Abstract
Carbon nanotubes and other possible causes of mesothelioma

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

Nonasbestos fibers in the lung tissue of patients with mesothelioma give a hint for mesotheliomagenicity of nanofibers
SY01-4
Rat ERC/mesothelin and early lesions of tumorigenic and non-tumorigenic fibrous materials including multi-wall carbon nanotube
Shuichi Adachi1, Miho Mizoi1, Sei-ichi Omura2, Takahiro Kobayashi2, Okio Hino3

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
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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 large temple of 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 Hypoo (354-430), quoted asbestos in their theological works. The Italian traveler Marco Polo (1254-1324) in the Milione (Travels of Marco Polo) describes asbestos 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 sftware of our Lord. In the most ancient tale of the Japanese literature, Jakeitori 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
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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 168, 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

In Vietnam, asbestos has been used since 1960s, average consumption 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 m²/year. According annual death record, there are around 150 death cases cause by membral cancers every year, there is including Mesothelioma. In order to clarify 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 among 150 reported death cases of membral cancers in community mortality registration in two years (2007-2008). 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).

**Panel Discussion**

**PD01-4**

**Pleural mesothelioma rates in South Africa: trends 1995-2006**

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**Introduction**

South Africa has mined and refined chrysolite, 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 to 2006, 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.

**Future burden of malignant mesothelioma in Western Australia**

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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 chrysolite and chrysotile-based goods particularly within developing countries; and (iii) general environmental exposure from naturally occurring asbestos or asbestos-form 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**

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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 sided, 1% are bilateral and 0.1% are midline with the location recorded as unknown. Overall the median survival is 267 days with a Y5S 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, outcome and the urgent priority for prevention of MM.
**PD02-1**

**Keynote Speaker**

**Mesothelioma at the intersection of law and medicine**

Steven Kazan
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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
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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 Kubota 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.
Panel Discussion

**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

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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

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Background and purpose: Building-up evidences suggests that SMRP carries a diagnostic and a prognostic value in MPM. Egypt suffers endemic asbestososis 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 parameters. Results: 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=86%, 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, epitheloid 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.
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between epigenetic and genetic alterations in

methyltransferase

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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, 5p21, and 5p22.
In addition, we observed prevalent gains at 1q23, 5p, 7q, and 8q24.

considering each probed locus, there were no instances of significantly
related 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% CI 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.
Round-table discussion

**RD01-4**

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

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William Travis1,2

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**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=39, 34.4%), 1.14 years, micropapillary (n=20, 8.6%, 3.5 years), tubulopapillary (n=51, 22.0%, 1.49 years), and trabecular (n=26, 16.4%, 2.58 years). Lymphatic/vascular proliferations were associated with pleomorphic subtype (p<0.001), lymphatic invasion (p<0.001), and vascular invasion (p=0.005) were significant in pleomorphic subtype compared with solid subtype. Moreover, lymphatic invasion correlated with lymph node metastasis (HR=1.68, p=0.001 and p<0.001, respectively). Moreover, lymphatic invasion correlated with lymph node metastasis (HR=1.68, p=0.001 and p<0.001, respectively). Moreover, lymphatic invasion correlated with lymph node metastasis (HR=1.68, p=0.001 and p<0.001, respectively).

**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.

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**RD01-5**

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

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**Background:** 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.

**Methods:** 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 epithelial mesothelioma, and none of sarcomatoid mesothelioma with anti-CD26 antibodies. Statistically significant correlation between CD26 antibody expression and similar results were also obtained with goat polyclonal anti-DPP4/CD26 antibody. These antibodies absorbed with solute 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 cell line 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.

**Conclusions:** We found either rabbit or goat polyclonal antibodies are suitable for immunohistochemical evaluation of membranous expression of CD26 in mesothelioma.

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**RD01-6**

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

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**Background:** Atypical mesothelial proliferation (AMP) is thought to represent in situ malignant 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 current available selection criteria by immunohistochemistry for humanized monoclonal anti-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.

**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 invasive mesothelioma (IM) over 16 months. Each case was assessed independently by two pathologists for AMP according to current available selection criteria by immunohistochemistry for humanized monoclonal anti-CD26 antibody (Novocastra, Newcastle, UK) and Dako’s EnVisionTM FLEX+ detection system (Dako, Carpinteria, CA).

**Conclusions:** These data indicate that immunohistochemistry for napsin A is a useful aid in separating adenocarcinoma of the lung from malignant mesothelioma.

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**RD01-7**

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

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**Background:** Differentiating malignant mesothelioma from adenocarcinoma, particularly adenocarcinoma of lung origin, remains a crucial but difficult histopathologic problem, that is often not easily resolved using the light microscope, 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.

**Conclusions:** 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%.
A standardized technique of radical pleurectomy for mesothelioma that achieves a macroscopic complete resection, regardless of tumor bulk

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


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 (Group 1, 81 patients, 69EPP and 12P/D) or not resected leaving a residual (Group 2, 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: 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 Group 1 and in 11 and 3 patients in Group 2 (p=0.02 and p=0.14). Two-year survival in group 1 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.

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

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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+HT, 22 had CHT+EPP and 68 underwent complete trimodal therapy.

Clinical Characteristics and survival:

<table>
<thead>
<tr>
<th></th>
<th>BSC (n=116)</th>
<th>CHT (n=266)</th>
<th>EPP (n=100)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (mean, range)</td>
<td>72 (49-87)</td>
<td>65 (39-89)</td>
<td>58 (36-69)</td>
</tr>
<tr>
<td>ECOG 0-1</td>
<td>90 (78%)</td>
<td>248 (93%)</td>
<td>99 (99%)</td>
</tr>
<tr>
<td>ECOG &gt;1</td>
<td>26 (22%)</td>
<td>18 (7%)</td>
<td>1 (1%)</td>
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<td>26 (22%)</td>
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<td>ECOG &gt;1</td>
<td>26 (22%)</td>
<td>18 (7%)</td>
<td>1 (1%)</td>
</tr>
<tr>
<td>Sphincter Hist.</td>
<td>84 (72%)</td>
<td>95 (73%)</td>
<td>81 (81%)</td>
</tr>
<tr>
<td>Clinical stage I-II</td>
<td>75 (65%)</td>
<td>145 (55%)</td>
<td>69 (69%)</td>
</tr>
<tr>
<td>Clinical stage III</td>
<td>25 (22%)</td>
<td>76 (29%)</td>
<td>27 (27%)</td>
</tr>
<tr>
<td>Clinical stage IV</td>
<td>16 (14%)</td>
<td>45 (17%)</td>
<td>4 (4%)</td>
</tr>
<tr>
<td>Median survival stage I-II</td>
<td>8.2 m</td>
<td>15.3 m</td>
<td>24.4 m</td>
</tr>
<tr>
<td>Median survival stage III</td>
<td>10.9 m</td>
<td>13.5 m</td>
<td>13.9 m</td>
</tr>
</tbody>
</table>

Our results reflect the typical clinical characteristics and outcomes with acceptable survival for patients with stage I/II disease undergoing EPP and poor survival for resected patients with stage III tumors. In stage III there was no survival benefit of EPP relative to nonsurgical therapy (Chemotherapy).
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 controversial, 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 Malignant Pleural 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. Additionally, the ERS TASK FORCE (guidelines published in March 2010, are the following: 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 cytologic 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). 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, additional 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% of 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 hybridization 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 region 9p21 are often inactivated by promoter methylation. Both genetic and epigenetic must be in the future useful and reliable tools in the identification of malignancies. References: 1) Schirpereel A, Astoul P, Baas P, Berghmans T, Claysen H, de Vuyt P, Diemelmann H, Galetou-Salle F, Leclercq C, Materin C, Hainaut P, van Houtte P, van Meerbeeck J, Waller D, Weder W. Guidelines of the European Respiratory Society and the European Society of Thoracic Surgeons for the management of malignant pleural mesothelioma. Eur Respir J. 2010 Mar;35(3):479-505. Epub 2009 Aug 28. 2) Husain AN, Colby TV, Ordonez NG, Krausz T, Cazzucchi C, Cogle PT, Chiniac LR, Chuang A, Galetou-Salle F, Gibbs AR, Gown AM, Haimmar SB, Litzky LA, Roggli VL, Travis WD, Wick MR. Guidelines for pathology diagnosis of malignant mesothelioma: a consensus statement from the International Mesothelioma Interest Group. Arch Pathol Lab Med. 2009 Sep;133(9):1214-22. 3) Savic S, Franco N, Barascud Ade V et al , Fluorescence in situ hybridization in the definitive diagnosis of malignant mesothelioma in effusion cytology. Chest, 2010;138:137-44.

Cytopathology-How reliable is it?

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

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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. Furthermore, immunohistochemical staining was carried out for a definitive diagnosis using the cell transfer method and/or the cell block method for immunohistochemical 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, immunohistochemical 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 0/5 (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 immunohistochemical 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.
**WS01-4**

**Cytopathology of desmoplastic malignant mesothelioma (DMM)**

Sadayuki Hiroi1, Susumu Tomimaga1, Sho Ogata1, Kensishi Urata2, Akira Hebisawa2, Toshiaki Kawai2

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**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-polygonal cells in small clusters. These cells had round nuclei and an pointed observation for biopsy are useful for accurate diagnosis.

**Conclusion**: SSMM and cytology and biopsy are useful for accurate diagnosis.

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**WS01-5**

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

Valentina Pinelli1, Lama Sakr3, P Roll1, Laurent Greillier4, Gian Pietro Marchetti1, Herve Dutau3, Andrei Robaglia3, P Cau1, Gian Franco Tassi1, Philippe Astouli2

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**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 thoracoscopcy 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.
Malignant Mesothelioma (MMe) is an aggressive cancer arising from mesothelial cells of the pleura, peritoneum, pericardium and tunica vaginalis tests. 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.
Integrated genomic analysis reveals BRCA1-associated Protein 1 (BAP1) as a commonly mutated gene in malignant pleural mesothelioma (MPM)

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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.

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

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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 SNVs 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.
Tumor microenvironment & Oncogenesis

WS03-1
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, alteration of heterozygity). Epigenetic modifications affect gene function by promoter hypermethylation, chromatin remodeling and changes in mRNA 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, growth 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 components 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
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PDGF-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 receptors. The PDGF-D effect was also prevented by knocking-down PDGF-β receptors or the PI3 kinase inhibitor wortmannin. The results of the present study represent a PDGF-D/PDGF-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 Toyooka1, Takafumi Kubo1, Kazunori Tsukuda1, Masakiyo Sakauchi1, Takuya Fukazawa2, Junichi Soh1, Hiroaki Asano1, Yasutomo Nasu3, Takumi Kishimoto4, Harvey Pass5, Hideki Matsu6, Nam-ho Huh7, Shinichiro Miyoshi1
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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-34a 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-34a 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 Hasina1, Ichiro Kawada1, Geetanjali Kanade1, Vidya Nallasura1, Rajani Kanteti1, Essam El-Hashani1, Maria Tretiakova2, Aliya Husain2, Wickii Vigneswaran1, Hedy Kindler1, Ravi Salgia1

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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 important in various tumour types. Our previously published work has shown that sarcomatoid 331, demonstrating that paxillin is highly overexpressed 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**

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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**

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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/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 IKKα, RIP, Bcl XL, Ripifyllin, 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.
WS04-1
Clinical management of diffuse malignant peritoneal mesothelioma
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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 perioperative hyperthermic intraperitoneal chemotherapy (HIPEC) 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.

WS04-2
Cancelled

WS04-3
Peritoneal mesothelioma
—Clinical and translational research—

WS04-4
Traslational of new prognostic biomarkers and therapeutic targets in clinical practice

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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 translational community on biological and therapeutic features. In this context, the combined approach of palliative surgery and systemic or intraperitoneal chemotherapy is associated to a median survival of about one year, ranging from 9 to 12 months. During the last two decades, few specialized centers have developed an innovative treatment consisting on Cytoreductive Surgery (CRS) with perioperative hyperthermic intraperitoneal chemotherapy (HIPEC) 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.
Identification and validation of new prognostic biomarkers and therapeutic targets

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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 therapeutic 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, 1.25-8.86; 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 XIAP) 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 suggest that strategies aimed at down-regulating survivin may provide a novel approach for the treatment of the malignancy.

More recently, we assessed 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 PDGFRα activation in half. The expression of the cognate ligands TGF-β, 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 STO cells. 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.
The Bcl-2 repertoire of mesothelioma spheroids underlies acquired apoptotic multicellular resistance

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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.

WS05-2

Keynote Speaker

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

WS05-3

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

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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. 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 caspase-3 activity. JNK1 siRNA, but not JNK2 siRNA, inhibited As2O3-induced ERK1/2 activation. JNK1 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
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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/BAX 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
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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 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 the reduced sensitivity to vinorelbine, suggesting that BRCA1 fission decreased mitochondrial apoptosis population and increased apoptosis in MM. To study whether Nix is required for SAHA-induced apoptosis, 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 (Nix siRNA). Attenuating Nix expression with siRNA reduced the apoptosis 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 microscopy. The overlapping of bright green (autophagy) and mito-red labeled mitochondria observed by confocal microscopy indicates the induction of mitophagy. 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
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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 clinical 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 c-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. Moreover, Vorinostat does not affect expression of other proteins involved in apoptotic pathway, such as Mcl-1, Bcl-2, Bcl-2, 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.
WS06-1
Chemotherapy alters cross-presentation of tumour antigen at the effector site

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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, affects 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 costimulatory molecules and their capacity to acquire particulate antigen in situ was not enhanced. Instead, gemcitabine chemotherapy reduced the proportion of CD11c+CD11b+Gr1+ 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

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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 MCM as the target antigen for one of our scFvs. Immunohistochemistry analysis of mesothelioma tissue microarrays confirmed that MCM 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 MCM 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-dependent cellular cytotoxicity against mesothelioma cells

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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 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 : target (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

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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 index with immunohistochemistry and flow cytometry, and K67 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 were depleted in lymphoid organs, thus leading to tumor cell lysis in CTL assay. However, cell killing 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. Conclusions: Administration of Treg depletion could dramatically inhibit tumor cell repopulation between cycles of chemotherapy, thus might be a potential approach to treatment of mesothelioma.

**WS06-5**

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

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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 administering the human MPM cell lines MSTD-211H and HIC-Z226 into the pleural cavity of immunodeficient nu/nu mice. Oncolytic vectors expressing green fluorescent protein (GFP) were administered as a bolus or repeatedly (three or six times) to the pleural cavity of the MPM-bearing mice. 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 cell lines already exhibited multiple tumor nodules in the pleural cavity on day 7, and all tumor-bearing mice died within 50 days (MSTD-211H) or 140 days (HIC-Z226). Administration of Bioknife-GFP significantly prolonged survival of mice bearing MSTD-211H and NC-Z226 (p < 0.001). GFP expression occurred almost exclusively in the tumors and only rarely in normal tissues. Anmunofluorescent 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 antigen receptor have anti-tumor effect in a murine mesothelioma model

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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 expression 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 and abrogate with suboptimal mesothelin expression. 2) mesoCAR mETs are a feasible first step in testing in 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-modulated oncolytic adenovirus

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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 HVS-thymidine kinase (TK) suicide gene, and which also carries the enhanced green fluorescence protein (GFP) 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

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**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 previously reported the significance of an immunological anti-cancer mechanism called antibody-dependent cellular cytotoxicity (ADCC) in colon and lung cancer cells. However, no published 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 cytometric analysis. The direct effect of cetuximab was evaluated by MTS assay. The ADCC activity of cetuximab was assessed by a 4h-51Cr release assay. The in vivo effect of cetuximab against mesothelioma was evaluated 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

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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 higher 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

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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.
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.

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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 fiber-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, TFIIID, 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.

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

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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

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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, however there was no evidence of 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-α-2b (supplied by Schering-Plough/Merck) instillation in MPM patients to see if Ad.IFN-α-2b 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-α-2b viral particles (vp) (1st cohort) or 3E11 Ad.IFN-α-2b 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-α-2b 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-α-2b levels in their serum/pleural fluid after each dose. Peak IFN-α-2b 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 showed strong 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)

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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(R)) 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, NYESO1, 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(R) cancer vaccine in combination with the standard pemetrexed /cisplatin chemotherapy.

WS08-3

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

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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 consistently 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 using 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.IFN 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

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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 Streptococcus 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

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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

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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 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

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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. 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 been rare, 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
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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 effusion 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.

Ref:

LS04
Pulmonary Fibrosis and Asbestos
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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.
unions and companies (responsible for engineering controls), reinforced Western countries will be highlighted. Management of asbestos-related workshops, is essential to prevent further release of asbestos into the environment. The dangers of asbestos have appeared in the press. There is substantial evidence that people have been exposed to asbestos fibers, and many articles about asbestos exposure are put in place. In Egypt, the incidence of MPM is rising dramatically and the median age has been reported to be below 50 years (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 R²=0.83 (p<0.0001) in 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 38,800 (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 95 (56-33) countries analyzed here represent 83% of the world’s population.

Mesothelioma, a global panoramic view
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More than 92,000 mesothelioma deaths (C45, ICD-10) have been recorded in the WHO Mortality Database from 1994-2008. The estimated 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 incidence of 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.

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**Global epidemiology of malignant pediatric mesothelioma**

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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 3 (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

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

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Background: 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 cardiology 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 pneumonectomy and others were diagnosed by the pathologic examination of biopsy, excision, or cytologic specimen. The pathologic subtypes were epithelial (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 in Korea.

**Prognostication of mesothelioma: Isolation without integration**

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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.
**SO2-1**

**Circulating microRNAs as markers of malignant mesothelioma**

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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 10^6 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 3.7 x 10^5, 1.9 x 10^6 and 1 x 10^6 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 miRNA associated with early MM has the potential to lead to improvements in treatment outcome for patients.

**SO2-2**

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

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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 36 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 new unique possibilities for the development of preventive and MM-targeting immuno-therapies.

**SO2-3**

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

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**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 malignant mesothelioma.**Methods/Results:** Pleuropneumonic tissue biopsies (n=49) were profiled using cDNA (Affymetrix) microarrays. Data were analysed with GeneSpring software and revealed FGF-9 (formerly called gilia-activating factor) as a novel candidate gene not previously associated with MM. FGF-9 is important in the pathobiology of MM and its four receptors (FGFR1-4) was detected by immunofluorescence and its four receptors (FGFR1-4) was detected by immunofluorescence. 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 immunohistochemistry 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.

**SO2-4**

**Diagnostic markers of mesothelioma: A systematic review**

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Many biomarkers have been evaluated in the diagnosis of MM, but currently there is no single marker with high diagnostic performance. We performed a systematic literature review on the diagnostic accuracy of markers in patients suspected for MM Materials and Methods: Medical 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 fulfilled the full text assessment, resulting in 92 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 control sample (mesothelioma patients instead patients suspected to have mesothelioma; more than 95%) and an unknown delay between the index and marker test (100%). SMIFR Ber-EP4, calretinin, CEA and EMA are the 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.
**SO3-1**

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

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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 (2q15.3-2q14.2), and extends over 1 Mb of genomic DNA. DPP10 binds specific voltage-gated potassium (K+) channels modulating their expression and biophysical properties. As a member of the “DPP-IV family 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.

**SO3-2**

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

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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.

**SO3-3**

Specific syndecan-1 domains regulate mesothelioma cell growth and migration

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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 cell lines with a full-length syndecan-1 construct and three truncated variants: 78 lacking the extracellular domain with the exception of the juxtamembrane DKKE sequence proposed to be essential for oligomerization; 77 lacking the whole extracellular domain; and RMKK 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 RMKK 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 decreases mesothelioma cell proliferation in two ways: the full-length syndecan-1 prolongs the G0/G1 phase, whereas the extracellular truncated variants 77 and RMKK 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.
SO3-4

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

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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 vivo 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<0.001) 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) 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.

SO3-5

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

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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 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 signaling 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 signaling 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.

SO3-6

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

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Background: Malignant pleural mesothelioma predilection site is the pleural parietal. 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 biphasic histopathological 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 high concordance on identical genes, pathways and gene ontology. Among the 784 overexpressed genes 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 TMY5, encoding tyrosinase 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 and B-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 MSKCC. 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.
The asbestos-related tumor malignant mesothelioma represents a significant clinical challenge. Not only can this tumor 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 biomarkers. However, studies world-wide have sought to establish a range of soluble markers including osteopontin, MFP, 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.

**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

**Methods:** Orthotopic MPM mice biologically recapitulating human disease were established by intrapleural injection of MPM cells secreting - soluble mesothelin-related peptide (SMRP) and osteopontin (OPN).

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

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Background: Expression of the excision repair cross-complementation group 1 (ERCC1) protein predicts response to platin-based chemotherapy and survival in lung cancer patients. The relevance of ERCC1 expression in Malignant Pleural Mesothelioma (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 cases. 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 24 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.

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

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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 (CellSearch ™), 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 significantly 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 area under curve (AUC-ROC) of 0.782 (95% confidence interval, 0.675 to 0.888; p<.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, 73 for stage II, 88 for stage III, and 117 for stage IV cases), and stage IV MPM cases showed a significantly higher mean CEC-count than stage I-III cases (117 and 67, respectively; p<.05).Conclusions: CEC-count is potentially useful clinical marker in the diagnosis of MPM, and also can be a marker of therapeutic effect.

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

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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 biopsy 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.

SO4-6

SO4-5

General Session

General Session
SO5 Novel targets

SO5-2 Activity and resistance to the pan-BCL-2 antagonist Obatoclax in malignant pleural mesothelioma

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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 amplified 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 at early timepoints. In a panel of 10 mesothelioma cell lines obatoclax was evaluated using Ren and MSTO-211 xenografts. Obatoclax treatment (8 mg/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.

SO5-3 Polycom repressive complex-2 is a novel target for mesothelioma therapy

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Background. Polycomb group proteins are global epigenetic gene silencers, which play key roles in the maintenance of stem cell pluripotency, multicellular transformation. 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, 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 EZH2 knockdown were more pronounced than EZH2 knockdown in the other MPM cell lines. 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.

SO5-1 Anti-Mesothelin Immunotoxin SS1P Plus Cisplatin And Pemetrexed For First Line Treatment of Mesothelioma

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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: Thirteen 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 25 μg/kg withdrew after only 1 cycle and this cohort was expanded to 5 patients. One patient treated at 55 μg/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=2), 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.
**SO5-4**

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

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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 cultured ala 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.

**SO5-5**

**The role of the hedgehog pathway in the development of malignant mesothelioma**

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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), Smootherned (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. This study is supported by MARF and NHMRC research grants.

**SO5-6**

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

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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 oncoproteins and tumour suppressor genes, could be one of those novel targets. Being overexpressed in various cancers (e.g. NSCLC, breast) its oncopegenes and tumour suppressor genes, could be one of those novel targets. Being overexpressed in various cancers (e.g. NSCLC, breast) its oncopogenes and tumour suppressor genes, could be one of those novel targets. Being overexpressed in various cancers (e.g. NSCLC, breast) its oncopogenes and tumour suppressor genes, could be one of those novel targets. Being overexpressed in various cancers (e.g. NSCLC, breast) its oncopogenes and tumour suppressor genes, could be one of those novel targets. Being overexpressed in various cancers (e.g. NSCLC, breast) its oncopogenes and tumour suppressor genes, could be one of those novel targets. Being overexpressed in various cancers (e.g. NSCLC, breast) its oncopogenes and tumour suppressor genes, could be one of those novel targets. YBX-1 knockdown has been shown to be associated with late stages of disease. To further investigate the role of YBX-1 in MM, 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 epithelial 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.
First-line treatment

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, 33% 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 pemetrexed has been tested in mesothelioma so far [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 thymidylate synthase, a key enzyme in the folate metabolism [15]. Its development was halted after the 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. Amrubin, 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.

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-arm 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 [3,13], 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%.
SO6-1
Inoperable malignant pleural mesothelioma: Phase II study of 1st line treatment with carboplatin and vinorelbine
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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 in 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 39% males, 59% had epithelial subtype, 55% had IMIG stage IV, and PS 1 and 2 occurred in 66% and 11%, respectively. Median no. of courses was 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 6 pts for toxicities of hypoalbuminemia (n=3) 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 0-198 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 trial pts in PS2 and age above70 years. The activity similar to that of other regimens combining platinum with drugs such as Pemetrexed, Raltitrexed, Gemcitabine, or Epirubicin in such patient populations.

SO6-2
Anti-mesothelin immunotoxin SS1P plus pemetrexed and carboplatin for first line treatment of mesothelioma
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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 has 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 front line treatment of pleural MMs. 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. All patients treated at 25µg/kg withdrew after only 1 cycle and this cohort was expanded to 3 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 peripheral neuropathy (n=3); 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. Median progression-free survival (PFS) for 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.

SO6-3
Phase II study of the combination of bevacizumab plus pemetrexed and carboplatin as first-line therapy in patients with malignant pleural mesothelioma (MPM)
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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.

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 enroll 77 pts. The accrual was reached on September 2012. Of the 77 eligible patients, 71 pts were available for a preliminary analysis. Pts characteristics were: M/F 46/25, median age 68 (range 40-78). EORTC prognostic stage good/poor 61/10. Histology was epithelioid in 37 pts (50%), mixt= 24 pts (32%), pleural sarcomatoid= 9 pts (12%). Partial response was achieved in 22/71 pts, for a response rate of 31%. 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 9 mo; 13 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, cases of bowel perforation, possibly related to treatment, were observed. All pts received front line treatment with BPC regimen. The combination of BPC pts is feasible, with acceptable toxicity, although bevacizumab-related treatment delays cannot be strictly ignored. For 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.

SO6-4
IFCT-GFPC-0701 MAPS trial, a multi-center randomized phase II-III trial of pemetrexed -cisplatin - carboplatin in patients with malignant pleural mesothelioma: preliminary results of the phase II trial
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Background: MPM median OS does not exceed 13 months with combined chemotherapy. In the APOLLO phase II study, the combination of carboplatin and pemetrexed is active and tolerable and feasible. The statistical endpoint of the phase II study was progression-free survival (PFS), and the phase III endpoint was overall survival (OS). The APOLLO phase II trial reached the predefined PFS. Updated toxicity data, PFS, DCR at 6 months and markers of progression will be presented at the meeting.

Methods: Eligible patients had unresectable histologically-proved MPM, PS 0-2, no upper age limit received 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.

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: 111 patients were enrolled within 24 months in 53 centers. Median age: 64.3 (34.7-75.3), M:F= 80:31, epithelioid:sarcomatoid/mixo= 90:21, WHO PS 0:1:2= 49:57:5. In the first 71 assessable patients 129 were observed. 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 9 mo; 13 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, cases of bowel perforation, possibly related to treatment, were observed. All pts received front line treatment with BPC regimen. The combination of BPC pts is feasible, with acceptable toxicity, although bevacizumab-related treatment delays cannot be strictly ignored. For 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.

General Session
**S07-1**

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

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**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 safety and effectiveness of SMRP in patients with MPM.

**Methods:** From January 2006 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.
SO7-2

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

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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 underevaluated.

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 were measured with the Human SM 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^2SM=13%, R^2MPF=13%). In the mesothelioma patients cohort, tumor stage predicted biomarker levels (R^2=10%). ROC regression analysis showed that age, GFR and tumor stage had an impact on the diagnostic performance of the biomarker levels. Multivariable Cox regression analysis revealed that performance status, tumor stage and histology were prognostic factors, in addition 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.

SO7-3

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

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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 (SM) and plasmatic osteopontin (pOPN) levels in 93 healthy asbestos-exposed subjects and 25 patients with epithelioid MPM. SM and pOPN median values in epithelioid MPM patients and healthy subjects were significantly different (p<0.0001). We compared sensitivity and specificity of SM 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 the AUC value (AUC values: pOPN 0.819, SM 0.808, pOPN + SM 0.933). This study demonstrated that the combined use of SM 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

SO7-4

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

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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. At the start of the study we established a cohort of asbestos-exposed workers, (i) developing a biospecimen bank, (ii) 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 between 45 and 65 years of age. Consenting participants completed questionnaires on demographics, employment and exposure histories, and health. Ten mL blood samples were collected. For the initial blood data collection, SMRP 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 ≤1.5nM (n=425, 94%)-the manufacturers 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). 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.

SO7-5

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

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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 (MESONIK 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**

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**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 98%, respectively.

Pure ADC-based diagnosis showed an optimal threshold of 0.00152 mm²/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 0.00125 and 0.00175 mm²/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**

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**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 patients with pleural fibroma and 16 benign pleural diseases. The sensitivity and specificity of PET/CT in differentiating benign from malignant pleural disease is 100% and 98%, respectively.

**Results:** 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 was no surgical proposed for macroscopically evidence of extended diseases and received chemotherapy. Thirty-seven patients had epithelioid subtype, 3 sarcomatous and 6 biphasics. Eighteen patients were no surgical proposed for macroscopically evidence of extended diseases and received chemotherapy.

**Conclusion:** In a multivariable analysis, high SUV tumors were associated with a 4.1 times greater risk of death than low SUV tumors (p < 0.001). In a better staging of disease and in a postoperative follow-up in MPM.

**S08-3**

**F-Fluorothymidine positron emission tomography (FLT PET) imaging in malignant pleural mesothelioma (MPM)**

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**PURPOSE**

18F-fluorodeoxyglucose (FDG) PET is emerging as a useful modality in prognostic and response assessment of MPM. 18F-Fluorothy咪dine (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 followed routine clinical care, including GT scans for response assessment at baseline and after cycles 1, 4 and 6. Patients were followed for progression and survival.

**Results:** 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 (x = 0.60 p<0.001), N (x = 0.41 p=0.002) and UICC T, N stage (x = 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. Consensus response after 1 cycle of chemotherapy was 80% complete response (80% FLT, 42% 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 was poor, predominantly because FLT changes post therapy appear less pronounced than FDG changes. Outcome data is being compiled.
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)

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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 (-4476.43,2657.39). The bias between pairs of observers ranged from 367mm 3 to 910mm 3, and the corresponding 95% limits of agreement for area differences were (-2645.24,1911.32) and (-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.

Computerized measurement of malignant pleural mesothelioma

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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 was 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 3 to 910mm 3, and the corresponding 95% limits of agreement for area differences were (-2645.24,1911.32) and (-4476.43,2867.39). The bias between the mean observer area and the computer area was 593mm 3, 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.
S09-2
A new electrocautery pleural biopsy technique using an insulated tip diathermic knife during semirigid pleuroscopy
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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 pleural fluid retention, suspected of MPM. Thoracoscopy under local anesthesia and video-assisted thoracoscopic surgery (VATS) were performed on 32 and 2 patients, respectively. VATS for pleural fluid retention. In 31 of 34 patients, a diagnosis of MPM was made by pleural examination. LTF-240) for NBI.

BF-F260) for AFI and a conventional white light thoracoscope (Olympus BF-F260). Blood vessels with irregular caliber. Punctate vessels were seen in 4 lesions under WL, and 14 in NBI. Our study demonstrated that NBI is useful in selecting optimal biopsy sites by highlighting punctate vessels or blood vessels with irregular caliber in the diagnosis of malignant pleural mesothelioma.

S09-3
Narrow band imaging: A new technology for the diagnosis of malignant pleural mesothelioma
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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 adhesions. 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
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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 thoracoscopy. 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 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.
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

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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 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 680 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: A total of 135 patients were evaluable for toxicity. There were few observed toxicities. 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.

Beyond first-line systemic therapy for MPM

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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.
S10-3
Randomized, multicentre phase IIIb study of ranpirnase + doxorubicin (DOX) versus DOX in patients with unresectable malignant mesothelioma (MM)


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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 (chemo naive and pretreated pts) who met the CALGB prognostic criteria group 1-4 (Mikulski, JCC 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 o 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 (95% CI:5%) in median OS using a two-sided logrank test (α=5%) with 90% power.

Results: Both arms were well balanced (DOX+ranpirnase/DOX: 203/201pts). Mean age 62.6±6.8 yrs; males 281/132; PS 0 52/60; PS 1 151/56. 40 pts (permetrexed 350/other chemotherapy 303/0), CALGB groups 14/14(1), 45/51(2), 117/115(3), 27/30(4). Investigator based assessment of progression was performed.

Between cycles 3 and 6 were performed with CT - scan. RESULTS: Since 2006 17 relapsed MPM patients were referred to second line 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 chemotherapy. Re-challenge with pemetrexed-based regimens appears as a feasible and safe treatment option for MPM patients after at least one line of chemotherapy

S10-5
Platinum-based doublet chemotherapy in pretreated Malignant Pleural Mesothelioma (MPM) patients: a mono-institutional experience

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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 no 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/m² 1.8 plus the alternative platinum compound against to cisplatin 75 mg/m² 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: 39-76); histology: 12 adenocarcinoma and 1 sarcomatoid, 1 pleural, 1-2 (1:2:1). The combination of gemcitabine with carboplatin/cisplatin was administered as second line treatment in 13(76%) patients, as third line in 4(24%) patients and as fourth line in 1 patient. Two patients 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 14 patients, 35.3% patients, median time-to-treatment failure: 15.5 months. Toxicity profile showed 2(14%) grade 4 and grade 3 thrombocytopenia, 6(43%) grade 3 leucopenia, 3(21%) grade 3 anemia, 1(7%) grade 3 proteinuria. Grade 3 non-hematological 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 third-line options for MPM relapsed patients after at least one line of chemotherapy.

S10-4
Vinorelbine (V) in pemetrexed-pretreated patients (PTS) with malignant pleural mesothelioma (MPM)

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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 and 8 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 43-62). EORTC prognostic score: 12 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 (0.6-10 months); median overall survival (OS) was 5.2months (0.6-40.3 months). Pts with a DC achieved a longer PFS (5.2 vs 1.4 months; p=0.001). A longer OS (8.3 vs 3.2 months; p=0.017). Pts with a good EORTC score showed an advantage 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 11 (25%) pts, thrombocytopenia in 4 (8.3%) pts. 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.

S10-6
Second-line chemotherapy in malignant pleural mesothelioma: Results of a retrospective multicenter study

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BACKGROUND: The standard of care in the first-line (FL) treatment of unresectable malignant pleural mesothelioma (MPM) is second-line (SL) chemotherapy. It is considered for pts with the optimal treatment has not been established. 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 chemotherapy. Re-challenge with pemetrexed-based regimens appears as a feasible and safe treatment option for MPM patients after at least one line of chemotherapy.

RESULTS: Since 2006 17 relapsed MPM patients were referred to second line 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 chemotherapy. Re-challenge with pemetrexed-based regimens appears as a feasible and safe treatment option for MPM patients after at least one line of chemotherapy.
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); 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); 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); T3 (PCI 21-30) and T4 (PCI 30-39). 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S12-1  
**Keynote Speaker**

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

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**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 29 months following RT.

**Conclusions:** IMRT is safe and effective in preventing recurrence and progression in localized mesothelioma.

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S11-3

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

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**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 leukopenia (9%). There was one febrile leukopenia 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 1st 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.
S12-2

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

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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 ethics board approved prospective study evaluating the feasibility of short accelerated neoadjuvant hemithoracic RT followed by EPP the next week for clinically resectable early stage MPM. The dose prescribed is 25 Gy/S x 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 (thrombosis of subclavian vein requiring anticoagulation; hemorthorax 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

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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) were taken. 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).

Results: 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

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Introduction: Radiotherapy 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 histologically 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.

Conclusion: 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.
S13-1
Final results of a phase II study of sunitinib as second-line therapy in malignant pleural mesothelioma
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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 x 28 days. Results: 46 pts were evaluated; 21 assessable for CT response: 20 without pleurodesis assessable on FDG-PET. Demographics: M/F (45:55); median age 67 (range 35-81); histology epithelial/sarcomatoid/mixed (78:18:4). Radiologic response: progressive disease (84%), stable disease (13%), complete response (3%). Correlative science: pre- and post-treatment median plasma VEGF (113 pg/ml), PDGF (12 ng/ml), serum CSF-1 (1518 pg/ml), and mesothelin-related protein (2.51 nM) were analyzed. Conclusions: Sunitinib is inactive in previously-treated MM; the 70 mg dose is poorly tolerated. Correlative markers will be evaluated for association with PFS and OS.

S13-2
A phase II study of dasatinib (D) in patients (pts) with previously treated malignant mesothelioma (MM): CALGB 30061
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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 was given orally. Medians: Age 66 (range 35-81); histology epithelial/sarcomatoid/mixed (78:18:4). Radiologic response: progressive disease (84%), stable disease (13%), complete response (3%). Correlative science: pre- and post-treatment median plasma VEGF (113 pg/ml), PDGF (12 ng/ml), serum CSF-1 (1518 pg/ml), and mesothelin-related protein (2.51 nM) were analyzed. 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 or with CBP501 in patients with advanced malignant pleural mesothelioma (MPM)
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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 antagonist. It inhibits several kinases which phosphorylate 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, 26 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.
S14-5

Keynote Speaker

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

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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 18F-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. Results Since 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. Conclusion We 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.
Feasibility of pleural intensity-modulated radiation therapy (IMRT) for malignant pleural mesothelioma (MPM)

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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 pleura without significant toxicity. We implemented a technique of delivering induction chemotherapy. 44% had unresectable disease while the other 56% underwent pleurectomy/dechoriation. Of 35 patients evaluable for acute toxicity, 7 (20%) had grade 3 or worse pneumonitis (including one death). 5 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/dechoriation followed by pleural IMRT. This approach seems to improve local control, particularly in patients who have undergone pneumonectomy.

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

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Purpose: To investigate combined modality treatment with neoadjuvant chemotherapy followed by extrapleural pneumonectomy(EPX) and adjuvant radiotheraphy 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, 5 of 15 patients underwent intaroperative 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 EPX. The mean age was 54 years (range 25 to 71). Pathological types were epithelial in 6, biphasic in 5, and sarcomatoid in 4. IMIG staging was stage I in 4, II in 3 and II in 1. T status was T1 in 7, T2 in 4 and T3 in 4. Node status was N0 in 9, N1 in 7, 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). Thirteen patients died 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 fatal 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 EPX.

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

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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-Strukwouk) and the addition of hyperthermia can be considered a good alternative (van der Zee). This is considered to be a standard procedure in the treatment 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 randomization 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.
Low-dose computed tomography as a screening tool for malignant pleural mesothelioma and early lung cancer in asbestos exposed individuals: The Toronto Experience

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Purpose: We established 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 parenchymal 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.

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

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The Eker (Tsc2 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 potentiation 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.

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

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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?

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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 asbestos-related disease.

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, rSM=0.87; rMPF=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 0.52 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

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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 Biomarker Study 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 (<1.5 nM) 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.
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 model organism.

Malignant pleural mesothelioma (MPM) is a highly aggressive tumor with a poor prognosis that may involve abnormalities in multiple signaling pathways. Protein kinase C (PKC) 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 PKCα, PKCβII 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 MPM) in humans. An increase in mortality from MPM is associated with asbestos exposure. Other animal mesothelioma models will also be discussed.

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

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Objective: To investigate the effect of intraperitoneal 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/ml) 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 intraperitoneally 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; plasmonic 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 with the cisplatin solution (p = 0.001, respectively). Animals treated with hyaluronate-chitosan-cisplatin had a tumour recurrence significantly lesser than animals treated with 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, which was maintained over time. Tumour-marked protein was completely eliminated after 72h. Conclusions: Polymeric films loaded with chemotherapeutic drugs were significantly effective in reducing tumour volume in a pleuripathology model treated with cisplatin/pemetrexed solution. Hyaluronate and hyaluronate-chitosan loaded with cisplatin showed significantly higher and more prolonged plasmatic drug concentrations than cisplatin solution without increasing toxicity.

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

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Novel treatments are urgently required for malignant pleural mesothelioma (MPM), a chemoresistant 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, 2981 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 triggered 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 downregulation of the autophagy pathway and the ribonucleotide reductase subunits, RRM1 and RRM2, and of the mTOR-p70S6K signaling pathway. Stable transfection of ASS1-CNA 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 spontaneously implanted MPM 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.

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

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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 MxetTag 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. MxetTag 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 MxetTag 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 hypothesis that the mechanism underlying the susceptibility of the mouse to asbestos and mesothelioma is a defect in the ability to mount an adequate inflammatory response.
S17-1

Multimodality treatment paradigm for mesothelioma

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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 more appropriate for locally aggressive disease. Combination chemotherapy with cisplatin and pemetrexed has shown superiorly over single agents. EPP after neo-adjuvant chemotherapy (HITHOC) has been shown to be both feasible and safe. Radiolucent 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/pemetrexed and radiotherapy

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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=0.05) 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 66.4 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)

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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 (20mg/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 chemoperusions 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 arrythmas 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) proved 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 experience with induction chemotherapy followed by extrapleural pneumonectomy for malignant pleural mesothelioma

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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). 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.

S18 Clinical trials

S18-1
Valproic acid plus doxorubicin: effective therapy for progressing mesothelioma. A ELCWP phase II study

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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 Feasibility study on multimodality therapy for MPM

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Backgrounds: Recent Japan 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 neoadjuvant 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-II 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/m2). 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.
S18-2
A Phase II Trial of anti-TGF-beta Monoclonal Antibody (GC1008) in Relapsed Malignant Pleural Mesothelioma (MPM)

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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 I clinical trials in cancer with minimal toxicity. We have opened a Phase II trial of GC1008 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 more 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

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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 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. ENDPOINTES We expect to screen 125 patients to enroll 63 patients with an ASS1-negative MPM from six cancer centers 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. CONCLUSION Targeting 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)

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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 progression-free survival 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 series achieved progression-free at 1 year. Toxicity was mild, with grade 3 or 4 hematologic toxicity occurring in 9.3% of patients. CONCLUSION: Retreatment 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

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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. Methods and materials: 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 complete, the drug was released in 1h. The high aqueous solubility of the drug and the hydrophilicity of the film components did not allow a control of the drug 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, 85% 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 efficiently delivered of a number of innovative anticancer compounds with a potential use in malignant mesothelioma.
S19-1
Prognostic factors in malignant mesothelioma

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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 a 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 marker beyond its prognostic potential. Novak et al. confirmed the prognostic value of FDG-PET in mesothelioma and constructed a prognostic nomogram including weight loss, pleurodesis and Total Glycolytic Volume to predict the survival of their patient population (Clin. Cancer Res. 2010). Richards et al. used their database on resectable epithelial mesothelioma to compare the Brigham and 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

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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

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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 1981-2009. 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. 580±20 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±9% of all nucleated cells. By flow cytometry, these tumor macrophages demonstrated an immunosuppressive phenotype with high expression of CD163, CD206, and the IL-4 receptor. The degree of macrophage infiltration negatively correlated with survival in non-epithelial (HR 1.12(1.021-1.218), 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(1.021-1.218), p=0.008]. Furthermore, macrophages infiltrating the tumor cell island component of non-epithelial tumors predicted overall survival (p=0.007), 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.
Validation of blood neutrophil-to-lymphocyte ratio as a prognostic factor in patients with malignant mesothelioma

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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: 68% epithelialoid, 34% non-epitheloid. At the time of report, 88% of patients were deceased. Median survival was 9.7 months (95% CI, 8.1-11.2). Female gender, epitheloid 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, 0.062, 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.

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

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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 tumours. 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 = 9), empyema (n = 4), bronchopulmonary fistula (n = 3), right heart failure (n = 2), pneumonia (n = 1), constrictive pericarditis (n = 1), acute pulmonary oedema (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-modality therapy in carefully selected MPM patients.

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

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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 carcinoid. 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 specific descriptors of the TNM classification 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**

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**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.

**Results:** 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. 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.

**Stage distribution, associated hazard ratios and median survival 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 N and grouping criteria would further improve staging accuracy of patients with biphasic tumors.

**Conclusions:** Proposed modifications to TNM staging criteria based on analysis of an independent cohort of MPM patients and suggests that modeling of individual N and grouping criteria would further improve staging accuracy of patients with biphasic tumors.

**S21**

**Chemotherapy**

**S20-3**

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

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**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 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 mediastral 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 (6/26 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 mediastral lymph nodes does not preclude 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.

**S21-1**

**In vitro chemoresistance to expanded drug panel in malignant pleural mesothelioma**


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**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 capcitabine 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 capcitabine 14% and 29%.

Low resistance to oxaliplatin was found in 40% and 29% epithelial and non-epithelial tumors, respectively; cisplatin 49% and 50%; cyclophosphamide 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 capcitabine 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, doxorubicin, and etoposide in both epithelial and non-epithelial tumors provide expanded treatment options based on resistance patterns for individual tumors.**
DRUG SENSITIVITY AND CYTOGENETIC CHANGES IN PRIMARY MALIGNANT MESOTHELIOMA CELLS

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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 4x24k 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.

A CLINICAL STUDY OF 34 MALIGNANT PLEURAL MESOTHELIOMA PATIENTS TREATED WITH PEMETREXED FROM NAGASAKI THORACIC ONCOLOGY GROUP

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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 pemetrexed in Nagasaki Thoracic Oncology Group. Results. Of the 34 patients, 28 were men and six were women with a median age 68 years old (range 53 to 81). 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 four patients (11.8%). Conclusions. 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.
Pemetrexed/carboplatin (AC) or pemetrexed/carboplatin (AP) as first line treatment of malignant pleural mesothelioma (MPM): tolerability and response rate in operable patients

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BACKGROUND: Tramodality 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 historically confirmed MPM, stage I-II, PS 0-1, received three cycles of pemetrexed 500mg/m² plus carboplatin AUC5 or cisplatin 75mg/m² 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%); 1 (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 groups. Grade 3 non-haematological toxicities were diarrhoea(3%) 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 78%, progressive disease 3% vs 4%. Patients in AC and AP groups showed: response 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 regiments 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.

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

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Background & Aims; The incidence of Malignant Pleural Mesothelioma (MPM) has been increasing since the 1960s and currently over 2000 new cases are reported each year in the UK. There are unlike many other cancer sites 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), knowledge is 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. Methods ChIMP (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. Results 888 patients 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 29% 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 experience 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.

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

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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) polymorphisms with the outcome of MPM patients treated with pemetrexed/carboplatin (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 mRNA expression and disease control (DC) to CP (odd ratio [OR], 4.2, 95%CI 2.12-14.3; p=0.001), longer PFS (95%CI 1.01-5.02, p=0.01), longer PFS (100% vs 60% at 12 months; HR:0.09, 95%CI, 0.02-0.43; p=0.023), or OS (18 vs 9 months; HR:0.22, 95%CI, 0.27-0.85, p=0.038; 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 (100% vs 60% at 12 months; HR:0.03-0.06, 95%CI, 0.01-0.58; p <0.001) and OS (12vs6 months; HR:0.17, 95%CI, 0.08-0.36; p <0.001). The higher tertile of TS mRNA expression also correlated significantly with a higher risk of progression-free survival (OR:2.55, 95%CI 1.003-6.43, p=0.04). Furthermore TS mRNA level and TS H-score confirmed their independent prognostic role for PFS and OS at multivariable analysis. In contrast, TS protein expression was not detected between ERCC1 protein expression, TS and ERCC1 polymorphisms, with clinical outcome. Conclusion; In our series of treated MPM patients, low TS protein and mRNA levels resulted significantly associated to response to treatment. Prospective trials for the validation of the prognostic/predictive role of TS in MPM patients treated with pemetrexed-based regimens are warranted.
Personalized medicine in malignant mesothelioma: Can TS and ERCC1 predict response to chemotherapy?

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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 and cis/pem chemotherapy responses to cis/platin in patients with MPM.

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 immunoreactive scores for TS and ERCC1 were 1.53 and 2.1 respectively. We found no significant association between TS or ERCC1 expression and radiographic response to pemetrexed and cisplatin respectively.

Conclusions: In contrast to one published report, TS and ERCC1 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.

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

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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 mechanisms(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 (88%). 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 expression which confers resistance to chemotherapeutic agents.

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

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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 Pleurex 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 Pleurex 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.
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

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Among 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%/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 yrs. (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 unrespectable patients (30.5%; 0.9-9.951%; 0.122.0, p = 0.0001). Median tumor volume was larger in unresectable patients (9.06 cc vs 41.5cc, p = 0.0025). Patients taking pain medication were more often unresectable (OR = 2.3, p = 0.0353). Unresectable patients had higher plate count (4.50, 0.8 ± 2.5, p = 0.0180). Resectability was not influenced by cell type. Overall survival was 11.9mos. Resectable patients survived longer than unresectable ones (11.7 mos vs 3.3 mos, p = 0.05) and 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. Overall, adenovascular chemotheraphy was a confounder of resectability. Lung volume 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 seems to be the best predictor in chemotherapy naive patients whereas lung volume predicts resectability post neoadjuvant treatment. These models are currently being validated in a prospective study.

Extrapleural pneumonectomy for malignant pleural mesothelioma results correlated to selection criteria

Cosimo Lequaglie, Gabriella Giudice, Rita Marasco, Margherita Garramone

Background: To show the role of selection criteria in extrapleural pneumonectomy (EPP) for malignant mesothelioma (MPM). Materials and methods: From January 2009, patients with potentially EPP subtypes were selected between 1999 and 2009. PS 0-1, Stages 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, age less 65 years, total coagulative necrosis a nd 1.56mm for total coagulative necrosis and 1.56mm (range 1.16-1.88) for subjacent changes. At 120W, depths were 0.249mm (range 0.217-0.289) for total coagulative necrosis and 1.56mm. At 80W, 0.358mm (range 0.329-0.385) for total coagulative necrosis and 1.56mm (range 1.16-1.88) for subjacent changes. At 120W, depths were 0.249mm (range 0.156-0.300) and 0.35mm (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 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 ablative of the visceral pleura may have a role as an adjunct to parietal pleurectomy in the treatment of pleural mesothelioma.

Integrating immunotherapies with debulking surgery to target residual and metastatic mesothelioma

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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. Debubling surgery (75%) in combination with the locally delivered TLR7 adjuvant molecule imiquimod (IMQ) induced a systemic, tumour-specific CD8+ T cell 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 Cases

Difficult Cases

Localized epithelial malignant mesothelioma

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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 complaint was increasing pain in the shoulder since more than a year. He was otherwise in a good condition for his age. A fine-needle biopsy showed malignant epithelial cells, and the patient was referred to thoracic surgery. An upper lobe resection was performed. An epithelioid 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-, Desmin-, Smoothelin-, CD99-, Synaptosin-, CD45-. Our pathologist’s final diagnosis: malignant epithelioid 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.

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

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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 polycyclic 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, perivesophageal, pericaval, peripheric 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.

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.

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

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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.
20 year-old female with inhibin-positive epithelioid mesothelioma

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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 Cases

Difficult Cases 02
Pathology-Ⅱ: Difficult cases: ask the expert

DC02-1
A rare case of papillary well-differentiated peritoneal mesothelioma with transition into diffuse malignant mesothelioma.

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Papillary well-differentiated peritoneal mesothelioma (PWDPM) is a rare subtype of diffuse malignant peritoneal mesothelioma (DDPM) 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 DDPM, 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 DDPM 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 cholecystectomy. Peritoneal disease involvement was scored as a peritoneal cancer index of 20/38. The microscopic residual tumor was treated by closed-abdomen HIPEC with cisplatin and doxorubicin.

Postoperative course was uneventful. Pathological examination of the post-surgical specimens showed coexistence of typical WPDPM and epithelial DDPM, with tubulo-papillary differentiation and deep tissue invasion. Immunohistochemical studies were positive for calretinin, cytokeratin 5/6 and WT-1, and negative for PECAM and BerE. On immunohistochemistry, the Ki-67 index revealed 2%.

CONCLUSION Differential diagnosis between PWDPM and DDPM 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

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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 asbestos exposure, and the cases were classified as probable, possible, or no exposure. Cytology specimens of pleural effusion of pleural MM 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 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 cholecystectomy. Peritoneal disease involvement was scored as a peritoneal cancer index of 20/38. The microscopic residual tumor was treated by closed-abdomen HIPEC with cisplatin and doxorubicin.

Difficult Cases

Difficult Cases 02
Pathology-Ⅱ: Difficult cases: ask the expert

DC02-3
KG case abstract for IMIG 2010 Difficult Cases: Ask the Experts

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A 29-year old woman without known asbestos exposure presented with pneumonia in December 2003. Chest CT revealed large left pleural and 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 (DDPM; PanK, calretinin, WT-1, and EMA positive). Further workup revealed a right effusion with pleural PET activity. Our team performed right pleuroscopy with biopsies confirming right epithelial dMMP (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, bivacitary intraoperative heated cisplatin and diaphragmatic reconstruction. Pathology confirmed left epithelial dMMP, 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 confirmed right epithelial dMMP, 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. Before 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.
**P01-1**

**Environmental asbestos related diseases: a South African experience**

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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. Methods: The 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. Results: There were 15,463 claimants on the database, of which 1% were confirmed environmental cases. Of these, 52% were diagnosed with malignant mesothelioma. Discussion: The 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**

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Objective: To describe mesothelioma incidence and survival in cancer networks and regions in England. Method: We 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; 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. There was 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**

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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).
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 cases of malignant pleural mesothelioma, diagnosed in the Trieste-marine, and one had been trader in marine setting. The latency periods occupational and environmental, have to be reconstructed. From the elapsed between first exposure to asbestos and diagnosis of for 23 years. Mild or late exposures to asbestos do not seem to explain in female population. In different districts of the city morbidity varied from of the third millennium (p<0.01). Incidence rate was noted as in male, so the most environmentally neglected districts, where enterprises of metallurgic and machine-building industry are situated. Conclusion: In epidemiology of malignant pleural mesothelioma (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%) 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 obtained by linking job histories using a restricted cubic-spline function in a non-conditional logistic regression model. The effect of temporal pattern was studied 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 (OR30vs7=0.08 [0.01-0.56]). These findings document the need to reduce 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. 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. 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 (OR30vs7=0.08 [0.01-0.56]). These findings document the need to reduce 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. 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.
P02-1

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

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Objectives: To describe 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.

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 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.

P02-2

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

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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 was 10 months. Survival rates for one, two, and three year are 35.7%, 14.3%, and 6.0%, 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 2003-2008 were combined separately.

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

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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 patients 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 candidates. 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 5 pathologists, 5 clinicians, 2 radiologists, and a fiber analyst. From April 2006 to March 2010, a total of 6,575 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.

Estimation of lifetime direct medical cost of pleural mesothelioma in Taiwan

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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 patents 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.
Conclusion: These results suggest that Crk is involved in cell motility, motility, adhesion on collagen I, anchorage-dependent and independent growth, and Rac activity in malignant mesothelioma cell lines. Rac activity by pull down assay and FRET based time-lapse analysis in or nucleus in malignant mesothelioma cells of surgical specimens and 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, and growth via activation of Rac in malignant mesothelioma cells. Additionally, overexpression of Cul4A protein was found in these mesothelioma cells. 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 p33-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.
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 lipooxygenase (LOX) pathways. These 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 95 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).

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.

P03-6
Expression and functional analysis of Hairy Enhancer of Split 1 (HES1) in human malignant mesothelioma cell lines

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Malignant pleural mesothelioma (MPM) is a highly aggressive neoplasm. Survival of patients with MPM is very poor because of inherent resistance to chemotherapy and detection of molecular aspects of MPM remains to be elucidated. To reveal genes relevant for MPM, we carried out oligonucleotide 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 may be 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/S 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.
Poster Discussion

P04 Oncogenesis- II

P04-1

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

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Background: Receptor tyrosine kinases (RTK) represent novel therapeutic targets for the treatment of malignancy. Using a phospho-RTK array strategy we have 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-SA cells. MPM expressed different isoforms of RON compared to benign pleural plaques: both benign pleural plaques and MPM expressed the shortform of RON (α-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, MST1/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

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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-8 (IL-8) 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 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 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, TGF-β receptors, and PDGFR-β, and 2) TGF-β-dependent proliferation and PDGFR-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

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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 nitrilacetate (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%; Crocidolite, 89%; Amosite, 100%) mapping to 9q32 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/#946; signal pathway, we try to find the important role of ctgf in asbestos-induced malignant mesothelioma.
Epigenetic inactivation of tumour suppressor genes by DNA methylation in malignant mesothelioma

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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 known TSGs (SFRP2, FBPI, Zic1, SLC19A3 and PCDH10) in established and primary MM cell lines upon treatment with the demethylating agent 5-azaC. The mRNA expression level of SFRP2, FBPI, Zic1 and SLC19A3 were then 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.

Genomic profile of human malignant pleural mesothelioma: A CGH-array comparison between primary tumors and cells in culture

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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 study 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.

Frequent deletions in 3p21.1 region in malignant mesothelioma cell lines established from Japanese patients

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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 homozgyous 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 homozgyous deletions in the 9p21 region. The minimal common region of the deletion was 36 kb, which carries the CDKN2A/p16 and CDKN2B/p15 genes. Homozgyous 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.
Knockdown of ZEB1, a master epithelial mesenchymal transition (EMT) inducing gene, suppresses growth of pleural mesothelioma cell lines

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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: 10 human MPM cell lines and one non-tumorigenic mesothelial cell line were used. Quantitative realtime 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 assays. Cell migration and invasion were measured using transwell chamber assays. E-cadherin expression was measured by immunohistochemistry.

Results: The knockdown of ZEB1 resulted in decreased cell proliferation, migration, and invasion of 18/22 cell lines tested. Elevated ZEB1 expression was confirmed in the 20 cell lines and was correlated with higher malignancy scores of MPM. Knockdown resulted in increased E-cadherin protein in MPM calls but not in H2052 cells. Cell proliferation was measured by WST-1 and clonogenic assays. Cell migration and invasion were measured using transwell chamber assays. E-cadherin expression was measured by immunohistochemistry.

Conclusion: These results suggest that ZEB1 serves as an attractive therapeutic target for MPM.

Association of asbestos exposure and cigarette smoking with gene abnormalities in lung adenocarcinomas in Japan

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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 FA1 (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 year) (zero, from 1 to 499 and 500 or more) and asbestos burden (AB) (zero, from 1 to 999 and 1000 or more).

RESULTS (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) hotspot mutations of p53 were observed among smokers, whereas no nonsynonymous 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. The incidence of p53 mutation increased in relation of both AB and smoking. 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.

Identification of tumor initiating cells in malignant pleural mesothelioma

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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 FAC5 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 mesothelioma progenitor cells we tested mesenchymal stem (MSC) markers in both models and found that SP cells were CD105+.

After implantation of sorted cells under the renal capsule of NOD/SCID mice the cells found to express the 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 mesothelin podoplanin markers, and by an important accumulation of HLA negative stromal cells. 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.
Analysis of early lesions in rats after intraperitoneal administration of nanofibers

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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.

Asbestos body analysis in patients with malignant pleural mesothelioma who underwent extrapleural pneumonectomy

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[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 method1). Scanning electron microscopic analysis was reported by us previously2).

[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 general population. A school teacher is classified as a job which involves a risk of asbestos exposure. However, the AB numbers of three

[Conclusions] The analysis of AB in the lungs of patients with MPM is useful to elucidate the carcinogenic nature of asbestos.

[References]

Trends in asbestos and nonasbestos fiber concentrations in the lung tissues of Japanese patients with mesothelioma

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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 increased from the 1970s (0.3 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.
P07-1
Human MT-2 cell line displays enhanced suppressive function by chronic exposure to asbestos
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Chronic exposure to asbestos results in malignant mesothelioma. As this disease has a long incubation period, CD4+CD25+ regulatory T (Treg) cell-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 μg/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 [3H]-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

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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 and low-dose 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 of 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 1 STAT3. Although cDNA array analysis showed differences of altered genes between two sublines, array CGH 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 of 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 1 STAT3. Although cDNA array analysis showed differences of altered genes between two sublines, array CGH.

P07-3

Suppressive effect of asbestos-exposure on the differentiation of human cytotoxic T lymphocytes, accompanied with decreases in IFN-γ and TNF-α

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[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 naïve CD8 T cells into CTL and functional properties of CD8 T cells from asbestos-exposed people with pleural plaque.

[Materials and Methods] CTL were induced by allogeneic mixed lymphocyte reactions (MLR). PBMCs from healthy volunteer (HV) were cultured with irradiated allogeneic PBMCs with chrysotile B (CB) or crocidolite (CR) asbestos at 5 μg/ml. After 7 days, cellularity, proliferation, apoptosis, cytokotoxicity, 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 and after stimulation with PMA and ionomycin.

[Results] CB suppressed the increase in cell-number of CD8 T cells during MLR, where allogeneic cytokotoxicity decreased, in contrast to no effect of CR. They showed decreases in GB T cells, IFN-γ, CD80 and CD86, but not IL-10, 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-γ T 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.

P07-4

Pleural mesothelioma instigates tumor associated fibroblasts to promote progression via malignant cytokine network

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Background: Regardless of the recent advance of chemotherapy combining cisplatin and pemetrexed, the prognosis of malignant pleural mesothelioma (MM) is still poor. We recently developed orthotopic implantation model of MPM focusing on tumor-associated fibroblasts (TAF) and orthotopic SCID mouse model in vivo. Immunohistochemistry of 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

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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 in MM and PBMCs cultured with CB, where the decrease was smaller than that NK cells in PBMCs cultured with CB, the decrease caused by delay of CD8+ T cell cytotoxicity, as well as by the direct effect of asbestos-exposure. Unlike MM-patients, NK cells isolated from PBMCs showed a decrease in NKP46 when cultured with CB, 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-12/70 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- 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.
**P08-1**

Mesothelioma xenografts developed in the immune deficient mice and the clinical relevance

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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 18-32 days (70-544) 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

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**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), intrapertitoneal (pseudorthotopic), 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 intrapertitoneal, 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 lymphangiosarcoma characteristic of MPM; facilitated accurate monitoring of tumor burden and progression by BLI; volumetric MRI, and SMRP (r=0.9, p<0.001); 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 visceral 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 lymphangiosarcoma, 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

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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.
In malignant mesothelioma, much of its benign counterpart, the cells may assume either an epithelial 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 invasiveness 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 facilitating new genetic changes. We have previously shown that specific break-points on chromosome 3 correlate to the degree of genomic facilitating new genetic changes. Array comparative microscopy results strengthen the same finding. The morphology and control and monotherapy in the EHMES-10 cell-bearing severe

The acquisition of new malignant traits such as drug resistance and invasiveness 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 facilitating new genetic changes. Array comparative microscopy results strengthen the same, 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.

Purpose: Malignant pleural mesothelioma (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 antibody, bevacizumab, in combination with pemetrexed against orthotopically implanted human pleural mesothelioma cells in severe combined immunodeficient mice

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Purpose: Malignant pleural mesothelioma (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 antibody, bevacizumab, in combination with pemetrexed against orthotopically implanted human pleural mesothelioma cells in 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.

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

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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 malignant 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.
P09-1
Expression of survivin and effects of survivin siRNA transfection on mesothelioma cell lines
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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 survivin siRNA transfection on mesothelioma cells.

Methods: Expression of survivin was analyzed in 2 mesothelioma 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 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?
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Immunohistochemical positivity of cytokeratin (CK) such as AE1/AE3, CAMS-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 a 72-year-old male. The whitish gelatinous tumor continuously encased the right lung, pericardium and diaphragm, and focally infiltrated into the right lung. Histologically, the tumor shows two components: one was a 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 CD10 and cytoplasmic, but not nuclear staining of WT-1. In conclusion, two tumors shared common immunohistochemical staining: positive for CD10 and cytoplasmic, but not nuclear staining of WT-1. Further studies of many CK-negative sarcomatoid mesotheliomas are necessary.

P09-3
Genomic gains and losses in malignant mesothelioma demonstrated by FISH analysis of paraffin-embedded tissues
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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 22q12(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 pathological 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.
Molecular pathology of lung carcinoma in asbestos-exposed workers compared to mesothelioma

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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 NSCLC 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 EGrF mutations were only present in NSCLC.

These results suggested 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.

Utility of immunohistochemistry in distinguishing between benign and malignant mesothelial proliferations

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[Abstract] 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 m RNA binding protein 3 (IMPS). 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, p53, Ki-67, GLUT-1 and IMP3). 3. 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. 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.

The trial of differentiation grading of epithelioid mesothelioma with reference to its clinicopathological significance

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[Introduction] Epithelioid mesotheliomas (EMs) often show various morphological features, including papillotubular, solid, microcystic, chord-like, signet-ring cell like, decidual 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 Methods] Fifty-three EMs were collected from Japanese medical institutes, and divided into three differentiation categories (i.e. well, moderate and poor) by focusing on “papillotubular” 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, 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

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**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 and patients MPM who were treated with extrapleural pneumonectomy, had complete pathologic staging, and were classified to stage II based on adjusted TNM classification. We evaluated histologic scoring and architectural characteristics. 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-year survival) than those with moderately-poorly differentiated tumors (18 months median, 0% 5-year survival). Using this algorithm, the number of cases and excluding cases that were not representative of the entire group, the accuracy of pretreatment biopsy was 79%.

**Conclusion:** The developed grading system 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

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**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%), 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 (68.4% p < 0.0001). The 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

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**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, AE1/AE3, Desmin, and negative markers CEA and Ber-Ep4, and, if needed, further markers were determined. Diagnoses were established by three pathologists, and compared to 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: The percentage of sarcomatoid component by light microscopy of each of the biphatic DMM specimens (average of 25.3 tumor-containing slides, range 11-79) was recorded by three pathologists. The average of the two readings and the pathologic stage (TNM, Sugarbaker and Bouchard) were correlated with overall survival. The mean follow-up period after surgery was 22.4 months. Biphatic DMM had a bimodal distribution: predominantly sarcomatoid (more than 50% sarcomatoid component, n=21) or predominantly epithelioid (less than 50% sarcomatoid component, n=21). Patients with predominantly sarcomatoid biphatic DMM had similar survival as patients with monophasic sarcomatoid DMM (10.4 and 9.1 months). Patients with predominantly epithelioid biphatic MM had a better survival (17.5 months). The extent of sarcomatoid component was significantly associated with worse overall survival (p < 0.0001). The patients with monophasic epithelioid DMM performed best; median survival of 39.5 months (p < 0.0001). In multivariate analysis, including sex, age, and pathologic stage, sarcomatoid differentiation and age was an independent prognostic indicator (p = 0.0001) and p = 0.001, respectively. Conclusion: Our results indicate that the extent of sarcomatoid component predicts overall survival in patients with biphatic 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 biphatic MM had an intermediate survival between monophasic epithelioid MM and biphasic sarcomatoid MM. Our data emphasize the importance of accurate histopathologic assessment and reporting of DMM.

**P10-5** Extent of the sarcomatoid component is an independent predictor of survival in malignant mesothelioma

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**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 biphatic 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 biphatic DMM specimens (average of 25.3 tumor-containing slides, range 11-79) was recorded by three 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 22.4 months. Biphatic DMM had a bimodal distribution: predominantly sarcomatoid (more than 50% sarcomatoid component, n=21) or predominantly epithelioid (less than 50% sarcomatoid component, n=21). Patients with predominantly sarcomatoid biphatic DMM had similar survival as patients with monophasic sarcomatoid DMM (10.4 and 9.1 months). Patients with predominantly epithelioid biphatic MM had a better survival (17.5 months). The extent of sarcomatoid component was significantly associated with worse overall survival (p < 0.0001). The patients with monophasic epithelioid DMM performed best; median survival of 39.5 months (p < 0.0001). In multivariate analysis, including sex, age, and pathologic stage, sarcomatoid differentiation and age was an independent prognostic indicator (p = 0.0001) and p = 0.001, respectively. Conclusion: Our results indicate that the extent of sarcomatoid component predicts overall survival in patients with biphatic 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 biphatic MM had an intermediate survival between monophasic epithelioid MM and biphasic sarcomatoid MM. Our data emphasize the importance of accurate histopathologic assessment and reporting of DMM.
Patterns of lymph node spread to N2 nodes predicts survival in patients with biphasic pleural malignant mesothelioma (MM)

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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 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?

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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

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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 ascertainment 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

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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.

CONCLUSIONS:
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

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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 1965 and was admitted to Chest CT because of familiar Alfa-1 Antitrypsin Deficiency. 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

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A 53yo otherwise healthy male presented with increasing shortness of breath, was found to have a right pleural effusion and underwent thoracoscopy for > two liters of thick yellow fluid, suspicious but nondiagnostic for malignancy. He underwent IVATS 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.

P12-1
Expression and localization of matrix metalloproteinase 9 (MMP-9) in mesothelioma cells and reactive mesothelial cells

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Objectives: Mesotheliomas occur in various forms in body cavity fluid. Immunohistochemical staining with a panel of antibodies is used to diagnose mesotheliomas 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. The study was performed by 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**

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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 mesotheliomum (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.

**P12-3**

**Molded mesothelioma cells with hump-like cytoplasmic process in effusion cytology**

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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 clusters, 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-**

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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 numbers of orangeophilic cells were counted in each specimen. 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 Gimsa 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 characteristic 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**

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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 OSPAC (p<0.001). Conclusion: Orangeophilic cells are 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-
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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 cytoplasm, (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 immunohistochemical 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 CEA, BerEp4, MOC31, TTF-1, and Napsin A. In our experiences, calretinin, D2-40, WT1, and WT2 are more recommended as mesothelioma markers, while CEA is one of essential adenocarcinoma markers especially in denying mesothelioma. EMA and/or desmin are useful in differentiation of mesothelioma from reactive mesothelia. We would 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
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Background: 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 epipleral pneumonectomy (EPP). A stamp-sized (5×4 cm) specimen including all thicknesses of the parietal pleura was collected. It contains not only nodule but normal pleura.

Results: One hundred twenty two patients underwent pleural biopsy. Seventy-two patients were diagnosed as malignant tumors. 66 MPM (45 epithelioid, 6 sarcoma, 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 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.

P13-1
Secretion of intelectin-1 from malignant pleural mesothelioma into pleural effusion
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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-invasive 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

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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 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 (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 system was established 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 controls, 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-systems constructed by using 20 mer antigenic peptides correlated with 2, but not at all against Gene-X. Furthermore, the serum antibody titers were lower and higher than the assumed cutoff of 460 pg/ml was statistically correlated with histological types (epithelioid type versus sarcomatoid type), IMIG staging (stage I, II versus III, IV), and to identify the antigens recognized by the antibodies.

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.

P13-3 Circulating tumor cells (CTCs) in the diagnosis of malignant pleural mesothelioma (MPM)

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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 CellSearch 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 35% (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; p<0.05), 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 suggests the need for more sensitive CTC-detection system. Acknowledgement: This study supported by “The Special Coordination Funds for Promoting Science, Sports, 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

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Introduction: Malignant pleural mesothelioma (MPM) is an aggressive tumor of mesothelial origin associated with asbestos exposure. As there is no sensitive test to conventional chemotherapy and radiotherapy so diagnosing MPM early is very important. Vascular endothelial growth factor (VEGF) is a 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 the diagnosis of 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

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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 (85%) 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.
**P14-1**

**Novel clinical role of angiotensin-1 in malignant pleural mesothelioma**

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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 angiotensin (Ang)-1 was correlated with beryllium-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 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 a prognostic factor.

**P14-2**

**Platelet-derived growth factor (PDGF) in pleural effusion of malignant pleural mesothelioma and cancerous pleurisy due to lung cancer**

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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=0.004). 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**

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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. Preliminarily, two parameters seem to be sufficient in final battery and its validation will be presented.

**Poster Discussion**
Poster Discussion

**P14-4**

**Soluble mesothelin for diagnosing malignant pleural mesothelioma: an individual patient data meta-analysis**

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**Background:** Soluble mesothelin is a serum biomarker of malignant mesothelioma. Its extensive diagnostic validation showed differences in reported diagnostic performance, hampering the interpretation of its actual diagnostic value. To address this, we aimed to perform a pooled analysis using 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 fitted to meta-regression models (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=296); 2) healthy asbestos-exposed (n=775); 3) individuals with a clinical anamnesis (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 the effects of age, sex and smoking status, the AUC of soluble mesothelin was 0.77 (95% CI: 0.74-0.80) in healthy controls, 0.68 (95% CI: 0.64-0.72) in healthy asbestos-exposed individuals, 0.67 (95% CI: 0.62-0.72) in individuals with a clinical anamnesis, and 0.75 (95% CI: 0.72-0.77) in lung cancer patients. Additional research will focus on case-specific covariates. Competing for all these covariates allows us to interpret and discuss the diagnostic potential of soluble mesothelin more accurately.

**Conclusions:** The IPD meta-analysis demonstrates the impact of age, sex and smoking status on the diagnostic performance of soluble mesothelin. Additional research will focus on case-specific covariates, and we speculate that both N-ERC and plasma OPN levels correlate 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 occupational 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) nM/L. On the basis of estimated fibres, three groups were distinguished: low (less than 12 fibres/cc/year), intermediate (12-25 fibres/cc/year) and high (more than 25 fibres/cc/year). Lightly higher SMRP levels were found in subjects with higher asbestos exposure than in the remaining subjects (0.58±0.4 vs. 0.53±0.4 nM/L; 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% CI: 1.02-1.44 for high compared to low exposure). 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.

**P15-1**

**Serum N-ERC as a useful biomarker for mesothelioma treatment**

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**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; stage/I/II/III/IV, 1/5/7/11; chemotherapy regimen, cisplatin + pemetrexed/ carboplatin + gemcitabine/ pemetrexed, 16/2/6, 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. Whole measurement of serum 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.
**Poster Discussion**

**P15-2**

**Megakaryocyte potentiating factor is effective for the differential diagnosis of malignant pleural mesothelioma**

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**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 = 93.3%, 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**

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**P15-4**

**Gene expression ratio-based diagnostic and predictive tests using fine needle aspiration biopsies in malignant pleural mesothelioma**

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**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.**

**Results:** Gene ratio tests comparing fixed and matched 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.  

**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. Our analysis 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.

**P15-5**

**Optimization of mesothelioma gene ratio tests for paraffin-embedded tissue**

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**Malignant pleural mesothelioma (MPM) is a highly lethal cancer with >90% 5-year mortality. A comprehensive pre-treatment staging strategy for uniting 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 > 80% concordance comparing predictive and diagnostic gene ratio tests comparing 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.**
P16.1
Computed tomographic assessment of apical involvement in clinical evaluation of malignant pleural mesothelioma

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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 log rank 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 preoperative CT scans. Among 102 epithelial cases, survival was significantly longer for the 54 cases without apical involvement (25.9 months median) than for the 48 with apical involvement (13.1 months, p=0.0005). Among 59 non-epithelial cases, there was no survival difference between the 22 cases without apical involvement (8.6 months median) and the 37 with apical involvement (8.0 months; p=0.3342). Median CT-estimated tumor volumes differ significantly between cases with apical involvement (median 589 cc, range 103-3416 cc) and those without apical involvement (230 cc, 1-2717 cc; p<0.0001). Median CT-estimated tumor volumes differ significantly between cases with apical involvement (median 589 cc, range 103-3416 cc) and those without apical involvement (230 cc, 1-2717 cc; p<0.0001) than for non-epithelial (477 (380-2830) vs 344 (34-2200) cc, p=0.0075) cases with and without apical involvement, respectively. Conclusions: CT-assessed tumor involvement of the apical pleural margin correlates with prognosis in epithelial, but not non-epithelial MPM. Positive CT 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

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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 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 post surgical evaluation to assess response to treatment.
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 plaques caused by asbestos exposure. Recently, we could develop a 3D display of pleural plaque by reconstructing MDCT data. This volumetric CT technique was found to improve the accuracy of plaque detection, and visualized the extent of plaques 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 plaque for the early detection of MPM. A case with MPM and two cases with benign pleural plaques were tried to perform PET/CT scan using. C-11-methionine. With use of AZE workstation (WS), a 3D image of the pleural plaque 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 plaque at the anterior chest wall. In the present study, we awaited for further clinical trials, however.

**P17-1**

**Physiologic and computed tomographic predictors of outcome following extrapleural pneumonectomy for mesothelioma**

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**Introduction:** Post-operative complications of extrapleural pneumonectomy (EEP) 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 EEP 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 MAP 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 EEP.
**P17-2**

**Assessment of therapeutic response using FDG PET in patients with malignant pleural mesothelioma**

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**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 SUVmax 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**

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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, posterolateral, 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 to 10mm, 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 levels in 17 patients, giving a total of 204 quadrants. Agreement between the two observers using a weighted Kappa with quadratic 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**

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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 YMESO14, 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 = 0.055) intrathoracic tumor foci in the YMESO14 group, (18.4 ± 6.3 vs 20.3 ± 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.
There were no severe postoperative complications and operative deaths. 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.

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=0-95.5 vs. unresectable: median=17.2%; range=3.6-41.4, p=0.0005), and significantly higher ipsilateral perfusion (resectable: median=28.35%; range=0-98.8 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=96) (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=0.0005, se=70%, sp=52%). Ipsilateral perfusion ≤25% of predicted was 3.1 times more likely to be unresectable (p=0.0006, 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 inverted to CT-derived tumor volume. A prospective study is required to validate whether preoperative V/Q can aid assessment of resectability prior to surgery.

Surgical treatment for malignant pleural mesothelioma

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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/decoration 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. Hyperthermochemo-therapy 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.

Role of pleural pressure control following extrapleural pneumonectomy during early postoperative days

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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 1065ml. 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, lidocain, 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 drained, the tube was clamped. The amount of hemorrhage on POD1, 2, 3 were 1095, 355, 375ml, respectively, and the tube was withdrawal on POD4. We succeed repetitive thoracostesis 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 50 cmH2O to -5 cmH2O, and it reached 0 cmH2O on POD23. 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 hemodynamics greatly.
Discrepancy between clinical and pathological stages in patients undergoing extrapleural pneumonectomy for malignant pleural mesothelioma

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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 2011 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 pathological stages in the above cases.

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.

BNP is a useful biomarker for cardiac condition after EPP

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Backgrounds: Extrapleural pneumonectomy (EPP) is frequently complicated with postoperative cardiac events such as atrial 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, 2 weeks after, and 4 weeks after EPP, respectively. All patients were given digoxin at a daily 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), 1519.9 (240-2661), and 454.8 (200-680). 

Conclusions: NT-proBNP peaked at 2 weeks after EPP. Patients with postoperative heart failure showed higher NT-proBNP value than patients without complication.
P19-1
Bronchopleural fistula after extrapleural pneumonectomy for pleural mesothelioma
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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 planned 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 lattisimus dorsi muscle flap. The third patient who developed empyma required open window thoracotomy followed by omentoplasty, 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
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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 2006; 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 injury (OR 3.01, CI95% [1.48-6.06]). 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
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Introduction: Extrapleural pneumonectomy (EPP) is a formidable surgical procedure for patients with malignant mesothelioma. 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 dysrhythmias (mainly atrial fibrillation), two patients suffered from diaphragnic hernia and 4 patients fell into the heart failure. Conclusions: The postoperative management of 25 patients received EPP for mesothelioma 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**

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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 kidney 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**

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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 LDMF 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 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**

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Aim of the study: Working out a new kind of complex treatment of local-expanded malignant pleural mesothelioma. Materials and methods. There were included 7 patients (2 male and 5 female) from 35 to 63 years (mean, 52.7) with malignant 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 mW/cm2) and intrapleural perfusion hyperthermo-chemotherapy (Cisplatin 200 mg/m2 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 mW/cm2) and intrapleural perfusion hyperthermo-chemotherapy (Cisplatin 200 mg/m2 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.
P20-1
Adjuvant chemotherapy subsequent to extrapleural pneumonectomy for patients with malignant pleural mesothelioma is slightly beneficial
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[目的] Trimodar 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 (MPM). However, such an aggressive 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 MPM.[患者] 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 supracavicular 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 Iib 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.[結果] 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 months. The other patient with peritoneal recurrence survived 2 years after EPP. One patient in stage Iib 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. [要約] 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
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Background: Tridrimonal 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%. 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). 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
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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, an adequate organ function (including predicted postoperative forced expiratory volume in 1 second >600ml/m²), and a clinical stage I-II, T1-3, 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 epithelial (n=14) or biphasic (n=7). The chemotherapeutic regimens included cisplatin/gemcitabine/vinorelbine (n=11), cisplatin/ pemetrexed (n=6) or cisplatin (n=4). At 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. The 2-year survival rate was 57.1% in patients with epithelioid tumors and 57.1% in patients with biphasic tumors. Postoperative recurrences 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.
Feasibility of establishing a multidisciplinary program devoted solely to the treatment of pleural diseases

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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 individual's 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 80 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.

The study of the malignant pleural mesothelioma case that survived more than two years

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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 stage 1b or 2. Two cases of the epithelial type were stage 3. Chemotherapy was performed in 5 cases, and neoadjuvant chemotherapy + extrapleural pneumonectomy was performed in 2 cases. The hemithoracic irradiation 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 stage 1 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.
Replication-competent retrovirus vector-mediated suicide gene therapy achieves significant therapeutic efficacy against human malignant mesothelioma xenografts

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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.

FGK45 immune-based treatment may cure mesothelioma in mice with or without combination with viral therapy

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**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.

Inhibition tumor growth of malignant pleural mesothelioma by adeno-associated viral type-8 vector expressing mda-7/IL-24

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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/IL-24 by single intramuscular injection. We generated type 8 AAV vector expressing secratable mda-7/IL24 (AAV8/8-md7a). In vitro studies showed that conditioned medium collected from C2C12 cells transduced with AAV8/8-md7a is able to suppress tumor growth of human MPM cell lines. We generated a MPM intra-peritoneal disseminated carcinoma model by injection of MX1-21H/Luc cells into BALB/c nude mice. After injection of AAV8/8-md7a 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 AAV8/8-md7a injected mice compared to control GFP expressing AAV injected mice (p<0.01). Survival effect was also detected in AAV8/8-md7a injected mice (p<0.01). These results demonstrated that single intramuscular injection of AAV8/8-md7a is useful for the gene therapy of MPM.
Poster Discussion

P21-4

VEGF targeting in mesothelioma treatment using an interleukin-6 signal inhibitor based on adenovirus gene delivery
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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 toclizumab, 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. A fiber-modified adenovirus vector (Ad5/3) was beneficial for gene transfer in mesothelioma cells. Because a fiber-modified adenovirus vector (Ad5/3) was beneficial for gene transfer in mesothelioma cells, the virus strategy 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
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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 therapies 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. 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 be a promising therapeutic approach and investigated a combination effect of pemetrexed and CRAd in several MPM cell lines.

P22-1

Ex vivo expansion of tumor-infiltrating lymphocytes for adoptive cell therapy: a potential therapeutic option for patients with malignant pleural mesotheloma
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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 in 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 (35Gy). 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 2.5 x 107 ± 1.5 x 107 (range: 4 x 107 - 5 x 107). 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.
Targeting macrophages as a novel therapeutic approach for malignant pleural mesothelioma

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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 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) polarized with M1 cytokines, suggesting that cytokine re-polarization of macrophages in mesothelioma tumors to an M1 phenotype could augment therapeutic efficacy.

Targeted imaging and therapy of malignant mesothelioma using novel internalizing antibodies

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Background: A panel of phase antibodies has been generated by selection of phase 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 Tc and related nanosize immunoposse 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°C for 1 h with M28, VAMT-1 or control cells (BPH-1), to assess the total cellular binding versus intracellular uptake. Cy5.2 labeled antibody was also used to monitor the in vitro intracellular uptake employing fluorescence microscopy. For in vivo studies, the immunoliposome and immunoliposome were 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 

Estrogen receptor beta exerts tumor repressive functions in human malignant pleural mesothelioma via EGFFR inactivation and affects response to gefitinib

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Background: The role of estrogen and estrogen receptors in oncogenesis has been investigated in various malignancies. Recently our group identified estrogen receptor beta (ERβ) 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 MMe cells and evaluated cell proliferation and EGF receptor (EGFR) activation. Results: Our data indicate that ERβ knockdown in ERβ 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 cavelin 1 that results in an altered internalization and in a subsequent reduced activation of EGFR-coupled signal transduction pathways. We also demonstrate that differential expression of ERβ influences MMe tumor cell responsiveness to the therapeutic agent: Gefitinib.Conclusion: This study describes a role for ERβ in the modulation of cell proliferation and Gefitinib 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.
P23-1

Angiogenic tissue response in patients with MPM after treatment with cisplatin, pemetrexed and axitinib

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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. 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 patients 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

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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, PDGFRB 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: YMESEO-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 MTSO-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-spectrum activity 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

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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 thoracic 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 EHMES1 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 inhibited 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.
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 IGFR1L, 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 IGFR1 inhibition in 13 MPM cell lines. Material and Methods: The RTK inhibitors (TKI) erlotinib (EGFR inhibitor), MK-0646 (IGFR-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 4/6 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 phosphorylated 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 IGFR1 as mediators of this effect in MPM. The characterization of the RTKs involved in this feedback activation could lead to new therapeutic strategies.

TS-1 suppresses the growth of malignant pleural mesothelioma cells co-expressing dihydroxypyrimidine dehydrogenase and thymidine phosphorylase in an orthotopic model

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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 dihydroxypyrimidine 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 orthotopic 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.
Poster Discussion

**P24-1**

Primary malignant peritoneal mesothelioma of the great omentum diagnosed by cytology of ascites: A case report

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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-labeled 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

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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. Case 2, a 67-year-old female came to us with abdominal distention, and despite chemotherapy, 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

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Benign multicystic mesothelioma is rare lesion mainly found in the pleural 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 up at our outpatient clinic. The pleural effusion was still uncontrollable in spite of several chemotherapy. Right pleural effusion has been pointed up at our outpatient clinic.

*Poster Discussion*
P24-4

Spontaneous chylothorax in malignant pleural mesothelioma

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Chylothorax occurs when the thoracic duct is damaged through a trauma or 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 Doxirubucine, 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 doxorubicin 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

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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 dyspnoea on exertion 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 mesothelioma, 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 thickened right diaphragm and irregularly decompresed 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 biopsied, 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 multilocyst formation, demonstrating abundant viscous liquid production and peculiar cytological findings

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Introduction: We report here a peculiar case of pleural malignant mesothelioma with multilocys 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 cytoplasts and atypical nuclei were scattered. The cells have an eccentric nucleus or occasionally have two or three nuclei. Some cell clusters contained collagenous stroma that appeared metachromatic on Giemsa stain. These cells were positive for calretinin, CK 5/6, D2-40 and CD68, 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.
P25-1
Collision tumor composed of malignant pleural mesothelioma and primary lung cancer: A case report and array-based study

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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 noncancerous 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

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We report a case of a malignant pericardial mesothelioma of the epithelioid type in a 38-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

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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 proportion 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 treatment of solitary pulmonary nodule in the upper lobe of the right lung. The nodule appeared 4 months after high orchiectomy 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-assisted 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 reflect its malignant nature and rapid invasiveness. Metastectomy for this disease 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.
Malignant pleural mesothelioma with long-term temporary tumor disappearance of a local relapse after surgery: a unique case report

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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.

A unique case of malignant pleural mesothelioma with angiosarcomatous differentiation

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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 anemic and required blood transfusion. A bronchoscopy, video-assisted thoracoscopic surgery (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 CKS5; they were negative for CD31 and TTF-1. The superficial component of the tumour contained a distinct proliferation of atypical epithelioid 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 a malignant epithelioid mesothelioma with angiosarcomatous differentiation rather than two synchronous tumours, namely a mesothelioma and angiosarcoma.

Acquired pemetrexed and carboplatin resistance in a long-term survivor of mesothelioma: a case report including gene expression findings

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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 25000 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 RNA 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 the 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.
Primary intra-hepatic malignant mesothelioma accompanying with multiple lymphadenopathies due to acid-fast bacilli: A case report

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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 lymphadenopathy 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 lymphnode lesions was a non-tumorous granuloma due to acid-fast bacilli. 18F FDG-PET examination was insufficient to differentiate the infectious lymphadenopathy from the malignant mesothelioma in this case.

Localized malignant pleural mesothelioma

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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.

A case report of recurrent malignant pleural mesothelioma with long-term disease control after extrapleural pneumonectomy

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We present a case of malignant pleural mesothelioma, who recurred 5 years after the initial treatment. The patient was 55-year-old man. He poitned 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 mass, 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 mesothelioma, 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).
Simultaneous presentation of pericardial mesothelioma and chronic lymphocytic leukemia

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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 suspected, 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 infiltrating 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 single-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 secondary malignancy.

Difficult case to diagnosis of malignant pleural mesothelioma by medical thoracoscopy

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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 fluid showed the mesothelial cells and macrophages. The pathology specimens revealed that the cysts were lined by mesothelial cells which reacted immunohistochemically positive for cytokeratin and negative for factor B. 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 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: MPM 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.