, Japan, [3]Department of Thoracic Oncology, Hyogo College of Medicine, Hyogo, Japan, [4]Division of Respiratory Medicine, Dept. of Internal Medicine, Hyogo College of Medicine, Hyogo, Japan

Backgrounds:
As extrapleural pneumonectomy (EPP) for malignant pleural mesothelioma (MPM) suffers from poor risk/benefit ratio, patient selection based on precise preoperative assessment is essential. In comparison with other solid tumors, however, clinical stage is often underestimated in MPM.
Methods:
Consecutive 35 MPM patients (15 right and 20 left side) enrolled for EPP in our institution from July 2004 to April 2010 were retrospectively reviewed. Preoperative chest CT, FDG-PET, and brain MRI were performed in all patients. Mediastinoscopy and/or laparoscopy were performed if necessary. We analyzed discrepancy between clinical and pathological stages in the above cases.
Results:
EPP was completed in 31 cases (88.5%), but thoracotomy revealed unresectable (T4) tumor in 4 cases (11.5%). Pathological stage proved to be the same as clinical stage in only 9 cases (25.7%). In no case clinical stage was overestimated than pathological stage. On the other hand, underestimation of clinical stage was seen in a total of 26 cases (74.3%) through T factor (23 cases, 65.7%) and N factor (14 cases, 40%).
Conclusions:
Even after vigorous preoperative assessment, underestimation of MPM stage is rather common. Possible underestimation should be taken into account in consideration of surgical indication for MPM.

P18-5

BNP is a useful biomarker for cardiac condition after EPP

Masaki Hashimoto[1], Teruhisa Takuwa[1], Yoshitomo Okumura[1], Seiji Matsumoto[1], Nobuyuki Kondo[1], Fumihiro Tanaka[1], Hayato Orui[1], Kazu Shiomi[1], Noriaki Tsubota[2], Kazuya Fukuoka[3], Takashi Nakano[3], Seiki Hasegawa[1]

[1]Department of Thoracic Surgery, Hyogo College of Medicine, Japan, [2]Department of Thoracic Oncology, Hyogo College of Medicine, Hyogo, Japan, [3]Division of Respiratory Medicine, Dept. of Internal Medicine, Hyogo College of Medicine, Hyogo, Japan

Backgrounds:
Extrapleural pneumonectomy (EPP) is frequently complicated with postoperative cardiac events such as artrial fibrillation and right heart failure.
Brain natriuretic peptide (BNP) and N-terminal prohormone BNP (NT-proBNP) are known as useful biomarkers for postoperative cardiac condition after major pulmonary resection. Therefore we hypothesized that the measurement of BNP or NT-proBNP may contribute to the management after EPP.
Methods:
In a total of 8 patients (4 right side and 4 left side), measurement of BNP or NT-proBNP was performed before, 2weeks after, and 4weeks after EPP, respectively. All patients were given digoxin at a dairy doses of 0.25mg when starting diet after EPP.
Results:
The mean values of NT-proBNP before, 2 weeks after, and 4 weeks after EPP were 72.75 (28-102), 1019.9 (240-3661), and 454.8 (200-680) . 3 cases complicated heart failure after EPP. NT-proBNP value was higher in three patients complicated with postoperative heart failure than in patients without complication ; 58.1 vs 81.5 before EPP, 1710 vs 605.9 at 2 weeks after EPP, and 587.5 vs 375 at 4weeks after EPP.
Conclusions:
NT-proBNP peaked at 2 weeks after EPP. Patients with postoperative heart failure showed higher NT-proBNP value than patients without complication. The measurement of NT-proBNP may be useful for evaluating cardiac condition after EPP.

P18-6

Intrapleural perfusion hyperthermo-chemotherapy with cisplatin in patients with malignant pleural mesothelioma

Hirozo Sakaguchi[1], Hironori Ishida[1], Hiroyuki Nitanda[1], Nobuhiro Yamazaki[1], Hiroyoshi Tusbochi[1], Nobuhiro Koyama[2], Hideo Oya[2], Yoshitake Murayama[2], Kunihiko Kobayashi[2], Koichi Kaneko[1]

[1]Department of General Thoracic Surgery,Saitama Medical University International Medical Center, Japan, [2]Department of Respiratory Medicine, Saitama Medical University International Medical Center, Saitama, Japan

Backgrounds: The preferred treatment for patients with malignant pleural mesothelioma (MPM) has not been determined. In the early stage, MPM reveals locally spread disease in the pleural cavity. Intensive local regulations are needed for multi-modality therapy, whether patients will be treated with EPP or not. In order to obtain adequate control of malignant effusion and expect temporary anti-tumor effect, we have introduced intrapleural perfusion hyperthermo-chemotherapy (IPHC) with cisplatin.**Patients and Methods:** Thirteen patients with MPM underwent IPHC. At the time of pleural incision biopsy with thoracoscope, perfusion was performed. Cisplatin was added when the temperature stabilized to a mean of 42.5° C. Dosages of cisplatin used were 80mg/m2. A IPHC was performed for 60 minutes under total lung ventilation. Complications, control of pleural perfusion, and treatment followed by IPHC were studied.**Results:** Median age was 67 years (range, 56-79). Eleven patients were male. Ten patients had epitherial tumor, and 3 had mixed histology. Nine had left-sided disease. There were no serious clinical complications associated with this procedure. Four had temporary mild rise of serum creatinin (Grage1) and 5 had mild nausea (Grage1). Duration of tube drainage were 7.0±0.8 day. The pleural effusion was well controlled in all patients. Adjuvant chemotherapies (CT) were performed immediately in all patients. Five patients were treated by EPP after 2 course of CT. Four of 5 were alive with no recurrence lesion (32M,31M,10M,2M after IPHC). Of six inoperable patients,4 were died with progression (24M,24M,14M,10M after IPHC). **Conclusions:** IPHC with cisplatin is easy to perform, and relatively safe. This method had brought an ideal pleural adhesion. IPHC may offer excellent local control for patients with MPM as a part of multi-modality therapy.

P19

Treatment-Ⅱ

P19-1

Bronchopleural fistula after extrapleural pneumonectomy for pleural mesothelioma

Kazu Shiomi[1], Nobuyuki Kondo[1], Seiji Matsumoto[1], Masaki Hashimoto[1], Teruhisa Takuwa[1], Yoshitomo Okumura[1], Hayato Orui[1], Fumihiro Tanaka[1], Munefumi Tomomatu[1], Noriaki Tsubota[2], Kazuya Fukuoka[3], Takashi Nakano[3], Seiki Hasegawa[1]

[1]Department of Thoracic Surgery, Hyogo College of Medicine, Japan, [2]Department of Thoracic Oncology, Hyogo College of Medicine, Hyogo, Japan, [3]Division of Respiratory Medicine, Dept. of Internal Medicine, Hyogo College of Medicine, Hyogo, Japan

Bronchopleural fistula (BPF) following extrapleural pneumonectomy for malignant pleural
mesothelioma(MPM) is not only a serious and frightening complication with a high mortality rate, but
associated with a prolonged hospital stay and the discontinuance of a planed therapy. Of 32 patients who had undergone the EPPs for MPM between April 2004 and March 2010, we experienced 3 cases of BPF. They were successfully treated by the different ways, respectively. One patients was treated with endoscopic submucosal injection of OK432 and basic fibroblast growth factor (bFGF). The second patients was treated by direct closure of bronchial stump with covering of latissimus dorsi muscle flap. The third patient who developed empyema required open window thoracotomy followed by omentplasty, muscle flap and thoracoplasty. Based on the above experience, we concluded that early intervention is essential, once BPF is suspicious after EPP.

P19-2

The first postoperative serum creatinine value predicts the development of sustained kidney injury in patients undergoing surgical treatment for malignant

K Annette Mizuguchi[1], Aya Mitani[1], Sushrut S Waikar[2], Christia Panizales[4], Gretchen Deluke[4], Joseph V Bonventre[2], Tamara R Tilleman[3], David J Sugarbaker[3], Gyorgy Frendl[1,4]

[1]Department of Anesthesiology, Perioperative and Pain Medicine, Brigham and Women's Hospital, USA, [2]Brigham and Women's Hospital, Renal Division, [3]Brigham and Women's Hospital, Department of Thoracic Surgery, International Mesothelioma Program, [4]Brigham and Women's Hospital, Surgical Critical Care Unit Translational Research Center

Cancer patients admitted to the intensive care unit with acute kidney injury (AKI) have higher mortality rates than non-cancer patients (Curr Opin Crit Care 2008; 14: 635-646; Crit Care 2005; 9: R700-709). However, it is unclear how AKI relates to the development of sustained kidney injury present 2-4 weeks after surgery. Sustained postoperative kidney injury has serious implications for cancer patients as many require additional treatment and follow-up diagnostic tests with potentially nephrotoxic agents. The identification of early biomarkers that predict sustained kidney injury is desirable as our current ability to detect AKI in a timely manner is limited. **Hypothesis**: small acute changes in serum creatinine (sCr) measured immediately after surgery and during the subsequent 24-48 hours, will predict development of sustained kidney injury. **Methods**: Retrospective study of patients who underwent extrapleural pneumonectomy.**Results**: Comparison of mean sCr values for patients who developed sustained kidney injury and those who did not develop it show a distinct time course and pattern of sCr elevation that persisted up to 100 days. We found that patients with a sCr increase >=0.1 mg/dl immediately after surgery are at increased risk of developing sustained kidney (OR 3.01, CI95% [1.49-6.06]). Similarly, sCr elevation of >=0.3 mg/dl during the first 24-hour and the subsequent 24-hour period reliably predicted sustained kidney injury.**Conclusions**: Small sCr changes in the immediate postoperative period are predictive of sustained kidney injury. As one of the earliest diagnostic tests described thus far in identifying patients prone to sustained kidney injury, our sCr elevation-based diagnostic strategy will enable enrollment of patients into well-powered interventional studies to test new strategies for the salvage of kidney function.

P19-3

Postoperative management of 25 patients undergoing extrapleural pneumonectomy for malignant mesothelioma

Takeshi Ide, Yuichirou Ikeda, Kenta Takeda, Shinichi Nishi

Division of Intensive Care Unit, Hyogo College of Medicine, Japan

Introduction: Extrapleural pneumonectomy (EPP) is a formidable surgical procedure for patients with malignant mesotherioma. The survival rate and the quality-of-life benefit may depend on the perioperative management. The characteristic postoperative complications in EPP are 1) high probability of dysrhythmias, 2) greater blood and fluid loss compared to simple pneumonectomy, 3) potential for hemodynamic instability related to cardiac herniation through pericardial window and its patch.
Methods: Twenty-five patients received the EPP and admitted to our ICU from 2004 to 2009 were retrospectively analyzed. The following data were collected: age, gender, the duration of mechanical ventilation, the administration duration of inotropic agents, the account of drainage and the characteristic postoperative complications.
Results: The average age was 61 years. The number of operated side was 12 on right and 13 on left. The length of stay in ICU was 4.3 days. The hours of mechanical ventilation were 29 hours. The administration duration of inotropic agents was 7.3 days. On postoperative first day, the account of drainage was 1025ml and the volume of infusion is 2755ml as crystalloid and 872ml as colloid. Eight in the 25 patients suffered from dysrhythmias (mainly atrial fibrillation), two patients suffered from diaphragmatic hernia and 4 patients fell into the heart failure.
Conclusions: The postoperative management of 25 patients received EPP for mesotherioma were evaluated. Compared to respiratory management, more delicate management of intravascular fluid and circulation were needed.

P19-4

Novel urinary biomarkers for the early detection of kidney injury following cytoreductive surgery and intracavitary cisplatin lavage for mesothelioma

Sushrut Waikar[1], Gyorgy Frendl[2,4], David Sugarbaker[3], Joseph Bonventre[1]

[1]Renal Division, Brigham&Women's Hospital, USA, [2]Department of Anesthesiology, Brigham&Women's Hospital, Boston, MA, [3]Department of Thoracic Surgery, International Mesothelioma Program, Brigham&Women's Hospital, Boston, MA, [4]STAR (Surgical Critical Care Translational Research) Center, USA

Acute kidney injury (AKI) is a common and severe postoperative complication in patients undergoing cytoreductive surgery with or without intracavitary cisplatin lavage for pleural mesothelioma. Serum creatinine (SCr) is the gold standard to diagnose AKI. However, in some cases SCr based diagnosis of AKI may take more than 24h after severe injury, leading to delayed diagnosis. A number of novel tubular injury biomarkers have been identified in animal models of ischemic and nephrotoxic kidney injury, and may permit early and accurate diagnosis of postoperative AKI. We have enrolled and measured urinary biomarkers pre- and post-operatively from 116 individuals undergoing cytoreductive surgery, 73 of whom received intracavitary cisplatin lavage (225 mg/m2). Post-operative AKI (defined as >50% rise in SCr) developed in 64 patients (29%), and 8 (7%) required renal replacement therapy. Peak post-operative urinary kidney injury molecule-1 (KIM-1) a type 1 transmembrane protein that is expressed at high levels in proximal tubule epithelial cells following ischemic or toxic injury were 22.7 ng/mg of creatinine in those with AKI and 7.8 ng/mg of creatinine in those without AKI. Other biomarkers currently under investigation include N-acetyl D glucosaminidase, neutrophil gelatinase-associated lipocalin, L-type fatty acid binding protein, interleukin 18, and vascular endothelial growth factor. We hypothesize that a panel of urinary tubular injury biomarkers will provide early and accurate diagnosis of AKI, enabling the prompt institution of renal protective strategies that would otherwise be significantly delayed using SCr for diagnosis.

P19-5

Replacement of the diaphragm by the latissimus dorsi muscle flap during mesothelioma resection

Tomasz Grodzki, Janusz Wojcik

Thoracic Surgery Department, Pomeranian Medical University, Szczecin, Poland

The goal was to assess the value of the latissimus dorsi muscle flap (LDMF) for diaphragmatic replacement during extended mesothelioma resection.26 patients of med. age 62 years were treated by extended resection due to malignant mesothelioma in the last 10 years. 8 of them had diaphragm replaced by the LDMF (5 on the right and 3 on the left). All others (control group: CG) had the diaphragm replaced by the artificial material. Technique of the mobilization of the flap was based on asymmetric division of the LDMF during posterolateral thoracotomy. The pedicled dorsal flap was transferred to the chest cavity through VIII or IX intercostal space and fixed to the ribs and pericardium by single stitches. An intraoperative and postoperative course was assessed and compared with CG regarding blood transfusions, complications related to the technique and functional recovery.There were no early postoperative deaths in both groups. The LMDF were planned for use in 10 cases but in two patients on the right side surgeon assessed their size as too small to provide replacement without tension and used artificial patch. All remaining 8 patients recovered without any severe complications related to the technique. 6 (75%) experienced lumbar subcutaneous haematoma (17%: 3 pts in CG). 2 patients (11%) from the CG experienced pleural empyema treated with good result. The average blood transfusion was 2 units (0 to 4, med. 2,2) and did not differ significantly from the CG (0 to 6, med. 2,3). The postoperative FEV1 and FVC decrease was similar in both groups. Conclusions: Replacement of the diaphragm by the pedicled LDMF is safe and does not affect postoperative functional recovery.

P19-6

Three modality treatment of malignant pleural mesothelioma

Evgeny Levchenko, Aleksey Barchuk, Konstantin Senchik, Mark Gelfond, Vasiliy Klimenko, Andrey Arseniev

Petrov Research Institute of Oncology, Russia

Aim of the study: Working out a new kind of complex treatment of local-expanded malignat pleural mesothelioma.Materials and methods. There were included 7 patients (2male and 5 female) from 35 to 63 years (mean, 52,7) with malignat pleural epithelioid mesothelioma T2-4N0-2M0. Treatment stages: at first, was performed video-thoracoscopy with photodynamic therapy (Fotoditazin 0,8-1,0 mg¥kg with semi-conduction laser length of wave 662 nm with power 300 mVt¥cm2) and intrapleural perfusion hyperthermo-chemotherapy (Cisplatin 200 mg¥ 90 min). A 28-34 days later 4 patients underwent extrapleural pneumonectomy and 2 underwent limited lung resection, pleurectomy¥decortications with intraoperative photodynamic therapy (Fotoditazin 0,8-1,0 mg¥kg with semi-conduction laser length of wave 662 nm with power 300 mVt¥cm2) and intrapleural perfusion hyperthermo-chemotherapy (Cisplatin 200 mg¥ 90 min). Results: Perioperative mortality rates was not observed. The median follow-up was 27.3 months from diagnosis. A median survival was 15 months. 1-year and 2-year survival rates were 71,4% and 22,9%, respectively. The conclusion: The role of that three-modality approach in treatment of malignant pleural mesothelioma is safe, but not clear and may warrant further research.

P20

Treatment-Ⅲ

P20-1

Adjuvant chemotherapy subsequent to extrapleural pneumonectomy for patients with malignant pleural mesothelioma is slightly benefical

Mamoru Ueda, Katsuhiko Aoyama, Kohei Tagawa, Masashi Goto, Kozo Asanuma

Department of Respiratory Surgery, Higashisaitama National Hospital, Japan

[Purpose] Trimodal treatment by a combination of preoperative chemotherapy, extrapleural pneumonectomy (EPP) and post-operative hemithoracic radiation is currently considered the standard therapy for resectable malignant pleural mesothelioma (MPP). However, such an aggresive protocol requires a long time of treatment and the outcome is reported to be poor. We have investigated the effectiveness of postoperative chemotherapy using cisplatin (CDDP) and pemetrexed (MTA) between 2007 and 2009 in six patients treated for MPP.[Patients] Four of these six underwent EPP with four courses of postoperative chemotherapy using CDDP and MTA. The other two patients were treated with chemotherapy alone because of supraclavicular or cervical lymph node involvement (Stage IV). Histopathological studies showed that five of six patients were diagnosed as having the epithelioid type while one was the biphasic type. The International Mesothelioma Interest Group classification of four resected was stage Ib in one and stage III in three. Four resected patients received first postoperative chemotherapy within sixty-six days (average 61 days) after EPP and they received four or more courses of chemotherapy uneventfully.[Results] 1.EPP with postoperative chemotherapy group : One patient was diagnosed as having local recurrence and died one year after EPP. Two were diagnosed as having regional recurrence (peritoneum and contralateral pericardium respectively) and one of two died after nine monthes. The other patient with peritoneal recurrence survived 2 years after EPP. One patients in stage Ib has survived without recurrence for one year after EPP to date. 2.Chemotherapy group : One patient died one year and four months after the initial chemotherapy and the other patient has survived six months to date. [Summary] Postoperative chemotherapy using CDDP and MTA is considered slightly effective for patients in stage III after EPP.

P20-2

Extrapleural pneumonectomy and medical treatment for malignant pleural mesothelioma (MPM): the role of pemetrexed

Matteo Incarbone[1], Giovanni Ceresoli[2], Edoardo Bottoni[1], Emanuela Morenghi[1], Paolo Zucali[3], Marta Scorsetti[3], Armando Santoro[3], Marco Alloisio[1]

[1]Thoracic Surgery, Istituto Clinico Humanitas, Italy, [2]Oncology, Humanitas Gavazzeni, Bergamo, Italy, [3]Oncology, Istituto Clinico Humanitas, Rozzano, Italy

Background: Trimodality therapy including neoadjuvant chemotherapy (CT), radical surgery and adjuvant radiotherapy (RT) has been proposed to improve survival in selected patients (pts) with MPM.**Methods**: Sixty consecutive pts submitted to extrapleural pneumonectomy (EPP) over a 9-year period were reviewed. Overall survival (OS) and disease-free survival (DFS) were analyzed, according to prognostic variables and to peri-operative treatments. **Results**: Forty-six pts were male (76.7%), with a median age of 61 years (range 35-72). Twenty-one pts (35%) had EPP alone; 24 (40%) had induction CT (in 20 cases including pemetrexed), followed by adjuvant RT in 13 cases; 10 pts had adjuvant RT only, and 6 pts received post-operative chemotherapy. Median hospital stay was 9 days (range 5-70). Thirty-day operative mortality was 1.7%; 27 pts (45%) had complications. Histology was epithelial in 49 cases (81.7%). Eleven pts were stage I-II (18.3%), 43 stage III (71.7%) and 6 stage IV (10%). In 35 patients nodes were negative (58.3%), 12 pts had pN1 (20%) and 13 pN2 disease (21.7%). Median OS (mOS) and DFS of the whole population were 22 and 11 months, respectively. OS was not influenced by stage, nodal status and histology in univariate analysis, but pts with pT1-3N0 epithelioid MPM had longer OS (mOS 29 vs 15 months, $p=0.04$). Pts treated with CT including pemetrexed had longer OS (mOS 29 vs. 14 months, $p=0.01$). Pts with epitheliod tumors and pT1-3N0 disease treated with pemetrexed-based CT had the longest OS (mOS 34 months, $p<0.001$).**Conclusions**: EPP can be performed with the same mortality of other major thoracic procedures, but the rate of complications remains high. Pemetrexed improves survival, especially in pts with pT1-3N0 epithelioid disease.

P20-3

Multimodality treatment with induction chemotherapy followed by an extrapleural pneumonectomy in patients with malignant pleural mesothelioma

Takuro Kometani, Kenji Sugio, Kaname Nosaki, Taro Ohba, Hiroaki Takeoka, Fumihiko Hirai, Hiromoto Kitajima, Masafumi Yamaguchi, Motoharu Hamatake, Takashi Seto, Yukito Ichinose

Department of Thoracic Oncology, National Kyushu Cancer Center, Japan

Purpose: Malignant pleural mesothelioma (MPM) remains associated with a poor outcome. We examined the results of multimodality therapy with cisplatin-based chemotherapy followed by extrapleural pneumonectomy (EPP) for MPM patients. **Patients and Methods:** Eligible patients had MPM of all histological types, an ECOG-PS of 0 to 1, adequate organ function (including predicted postoperative forced expiratory volume in 1 second $>600ml/m^2$), and a clinical stage T1-T3, N0-2, M0 disease that was considered completely resectable. Induction chemotherapy consisted of a cisplatin-based regimen followed by EPP. All patients with induction chemotherapy followed by surgery for MPM at our institution from 1995 through 2008 were retrospectively reviewed. **Results:** A total of 21 patients were suitable candidates. The patient demographics were as follows: median age, 54 years, male/female, 19/2, right/left, 12/9, PS 0/1, 15/6, clinical stage I/II/III, 11/7/3. The histology was epithelioid (n =14) or biphasic (n =7). The chemotherapeutic regimens included cisplatin/gemcitabine/vinorelbine (n=11), cisplatin/pemetrexed (n=6) or cisplatin (n =4). All 21 patients were intended for surgery. Sixteen patients (76.1%) underwent complete resection after induction chemotherapy. The postoperative mortality rate was 4.8%, and 5 patients (23.8%) had major postoperative complications. The pathological stage was stage I in 3 patients, stage II in 3, stage III in 10, and stage IV in 5. The overall median survival was 29.4 months, and the 2-year survival rate was 70.6%. In EPP patients, the overall median survival was 29.9 months and the 2-year survival rate was 75.0%. The 2-year survival rate was 88.9% in patients with epithelioid tumors and 57.1% in patients with biphasic tumors. Postoperative recurrence occurred in 11 patients (local in 10 and distant in 1), and the 2-year disease-free survival rate was 40.0%. **Conclusion:** Multimodality treatment with induction chemotherapy followed by EPP appears to be a feasible treatment, and it also contributes to a favorable outcomes in patients with MPM.

P20-4

Cancelled

P20-5

The study of the malignant pleural mesothelioma case that survived more than two years

Osamu Kawamata

Department of Surgery, Onomichi Municipal Hospital, Japan

Purpose: It is difficult to perform trimodality therapy in malignant pleural mesothelioma all cases, and we review our hospital long-term survival patients and think about a strategy by the practical medical treatment. Subject: 16 patients who made a definite diagnosis by thoracoscope biopsy in our hospital by April, 2008, and there were 15 male, 1 female(mean age, 63.8 years). There were eight epithelial types, 5 biphasic types and 3 sarcomatous types. 7 of 16 cases survived more than two years. Six cases of the epithelial type and one case of the biphasic type survived more than two years, but three sarcomatous types died for all cases less than 1 year. 5 of 7 cases were stage1b or 2. Two cases of the epithelial type were stage3. Chemotherapy was performed in 5 cases, and neoadjuvant chemotherapy+extrapleural pneumonectomy was performed in 2 cases. The hemithoracic radiation therapy did not enforce all cases. We performed neoadjuvant chemotherapy+extrapleural pneumonectomy in another case of the biphasic type, but died in one year. The case that survived more than three years is one case out of 7, and now 2 cases are living. Conclusion: It seemed that chemotherapy could expect survival more than two years for stage1 and 2 in epithelial type and biphasic type. It seemed that we could expect long-term survival when we performed extrapleural pneumonectomy for the case in epithelial type that a tumor reduced by chemotherapy. As for the biphasic type and the sarcomatous type, there were much progress cases and this seemed to be an unfavorable cause. The progress case cannot expect the life lengthening by the current treatment, and it seemed that it will be necessary to develop new treatment.

P20-6

Feasibility of establishing a multidisciplinary program devoted solely to the treatment of pleural diseases

Melissa Culligan, Daniel Sterman, James Stevenson, Keith Cengel, Stephen Hahn, Stephen Albelda, Corey Langer, John Hansen-Flashen, Joseph Friedberg

University of Pennsylvania, USA

Background: Pleural cancers, mesothelioma in particular, are cancers for which a nihilistic attitude generally pervades and no single therapeutic modality is effective. Innovative treatments are offered at the rare institutions where there is an individual with an interest in pleural cancers and those treatments tend to reflect that individuals focus. To the best of our knowledge, a true multidisciplinary program, dedicated solely to the treatment of pleural diseases, did not exist.Purpose: The purpose of this project was to determine if it would be possible to establish and sustain a multidisciplinary program devoted solely to treating patients with pleural diseases, the majority of whom presented with mesothelioma. Methods: In June 2008, The Penn Mesothelioma and Pleural Disease Program was formed. A mission statement was written and a morning biweekly meeting was established with representation from thoracic surgery, pulmonary medicine, medical oncology, radiation oncology, nursing and specialists from thoracic oncology research, gene therapy and photodynamic therapy. Patients, either new or established, were presented at the meeting, seen by the relevant specialists and then discussed again for a consensus recommendation.Results: In the first 6 months, 45 patients were evaluated by the Program team and the subsequent 6 month number of patients was 52, 64 and 72. The volume of patient presentations mandated increasing the frequency of meetings to weekly after one year. Approximately 90 percent of the patients presented with mesothelioma. Conclusion: In a tertiary care center, with a nucleus of individuals interested in pleural diseases, it is possible to form and sustain this type of program. Feedback, from both patients and referring health professionals, has been very positive. The program has resulted in enrollment of patients in protocols, plans for collaborative grant applications and collaborative clinical trials, all directed at moving the field forward and offering patients better care.

P21

Novel therapeutics- Ⅰ

P21-1

Replication-competent retrovirus vector-mediated suicide gene therapy achieves significant therapeutic efficacy against human malignant mesothelioma xenografts

Shuji Kubo[1], Yoshiko Kawasaki[1], Norie Yamaoka[1], Nobuyuki Terada[2], Haruki Okamura[1], Noriyuki Kasahara[3]

[1]Laboratory of Host Defenses, Institute for Advanced Medical Sciences, Hyogo College of Medicine, Japan, [2]Department of Pathology, Hyogo College of Medicine, Nishinomiya, Japan, [3]Department of Medicine, David Geffen School of Medicine, University of California Los Angeles, USA

Replication-competent retrovirus (RCR) vectors have been shown to achieve significantly enhanced tumor transduction efficiency and therapeutic efficacy in various cancer models. We and others have previously engineered RCR vectors for highly efficient delivery of suicide genes, and as the virus is intrinsically incapable of infecting post-mitotic normal cells, retrovirus spread after intratumoral injection is highly restricted to tumor tissue, particularly in immunocompetent hosts. In the present study, we hypothesized that RCR vector-mediated suicide gene therapy could be effectively applied to the treatment of malignant mesothelioma, a highly aggressive tumor with poor prognosis.

RCR vectors successfully infected and efficiently replicated in human malignant mesothelioma cell lines, as compared to non-malignant transformed mesothelial cells. In mice with pre-established subcutaneous tumor xenografts, the RCR-GFP showed robust spread throughout entire tumor masses by Day 12 after intratumoral administration of 1 x 104 total infectious units per 100 ul inoculum. Notably, no RCR infection was detectable in adjacent normal tissue. RCR-yCD showed efficient transmission of the yeast cytosine deaminase (yCD) suicide gene associated with replicative spread of the virus, resulting in efficient killing of malignant mesothelioma cells in a 5FC-dose dependent manner in vitro. After intratumoral injection of RCR-yCD followed by intraperitoneal administration of 5FC prodrug, RCR vector-mediated suicide gene therapy achieved significant inhibition of subcutaneous tumor growth, and significantly prolonged survival in the disseminated peritoneal model of malignant mesothelioma.

These data indicate that RCR vector-mediated suicide gene therapy may represent a highly useful new treatment strategy for malignant mesothelioma.

P21-2

FGK45 immune-based treatment may cure mesothelioma in mice with or without combination with viral therapy

Wei Xia[1], Agatha Labrinidis[2], Andreas Evdokiou[2], Mark Holmes[1,3], Paul Reynolds[1,3]

[1]Hanson Institute, Royal Adelaide Hospital, SA, Australia, [2]Discipline of Orthopaedics and Trauma, University of Adelaide, SA, Australia, [3]Department of Medicine, University of Adelaide, SA, Australia

Introduction Malignant pleural mesothelioma is not rare, and it is dramatically increasing worldwide. The clinical outcome for patients with this disease is extremely poor. Immune-based therapies for mesothelioma are conceptually attractive but to date have limited efficacy. We hypothesised that virus-induced inflammation and tumour cell destruction might enhance therapy. Immune-based (FGK45) and viral (Ad5Δ24RGD) strategies were assessed.

Methods Mouse cell line AE17 was stably transfected with a construct containing the luciferase reporter gene (AE17-SFG). C57Black6 mice were injected intrapleurally with AE17-SFG cells. When all mice developed detectable tumours, PBS, FGK45 alone or FGK45 combined with Ad5Δ24RGD were given to C57Black6 mice. Tumour growth in the pleural space was detected by systemic administration of luciferin followed by light detection with the Xenogen camera.

Results In AE17-SFG mouse model, all mice in the PBS group developed massive detectable tumours. 10 of 10 mice treated with PBS were sacrificed by Day 33 because of tumour burden. On Day 40, there was 1 detectable tumour out of 10 in the FGK45 treated group, 3 out of 10 in Adwt+FGK45 or AdΔ24RGD+FGK45 (1 mouse was sacrificed). On Day 68, there were no detectable tumours in any remaining mice in treatment groups.

Conclusions FGK45 treatment can effectively treat mesothelioma cell growth in the pleural space of mice with some potential cures. Combination of FGK45 and Ad5Δ24RGD has not showed improvement over FGK45 alone.

P21-3

Inhibition tumor growth of malignant pleural mesothelioma by adeno-associated viral type-8 vector expressing mda-7/IL-24

Yuji Minegishi[1], Koichi Miyake[2], Akinobu Yoshimura[1], Akihiko Gemma[1], Takashi Shimada[2]

[1]Department of Internal Medicine, Division of Pulmonary Medicine, Infectious disease, and Oncology, Nippon Medical School, Japan, [2]Department of Biochemistry and Molecular Biology, Division of Gene Therapy Research, Center for Advanced Medical Technology, Nippon Medical School, Tokyo, Japan

Malignant pleural mesothelioma (MPM) is a rare but lethal cancer associated with asbestos exposure. Melanoma differentiation-associated gene-7/interleukin-24 (mda-7/IL-24) not only induces apoptosis but also has immune modulatory and anti-angiogenic properties as well as potent antitumor bystander effects. We examined a feasibility of adeno-associated virus (AAV) vector mediated gene therapy for MPM using mda-7/IL24 by single intramuscular injection. We generated type 8 AAV vector expressing secretable mda-7/IL24 (AAV2/8-mda7). In vitro studies showed that conditioned medium collected from C2C12 cells transduced with AAV2/8-mda7 is able to suppress tumor growth of human MPM cell lines. We generated a MPM intra-peritoneal disseminated carcinoma model by injection of MSTO-211H/Luc cells into BALB/c nude mice. After injection of AAV2/8-mda7 into the right quadriceps muscle of the MPM model mice, tumor cell growth was monitored by a real-time in vivo imaging analyze system (IVIS). Suppression of tumor growth was observed in AAV2/8-mda7 injected mice compared to control GFP expressing AAV injected mice ($p<0.01$). Survival effect was also detected in AAV2/8-mda7 injected mice ($p<0.01$). These results demonstrated that single intramuscular injection of AAV2/8-mda7 is useful for the gene therapy of MPM.

P21-4

VEGF targeting in mesothelioma treatment using an interleukin-6 signal inhibitor based on adenovirus gene delivery

Yasuo Adachi, Norihiro Nishimoto

Laboratory of Immune Regulation, Wakayama Medical University, Japan

We previously reported that interleukin-6 (IL-6) induces both tumor cell growth and VEGF production in malignant mesotheliomas. It has been reported that VEGF induces angiogenesis and acts as a mitogen for mesothelioma cells. These results suggested that an anti-IL-6 approach should be considered as a feasible therapeutic candidate for mesotheliomas.We also developed a new receptor inhibitor of IL-6 (NRI) by genetically engineering tocilizumab, a humanized anti-IL-6 receptor monoclonal antibody, into single chain fragment format. Since NRI is encoded on a single gene, it is applicable to a gene delivery system using virus vehicles. In this study, we report VEGF targeting through NRI expression based on adenovirus-mediated gene delivery in mesothelioma cells.Materials and Methods: We constructed a NRI expression vector in the context of a tropism modified adenovirus vector that had enhanced infectivity in mesothelioma cells. Because a fiber modified adenovirus vector (Ad5/3) was beneficial for gene transfer in mesothelioma cells.Results: This virus effectively induced NRI secretion from mesothelioma cells (H2052). This virus also significantly reduced the VEGF production in H2052.Discussion: These results indicate that NRI shows potential as an agent in the treatment of mesotheliomas, with respect to the VEGF suppression. Since mesothelioma is a highly malignant neoplasm, many innovative approaches, including virus-based treatments, have been reported. However, the recruitment of cytokines induced by virus infection is a possible reason that undermines the efficiency. Thus, the virus strategy combined with anti-IL-6 is a promising approach in achieving better control of the immune system. In addition, because angiogenesis is one of the main progression factors for tumor growth, a virus approach combined with anti-angiogenic agents is a rational application.

P21-5

The antitumor effect of pemetrexed combined with conditionally replicative adenovirus against human malignant pleural mesothelioma cell lines

Noriaki Nakagaki, Koichi Takayama, Miiru Izumi, Kentaro Tanaka, Junko Hisasue, Yoichi Nakanishi

Research Institute for Diseases of the Chest, Kyushu University, Japan

Malignant pleural mesothelioma (MPM) is intractable malignancy that shows resistance to chemotherapy and radiotherapy. It is estimated that MPM will increase in future because of asbestos consumption past several decades in Japan. So, new therapy is desired for MPM. Pemetrexed is a novel multitargeted antifolate agent that has shown antitumor activity in various types of solid tumors, including MPM, and the combination of pemetrexed and platinum is one of the standard therapy for MPM. On the other hand, adenovirus vector is widely used in gene therapy, and it is reported that conditionally replicative adenovirus (CRAd) has an antitumor effect in various malignancies. CRAd using tumor specific promoter can replicate in only tumor cells, and a large number of replicated adenoviruses in tumor cells induce cell lysis. The progeny CRAds released from lytic tumor cells infect surrounding intact tumor cells. Consequently, CRAd has selective cell killing effect to tumor cells. We manufactured CRAd which possesses telomerase promoter, and confirmed the antitumor effect in MPM in vitro and in vivo. So we hypothesized combination therapy of pemetrexed and CRAd can be a promising therapeutic approach and investigated a combination effect of pemetrexed and CRAd in several MPM cell lines.

P22

Novel therapeutics-Ⅱ

P22-1

Ex vivo expansion of tumor-infiltrating lymphocytes for adoptive cell therapy: a potential therapeutic option for patients with malignant pleural mesothelioma

Masaki Anraku[1,2], Linh Nguyen[3], Nicole Liadis[3], Jessica Nie[3], Pei Hua Yen[3], Licun Wu[1], Pamela Ohashi[4], Marc de Perrot[1,2]

[1]Latner Thoracic Surgery Laboratories, Toronto General Research Institute, University of Toronto, Canada, [2]Division of Thoracic Surgery, Toronto General Hospital, University of Toronto, [3]Campbell Family Cancer Research Institute, University Health Network, [4]Departments of Immunology and Medical Biophysics, University of Toronto, Canada

Objective: Malignant pleural mesothelioma (MPM) remains associated with poor outcome despite aggressive treatment with chemotherapy, radiotherapy, and surgery. Recent trials of Adoptive Cell Therapy (ACT) have shown encouraging clinical response rates in solid malignancies. One promising protocol is based on the *ex vivo* expansion of tumor-infiltrating lymphocytes (TILs) and subsequent autologous infusion of TILs with anti-tumor reactivity. This pilot study evaluated whether TILs from MPM can be expanded ex vivo and therefore potentially represent a novel therapeutic approach for MPM. **Methods:** Cells from MPM tumors obtained at the time of surgery were cultured in medium containing the T cell growth factor interleukin-2. Cell populations from established cultures were assessed by flow cytometric analysis. **Results:** We obtained tumor samples from 9 patients with MPM [5 extrapleural pneumonectomy (EPP), 2 pleurectomy/decortication and 2 biopsy]. TIL growth was observed from 8 of the 9 MPM tumors. The one tissue sample that did not yield TILs was from a case that underwent EPP 5 days after induction intensity modulated radiation therapy (30Gy). Immunohistochemistry on this sample revealed only fibrous tissue and the absence of lymphocytes. The average total number of TILs obtained within 4 weeks of culture from the 8 patients with successful cultures was $2.2 \times 10^8 \pm 1.5 \times 10^8$ (range: 4×10^7 - 5×10^8). Flow cytometry confirmed that the cultures were mainly comprised of T cells (79% ± 17% $CD3^+CD56^-$ cells) and that the proportion of $CD4^+$ "helper " type cells and $CD8^+$ "cytotoxic " type cells was heterogeneous among independent cultures (54% ± 30% $CD4^+$; 26% ± 20% $CD8^+$). **Conclusions:** TILs from MPM can be expanded to numbers suitable for current ACT protocols. Studies to evaluate the reactivity of these TILs are underway. This preclinical work presents ACT as a potential treatment option for patients with MPM.

P22-2

Targeting macrophages as a novel therapeutic approach for malignant pleural mesothelioma

Nikita Kolhatkar[1], Magnus Johansson[1], Dario Barbone[3], Adam Yagui-Beltran[2], Raphael Bueno[5], David J. Sugarbaker[5], David M. Jablons[2,4], Lisa M. Coussens[1,4], V. Courtney Broaddus[3]

[1]Department of Pathology, University of California, San Francisco, USA, [2]Department of Surgery, University of California, San Francisco, CA, [3]Lung Biology Center, University of California, San Francisco, CA, [4]Helen Diller Family Comprehensive Cancer Center, University of California, San Francisco, CA, [5]Division of Thoracic Surgery, Brigham and Women's Hospital, Harvard Medical School, Boston, MA, USA

Mesothelioma is a life-threatening tumor, induced by inhalation of asbestos fibers, which is largely resistant to most chemotherapeutic approaches. One feasible approach could be to harness the power of the immune system to increase the chemosensitivity of mesotheliomas. Using a combination of immunohistochemistry and flow cytometry to analyze the leukocyte compositions of human mesotheliomas, we have found that 1) epithelioid and mixed mesothelioma tumor subtypes have a higher degree of immune cell Infiltration, when compared to sarcomatous tumors, and 2) mesothelioma tumors have large infiltrations of macrophages (31 +/- 4.6% of the inflammatory cell population (CD45+)). Indeed, the percentage of macrophages in mesothelioma exceeded that found in other thoracic malignancies thus far evaluated (NSCLC cancer, 9%; esophageal, 4%). In view of recent data indicating that macrophages can be targeted therapeutically to minimize some aspects of cancer development, we investigated whether macrophages could be targeted to enhance chemosensitivity of human mesotheliomas. To address this question, we adapted a 3-dimensional spheroid growth model, enabling heterotypic culture of mesothelioma cells with macrophages. We found that mesothelioma chemoresistance can be lowered by co-incubation with macrophages. However, the magnitude of the response was dictated by macrophage phenotype. Macrophage phenotype and bioactivity is modulated by Th1 versus Th2 cytokine exposure that in turn regulate either an M1 (IFN-gamma & LPS) or M2 (IL-4) phenotype. M1-polarized macrophages increased the response of malignant mesothelioma spheroids to pro-apoptotic agents, such as TRAIL plus anisomycin. Furthermore, our preliminary data indicate that primary human tumor-associated macrophages, isolated from malignant mesotheliomas, have similar pro-apoptotic effects when polarized with M1 cytokines, suggesting that cytokine re-polarization of macrophages in mesothelioma tumors to an M1 phenotype could augment therapeutic efficacy.

P22-3

Targeted imaging and therapy of malignant mesothelioma using novel internalizing antibodies

Jiang He[1,4], Arun K Iyer[1], Xiaodong Zhu[2], Xiaoli Lan[1], Jinjin Feng[1], Yang Su[1], Xiaoju Zhang[2], Yue Liu[2], Youngho Seo[1], Henry F VanBrocklin[1,4], Courtney V Broaddus[3,4], Bin Liu[2,4]

[1]Department of Radiology & Biomedical Imaging, University of California San Francisco, USA, [2]Dept. of Anesthesia, University of California San Francisco, San Francisco, CA, United States, [3]Lung biology center, University of California San Francisco, San Francisco, CA, United States, [4]Helen Diller Family Comprehensive Cancer Center, University of California San Francisco, San Francisco, CA, United States

Background: A panel of phage antibodies has been generated by selection of phage antibody display libraries against live mesothelioma cells. These human antibodies are attractive candidates for the development of targeted imaging and therapeutics. The objective of this study was to evaluate the tumor targeting of a novel rapidly internalizing human single chain antibody fragment (scFv) labeled with ^{99m}Tc and related nanosize immunoliposome labeled with 111In in a murine model bearing mesothelioma tumors of both epithelioid and sarcomatoid origins.Methods:For in vitro studies, the radiolabeled antibody or immunoliposome was incubated at 37^{o}C for 1 h with M28, VAMT-1 or control cells (BPH-1), to assess the total cellular binding versus intracellular uptake. Cy5.5 labeled antibody was also used to monitor the in vitro intracellular uptake employing fluorescence microscopy. For animal studies, the radiolabeled antibody or immunoliposome was administered to athymic mice bearing both M28 and VAMT-1 tumors, and imaged with a small animal-SPECT/CT with concomitant biodistribution. Results: The in vitro cell culture results showed that both ^{99m}Tc labeled antibody and ^{111}In labeled immunoliposome M40 could bind and internalize selectively into both M28 and VAMT-1 tumors with approximately 70-90 % of total cell accumulation accounted for internalization in mesothelioma cells. The in vivo studies also showed rapid and specific targeting into both epithelioid (M28) and sarcomatoid (VAMT-1) subtypes of mesothelioma tumors, with the clear SPECT/CT images showing significant tumor uptake in both subtypes of mesothelioma tumors as early as 1 h p.i. for ^{99m}Tc labeled antibody and 24 h for ^{111}In labeled immunoliposome. Conclusion: This study demonstrated the potential of these antibodies as a versatile targeting ligand for imaging and therapy for both subtypes of mesothelioma either in native format or along with nanoparticles, warranting further investigation.

P22-4

Cancelled

P22-5

Estrogen receptor beta exerts tumor repressive functions in human malignant pleural mesothelioma via EGFR inactivation and affects response to gefitinib

Giulia Pinton[1], Warren Thomas[2], Paolo Bellini[1], Arcangela Manente[1], Roberto Favoni[3], Brian Harvey[2], Luciano Mutti[4], Laura Moro[1]

[1]DiSCAFF, University of Piemonte Orientale "A: Avogadro", Italy, [2]Department of Molecular Medicine, Royal College of Surgeons in Ireland and Education and Research Centre, Beaumont Hospital, Dublin 9, Ireland, [3]Laboratory of Experimental Pharmacology, National Cancer Institute, Genoa, Italy, [4]Italian Group for the Study and Therapy for Mesothelioma (G.I.Me), Italy

Background: The role of estrogen and estrogen receptors in oncogenesis has been investigated in various malignancies. Recently our group identified estrogen receptor beta (β) expression as an independent prognostic factor in the progression of human Malignant Pleural Mesothelioma (MMe), but the underlying mechanism by which ERβ expression in tumors determines clinical outcome remains largely unknown. This study is aimed at investigating the molecular mechanisms of ERβ action in MMe cells and disclosing the potential translational implications of these results. Methods: We modulated ERβ expression in REN and MSTO-211H MMe cell lines and evaluated cell proliferation and EGF receptor (EGFR) activation. Results: Our data indicate that ER β knockdown in ER positive cells confers a more invasive phenotype, increases anchorage independent proliferation and elevates the constitutive activation of EGFR-coupled signal transduction pathways. Conversely, re-expression of ERβ in ER negative cells confers a more epithelioid phenotype, decreases their capacity for anchorage independent growth and down-modulates proliferative signal transduction pathways. We identify a physical interaction between ERβ, EGFR and caveolin 1 that results in an altered internalization and in a selective reduced activation of EGFR-coupled signaling, when ERβ is over-expressed. We also demonstrate that differential expression of ERβ influences MMe tumor cell responsiveness to the therapeutic agent: Gefitinib.Conclusions: This study describes a role for ERβ in the modulation of cell proliferation and EGFR activation and provides a rationale to facilitate the targeting of a subgroup of MMe patients who would benefit most from therapy with Gefitinib alone or in combination with Akt inhibitors.

P23

Novel therapeutics-Ⅲ

P23-1

Angiogenic tissue response in patients with MPM after treatment with cisplatin, pemetrexed and axitinib

Wieneke Buikhuisen, Marion Scharpfenecker, Paul Baas
Netherlands Cancer Institute, The Netherlands

The vasculature in malignant pleural mesothelioma (MPM) is considered an important treatment target. Since standard treatment with platinum and antifolate does not lead to long survival, we aimed at additionally targeting one of the major regulators of blood vessel formation, vascular endothelial growth factor receptor 2 (VEGFR2) with the small tyrosine kinase inhibitor axitinib (Pfizer). VEGFR2 levels and activity and protein expression and expression of its ligand vascular endothelial growth factor (VEGF) were analyzed in patient biopsies to evaluate the treatment response in a feasibility study. M and M: Tumour tissue was obtained by thoracoscopy from five patients who received cisplatin 75 mg/m2, pemetrexed 500 mg/m2 (q3), and daily 2x 5 mg axitinib orally. Before treatment and after three treatment courses, material was collected and either snap frozen or embedded in paraffin. Biopsies were analysed for VEGFR2, phosphorylated VEGFR2 and VEGF by Western blotting. Immunohistochemistry was used to detect expression levels of VEGFR2 in the respective cells types.Results: Epithelial type MPM was observed in four patients who all showed either stable disease (SD) or partial response (PR). One patient had mixed type MPM and showed progression during treatment. Patients with PR or SD displayed decreased or constant expression and activity of VEGFR2. VEGF expression levels followed a similar pattern. In biopsies of the patient with tumour progression, higher VEGFR2 levels and activity and higher VEGF protein expression after treatment were measured. VEGFR2 immunohistochemistry showed strong staining of tumour cells and blood vessels before treatment. VEGFR2 positive areas and staining intensity were clearly reduced in some of the patients. Conclusions: We show that VEGFR2 protein expression and activity and VEGF protein levels correlate with treatment response. We will further extend our findings in a randomised phase 2 study of chemotherapy +/- axitinib and investigate additional effects of this treatment approach.

P23-2

TSU-68 suppresses progression of malignant pleural mesothelioma through inhibiting angiogenesis in SCID mice

Hisatsugu Goto[1], Trung Van[2], Takuya Kuramoto[2], Soji Kakiuchi[2], Seidai Sato[1], Makoto Tobiume[1], Atsuro Saijo[1], Syo Tabata[2], Shin-Ichi Akiyama[2], Yasuhiko Nishioka[1], Saburo Sone[1,2]

[1]Department of Respiratory Medicine & Rheumatology, Institute of Health Biosciences, The University of Tokushima, Japan, [2]Department of Medical Oncology, Institute of Health Biosciences, The University of Tokushima, Japan

Background: Malignant pleural mesothelioma (MPM) is a highly lethal neoplasm, for which the therapeutic options are limited. Pemetrexed combined with cisplatin, which has been approved, still has poor overall outcome. Molecular targeted therapies represent a promising strategy for overcoming limitation in therapy of malignant neoplasm including MPM. TSU-68 (SU6668), a tyrosine kinase inhibitor targeting VEGFR2, PDGFRβ and FGFR1 can inhibit the growth of various tumors. However, the efficacy of TSU-68 on MPM has not been investigated. Purpose: Investigate the therapeutic efficacy of TSU-68 on the progression of human MPM cells in an orthotopical implantation model.Method: Y-MESO-14 cells (expressing high level of VEGF and low level of bFGF, kindly provided by Drs Taniguchi and Sekido, Aichi Cancer Research Institute, Japan) and MSTO-211H cells (expressing low level of VEGF and high level of bFGF) were orthotopically inoculated into thoracic cavities of SCID mice. From day 7 after inoculation, mice were treated with either TSU-68 (200mg/kg/day, kindly supplied by Taiho Pharmaceutical Co. Ltd.) or vehicle for two weeks. At the end of treatment, mice were sacrificed for analyzing the characteristics of thoracic tumors and pleural effusion. Result: Treatment with TSU-68 potently inhibited the progression of both MPM cell lines and markedly prolonged mouse survival, which was associated with decreased numbers of tumor-associated vessels and proliferating MPM cells in the tumor. Conclusion: These results strongly suggest broad-spectrumactivity of TSU-68 against MPM with different proangiogenic cytokine production profiles in humans.

P23-3

SOCS-3 protein exhibits preclinical anti-tumor activity in malignant pleural mesothelioma

Kota Iwahori[1,2], Satoshi Serada[1], Kazuki Shimada[1,2], Hidekazu Suzuki[3], Tomonori Hirashima[3], Ichiro Kawase[3], Tetsuji Naka[1]

[1]Laboratory for Immune Signal, National Institute of Biomedical Innovation, Japan, [2]Department of Respiratory Medicine, Allergy, and Rheumatic Diseases, Osaka University Graduate School of Medicine, [3]Department of Thoracic Malignancy, Osaka Prefectural Medical Center for Respiratory and Allergic Diseases, Japan

Background: Levels of IL-6 protein in pleural fluid of MPM patients have been reported to be markedly high and various studies indicate a pathogenic role for IL-6 in the progression of this disease. The IL-6/JAK/STAT3 signaling pathway may therefore represent a novel therapeutic target in patients with MPM. The suppressor of cytokine signaling 3 (SOCS3) protein is a negative regulator of this signaling pathway, however the therapeutic potential of SOCS3 delivery in MPM has not been previously explored. In this report we have evaluated the therapeutic potential of SOCS-3 gene delivery as a novel treatment for MPM. Methods: IL-6 protein levels in 24-hr culture supernatants of MPM cell lines were quantitated by ELISA. MPM cell lines were transiently transfected with a replication-defective recombinant adenoviral vector expressing SOCS3 (AdSOCS3). To investigate a cancer inhibitory effect of SOCS3 gene delivery in vivo, AdSOCS3 was injected intrathoracically in nude mice 7, 14 and 21 days following implantation of MPM cells into the thoractic space. Twenty-eight days after cell inoculation, tumors in the thoracic spaces were removed and weighed.Results: In MPM cell lines, the highest levels of IL-6 secretion were observed in cultured H226 and EHMES-1 cells. AdSOCS3 inhibited the growth of cultured H226 and EHMES1 cells. Injection of AdSOCS3 into the thoracic cavity of a mesothelioma xenograft mouse model significantly inhibited tumor growth compared with control AdLacZ-injected mice. Thus, AdSOCS3 exhibited potent anti-tumor activity in a mesothelioma xenograft mouse model.Conclusions: We demonstrate that SOCS-3 gene delivery significantly inhibits the growth of cultured MPM cell lines and strongly inhibits tumor growth in a mesothelioma xenograft mouse model. Importantly, SOCS-3 gene delivery may represent a novel and effective therapeutic strategy for the treatment of human MPM.

P23-4

MET, EGFR and IGF1R are involved in feedback activation of AKT arising from inhibition of mTOR in malignant pleural mesothelioma (MPM): rationale for inhibitor combinations

Marie Brevet, Shigeki Shimizu, Christine Zhou, Adam Olshen, Valerie Rusch, Marc Ladanyi

Memorial Sloan Kettering Cancer Center, USA

Background: NF2 inactivation is a major genetic alteration in MPM and has recently been causally associated with activation of the mTOR pathway. In other cancers, inhibition of the mTOR pathway by rapamycin is associated with AKT feedback activation. This paradoxical AKT activation is usually mediated by IGF1R, but may be mediated by other RTKs. EGFR and MET are frequently expressed and activated in MPM. To define the therapeutic potential of mTOR inhibition in MPM, we examined Rapamycin with and without MET, EGFR and IGF1R inhibition in 13 MPM cell lines. Material and Methods : The RTK inhibitors (TKI) erlotinib (EGFR inhibitor), MK-0646 (IGF-1R inhibitor, gift of Merck) and PHA-665752 (MET inhibitor, gift of Pfizer) were tested in combination with Rapamycin on 13 MPM cell lines. Results : Rapamycin induces AKT activation in 10/13 MPM cell lines cultured in presence of 10%FCS. This feedback activation disappears when the cells are cultured without FCS. MK-0646 + Rapamycin induced a significant decrease of cell proliferation in 6 cell lines (compared to either alone), with decreased phospho-AKT in 4/6. Erlotinib + Rapamycin induced a significant decrease of cell proliferation in 7/13 cell lines (compared to either alone), with decreased phospho-AKT in 2/7. PHA-665752 + Rapamycin induced a significant decrease of cell proliferation in 6/13 cell lines (compared to either alone), with decreased phospho-AKT in 2/6. By ELISA, sensitivities to the inhibitors of IGF-1R, EGFR, and MET were not correlated with total or phospho- IGF-1R, EGFR or MET, respectively. Discussion : In MPM as in other cancers, the inhibition of mTOR by rapamycin induces AKT feedback activation which can be blocked by TKIs. Our results identify MET, EGFR and IGF1R as mediators of this effect in MPM. The characterization of the RTKs involved in this feedback activation could lead to new therapeutic strategies.

P23-5

RNAi-based screening of potential chemosensitising targets for malignant mesothelioma

Lyn Schedlich[1,2], Michaela Kirschner[1], Vandana Relan[2], Kwun Fong[2], Rayleen Bowman[2], Nico van Zandwijk[1], Glen Reid[1]

[1]Asbestos Diseases Research Institute, Bernie Banton Centre, The University of Sydney, Australia, [2]The Prince Charles Hospital, University of Queensland, QLD, Australia

Malignant mesothelioma (MM) is an asbestos-related tumour involving the membrane lining of serosal cavities. Australia suffers the highest incidence of this almost invariably fatal disease, a legacy of the mining of asbestos and its widespread use in the construction industry. Despite modest improvements in treatment, the prognosis remains poor, in part because of the genetic heterogeneity of MM tumours and their intrinsic resistance to chemotherapy. Therefore, the aim of this study is to identify novel growth inhibitory and chemosensitising targets using a rational RNAi-based screening approach. Gene targets were selected on the basis of known overexpression in MM cells and tumours and known involvement in abnormal tumour cell metabolism or drug resistance in MM or other tumour types. The primary readout for the screening process was inhibition of cell growth determined by quantifying total DNA using a SYBR Green based assay. Transfection of primary cells was optimised using siRNAs targeting RRM1, a gene essential for cell growth. In the cell lines tested, silencing RRM1 gene expression led to greater than 95% knockdown of RRM1 mRNA and between 70-80% growth inhibition. In initial screening experiments, silencing the genes encoding Chk1 and survivin inhibited the growth of primary MM cells. Chk1 is a key protein controlling the G2/M checkpoint and DNA repair and survivin functions as a key regulator of mitosis and programmed cell death. Both are targets of small molecule inhibitors currently undergoing clinical trials. Future experiments will screen and validate potential chemosensitising targets, using synthetic lethal screens combining gene knockdown with chemotherapeutic agents used to treat MM. This work has the potential to identify chemosensitising genes that are targets in their own right, or can enhance the efficacy of currently used chemotherapeutic agents.

P23-6

TS-1 suppresses the growth of malignant pleural mesothelioma cells co-expressing dihydropyrimidine dehydrogenase and thymidine phosphorylase in an orthotopical model

Trung Van[1], Masaki Hanibuchi[2], Hisatsugu Goto[2], Soji Kakiuchi[1], Takuya Kuramoto[1], Seidai Sato[2], Makoto Tobiume[2], Atsuro Saijo[2], Sho Tabata[1], Yasuhiko Nishioka[2], Shin-Ichi Akiyama[1], Saburo Sone[1,2]

[1]Department of Medical Oncology, Institute of Health Biosciences, The University of Tokushima, Japan, [2]Department of Respiratory Medicine & Rheumatology, Institute of Health Biosciences, The University of Tokushima, Japan

Introduction: Malignant pleural mesothelioma (MPM) is an aggressive fatal malignancy. Although several chemotherapeutic agents have been tested for the treatment of this disease, the benefit of these treatments remains poor. TS-1, which contains pro-drug of 5-FU and active modulator CDHP, has been reported as an effective antineoplastic agent against various types of cancers such as colon-gastric cancers which often highly express dihydropyrimidine dehydrogenase (DPD). However, the therapeutic efficacy of TS-1 on MPM has not been sufficiently addressed.Method: Three different human mesothelioma cell lines Y-MESO-14 (kindly provided by Drs Taniguchi and Sekido, Aichi Cancer Center Research Institute, Japan), NCI-H290 and MSTO-211H were used in an orthotopical implantation model. Human MPM cells were injected into mouse thoracic cavity. After cell inoculation, tumor-bearing mice were orally administrated with TS-1 (10mg/kg) or vehicle for 10 days (day 11 to day 20) or 14 days (day 7 to day 20). Mice were killed on day 21, and the tumor weight and the volume of pleural effusion was assessed. Result: In vivo data showed that treatment with TS-1 significantly reduced the tumor weight and pleural effusion produced by Y-MESO-14 cells. In addition, treatment with TS-1 prolonged the survival period of Y-MESO-14 cells-bearing mice. Moreover, in vitro MTT assay showed that the combination of 5-FU and CDHP was more effective than 5-FU alone in inhibiting MPM cell proliferation. This combination was most effective in Y-MESO-14 cells, which co-expressed high protein level of DPD and thymidine phosphorylase (TP).Conclusion: Our data suggest that TS-1 might present advance in the development of chemotherapeutic treatment of MPM that especially express both DPD and TP.

P24

Case report- I

P24-1

Primary malignant peritoneal mesothelioma of the great omentum diagnosed by cytology of ascites: A case report

Kayo Inoue[1], Hiroshi Tsubamoto[1], Riya Sakane[1], Riichiro Kanazawa[1], Shinji Komori[1], Takashi Nakano[2], Seiichi Hirota[3]

[1]Department of Obstetrics and Gynecology, Hyogo College of Medicine, Japan, [2]Department of Internal Medicine, Hyogo College of Medicine, [3]Department of Pathology, Hyogo College of Medicine, Japan

Malignant peritoneal mesothelioma (MPM) is the differential diagnosis of malignant ascites when radiological and endoscopic examinations fail to identify the primary lesion. MPM is a rare tumor, the annual female mortality rate of which is less than 30 in Japan, and only a few cases of primary MPM of the greater omentum have been reported in the literature. We report a case of MPM preoperatively diagnosed by cytology of ascites. A 32-year-old woman presented with amenorrhea and was found to have ascites. The serum CA125 level was 129 IU/mL. Transvaginal ultrasonography showed a moderate amount of ascites without any abnormal findings of her uterus, adnexa or pelvic peritoneum. Contrast enhanced CT scan of the abdomen demonstrated thickening and increased density of the omentum. Endoscopic examinations of the colon and stomach were normal. PET/CT detected no significant 18F-labled fluorodeoxyglucose uptake. Transvaginal centesis revealed light brown viscous ascites, overlapping marimo-like cell clusters which were stained strongly for carletinin, cytokeratin 5/6 and D2-40, consistent with MPM. On laparoscopic exploration, the only abnormal finding was thickening of the greater omentum that was biopsied, and histopathologic diagnosis of MPM was confirmed. At subsequent laparotomy, total omentectomy and biopsy of the abdominal peritoneum were performed. Histopathologic analysis of the biopsied specimens of the peritoneum with normal gross appearance revealed MPM. The patient refused chemotherapy, and has been closely followed up at our outpatient clinic.

P24-2

Malignant mesothelioma of the peritoneum: Case reports and immunohistochemical findings including Ki-67 expression

Hiroshi Hirano[1], Takashi Fujisawa[2], Koichi Maekawa[2], Eriko Ohkubo[3], Akira Okimura[1], Kozo Kuribayashi[4], Takashi Nakano[4], Keiji Nakasho[5], Takashi Nishigami[5], Nobuyuki Terada[5]

[1]Department of the Pathology, Nippon Steel Hirohata Hospital, Japan, [2]Department of Internal medicine, Nippon Steel Hirohata Hospital, [3]Department of the Pathology, Takasago Munincipal Hospital, [4]Division of Respiratory Medicine, Department of Internal medicine, Hyogo College of Medicine, [5]Department of the Pathology, Hyogo College of Medicine, Japan

A malignant mesothelioma (MM) is an aggressive neoplasm, though some patients have shown long-term survival, while factors related to survival remain uncertain. We present 3 cases of MM of the peritoneum including autopsy results, in which factors related to long-term survival were investigated. Case 1 was a 69 year-old male who died 6 years after the initial diagnosis. In Case 2, a 67-year-old female came to us with abdominal distention, and despite chemotherapy, died 9 months after the initial diagnosis. The patient in Case 3 was a 68-year-old male who also had abdominal distention, and died 9 months after the initial diagnosis. We studied the clinicopathological appearance and performed immunohistochemical staining including Ki-67 Labeling index (Ki-67 LI) in primary and metastatic sites of these cases. The histological findings of Case 1 indicated epithelioid type, while case 2 and 3 were biphasic type. Immunohistochemical results were consistent with MM. The Ki-67 LI value for both primary and metastatic sites of case 1 was significantly lower than those in Case 2 and 3. We consider Ki-67 LI to be a useful prognostic indicator for MM of the peritoneum.

P24-3

Pleural multicystic mesothelioma : Report of a case

Takekazu Iwata[1,2], Kenzo Hiroshima[3], Taiki Fujiwara[1], Michio Fujino[1], Shinji Matsumoto[4], Akiko Oogami[4], Toshikazu Yusa[5], Kazuki Nabeshima[4], Hisami Yamakawa[1], Ichiro Yoshino[2]

[1]Department of Thoracic Surgery, National Hospital Organization Chiba-East National Hospital, Japan, [2]Department of General Thoracic Surgery, Graduate School of Medicine, Chiba University, Chiba, Japan, [3]Department of Pathology, Tokyo Women's Medical University Yachiyo Medical Center, Chiba, Japan, [4]Department of Pathology, Fukuoka University Hospital, Fukuoka, Japan, [5]Department of Respiratoy Surgery, Chiba Rosai Hospital, Chiba, Japan

Benign multicystic mesothelioma is rare lesion mainly found in the peritoneal cavity and controversial whether its entity is neoplastic or reactive. Only one case of the pleural lesions has been ever reported. We here report a rare case of recurring multicystic mesothelioma arising in the pleural cavity of a 72-year-old Japanese woman. The patient has no history of asbestos exposure. Right pleural effusion has been pointed by chest x-ray since 2004. In 2006, the pleural effusion increased and concentration of hyaluronic acid in the effusion was high (132,000 ng/ml). Malignant mesothelioma was suspected and the pleural biopsy under VATS was performed. Biopsy revealed no cystic lesions or neoplasms. The effusion was still uncontrollable in spite of several chemical pleurodesis. In 2008, she admitted our institution because of right pneumothorax. Chest CT revealed multiple cystic lesions in the right thoracic cavity with pnemothorax. VATS revealed that pneumothorax was due to rupture of a bullous lesion in the lung, and the cystic lesions filled with yellow fluid were arising from parietal pleura. The pleural effusion decreased after the resection of these cysts. However the effusion increased again in 2010. The concentration of hyaluronic acid was 255,000ng/ml. Atypical cells were observed in the effusion at this time. Recurrence of the same cystic lesions was confirmed at thoracotomy and we resected them again. Histology of the surgical samples showed multiple cystic spaces lined by a single layer of cuboidal cells and there was no stromal invasion. Immunohistochemical staining showed that they were of mesothelial origin, and Ki-67 labeling index of the tumor cells was 0.4%. Fluorecence in situ hybridization assay revealed no chromosomal aberrations with loss of 9p21. We diagnosed this case as multicystic mesothelioma. No stromal invasion and low proliferative activity of the tumor suggest that these are reactive process.

P24-4

Spontaneous chylothorax in malignant pleural mesothelioma

Gunnar Hillerdal

Lung Department, Karolinska Hospital, Sweden

Chylothorax occurs when the thoracic duct is damaged through a trauma, such as surgery, or in a malignant disease, usually a lymphoma, or in rare cases with some disease of lymph vessels. In pleural carcinoses chylothorax is rare. In mesothelioma, cases occur after surgery, but very rarely in non-surgical cases. We have recently seen two cases, with chylothorax on the ipsilateral side.Case 1: A 57-year-old ex-insulator was referred in November, 2004, with pain from the right hemithorax and dyspnoea. Investigation revealed a malignant mesothelioma, and Pemetrexed-Carboplatin 6 courses gave a very good response. There was no progression until September, 2007, due to high creatinine he was treated with Liopsomozed Doxirubicine, 4 courses, and there was stable disease until July, 2008, when due to new progression Vinorelbine was given for three courses, again with stable disease. In January, 2009, progression and pleural fluid on LEFT side, which was shown to be chylus. The condition did not allow any further treatment and a PleurX catheter was inserted, with good palliation. After three months the catheter was removed. The patient is still alive, more than five years after diagnosis.Case 2: A 69-year old former dock-yard worker presented with right-sided fluid and pleural thickening in February, 2008. Pemetrexed-Carboplatin was given, but after four courses ascites developed and treatment was stopped. In July, left-sided fluid developed and was found to be a chylothorax. A combination therapy including gemcitabine and liposomozed doxirubicin was tried, but the patient deteriorated and died on Christmas Eve, 2008. The left-sided fluid did not recur, nor the ascites.

P24-5

A case of bilateral, simultaneous pleural mesothelioma

Takao Morohoshi[1], Yukio Tsuura[2], Shoutarou Tsuji[3], Youhei Miyagi[3], Akiko Shoutsu[4], Keita Fujii[1], Mayumi Hori[2]

[1]Division of Surgery, Chest Disease Center, Yokosuka Kyousai Hospital, Japan, [2]Department of Pathology, Yokosuka Kyousai Hospital, Yokosuka, Japan, [3] Division of Cancer Therapy, Kanagawa Cancer Center Research Institute, [4]National hospital Organization, Yokohama Medical Center, Japan

We present a case of malignant pleural mesothelioma, who had diagnosed ipsilateral early primary malignant mesothelioma, simultaneously. The patient was 69-year-old man. He had complained of dyspnea on exersion for 3 months.Chest X ray and computed tomography scan demonstrated right pleural effusion and diffusely thickened right parietal pleura. Ultrasound guided pleural biopsy revealed malignant pleural masothelioma, epithelioid type. The TNM stage was cT2N0M0 stage 2, in International Mesothelioma Interest Group (IMIG) staging system. Three courses of cisplatin at 75 mg/m2 plus pemetrexed at 500mg/m2 were administrated every 6 to 8 weeks. After this treatment, all of the lesions shrank, and the best overall response was minor response (MR) in Response Evaluation Criteria in Solid Tumors (RECIST), in another hospital. The patient was referred to our hospital, intended to undergo total excision of tumors, extra-pleural pneumonectomy, 3 months after diagnosed as mesothelioma. The CT scan showed thichened right diaphragm and irregularly decompressed liver, irregularly thickened left parietal pleura, and pretracheal lymphnode swelling measured as 7 mm in longitudinal diameter. Laparoscopy, mediastinoscopy, and left thoracoscopy were carried out subsequently, to examine the invasion to the liver, lymphnode metastasis of mesothelioma, contralateral lesion of the tumor. Neither tumor invasion to the liver, nor lymphnode metastasis was found out. The irregularly thickened left parietal pleura was biopsized, and was diagnosed as malignant pleural mesothelioma, simultaneous ipsilateral, early stage of primary mesothelioma. Immunohistochemically, the tumor was positive for calretinin, glut-1, intelectin1. We, patient and his family resined surgical operation, after diagnosis. He received 2 courses chemotherapy at prior hospital after discharged our hospital.

P24-6

A case of pleural malignant mesothelioma with multicyst formation, demonstrating abundant viscous liquid production and peculiar cytological findings

Hiroshi Sonobe[1], Kyoko Kaihara[1], Noriyuki Fujimura[1], Toshiyuki Habara[1], Toshiaki Kamei[2]

[1]Department of Laboratory Medicine, Chugoku Central Hospital, Japan, [2]Department of Pathology, Yamaguchi Grand Medical Center, Hofu, Japan

Introduction: We report here a peculiar case of pleural malignant mesothelioma with multicysts that consisted of foamy tumor cells and abundant viscous liquid production. Clinical History: An 83-year-old woman had worked as a teacher of home economics from 17 to 28 years of age. For recent several year, she underwent treatment for chronic bronchitis, but her coughing gradually worsened. Chest X-ray or CT showed pleural thickening in the right lower lung field with pleural effusion. From cytological findings, the diagnosis of malignant mesothelioma was made. Despite chemotherapy, the patient died 2 months later. Cytological Findings: Against a mucous-like background, clusters of large round or polygonal epithelioid cells with rich foamy cytoplasm and atypical nuclei were scattered. The cells have an eccentric nucleus or occasionally have two or three nuclei. Some cell clusters contained collagneous stroma that appeared metachromatic on Giemsa stain. These cells were positive for calretinin, CK 5/6, D2-40 and EMA, but not for CEA or Ber-Ep4. Autopsy findings: In the right thoracic cavity, a large volume of viscous fluid compressed the right lung. Both parietal and visceral pleurae were thickened with multiple cysts. Microscopically, tumor cells were round or polygonal, sometimes foamy or clear with atypical nuclei, growing in an alveolar pattern and forming multilocular cystic spaces filled with mucoid fluid. Immunohistochemical results were the same as those found on immunocytochemistry described above. These findings confirmed the cytological diagnosis of malignant mesothelioma. The pleural tumor invaded a portion of the posterior mediastinum but did not demonstrate remote metastatic tumors. Discussion and Conclusion: The present tumor showed peculiar cytological findings as described above, and the diagnosis was difficult. To establish the correct diagnosis, pathologists and cytological technicians must be aware of the existence of such a tumor, and must be able to perform immunocytochemistry effectively.

P25

Case report-Ⅱ

P25-1

Collision tumor composed of malignant pleural mesothelioma and primary lung cancer: A case report and array-based study

Yukiko Tabata[1,2], Akira Takasawa[1], Yataka Hatanaka[1], Mishie Tanino[3], Katsuji Marukawa[1], Hiromi Kanno[3], Taisuke Harada[1], Kanako Kubota[1], Hironori Haga[1], Yasuhiro Hida[2], Kichizo Kaga[2], Yoshihiro Matsuno[1]

[1]Department of Surgical Pathology, Hokkaido University Hospital, Japan, [2]Department of Surgical Oncology, Hokkaido University Hospital, [3]Laboratory of cancer research, Department of pathology, Hokkaido University graduate school of medicine, Japan

Asbestos exposure is an important risk factor for development of malignant pleural mesothelioma (MPM) and lung cancer. There have been only twenty reported cases of MPM complicated by primary lung cancer, of which only two cases involved collision tumors. Here we report the third such case of collision tumor in a patient with a history of asbestos exposure, which to our knowledge is the first case for which DNA microarray has been applied for characterization.
The two tumors had collided within the same lobe of the lung, and extrapleural pneumonectomy was performed. A high concentration of asbestos bodies was found in the lung tissue. Histopathologically, epithelioid mesothelioma was detected in the pleural tumor and pulmonary adenocarcinoma was observed in the left upper lobe of the lung. Immunohistochemical analysis revealed that the mesothelioma cells were positive for calretinin, podoplanin and p16, whereas the adenocarcinoma cells were positive for TTF-1 and epithelial antigen (Ber EP4). The mesothelioma cells showed positivity for EMA in the cytoplasm, whereas the adenocarcinoma cells showed positivity in the cell membrane.
The two different lesions in this case were considered to be asbestos-related malignancies with completely the same genetic and environmental background. Therefore, comprehensive analysis by DNA microarray was conducted using formalin-fixed, paraffin-embedded tissues of the MPM and adenocarcinoma lesions, and also the non-cancerous part of the lung. In the MPM we identified 54 genes that were highly expressed relative to the adenocarcinoma, including the well-known mesothelioma markers calretinin, CK5 and podoplanin, whereas the adenocarcinoma showed 40 genes that were highly expressed relative to the MPM, including SP-C.
In this investigation, we identified a difference in gene expression between MPM and adenocarcinoma with the same genetic background and history of asbestos exposure. This study is currently ongoing and updated results will be reported at this meeting.

P25-2

Post-irradiation pericardial malignant mesothelioma: report of an autopsy case and review of the literature

Matyas Bendek[1,3], Miroslaw Ferenc[2], Nikolaus Freudenberg[3]

[1]Department of Pathology, Szt. Borbala Hospital, Hungary, [2]Herzzentrum Bad Krozingen, [3]Institute of Pathology, Freiburg University Medical Center, Freiburg, Germany

We report a case of a malignant pericardial mesothelioma of the epithelioid type in a 39-year-old man. He had a history of nodular sclerosing Hodgkin's disease treated with irradiation of the cervical and mediastinal regions 24 years before, and of infarction of the anterior wall of the left ventricle, after which a percutaneous coronary intervention was carried out 7 years previously. He was admitted to a cardiology unit with progressive dyspnea. On examination, a hemorrhagic pericardial fluid collection of 600 ml was detected which was successfully drained. On the next day, the patient developed an electromechanical dissociation suggesting a pericardial tamponade, which was followed by circulatory arrest. At autopsy, the pericardial sac was found to contain 300 ml of partly clotted blood. The epicardial surface showed a diffuse thickening, suggesting a chronic fibrous pericarditis without a macroscopically evident distinct tumor mass. A rupture measuring 0.4 cm in diameter was detected in the right ventricular free wall, 1 cm below the level of the tricuspid valve. The diagnosis of a diffusely growing, malignant mesothelioma of the epithelioid type was made on the basis of histological and immunohistochemical examination of the thickened pericardium.

P25-3

Pulmonary metastases from malignant mesothelioma of the tunica vaginalis testis: A case report

Tomohito Saito, Takahito Nakano, Hiroyuki Kaneda, Ken-ichiro Minami, Toshifumi Konobu, Yukihito Saito

Department of Thoracic and Cardiovascular Surgery, Kansai Medical University Hirakata Hospital, Japan

Malignant Mesothelioma of the tunica vaginalis testis is rare entity. From 1966 to 1999, only 73 case reports had been published worldwide. In Japan, 21 cases had been reported between 1977 to 2004. Its proprtion of malignant mesothelioma of all sites is reportedly estimated 0.3% to 0.9%. According to Plas and colleagues, pulmonary metastasis arises in 9.7% of the cases. A 67-year old Japanese man was referred for further investigation and teatment of solitary pulmonary nodule in the upper lobe of the right lung. The nodule appeared 4 months after high orchidectomy for malignant mesothelioma of the tunica vaginalis testis, and follow up computed tomography of the chest showed its rapid growth. The tumor volume doubling time (TVDT) of the nodule was estimated about 29 days. Through video-assissted thoracoscopic surgery, the lung nodule was resected and pathological investigation revealed pulmonary metastasis of malignant mesothelioma of the tunica vaginalis testis. The Ki-67 index was about 70%. One month after VATS, another pulmonary nodule developed in the right upper lobe. We decided to launch cytotoxic chemotherapy using pemetrexed-containing regimen based on recognition of systemic disease. Estimated TVDT of the pulmonary nodule of this patient was far shorter than any other origin except testis which had been reported by and Fliberg and colleagues. These clinicopathological feature of our case would reflected its malignant nature and rapid invasiveness. Metastectomy for this diseas might be performed for the purpose of diagnosis rather than treatment. Further investigation should be necessary to confirm a clinical efficacy of pemetrexed-based regimen on treatment of malignant mesothelioma of the tunica vaginalis testis.

P25-4

Malignant pleural mesothelioma with long-term temporary tumor disappearance of a local relapse after surgery: a unique case report

Masahiko Higashiyama[1], Jiro Okami[1], Toshiteru Tokunaga[1], Jun Maeda[1], Ayako Fujiwara[1], Fumio Imamura[2], Ken Kodama[1]

[1]Department of Thoracic Surgery, Osaka Medical Center for Cancer and Cardiovascular Diseases, Japan, [2]Department of Respiratory Medicine, Osaka Medical Center for Cancer and Cardiovascular Diseases, Osaka, Japan

There have been few reports of spontaneous regression of malignant pleural mesothelioma, but the mechanism for this is still unknown. We present a rare case report on a patient with malignant pleural mesothelioma showing long-term, but temporary, tumor disappearance in a local relapse after surgery. Case Presentation: A 73-year-old man presented with malignant pleural mesothelioma in the right thoracic cavity. Only pleurectomy was performed because of carina and esophagus involvement (IMIG staging: T4N0), and as expected, the tumor locally relapsed with increasing chest pain. However, the symptoms suddenly improved while the tumor was apparently reduced, and spontaneous tumor regression was initially considered. The patient confessed that he had self-administered a mushroom extract with alternative parasympathetic nerve stimulation therapy thereafter. The complete disappearance of the tumor was clinically achieved during a 29-month follow-up, but in site of continuous self-treatment, tumor recurred again in the local site. Uniquely, the patient is living for 30 months without receiving aggressive treatment after re-relapse. This is the first report describing a malignant pleural mesothelioma patient in Japan showing long-term complete but temporary disappearance of a local relapse after surgery. This event was a tumor regression possibly due to an immunological effect of combined complementary and alternative therapy.

P25-5

Acquired pemetrexed and carboplatin resistance in a long-term survivor of mesothelioma: a case report including gene expression findings

Oluf Roe[1], Endre Anderssen[2], Tore Amundsen[3], Helmut Sandeck[4], Kristin Nesgard[5], Sten E Erlandsen[6], Stein Sundstroem[1]

[1]Department of Oncology, St. Olavs Hospital, University Hospital of Trondheim, Norway, [2]Harvard Medical School, Boston, USA, [3]Dept. of Pulmonary Medicine, St. Olavs Hospital, University Hospital of Trondheim, Norway, [4]Dept. of Laboratory Medicine, Childrens and Womens Health, St. Olavs Hospital, University Hospital of Trondheim, Norway, [5]Dept. of Radiology, St. Olavs Hospital, University Hospital of Trondheim, Norway, [6]Dept. of Cancer Research and Molecular Medicine, Norwegian University of Science and Technology, Trondheim, Norway

Aims: Malignant mesothelioma has a median survival of one year. We report an exceptional case, a 42-year old woman that presented with advanced mediastinal/pleural epithelial mesothelioma and survived for five years and nine months after 40 cycles of Pemetrexed/Carboplatin. Her clinical course was correlated to gene profile, protein expression of the tumour and serum biomarker expression. **Methods:** The patient was included in our microarray study in 2003, where mesothelioma samples were correlated to normal parietal pleura samples (Roe et al, PLoSOne, 2009). At progression, five years later, we did a rebiopsy, isolated RNA and analysed case and control samples with Illumina BeadChip Kit of 25 000 genes. Cell specific expression of proteins encoded by selected genes was analysed by immunohistochemistry. Serum CA125, CYFRA21-1 and SMRP levels were determined from every hospitalisation. **Results:** After resistance onset, histology and routine immunohistochemistry remained virtually unchanged. However, in the biopsy material 241 overexpressed and 289 downregulated genes were identified. Metabolic processes was the largest gene entity whereof 46 genes were involved in purine and pyrimidine metabolism. There were, among others, enrichments of tRNA amino-acylation (7.5-fold), response to DNA damage (3.5-fold), and DNA replication and repair genes (2.5-fold). TYMP gene encoding the potent angiogenic and thymine-producing enzyme thymidine phosphorylase was overexpressed. CHK1 protein expression increased from zero to 70% of the cells when resistance ensued. TYMS gene expression did not increase, but expression of encoded thymidylate synthetase, an important predictor of antifolate resistance, increased from <1% to 15% of tumour cells. Serum biomarkers CA125 and SMRP followed the clinical/radiological course of response and progression, CYFRA21-1 did not. Mesothelin in tumour was unchanged. **Conclusion:** There were both gene/protein expression and serum biomarker changes related to acquired Pemetrexed resistance. A gene/immunoprofile of Pemetrexed resistance in mesothelioma may be derived from future study of more cases.

P25-6

A unique case of malignant pleural mesothelioma with angiosarcomatous differentiation

Astero Klabatsa[1], Krishna Dulay[2], Jeremy P Steele[1], Robin M Rudd[1], Andrew G Nicholson[3], Michael T Sheaff[2]

[1]Department of Medical Oncology, St Bartholomews Hospital, Barts and The London Queen Marys Medical School, UK, [2]Department of Histopathology, Division of Cellular Pathology, Royal London Hospital, London, UK, [3]Department of Histopathology, Royal Brompton and Harefield NHS Foundation Trust, London, UK

We describe the case of an 82-year-old man who presented with left-sided chest pain and a gradual onset of breathlessness over several weeks. Computerised tomographic scans showed a large left pleural effusion, a uniformly collapsed left lung and thickened pleura in the left basal region. He was also found to be anaemic and required blood transfusion. A bronchoscopy, video-assisted thoracoscopy (VATS), pleural biopsy and talc pleurodesis were performed. Histological examination of lung and pleural biopsies taken during the procedure revealed an infiltrative tumour composed of atypical epithelioid and spindle shaped cells arranged in sheets, small clusters and occasional micropapillae. A marked degree of nuclear atypia and atypical mitoses were noted. These tumour cells were positive for immunohistochemical markers calretinin, WT1 and CK5/6; they were negative for CD31 and TTF-1. The superficial component of the tumour contained a distinct proliferation of atypical endothelial cells, many of which were forming irregular vascular spaces. These blended in with the more epithelioid regions described above. These cells were positive for factor VIII related antigen, CD34 and CD31. They were negative for the mesothelial markers. Occasional cells at the interface expressed a combination of markers.The features were reported to be those of an epithelioid malignant mesothelioma. Due to the presence of atypical endothelial cells however the possibility of a coincidental angiosarcoma could not be excluded. After further consultation, the overall features were considered to be those of a malignant mesothelioma with angiosarcomatous differentiation rather than two synchronous tumours, namely a mesothelioma and angiosarcoma.

P26

Case report-Ⅲ

P26-1

Primary intra-hepatic malignant mesothelioma accompanying with multiple lymphadenopathies due to acid-fast bacilli: A case report

Noriko Inagaki, Toshiki Shimizu, Akira Hyoh, Naoko Murakami, Makoto Ogata, Hiroyuki Sugimoto, Ryuichi Amakawa, Shosaku Nomura

The First Department of Internal Medicine, Kansai Medical University, Japan

A 68-year-old woman visited to our hospital for the investigation of a prolonged low-grade fever and multiple lymph-node swellings. A computed tomography (CT) revealed axillary, mediastinal and abdominal para-aortic lymph-node swellings in addition to the intra-hepatic tumor with a diameter of 30 mm. There was no evident finding of pleural effusion, ascites, pleural thickening or peritoneal tumor. The histological findings of fine-needle biopsy specimen of the liver tumor showed the tumor cells staining positive for Alcian-blue and PAS staining. In addition, the results of immunohistochemical staining clearly showed the tumor cells staining positive for Calretinin, WT-1 and D2-40. The findings of 18F-fluorodeoxyglucose (FDG) positron emission tomography (PET) showed no significant accumulation of FDG except for the lymph-nodes and the liver tumor. However, the histological finding of the biopsy specimen of the axillary gland showed an epithelioid granuloma with acid-fast bacilli. Thus, we made the final diagnosis of a primary intra-hepatic malignant mesothelioma accompanying with lymphadenopaty due to acid-fast bacilli. Unfortunately, a hepatic rupture due to a rapid growth of liver tumor was occurred and the general condition of the patient was deteriorated. Thus, we could not carry out any further investigation or treatment in this case.Primary intra-hepatic malignant mesothelioma is an extremely rare tumor. To our knowledge, only four other cases of intra-hepatic malignant mesothelioma have been reported. Except for our case, all the other reported cases were solitary tumor localized in liver at the time of an initial diagnosis. On the other hands, multiple lymphadenopathies were observed in addition to the liver tumor, in our case. However, we revealed that the one of these lymphnode lesions was a non-tumorous granuloma due to acid-fast bacillus. 18-F FDG-PET examination was insufficient to differentiate the infectious lymphadenopathy from the malignant mesothelioma in this case.

P26-2

Localized malignant pleural mesothelioma

Shusai Yamada[1], Kazuya Fukuoka[1], Chiharu Tabata[1], Kozo Kuribayashi[1], Kunihiro Tamura[1], Aki Murakami[1], Asuka Okada[1], Akihiro Yasumitsu[1], Takayuki Terada[1], Ikuko Torii[3], Fumihiro Tanaka[2], Tohru Tsujimura[3], Seiki Hasegawa[2], Noriaki Tsubota[4], Takashi Nakano[1,4]

[1]Division of Respiratory Medicine, Department of Internal Medicine, Hyogo College of Medicine, Japan, [2]Department of Thoracic Surgery, Hyogo College of Medicine, JAPAN, [3]Department of Thoracic Oncology, Hyogo College of Medicine, JAPAN, [4]Department of Pathology, Hyogo College of Medicine, JAPAN

Malignant pleural mesothelioma typically shows either diffuse tumors or multiple pleural nodules. Localized malignant pleural mesothelioma is rare. We report a case of radically resected localized malignant pleural mesothelioma.In April 2008, a 55-year-old previously healthy female, non-smoker, presented with right side chest pain. She had environmental asbestos exposure because she lived near the asbestos factory in Amagasaki, Japan, ever since she was born. The chest X-ray showed a mass shadow with a smooth surface in right upper lung fields. The chest computed tomography (CT) scan showed a 3.5 cm extra pleural mass with a smooth surface, located in the right posterior chest wall. Surgical resection was performed for an extra pleural tumor on May 1st. The final diagnosis was localized malignant pleural mesothelioma, epithelioid type.

P26-3

A case report of recurrent malignant pleural mesothelioma with long-term disease control after extrapleural pneumonectomy

Keita Fujii[1], Takao Morohoshi[1], Yukio Tsuura[2], Mayumi Hori[2], Minoru Okouchi[3], Haruhiko Furusawa[3], Akiko Shoutsu[4]

[1]The Division of Surgery, Chest Disease Center, Yokosuka Kyosai Hospital, Japan, [2]The Department of Pathology, Yokosuka Kyosai Hospital, Kanagawa, Japan, [3]The Division of Internal Medicine, Chest Desease Center, Yokosuka Kyosai Hospital, Kanagawa, Japan, [4]National Hospital Organization Yokohama Medical Center, Japan

We present a case of malignant pleural mesothelioma, who recurred 5 years after the initial treatment. The patient was 55-year-old man. He poited out left pleural effusion for 25 months without any symptoms. Chest X-ray and computed tomography scan demonstrated left pleural effusion and partly thickened left parietal pleura. Thoracoscopic pleural biopsy revealed malignant pleural masothelioma, epithelioid type. The TNM stage was cT2N0M0-stage II, in International Mesothelioma Interest Group (IMIG) staging system. He underwent left extrapleural pneumonectomy successfully. The postoperative pathological diagnosis was malignant pleural masothelioma, epithelioid type, pT2N0M0- stage II (IMIG). The surgical margin of the resected specimen was negative for tumor. He rejected our proposal of adjuvant chemo/radiotherapy and was followed up every 3 months, examined by Labo. Chest X-ray. He underwent CT scan of the chest and abdomen every 6months. He came to our hospital with complaints of back and right lower quadrant abdominal pain 58 months after the surgery. He was diagnosed as diverticulitis of ascending colon with localized peritonitis, and underwent ileo-cecal resection successfully. He discharged 9th postoperative day. He admitted under the diagnosis of acute abdomen and underwent laparotomy. Many nodules and ascites are found in the abdominal cavity. Biopsy specimen of the nodules were recurrent malignant pleural mesothelioma. Chemotherapy was started on 28days after the operation. The regimen was administration of pemetrexed (PEM) only at 500mg/m2. every 3 weeks. After one course, the performance status improved from 2 to 0, appetite increased, and he discharged 43days after the operation. After eight courses, ascites disappeared. After this treatment , ten courses of PEM administration,the best overall response was partial response (PR) in Response Evaluation Criteria in Solid Tumors (RECIST).

P26-4

Simultaneous presentation of pericardial mesothelioma and chronic lymphocytic leukemia

Takuya Oyakawa[1], Yasuo Iwamoto[1], Shinzirou Sakamoto[1], Zyunya Inata[1], Souichi Kitaguchi[1], Toshihiko Suzuki[2], Ryou Mouri[2], Shinichi Takada[3], Hideo Yoshida[2]

[1]Respiratory Medicine, Hiroshima City Hospital, Japan, [2]Cardiovascular surgery, Hiroshima City Hospital, Hiroshima, Japan, [3]Pathology, Hiroshima City Hospital, Hiroshima, Japan

A 34-year-old man, ex-smoker, without exposure of asbestos, suffered from shortness of breath and systemic edema. Past medical history was Chronic lymphocytic leukemia(CLL) diagnosed 15 years before, and treated with fludarabine/cyclophosphamide. Chest CT showed an abnormal shadow around the heart with mediastinal lymphadenopathy and bilateral pleural effusion. Echocardiogram showed diastolic dysfunction and there was a thickened circumference of pericardium without pericardial effusion. Constrictive pericarditis was susupected, and treatment with diuretics was initiated. His symptoms gradually progressed. Three months later he was referred to our hospital for pericardectomy. Pericardectomy were planned to be performed through a median sternotomy. The pericardial cavity was filled with rubbery small nodules, adhering firmly to the heart. Light microscopic findings showed the pattern of tumor consisting with glandular structure of epithelial cells and small lymphocytes infiltration in the adipose tissue. On immunohistochemical staining, the tumor cells were positive for calretinin and cytokeratin AE1/AE3 and negative for carcinoembryonic antigen, the small lymphocytes were consistent with CLL immunohistochemically (CD5 CD20 positive; CD3 Cyclin D1 negative), and the diagnosis of mesothelioma and CLL was made. One month after operation, chemotherapy with carboplatin AUC 4 plus pemetrexed 500mg/m2 every 21 days with usual vitamin supplementation was administered. He developed febrile neutropenia and grade 4 thrombocytopenia with one cycle and radiological assessment by CT after one cycle treatment showed stable disease. Presently he is on sigle-agent pemetrexed. Primary pericardial mesothelioma is a rare malignancy. To our knowledge, the increased risk for second primary mesothelioma after radiation treatment for Hodgkin lymphoma have been reported, there is no report of pericardial mesothelioma after chemotherapy alone. The appearance of this rare secondary malignancy in a long-term survivor of CLL emphasize the importance of continuous perusal for early detection of second malignancy.

P26-5

Ultrasonography guided drainage and hormonal therapy in the treatment of recurrent benign multicystic peritoneal mesothelioma : a case report

Salih Emri[1], Pervin Hurmuz[2], Fatma Aktepe[3], Omer Gunhan[4], Devrim Akinci[5], Ali Ayhan[6]

[1]Department of Chest Diseases, Hacettepe University Faculty of Medicine, Turkey, [2]Department of Radiation Oncology, Trabzon Numune Education and Research Hospital, Trabzon, Turkey, [3]Department of Pathology, Afyon Kocatepe University Faculty of Medicine, Afyon, Turkey, [4]Gulhane Military Medical Academy, Department of Pathology, Ankara, Turkey, [5]Department of Radiology, Hacettepe University Faculty of Medicine, Ankara, Turkey, [6]Department of Obstetrics and Gynecology, Baskent University Faculty of Medicine, Ankara, Turkey

Introduction: Benign multicystic peritoneal mesothelioma(BMPM) is a rare condition affecting primarily the females of the reproductive age.Its pathogenesis is unknown.Surgery is the basis of treatment but the local recurrence rate is high after surgery. Case presentation: A 29 years old female patient presented with the complaint of abdominal pain that was aggravated with menses.Previously she had two pelvic cyst excision operations in the subsequent 2 years. Pathological examination of the specimens revealed that the cysts were lined by mesothelial cells which reacted immunohistochemically positive for cytokeratine and negative for factor 8.These findings were consisted with the diagnosis of benign multicystic mesothelioma.Two years after the last operation she applied to our hospital with the complaints described above.Her abdominal tomography revealed 12x6x8 cm lobulated cystic mass extending from right side of the uterus to the anterior abdominal wall.The intra abdominal cyst was drained by the ultrasonography guidance.The cytology of the fluid showed the mesothelial cells and macrophages.The pathology specimens of the previous operations were re-examined.The mesothelial cells showed estrogen receptor nuclear positivity and progesterone receptor negativity.Then the patient was put on hormonal treatment.She was given 3.6 mg goserelin acetate subcutaneously each month for 4 years.She tolerated the treatment well without toxicity.The patient is under control for her bone mineral density.On her 4th year follow up she is alive without any evidence of disease either clinically or radiologically. Conclusion: BMPM is a benign condition with a high local recurrence rate after surgery. Rather than repeated surgeries after each recurrence,symptomatic control can be achieved by non-invasive methods.Ultrasonography guided drainage seems to be valid option for this group of patients.Patients with hormone sensitive disease may be candidates for hormonal treatment.

P26-6

A difficult case to diagnosis of malignant pleural mesothelioma by medical thoracoscopy

Yumeko Hayashi[1], Yoshiki Ishii[1,2]

[1]Department of Pulmonary Medicine and Clinical Immunology, Dokkyo Medical University School of Medicine, Japan, [2]Respiratory Endoscopy Center, Dokkyo Medical University School of Medicine

Diagnostic efficacy of malignant pleural mesothelioma (MPM) by cytology of pleural effusion and transdermal pleural biopsy is insufficient. On the other hand, pleural biopsy using a medical thoracoscopy under local anesthesia increases diagnostic yield because it can perform under direct vision and be valuable in obtaining the sufficient specimen for pathological diagnosis. Although almost of cases with MPM can be diagnosed using medical thoracoscopy, there are a few difficult cases to diagnose. We present such a difficult case with MPM. We performed medical thoracoscopy for 14 patients with MPM from 2006 to 2010. Only one case required re-examination. Patient was 77 years-old male who were exposed to asbestos for 30 years as a carpenter. He was admitted to our hospital with a complaint of right chest pain and dyspnea. Chest x-ray showed right massive pleural effusion. Tharacentesis revealed that effusion was exudate with lymphocyte dominance and negative for cytology. Medical thoracoscopy showed severe fibrinous adhesion and thickening of pleura. There were no specific lesions suggestive for MPM. Pleural biopsy specimen from thickened pleura showed equivocal finding. Repeated biopsy of outer part of parietal pleura from the insertion port of thoracoscopy revealed sarcomatous type of MPM. To obtain the accurate diagnosis of sarcomatous MPM, sufficient biopsy from whole layer of pleura including an extrapleural fat layer should be performed. In case of medical thoracoscopy under local anesthesia, an electrocautery whole-layer pleural biopsy using an insulated-tipped diathermic knife (IT-knife) is useful.