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## NURSING PROGRAM

**WEDNESDAY, SEPTEMBER 12, 2012 08:00-17:00**

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NS1.2: BWH THORACIC NURSING CARING PRACTICES UNCOVERED: LINKING CARING THEORY TO CARING PRACTICES

Kathleen Murphy, Carol M. Corbett
Brigham And Women’s Hospital/UNITED STATES OF AMERICA

BWH Thoracic Surgery Conference Planning Committee Members embarked on an exploration of nursing practices used to care for the mesothelioma patient and family. In addition to a comprehensive literature search, nurses reflected on patient care experiences using guided meditation, narrative work, and story telling. Using these methods, recurrent themes emerged that illuminated the BWH specialized thoracic nursing care practices which support excellent care for mesothelioma patients and families. This nursing conference content will highlight these caring practices over each stage of recovery. This talk will include discussion of identified nursing care themes, and their proposed link to the Caritas Processes described in Jean Watson’s Caring Theory.

Disclosure: No significant relationships.

NS2.1: SPECIALIZED NURSING CARE IN THE ICU: DELIVERING HOPE AND POSITIVITY

Kathryn Andrews, Paulette Puleo, Chantal Scutt, Louise Caperelli White
Brigham And Women’s Hospital/UNITED STATES OF AMERICA

Malignant Pleural Mesothelioma as a disease process presents many challenges. Patients come to BWH for treatment with the hopes of increasing quality and quantity of life. Nurses in the Thoracic Intensive Care Unit are highly skilled in the care of the patient in the post-op critical care period. The two operative procedures for the treatment of mesothelioma; extrapleural pneumonectomy and pleurectomy/decortication and their nursing care will be described. While some of the patients follow a routine postoperative course, there are others whose course is more prolonged and complicated. Two case studies will highlight the use of hope and positiveness in the recovery of this patient population. Kaye Herth’s Hope Index and Barbara Frederickson’s Broaden and Build Theory of Positive Emotion are helpful as references for our nursing practice.

Disclosure: No significant relationships.

NS2.2: SPECIALIZED NURSING PRACTICES IN THE TICU: ESTABLISHING TRUST AND GUIDING RECOVERY

Teresa Breslin, Judy Finn, Rebecca Guertin, Melanie Smith, Maureen Tapper
Brigham And Women’s Hospital/UNITED STATES OF AMERICA

Thoracic Intermediate Care Unit (TICU) nursing care practices are directed towards guiding the postoperative recovery of the mesothelioma patient. Low nurse to patient ratios (1:2), combined with up to date bedside technology and monitoring tools promote nursing care interventions reflective of ‘Knowing’ the patient and family, and serve as a foundation for establishing trust. Description of the standardized yet individualized nursing interventions; the ‘TICU Commandments’ that are directed at specified patient outcomes that promote recovery will be discussed. Interdisciplinary collaboration is a cornerstone of TICU nursing care. Daily Thoracic Surgery multidisciplinary rounds provide for an on unit discussion of the mesothelioma patient’s daily goals as well as post hospital care needs. Nursing representation in the weekly Thoracic Surgery Quality Assurance meeting allows for timely communication and ongoing care improvement efforts.

Disclosure: No significant relationships.

NS3.1: IMP SUPPORT TEAM: CHAPLAINCY AND SOCIAL WORK

Virginia Hemani, George Winchester Sj, Charlene Haouiliya
Brigham And Women’s Hospital/UNITED STATES OF AMERICA

A diagnosis of Mesothelioma can be a frightening, lonely and unexpected experience; often coming as a shock to an otherwise healthy person. A cancer diagnosis affects patients, families, loved ones and friends. Its impact is not only physical, but also emotional, spiritual and financial. It is normal to worry about how this diagnosis will affect you, your life and your family.

Disclosure: No significant relationships.
The International Mesothelioma Program treats patients from all parts of the country as well as world-wide. Being far away from home adds another layer of difficulty. It means being away from supports, securing local accommodations in Boston, and all the while still coping with a life-threatening illness.

To support patients and families through this process, which at the onset can feel overwhelming, there is a dedicated Mesothelioma Support Team consisting of social work, chaplaincy, a patient liaison and housing coordinator. These professionals provide counseling, emotional and spiritual support as well as practical help with housing and financial needs to patients as well as caregivers.

Disclosure: No significant relationships.

NURSING SESSION 3   SEPTEMBER 12, 2012 13:15-14:15

NS3.2: NURSING SUPPORT: EASING THE BURDEN OF PATIENTS AND FAMILIES

Joanne Hall, Robin Kaufman, Nancy Roy, Bernadette White
Brigham And Women’s Hospital/UNITED STATES OF AMERICA

Thoracic Intermediate Care Unit nursing practice encompasses patient advocacy, establishing trust, respecting patient autonomy, and knowing the patient. These themes are central to expert Thoracic nursing practice, and have emerged through narrative work of staff nurse members of the conference planning committee. A case study presentation will explicate supportive Thoracic nursing care practices during the inpatient hospitalization of a patient with newly diagnosed mesothelioma. This skit like format coupled with the nurses reflective voice highlights notable moments in practice including patient teaching, emotional support, and specialized knowledge. The essence of the patient’s experience is highlighted and captured through practical and experienced staff familiar with the patient’s underlying pathology and resulting surgery.

Disclosure: No significant relationships.

NURSING SESSION 4   SEPTEMBER 12, 2012 14:30-17:00

NS4.1: REFLECTIONS OF MESOTHELIOMA PATIENTS AND FAMILIES; IMPACT OF NURSING CARE

Meg Meccariello, Katherine Almeida
Brigham And Women’s Hospital/UNITED STATES OF AMERICA

Patients and their family care givers can offer nurses valuable information about their lived experience. In this session, a Brigham & Women’s Hospital mesothelioma patient and family caregiver will speak to us about what nursing care practices were meaningful and offer suggestions on those that can be improved.

Disclosure: No significant relationships.
IA.2: EARLY DETECTION OF MALIGNANT MESOTHELIOMA IN ASBESTOS-EXPOSED INDIVIDUALS WITH A NONINVASIVE PROTEOMICS-BASED SURVEILLANCE TOOL

Rachel M. Ostroff1, Michael Mehan1, Alex Stewart1, Deborah Ayers1, Edward Brody1, Stephen Williams1, Stephen Levin1, Brad Black1, Michael Harbut2, Michele Carbone2, Chandra Goparaju3, Harvey Pass3
1Somalogic, Inc., Boulder/CO/UNITED STATES OF AMERICA, 2Mt. Sinai Medical Center, New York/NY/UNITED STATES OF AMERICA, 3Libby Mt Center For Asbestos Related Diseases/MIT/UNITED STATES OF AMERICA, 4Oncology, Karmanos Cancer Institute, Detroit/MI/UNITED STATES OF AMERICA, 5Center For Occupational And Environmental Medicine, Providence Hospital, Southfield/MI/UNITED STATES OF AMERICA

Background: Malignant mesothelioma (MM) is an aggressive, asbestos-related pulmonary cancer that is increasing in incidence. Because diagnosis is difficult and the disease is relatively rare, most patients present at a clinically advanced stage where possibility of cure is minimal. To improve surveillance and detection of MM in the high-risk population, we completed a series of clinical studies to develop a noninvasive test for early detection.

Methods: We conducted multi-center case-control studies in serum from 117 MM cases and 142 asbestos-exposed control individuals. Biomarker discovery, verification, and validation were performed using SOMAmer proteomic technology, which simultaneously measures over 1000 proteins in unfractionated biologic samples.

Results: Using univariate and multivariate approaches we discovered 64 protein biomarkers and derived a 13-marker random forest classifier with an AUC of 0.99 ± 0.01 in training, 0.98 ± 0.04 in independent blinded verification and 0.95 ± 0.04 in blinded validation studies. Sensitivity and specificity at our pre-specified decision threshold were 97%/92% in training and 90%/95% in blinded verification. This classifier accuracy was maintained in a second blinded validation set with a sensitivity/specificity of 90%/89% and overall accuracy of 92%. Sensitivity correlated with pathologic stage; 77% of Stage I, 93% of Stage II, 96% of Stage III and 96% of Stage IV cases were detected. An alternative decision threshold yielding 98% specificity would still detect 60% of MM cases. In a paired sample set the classifier AUC of 0.99 and 91%/94% sensitivity/specificity was superior to that of mesothelin with an AUC of 0.82 and 66%/88% sensitivity/specificity. The biomarker panel consists of both inflammatory and proliferative proteins, processes strongly associated with asbestos-induced malignancy.

Conclusion: The SOMAmer biomarker panel discovered and validated in these studies provides a solid foundation for surveillance and diagnosis of MM in those at highest risk for this disease.

Disclosure: RO, MM, AS, DA, EB, and SW are employees of SomaLogic.

IA.3: IDENTIFICATION OF AN AUTOANTIBODY PANEL AS A POTENTIAL SIGNATURE OF MALIGNANT PLEURAL MESOTHELIOMA

Siyu C. Zhang1, Xinho Zhang1, Felix Fernandez-Madrid2, Harvey Pass1, Ann G. Schwartz3, Michael Harbut4
1Medstar Program, Irvin D. Reid Honors College, Wayne State University, Detroit/MI/UNITED STATES OF AMERICA, 2Internal Medicine, Wayne State University, Detroit/MI/UNITED STATES OF AMERICA, 3Cardiothoracic Surgery, New York University Medical Center, New York/NY/UNITED STATES OF AMERICA, 4Oncology, Karmanos Cancer Institute, Wayne State University, Detroit/MI/UNITED STATES OF AMERICA, 5Center For Occupational And Environmental Medicine, Providence Hospital, Southfield/MI/UNITED STATES OF AMERICA

Background: Malignant pleural mesothelioma (MPM), associated with occupational asbestos exposure, is a deadly disease with no effective treatment due to its high resistance to chemo-radiotherapy. Molecular mechanisms responsible for its chemo-radiotherapeutic resistance are complicated and undefined. The presence of side population cells (SP cells) in tumors is a well-accepted explanation for their anti-cancer drug resistance and tumor relapse. SP cells are reported to be enriched in cancer stem cells (CSCs). Therefore, monitoring SP cells may predict both CSC-associated aggressiveness and SP-associated chemo-radio-therapeutic resistance. A panel of autoantibodies against SP-associated autoantigens may be an invaluable signature for early diagnosis and prediction of drug resistance and MPM relapse.

Methods: In the present study, Hoechst 33342 SP cell analysis was used to isolate SP cells from the H2714 MPM cell line. A T7 phage MPM SP cDNA library was constructed in order to identify SP-associated autoantigens using biopanning techniques with sera from patients with asbestos exposure or MPM. The enrichment of SP-associated autoantigens after biopanning was tested using plaque lift assay and immunochemical detection. The putative SP-associated phage clones were collected for PCR and sequencing analysis. Identities of those selected autoantigens were revealed through the sequence BLAST program. Functional annotation of SP-associated autoantigens was analyzed by DAVID Bioinformatics.

Results: We have identified 300 putative SP-associated phage clones after biopanning enrichment using sera of patients with asbestos exposure and MPM. Sequencing analysis revealed that a total of 29 clones carrying SP-associated autoantigens were in frame and unique. Table 1 shows the differential autoantigens identified in the sera of patients with asbestos exposure and MPM, which summarizes BLAST results of the SP-associated autoantigen clones. Functional annotation analysis indicates that 11 autoantigens identified by the sera of asbestos exposed individuals are related to acetylation, nucleotide binding and coupled ATPase activity, and that 18 autoantigens identified from the sera of MPM are involved in ribosome acetylation and WD 40 repeats, indicating that generating autoantibodies against autoantigens involved in acetylation may be a common feature of asbestos-related disease. Table 1. Differential autoantigen panels identified in the sera of patients with asbestos exposure and MPM.
Conclusion: Differences in autoantibody signatures between the sera of patients with asbestos exposure and MPM may provide useful tool for early diagnosis of MPM. Autoantibodies against SP-associated MPM autoantigens may be used to construct an invaluable panel for detecting the existence of an SP fraction within MPM, monitoring anti-cancer drug resistance, and predicting cancer relapse of MPM.

Disclosure: No significant relationships.

SESSION IA  BIOMARKERS FOR MESOTHELIOMA DETECTION, DIAGNOSIS AND PROGNOSIS  SEPTEMBER 12, 2012 10:00-11:30

IA.4: DETECTION OF CIRCULATING TUMOR CELLS IN PERIPHERAL BLOOD OF PATIENTS WITH MALIGNANT PLEURAL MESOTHELIOMA

Jacques Raphael1, Christophe Massard1, Françoise Farace2, Cwenaël Le Teuff3, Jacques Margery4, Fanny Billiot4, Antoine Hollebecque2, Benjamin Besse1, Jean-Charles Soria1, David Planchard1

Thoracic Group, Inserm U818, Institut Gustave Roussy, Villejuif/FRANCE, 1Department Of Medical Oncology, Institut Gustave Roussy, Villejuif/FRANCE, 2Translational Research Laboratory, Institut Gustave Roussy, Villejuif/FRANCE, 3Department Of Statistics And Epidemiology, Institut Gustave Roussy, Villejuif/FRANCE, 4Pulmonary Department, Percy Hospital, Paris/FRANCE

Background: The independent prognostic value of Circulating Tumor Cells (CTC) level has been demonstrated in patients with advanced breast, prostate and colorectal cancers. There is currently very few data on Malignant Pleural Mesothelioma (MPM) and CTC. We investigated whether the presence of CTC was correlated with prognosis factors and treatment efficacy in MPM patients.

Methods: Patients (pts) with MPM in progression were enrolled before any new line of treatment in a prospective monocentric study. CTC detection was made on peripheral blood samples (7.5ml) using the “CellSearch” assay according to the manufacturer’s protocol. The correlation between the presence of CTC and known worse prognosis factors was assessed using the X2 test. Progression Free Survival (PFS) was defined as the time from diagnosis until first progression (PFS1) and as the time from CTC measure until progression or death (PFS2). Comparison of PFS value according to CTC detection was performed using the log-rank test. The cut-off date of the analysis was May 2012.

Results: Twenty-five MPM pts with a median follow-up of 4.2 months were included. The median age and sex ratio (M/F) were 65 years old and 2.1 respectively. Eighty-four percent of pts had an epithelioid MPM, 64% had a stage 4 disease, 60% had an anemia, a thrombocytosis or a leucocytosis at the time of inclusion. All pts except one had an Eastern Cooperative Oncology Group performance status (ECOG) < 2 and 64% received more than one line chemotherapy. CTC were detected in 48% of pts (n=12) with a median level of 1.5 (0-36). No significant correlation was observed between the presence of CTC and a metastatic disease, an ECOG > 1, the presence of anemia, leucocytosis, or thrombocytosis and the non-epithelioid type. The median PFS (1 and 2) were 17.9 (95%CI= [10.1-24.0]) and 2.5 (95%CI=[ 1.3-3.5]) months respectively. CTC detection was not a significant predictor of PFS 2 (p=0.27).

Conclusion: Detection of CTC has been done in a small cohort of MPM patients. Their detections could be an important tool though we were not able to demonstrate a significant prognostic value or a difference in PFS between CTC levels. Further analyzes are in progress, and updated results will be presented in September.

Disclosure: No significant relationships.

SESSION IA  BIOMARKERS FOR MESOTHELIOMA DETECTION, DIAGNOSIS AND PROGNOSIS  SEPTEMBER 12, 2012 10:00-11:30

IA.5: THE PROGNOSTIC ROLE OF EXPRESSION OF POLO-LIKE KINASE 1 (PLK1) AND CELL DIVISION CONTROL 2 (CDC2), TWO POTENTIAL THERAPEUTIC TARGETS IN MALIGNANT PLEURAL MESOTHELIOMA

Anthony Linton1, Kim Griggs2, Steven C. Kao3, Janette Yardy3, Stephen Clarke4, Douglas Henderson4, Brian C. McCaugha5, Nico Van Zandwijk6, Sonja Klebe6, Glen Reid1

1Asbestos Diseases Research Institute, Concord/NSW/AUSTRALIA, 2Flinders Medical Centre, Adelaide/AUSTRALIA, 3Concord Repatriation General Hospital, Concord/AUSTRALIA, 4Medical Oncology, Royal North Shore Hospital, St Leonards/NSW/AUSTRALIA, 5Cardiothoracic Surgical Unit, Royal Prince Alfred Hospital, The Baird Institute And Faculty Of Medicine, University Of Sydney, Newtown/AUSTRIAN, 6Department Of Anatomical Pathology, Flinders University, Adelaide/AUSTRALIA

Background: PLK1 and CDC2 play important roles in cell cycle regulation. Elevated levels in several tumours have been associated with poor prognosis. Previous gene expression profiling studies revealed both targets to be upregulated in malignant pleural mesothelioma (MPM). We aimed to assess the effects of target knockdown on MPM cell growth and to determine whether the pattern of PLK1 and CDC2 expression is associated with overall survival (OS) in MPM patients.

Methods: Patients with a confirmed pathological diagnosis of MPM who underwent extrapleural pneumonectomy (EPP), or pleurectomy/decortication during 1991-2009 were identified from a prospective Royal Prince Alfred Hospital database. Tissue microarrays were constructed and PLK1 and CDC2 immunohistochemistry performed. OS was calculated from date of diagnosis. The prognostic role of PLK1 and CDC2 was reviewed (mean score from assessable cores as percentage of tumour cells labelled), adjusting for known prognostic factors including age, gender and histological subtype, using a Cox regression multivariate model. To assess target knockdown, MPM cell lines were transfected with siRNA specific for PLK1 or CDC2, or the small molecule drugs BI 2536 and Roscovitine, and effects on proliferation measured.

Results: 155 patients with available tissue were identified: 79% male; median age 61 (range 22-83); 52% EPP. Histological subtypes included: 68% epithelioid, 24% biphasic; 8% sarcomatoid. Median PLK1 expression was 3% (Range 0-42.5%). Median CDC2 expression was 15.8% (Range 0.5-96%). The median OS was 14.3 months (95% CI: 10.6-18.0). On univariate analysis, greater PLK1 expression (hazard ratio (HR) 1.90 for each 0.5–96%), age (HR 1.38 for each 10 year increase, p <0.001) and male gender (HR 1.53 (95%CI: 1.1-2.1)) were significant predictors of OS. On multivariate analysis, an elevated PLK1 expression was significantly associated with worse overall survival (HR 2.43, 95% CI: 1.30-4.60, p = 0.005). CDC2 expression was not significantly associated with survival on univariate analysis (HR 1.94, 95% CI: 0.82-4.60, p = 0.136).

Conclusion: Elevated levels of PLK1 are associated with worse prognosis in malignant pleural mesothelioma. Our findings support the potential of targeting PLK1 for therapeutic benefit in malignant pleural mesothelioma.

Disclosure: No significant relationships.
IA.6: AN UNEXPECTED PREDICTIVE ROLE OF RRM1 IN MALIGNANT PLEURAL MESOTHELIOMA TREATED WITH CISPLATIN AND VINORELINE.

Zarah G. Zimling1, Eric Santoni-Rugiu1, Cecilia Bech1, Jens B. Soerensen1
1Oncology, 5074, University Hospital Of Copenhagen, Copenhagen/DENMARK, 2Pathology, University Hospital Of Copenhagen, Copenhagen/DENMARK

Background: Ribonucleotide reductase (RRN) is an essential enzyme for DNA synthesis that converts ribonucleoside di-phosphates into deoxyribonucleoside di-phosphates. The enzyme consists of a large sub-unit (RRM1) and a small sub-unit (RRM2). Overexpression of RRM1 is conventionally associated with resistance towards the chemotherapeutic agent gemcitabine, which is a potent inhibitor of RNR. Our group have recently finished a study on the predictive value of immunohistochemically detected RRM1 in a cohort of non small-cell lung-cancer (NSCLC) patients from a randomized phase III trial comparing triplet-chemotherapy (paclitaxel, cisplatin, gemcitabine) to standard doublet-therapy (cisplatin, vinorelbine). We found that increased expression of RRM1 was associated with significantly decreased progression-free survival (PFS) and overall survival (OS) only in the patients receiving cisplatin-vinorelbine therapy. This finding was unexpected, since vinorelbine is a spindle-toxin and resistance towards this agent has never been associated with RRM1 over-expression. It has however been shown that vinorelbine can reduce the repair of radiotherapy-induced DNA damage in small-cell lung-cancer cell-lines, pointing to a possible interaction between vinorelbine and the DNA repair system. To further investigate this finding we tested the association between immunohistochemically detected RRM1 expression and outcome in a cohort of patients with malignant pleural mesotheliomas (MPM) treated with cisplatin and vinorelbine.

Methods: Fifty-four consecutive patients with MPM, were enrolled between February 2003 and September 2006 into a phase II trial with cisplatin and vinorelbine. The formalin-fixed paraffin-embedded bioptic samples were used for IHC. In short, tissue sections (4µm) from five different areas were stained with an antibody against RRM1. A semi-quantitative score was given to four different regions of the sections: nuclear, cytoplasmic, plasma membrane, and nucleolus. The score was given on a scale from 0 to 3.

Results: Sixty-six patients had enough tumor tissue for IHC. The upper quartile H-score (≥1.93; p = 0.004) were associated with worse outcomes. CDC2 expression was not associated. On multivariate analysis, PLK1 expression (HR 1.87; 95% CI 1.23-2.83; p=0.003), subtype (HR 3.05; 95% CI 2.08-4.46; p=0.001) and age (HR 1.22; 95% CI 1.01-1.48; p=0.036) remained significant. In mesothelioma cell lines, knockdown of either PLK1 or CDC2 led to siRNA dose-dependent growth inhibition. Experiments with small molecule inhibitors of PLK1 (BI 2536) and CDC2 (Roscovitine) also inhibited proliferation.

Conclusion: We have identified PLK1 expression as an independent prognostic factor in MPM. Furthermore, inhibition of PLK1 and CDC2 is growth inhibitory in MPM cells, suggesting that PLK1 and CDC2 have potential as targets for therapeutic intervention in MPM.

Disclosure: No significant relationships.
identify all the MPM samples. When we performed the sequential combination of the binary gene ratio tests using the test set we found that 90 of 100 MPM samples were correctly called as MPM, and 1 normal pleura sample and 1 sarcoma were incorrectly classified as MPM. Additional review of the MPMs called not-MPM showed that in 2 MPMs the actual specimens used for the RT-PCR had 0% tumor content. The overall diagnostic sensitivity of the 26-gene signatures in the test set analyzed by RT-PCR was 92%, whereas the specificity was 97%.

**Conclusion:** Our results indicate that tests generated from a relatively small number of genes are able to accurately distinguish MPMs from other thoracic samples supporting our hypothesis that the gene ratio tests could provide a useful clinical adjunct in the diagnosis of MPM.

**Disclosure:** No significant relationships.
SESSION IB  CHEMOTHERAPY FOR MESOTHELIOMA  SEPTEMBER 12, 2012 10:00-11:30

IB.1: UPDATED ANALYSIS OF EORTC 08983: A RANDOMIZED TRIAL OF RALTITREXED AND CISPLATIN VERSUS CISPLATIN IN MALIGNANT PLEURAL MESOTHELIOMA

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Background: EORTC 08983 randomised 250 chemotherapy-naive patients with advanced pleural mesothelioma to receive cisplatin 80 mg/m² IV on day 1, alone (arm A) or combined with raltitrexed 3 mg/m² IV (arm B) (Van Meerbeeck, j Clin Oncol 2005; 23:6881-9).

Methods: An unplanned updated overall survival (OS) analysis based on the data as of May 5, 2011. OS is defined as time from randomisation to death from any cause. Adjusted indirect comparison of outcomes with EMPHASIS trial (Vogelzang, 2003)

Results: After a median follow up of 98 months, 118/124 participants in arm A and 115/126 in arm B were deceased, an increase of 23 deaths (10 and 13) compared to the previous final analysis. Seventeen (6 and 11) patients were censored at the last date known to be alive: 12 (4 and 8) had their survival status not updated due to sites not responding, 2 in arm B are alive and 3 (2 and 1) are lost to follow up. Progressive disease was the cause of death in 87% of deaths in both arms. The median survival time in arm B is 11.4 months (95% CI 9.5 - 13.7) compared to 8.8 months (7.7-10.7) in arm A. The hazard ratio is 0.77 (95% CI 0.6 -1.0) in favour of arm B.

The p-value for the log-rank test is 0.0491, which is still below the nominal cut-off value of 0.049266 alpha level that was used at the time of the final analysis. The adjusted indirect comparison of response rates, progression and overall survival is 0.56 (95% CI 0.24-1.30), 1.15 (0.82-1.61) and 0.99 (0.69-1.41), respectively.

Conclusion: The results of this updated analysis 1. confirm the superior efficacy of the raltitrexed/cisplatin combination over cisplatin alone and 2. suggest an equipoise between raltitrexed and pemetrexed in combination with cisplatin, in the first line palliative treatment of patients with malignant pleural mesothelioma.

Disclosure: No significant relationships.

SESSION IB  CHEMOTHERAPY FOR MESOTHELIOMA  SEPTEMBER 12, 2012 10:00-11:30

IB.2: UPDATED RESULTS OF THE PHASE II CLINICAL TRIAL OF THE ANTI-MESOTHELIN MONOClonAL ANTIBODY AMATUXIMAB IN COMBINATION WITH PEMETREXED AND CISPLATIN FOR FRONT LINE THERAPY OF PLEURAL MESOTHELIOMA AND CORRELATION OF CLINICAL OUTCOME WITH SERUM MESOTHELIN, MPF AND CA-125

Raffit Hassan¹, Dan O’Shannessy¹, T. Jahan², H. L. Kindler³, L. Baethenova⁴, M. Reck⁵, Ira Pastan⁷, P Fatato⁸, J. Parno⁹, L. Bazhenova⁵, M. Reck⁶, Ira Pastan⁷, P Fatato², J. Maltzman B. Wallin J. Parno is an employee of United BioSource Corporation. The other authors have no disclosures.

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Background: Amatuximab (MORAb-009) is a chimeric monoclonal antibody to mesothelin, a cell surface glycoprotein highly expressed in malignant mesothelioma. Based on the results of amatuximab in a phase I clinical trial and pre-clinical studies showing synergy in combination with chemotherapy, a single arm phase II study of amatuximab plus pemetrexed (P) and cisplatin (C) was initiated in chemotherapy naive patients with unresectable malignant pleural mesothelioma (MPM). Serum was collected during the study and evaluated for potential biomarkers.

Methods: Eligibility criteria included patients with unresectable epithelial or biphasic MPM, no prior chemotherapy and Karnofsky Performance Status (KPS) >70%. A new Morphotek developed Mesothelin and MPF assay was assessed as part of an exploratory end point for the clinical trial.

Results: From Feb. 2009 to Oct. 2010, 89 pts. were enrolled at 26 global sites. Median pt age = 67 yrs. (range 46-80), 78% male, 70% with KPS >90%, 89% epithelial MPM, 11% biphasic MPM and 88% had stage III/IV disease. In addition to the expected toxicity from PC, hypersensitivity reactions (12.4%; Grade 3/4=4.5%) from amatuximab were noted. Of the 77 evaluable pts 39% had a partial response, 51% stable disease and 10% progressive disease. The median PFS and OS was 6.1 months and 14.5 months respectively. Baseline serum mesothelin, megakaryocyte potentiating factor (MPF) and CA-125 were assessed on all subjects. Baseline mesothelin and MPF were found to be highly correlated with a correlation coefficient (r) =0.77, p=0.001. Using maximally selected chi square methodology, values of 33.14 ng/mL and 4.7 ng/mL were identified as optimal cut points in the determination of OS response for mesothelin and MPF respectively. MPF was also assessed over time and its decline was found to have modest correlation with tumor response (r=0.4; p=0.008), however no such correlation was noted with its rise and tumor progression (r =0.19; p=0.186). For CA-125, when using maximally selected chi square methodology, a value of 6 U/mL was shown to be an optimal cut point in the determination of OS response, with the median OS of 20.7 months in pts with baseline CA-125 less than 6 U/mL versus 13.3 months in pts with CA-125 greater than 6 U/mL.

Conclusion: Amatuximab in combination with P and C was generally well-tolerated with an objective response rate by independent radiological review of 39% and median OS of 14.8 months. Exploratory evaluation of potential biomarkers shows that baseline Mesothelin and MPF are highly correlated. In addition, baseline mesothelin, MPF and CA-125 levels were associated with improved OS and will be useful in patient selection and follow up in future studies.

Disclosure: The following authors are employees of Morphotek Inc. D. O’Shannessy P. Fatato J. Maltzman B. Wallin J. Parno is an employee of United BioSource Corporation. The other authors have no disclosures.
IB:3: RANOMIZED PHASE II TRIAL OF PEMETREXED/CISPLATIN WITH OR WITHOUT CBP501 IN PATIENTS WITH ADVANCED MALIGNANT PLEURAL MESOTHELIOMA (MPM)

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Background: CBP501, a synthetic duodecapeptide, is a novel compound with a dual mechanism of action: 1) CBP501 increases cisplatin influx into tumor cells through an interaction with calmodulin, enhancing cisplatin cytotoxicity, and 2) CBP501 affects cell cycle progression by abrogating DNA repair at the G2 checkpoint. In Phase I clinical trials of CBP501 alone or in combination with cisplatin, the most common toxicity was infusion-related urticaria managed with prophylactic antihistamines. Activity of CBP501 plus cisplatin was observed in patients with ovarian cancer and mesothelioma, including some patients previously treated with cisplatin. These data prompted this randomized phase II trial in MPM.

Methods: Chemotherapy naïve patients with unresectable MPM were stratified by histology (epithelioid vs other) and performance status (PS 0-1 vs 2) and randomized 2:1 to Arm A: pemetrexed/cisplatin plus CBP501 25 mg/m2 IV (42 pts planned), or Arm B: pemetrexed/cisplatin alone at standard doses (21 pts planned). Patients continued on treatment until progression or intolerance; no maintenance single-agent therapy was endorsed. The primary endpoint was progression free survival (PFS) in Arm A; if > 23 of the 42 patients remained free of progression more than 4 months, the combination would be deemed worthy of further study. In addition to standard CT imaging to assess response and PFS, PET scans, pulmonary function tests, and mesothelin levels were performed.

Results: Enrollment was completed in October 2011. 65 pts from 14 institutions were randomized, and 63 were treated. Patient characteristics in the two arms were similar in Arms A/B: median age 62/66, 80/87% male, 75/70% epithelioid histology. Grade 3/4 treatment-related toxicities were uncommon, no different than expected from standard chemotherapy, and comparable in the two arms. 53% of patients treated with CBP501 had infusion reactions, all grade 1-2. Preliminary progression free survival (PFS) data are available based on investigator assessments, which incorporates both radiologic and clinical progression. 27 patients (68%) in Arm A and 14 (61%) in Arm B remained free of progression greater than 4 months. The median PFS was 5.9 mo (95% CI 4.3-9.1) in Arm A, 4.7 mo (3.7-5.9) in Arm B. The investigator-assessed best response as determined using modified RECIST in the treated population was 16/40 (40%; 95% CI 25-57) in Arm A, and 4/23 (17%; 5-39) in Arm B.

Conclusion: This randomized phase II trial met its primary endpoint. Response rate and median progression free survival favored the triplet combination arm. Updated progression data, including results from an independent radiologic review will be provided at the meeting.

Disclosure: No significant relationships.

IB:4: A PASE IB CLINICAL TRIAL OF CISPLATIN AND PEMETREXED IN COMBINATION WITH THE CD40 ACTIVATING ANTIBODY CP-870,893 IN MALNANT MESOTHELIOMA (MPM)

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Background: CD40 is a costimulatory molecule expressed by antigen presenting cells. Murine data supports synergy between cytotoxic chemotherapies, including pemetrexed, and CD40 activation in a mesothelioma mouse model. The purpose of this study was to determine the maximum tolerated dose and safety profile of the CD40 activating antibody CP-870,893 in patients with advanced MPM, to explore immunological biomarkers of activity, and to provide preliminary efficacy information.

Methods: Eligible patients had advanced MPM, were planned for first-line palliative chemotherapy with cisplatin and pemetrexed, had ECOG PS 0-1 and adequate organ function. Patients received cisplatin (75mg/m2) and pemetrexed (500mg/m2) day 1 and CP-870,893 in escalating doses from 0.1mg/kg/day 8 of a 21 day cycle for a maximum of 6 cycles. Stable or responding patients continued maintenance CP-870,893 D1 q21d for a maximum of 6 further cycles. A standard 3+3 phase I trial design was used. Toxicity was assessed weekly using NCI CTCAE V3.0. Immunology biomarkers were obtained at baseline and weekly throughout treatments. CT imaging for response was performed at baseline and 6-weekly with measurement by Modified RECIST.

Results: From March 2010, 15 patients received combination therapy at three dose levels of CP-870,893. 3 patients were treated at dose level 1 (0.1mg/kg) with no dose limiting toxicities (DLT). 3 patients were treated at dose level 2, with 2 DLTs (one episode of hyponatremia and confusion; one episode of splenic infarction, both occurring subsequent to cycle 1). 3 patients were treated at dose level 1.5 (0.15 mg/kg) with no DLTs observed, and an additional 6 patients were then accrued to an expansion cohort at this dose level. The best radiological response was partial response in 6 patients (40%), and stable disease in 9 patients (60%). As of May 2011, median survival is 12.9 months with 8/15 patients deceased. Two patients are continuing single agent treatment. Two patients showed progression followed by subsequent prolonged stabilisation of disease (>24 months). All patients experienced some grade of cytokine release syndrome (CRS) (grade 1-2) following infusion of study drug during at least one treatment cycle. Management of CRS required patient and nursing staff education but was readily managed using parenteral antihistamines and pethidine. Updated survival data, safety data and results of immunology studies will be presented.

Conclusion: A combination of cisplatin, pemetrexed, and CD40 activation with CP-870,893 can be safely administered in patients with advanced MPM, and shows objective response activity similar to cisplatin and pemetrexed alone but with a signal for a delayed and prolonged response in some patients. Further trials of this combination are warranted but should include a chemotherapy-alone control arm.

Disclosure: No significant relationships.
IB.5: IFCT-GFCP-0701 MAPS TRIAL, A MULTICENTER RANDOMIZED PHASE III TRIAL OF Pemetrexed-CISPLATIN WITH OR WITHOUT BEVACIZUMAB IN PATIENTS WITH MALIGNANT PLEURAL MESOTHELIOMA (MPM)

Arnaud Scherpereel1, Julien Mazieres1, Jacques Margery1, Laurent Greillier1, Denis Moro-Sibilot1, Jean-jacques Parienti2, Valerie Coumont3, Alain Riviere4, Isabelle Monnet4, Olivier Molinier5, Herve Lena6, Sylvie Friard7, Jean-paul Duhamel8, Clarisse Audigier-Valette4, Gilles Robinet9, Christian Creveuil9, Catherine Ligeza-Poison10, Philippe Astoul11, Franck Morin11, Gerard Zalcman12

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Background: MPM median OS does not exceed 13 months with pemetrexed/CDDP doublet. U.S. Intergroup phase II trial of gemcitabine/CDDP, with or without bevacizumab, gave an appealing 15.6 months median OS in the bevacizumab arm. French Intergroup aimed to test pem/CDDP, with or without bevacizumab, in a randomized phase III trial.

Methods: Eligible patients had unresectable histologically proved MPM, no prior chemo, PS 0-2, no thrombosis, nor bleeding. Primary endpoint: Primary outcome is overall survival; secondary endpoint is Progression-Free Survival. Patients received pem 500 mg/m², CDDP 75 mg/m² (PC), at D1, and vitamin B12 + B9 substitution, with (arm B) or without bevacizumab (arm A), 15 mg/kg Q3D, for 6 cycles. Arm B non- progressive patients received bevacizumab maintenance therapy until progression or toxicity. 445 patients were to be recruited during a period 48 months, with at least 24 months of follow-up, and 385 events (deaths), will be needed to assure a power of 80% and detect at least a 4.3 months of median survival increase.

Results: This hypothesis leads to a Hazard Ratio (HR) of 1.33 and a 3-years survival of 14.7% in control arm and 23.6 % in experimental arm, with an absolute difference of 8.9% in survival rates. Accrual status: The first patient was included on February 08. On April 30, 2012, 280 patients from 85 French centers had been enrolled.

Conclusion: The end of accrual can be expected for September 2013. Ancillary study: For molecular biomarker analyses, thoracoscopic tissue specimens (TS, ERCC1, MSH2, TUBB3, NF2, p16, RASSF1A methylation, 15 microRNAs) and blood samples (micro-RNAs, VEGF, osteopontin, SMRP) at diagnosis are centrally collected. Finally, a prospective study comparing PET-CT to standard CT with central blinded analysis, is currently on-going for evaluation of response, and accuracy of modified RECIST criteria for mesothelioma.

Disclosure: Research grant to AS lab and travel grant for IMIG 2012 meeting (A Scherpereel)

IB.6: VALIDATION OF PFS FOR THE COMBINATION OF BORTEZOMIB (VELCADE) AND CISPLATIN AS FIRST LINE TREATMENT OF MALIGNANT PLEURAL MESOTHELIOMA (MPM) EORTC 08052.

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Background: Cisplatin is one of the most active drugs available in MPM while bortezomib has shown some activity in single agent phase II studies against MPM. This was a prospective phase II study of cisplatin and bortezomib (CB) in the first line treatment of MPM.

Methods: Patients with histological proven MPM, with performance status (PS) 0/1, were eligible. The doses were cisplatin 75mg/m² /3 wks and bortezomib 1.3mg/m² /day 1, 4, 8, 11 every 3 wks. The primary end-point was progression free survival rate at 18 wks (PFSR=18). The 2 stage Simon design (a=0.1; b = 0.05, P0=0.50 and P1=0.675) was used. The values of P0 and P1 are based on results from previous EORTC LCG studies in malignant mesothelioma. Data from “negative” single agent, “positive” single agent and combination trials show that insufficient clinical activity, moderate clinical activity and significant clinical activity respectively correspond to 18 week PFSR rates of 40.1%, 51.4% and 67.2%. In the first step of the study 37 eligible patients were planned. If more than 19 patients were alive and free of progression at 18 wks the total sample size was increased to 76 eligible patients.

Results: Between 2007 and 2010 82 patients were entered. The median follow-up time is 32.3 months The median age was 55 years (range: 22-77yrs), male/female: 55/27 , PS 0/1: 6/73, Stage T1: 10%; T2: 42%, T3: 25%, T4: 23% and N0: 57%; N1: 4%; N2: 33%; N3: 6%. The median number of cycles received was 4 and 38% received 6 cycles. Cisplatin/bortezomib dose intensity was 98/ 80%. Toxicity (grade 3/4): neutropenia 10%, thrombocytopenia 11%, anaemia 1%. Grade 3-4 hyponatraemia/bortezomib dose intensity was 98/ 80%. Toxicity (grade 3/4): neutropenia 10%, thrombocytopenia 11%, anaemia 1%. Grade 3-4 hyponatraemia/hypokalaemia occurred in 46/ and 17%. Grade 2 tinnitus, grade 3 fatigue occurred in 16%, and 12%, of patients. Motor/sensory/other neurotoxicity was grade 1: 6/28%/7%, grade 2: 2/26%/2% and grade 3: 1/7% respectively. There were 2 toxic deaths at 32 and 74 days due to acute pneumonitis and cardiac arrest. The PFRS-18 (including symptomatic progression) was 53% (80% confidence intervals, CI, 42-64%). The overall survival was 13.5 months (95% CI 10.5-15) with 56% (95% CI 44-66%) alive at 1 year. The PFS was 5.1 months (95% CI 3.3-6.5).

Conclusion: On the basis of the PFRS-18, the null hypothesis could not be rejected, CB gave predictable toxicity and was considered of moderate activity in MPM.

Disclosure: No significant relationships.
Sloan-Kettering Cancer Center, New York/NY/UNITED STATES OF AMERICA; Department Of Radiology, Memorial Sloan-Kettering Cancer Center, New York/NY/UNITED STATES OF AMERICA

**Background:** After initial treatment with pemetrexed/platinum, no second-line therapy has established efficacy for MPM. Vinorelbine is listed in the NCCN guidelines as a potential treatment option for these patients based on a subgroup analysis from a first-line randomized trial (Muers, Lancet 2008) and on a single-arm, single-center phase II study (Stebbing, Lung Cancer 2009). In the latter trial, 16% of 63 patients had a partial response to vinorelbine, but these patients had not received prior treatment with pemetrexed, and over half experienced grade 3-4 toxicity. To augment the existing data, we examined our institutional experience using vinorelbine to treat patients with previously treated MPM.

**Methods:** We reviewed the records of all patients treated at Memorial Sloan-Kettering Cancer Center with vinorelbine as second- or third-line therapy for MPM between 2003 and 2010. In all cases, vinorelbine was administered at a dose of 25 mg/m^2 days 1 and 8 in a 3-week cycle. CT scans were generally performed after every two cycles. Imaging studies were reviewed according to the modified RECIST criteria.

**Results:** Forty-five patients were identified, including 24 treated in 2nd line and 21 in 3rd line. Patient characteristics: male 76%; median age 66 (range 41-85); epithelial 64%, sarcomatoid 19%, mixed 17%; 47% reported asbestos exposure. Treatment prior to vinorelbine included: surgery 47% (20% EPP, 27% P/D); radiation 31%; first-line therapy with pemetrexed/platinum 80%, gemcitabine/platinum 13%, and 7% other (1 patient pemetrexed alone, 2 patients on clinical trial with PDX). Table 1 summarizes the results. No complete or partial responses were achieved (95% C.I. 0-8%). Twenty patients (44%) had stable disease for a median of 2.5 months. Only 16 patients received more than 2 cycles. Seventeen patients (38%) experienced at least one episode of grade 3-4 toxicity, most commonly (>4%) neutropenia, anemia, fatigue, neutropenic fever, nausea, and vomiting.

<table>
<thead>
<tr>
<th>Table 1</th>
<th>All(N=45)</th>
<th>Second-Line(N=24)</th>
<th>Third-Line(N=21)</th>
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<tr>
<td>Response Rate</td>
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<tr>
<td>* Complete response</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
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<tr>
<td>* Partial Response</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>* Stable disease</td>
<td>20 (44%)</td>
<td>9 (38%)</td>
<td>11 (52%)</td>
</tr>
<tr>
<td>* Progression of disease</td>
<td>25 (56%)</td>
<td>15 (62%)</td>
<td>10 (48%)</td>
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<tr>
<td>Median OS (months)</td>
<td>5.4</td>
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<tr>
<td>Grade 3 or 4 hematologic toxicity</td>
<td>12 (27%)</td>
<td>4 (17%)</td>
<td>8 (38%)</td>
</tr>
<tr>
<td>Grade 3 or 4 non-hematologic toxicity</td>
<td>15 (33%)</td>
<td>7 (29%)</td>
<td>8 (33%)</td>
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</tbody>
</table>

**Conclusion:** We observed no responses amongst the 45 patients in this retrospective cohort, which excludes an 8% response rate. The rate of stable disease might suggest some level of activity, and thus it remains a reasonable standard therapeutic option. However, the survival rate was comparable to that of the placebo arm in the vorinostat phase III trial (Krug, ECCO/ESMO 2011). This lack of activity supports the use of a placebo control arm in randomized second-line MPM trials.

**Disclosure:** No significant relationships.
IC.1: ERK SIGNALING AND MESOTHELIOMA

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ERKs (Extracellular Signal-Regulated Kinases) are a family of Mitogen Activated Protein Kinases (MAPKs) that are linked causally to cell proliferation and death in mesothelial and respiratory epithelial cells by asbestos and other pathogenic minerals. ERK pathways involve a cascade of phosphorylation events that can be stimulated by oxidative stress, dimerization or activation of the EGFR, activation of B-integrins, Simian Virus 40, and chemotherapeutic drugs. Activation of ERKs culminates in induction of Activator Protein-1, a heterodimeric transcription factor comprised of members of the c-Fos and c-Jun proto-oncogene families that governs transcription of a number of key genes in cell proliferation, transformation and malignancy. These genes and key ERK family members (ERK1, ERK2, ERK5) have been studied by our lab in mesothelial cells and mesotheliomas as they govern proliferation, migration, apoptosis and chemoresistance of MMs. Moreover, the ERK pathway is closely linked to the AKT tumor survival pathway and is the target of combination therapies in MMs and other tumors as both ERKs and AKT levels are linked causally to cell proliferation, dimerization or activation of the EGFR, activation of B-integrins, Simian Virus 40, and chemotherapeutic drugs. Activation of ERKs culminates in induction of Activator Protein-1, a heterodimeric transcription factor comprised of members of the c-Fos and c-Jun proto-oncogene families that governs transcription of a number of key genes in cell proliferation, transformation and malignancy. These genes and key ERK family members (ERK1, ERK2, ERK5) have been studied by our lab in mesothelial cells and mesotheliomas as they govern proliferation, migration, apoptosis and chemoresistance of MMs. Moreover, the ERK pathway is closely linked to the AKT tumor survival pathway and is the target of combination therapies in MMs and other tumors as both ERKs and AKT levels are constitutively upregulated in tumor cells. Understanding the roles of individual ERKs, their substrates, and cross-talk between pathways is critical to designing novel therapeutic strategies for MMs.

Disclosure: No significant relationships.

IC.2: ROLE OF PAXILLIN IN MESOTHELIOMA: EXPRESSION, MUTATION AND FUNCTIONAL ANALYSIS

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Background: The treatment of mesothelioma has proven difficult with surgical intervention and radiation therapy due to its heterogeneous and invasive nature. Chemotherapy has been the main therapeutic option for many patients. More effective treatment can be achieved with targeted interventions against receptor tyrosine kinases, ligands and other intracellular proteins that modulate tumor cell growth, invasion and metastasis. We have been studying paxillin and FAK in mesothelioma in order to analyze their role in modulating cellular mechanisms in this cancer. Paxillin is a focal adhesion phosphoprotein localized to the cytoskeleton that plays a role in signal transduction, regulation of cell morphology, and the recruitment of structural and signaling molecules to focal adhesions and is involved in motility and migration of tumor cells. It has been shown that the modulation of tyrosine phosphorylation of paxillin regulates both the assembly and turnover of adhesion sites and phosphorylated paxillin enhances lamellipodial protrusions whereas non-phosphorylated paxillin is essential for fibrillar adhesion formation. FAK, a focal adhesion kinase, preferentially interacts with paxillin and controls important biological events, including cell migration, proliferation, and survival. We hypothesized that paxillin and FAK play important roles in mesothelial cell migration and thus studied the genetic aberrations, expression, and functionality of these molecules in mesothelioma.

Methods: We analyzed the expression of paxillin and FAK by IHC on 50 epithelioid, 16 sarcomatoid mesothelioma, and 1 mixed tumor tissues and compared them to 40 normal adjacent lung parenchyma. Mutational analysis of paxillin was performed on tumor DNA. In vitro functional analysis was performed on cells overexpressed with paxillin and its mutants as well as cells with silenced paxillin. In vitro growth assays and confocal imaging to define cellular motility events were performed.

Results: We found an increase in paxillin and FAK expression in mesothelioma compared to normal adjacent lung (p<0.01). Using automated cellular imaging system (ACIS) the immunostained slides were quantified and the analysis of the intensity showed that normal lung had IODs of 118, 13, 45 for paxillin, pFAK and FAK respectively, whereas epithelioid mesothelioma had IODs of 268, 14 and 273 and sarcomatoid had IODs of 331, 12 and 218. We also detected paxillin A127T mutation in mesothelioma patient samples. Live-cell imaging studies revealed that in comparison to wild-type, mutant A127T confers a) increased lamellipodia and filopodia formation b) enhanced mobility c) increased focal adhesion formation and d) increased cell displacement in transiently transfected HEK-293 cells. A mesothelial cell line with silenced paxillin showed increased sensitivity to treatment with cisplatin compared to another that did not respond similarly, emphasizing the heterogeneity of mesothelial tumors types.

Conclusion: Paxillin and FAK are highly upregulated in sarcomatoid and epithelioid mesothelioma. Unique mutations were also found. Paxillin mutations lead to differential processing of lamellipodia and filopodia. We believe that FAK and paxillin are important molecules in malignant mesothelioma and their biological function and therapeutic potential need to be explored further.

Disclosure: No significant relationships.

IC.3: INHIBITION OF RON (MST1R) REDUCES THE PROLIFERATIVE AND MIGRATION CAPACITY OF MESOTHELIOMA CELLS.

Anne-Marie Baird1, Kenneth J. O’Byrne2, David Easty3, Alex Soltermann1, Daisuke Nonaka4, Dean Fennell5, Luciano Mutti6, Harvey Pass7, Isabelle Opitz2, Dearbhaile O’Donnell1, Steven Gray1
1Clinical Medicine, St. James’s Hospital/Trinity College Dublin, Dublin/Ireland, 2Clinical Medicine, Trinity College Dublin/St. James’s Hospital, Dublin/Ireland, 3Department Of Pathology, New York University Medical Center, New York/UNITED STATES OF AMERICA, 4MRC Toxicology Unit, University Of Leicester, Leicester/UNITED KINGDOM, 5Department Of Medicine, Vercelli Hospital, Vercelli/ITALY, 6Cardiothoracic Surgery, Nyu Langone Medical Center, New York/NEW YORK/UNITED STATES OF AMERICA, 7Division Of Thoracic Surgery, University Hospital Zurich, Zurich/SWITZERLAND

Background: The treatment of mesothelioma has proven difficult with surgical intervention and radiation therapy due to its heterogeneous and invasive nature. Chemotherapy has been the main therapeutic option for many patients. More effective treatment can be achieved with targeted interventions against receptor tyrosine kinases, ligands and other intracellular proteins that modulate tumor cell growth, invasion and metastasis. We have been studying paxillin and FAK in mesothelioma in order to analyze their role in modulating cellular mechanisms in this cancer. Paxillin is a focal adhesion phosphoprotein localized to the cytoskeleton that plays a role in signal transduction, regulation of cell morphology, and the recruitment of structural and signaling molecules to focal adhesions and is involved in motility and migration of tumor cells. It has been shown that the modulation of tyrosine phosphorylation of paxillin regulates both the assembly and turnover of adhesion sites and phosphorylated paxillin enhances lamellipodial protrusions whereas non-phosphorylated paxillin is essential for fibrillar adhesion formation. FAK, a focal adhesion kinase, preferentially interacts with paxillin and controls important biological events, including cell migration, proliferation, and survival. We hypothesized that paxillin and FAK play important roles in mesothelial cell migration and thus studied the genetic aberrations, expression, and functionality of these molecules in mesothelioma.

Methods: We analyzed the expression of paxillin and FAK by IHC on 50 epithelioid, 16 sarcomatoid mesothelioma, and 1 mixed tumor tissues and compared them to 40 normal adjacent lung parenchyma. Mutational analysis of paxillin was performed on tumor DNA. In vitro functional analysis was performed on cells overexpressed with paxillin and its mutants as well as cells with silenced paxillin. In vitro growth assays and confocal imaging to define cellular motility events were performed.

Results: We found an increase in paxillin and FAK expression in mesothelioma compared to normal adjacent lung (p<0.01). Using automated cellular imaging system (ACIS) the immunostained slides were quantified and the analysis of the intensity showed that normal lung had IODs of 118, 13, 45 for paxillin, pFAK and FAK respectively, whereas epithelioid mesothelioma had IODs of 268, 14 and 273 and sarcomatoid had IODs of 331, 12 and 218. We also detected paxillin A127T mutation in mesothelioma patient samples. Live-cell imaging studies revealed that in comparison to wild-type, mutant A127T confers a) increased lamellipodia and filopodia formation b) enhanced mobility c) increased focal adhesion formation and d) increased cell displacement in transiently transfected HEK-293 cells. A mesothelial cell line with silenced paxillin showed increased sensitivity to treatment with cisplatin compared to another that did not respond similarly, emphasizing the heterogeneity of mesothelial tumors types.

Conclusion: Paxillin and FAK are highly upregulated in sarcomatoid and epithelioid mesothelioma. Unique mutations were also found. Paxillin mutations lead to differential processing of lamellipodia and filopodia. We believe that FAK and paxillin are important molecules in malignant mesothelioma and their biological function and therapeutic potential need to be explored further.

Disclosure: No significant relationships.
Background: RON (MST1R) is a member of the MET family and has a putative role in several cancers. The receptor has tyrosine kinase activity and consists of an alpha and a beta chain. The only ligand recognised to bind MSTIR is the serum protein heterodimer, macrophage stimulating protein (MSP). The MSP-RON signalling pathway has been implicated in a variety of cellular functions such as macrophage activity and wound healing. We have previously identified MSTIR/RON as frequently activated in MPM, and high positivity for RON staining was an independent predictor of favourable prognosis. This study aimed to further examine the MSP-RON axis in MPM.

Methods: A panel of mesothelioma cell lines were screened for the expression of MSP and MSTIR at the mRNA and protein level. The proliferative response of Ju77, H226 and Met5A (non-malignant transformed human pleural mesothelial cells) to recombinant MSP treatment was determined. A phospho-kinase proteome profiler array was utilised to detect the downstream signalling pathways activated upon MSP stimulation. Proliferation, migration and apoptotic assays were performed using MSP and two MSTIR/RON inhibitors (a) a pre-clinical monoclonal antibody (RON8, lclone) and (b) a small molecule inhibitor. In addition, a series of MPM TMAa were stained for MSP and macrophage (CD68) markers.

Results: MSP and MSTIR expression varied between the mesothelioma cell line panel at both the mRNA and protein level. Treatment with recombinant MSP reduced the proliferative capacity of the Met5A cell, with a modest effect on the Ju77 MPM line. However, MSP stimulation modified the expression of the SRC family of kinases. In terms of targeting MSTIR/RON, the small molecule inhibitor resulted in a significant decrease in proliferation and migration. Although treatment with RON8 had no effect on proliferation, it did affect the migration capacity of the MPM cells. High expression of MSTIR/RON or MSP correlated with better survival by univariate analysis. In multivariate analysis, MSP was identified as an independent prognosticator for survival in MPM. Likewise we observed no correlation with macrophage (CD68) staining and survival.

Conclusion: The RON (MSTIR) receptor comprises of a number of different isoforms, the most common of which are fR0N (full length) and sf (short form). Our study results indicate that although high levels of RON and MSP correlate with increased survival, our in vitro observations would indicate that this may be isoform dependant. Experiments are ongoing to further elucidate the RON-MSP axis in MPM, including in vivo studies.

Disclosure: No significant relationships.

SESSION IC  GENE REGULATION AND MESOTHELIOMA PATHOGENESIS 1
SEPTEMBER 12, 2012 10:00–11:30

IC.4: THE ROLE OF FIBROBLAST GROWTH FACTOR-9 IN THE REGULATION OF MALIGNANT MESOTHELIOMA TUMOUR GROWTH AND THE TUMOUR-SPECIFIC IMMUNE RESPONSE

Sally M. Lansley1, Ai Ling Tan1, Julius Varano1, Sophia P. Karabela1, Georgios T. Stathopoulos1, Jenette Creaney2, Y C G. Lee4
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Background: Identifying key molecules in the pathobiology of MM is needed to develop new therapies and biomarkers. Fibroblast growth factor-9 (FGF-9) is an exciting and novel target uncovered from our global gene profiling of human MPM samples. Recently FGF-9 has been implicated in cancer development and neoplastic transformation of embryonic fibroblasts. We have verified over-expression of FGF-9 in MPM over other cancers and benign pleuritis in five separate cohorts of human pleural tissues and effusions. Our preliminary in vitro work demonstrated that FGF-9 induces mesothelioma cell proliferation and matrix invasion. We therefore hypothesised that antagonising FGF-9 may reduce tumour aggressiveness, growth and induce tumour regression in vivo.

Methods: To study the ‘necessity’ of FGF-9 in MPM development in vivo we transfected the mouse MM cell line, AB1, with shRNA directed against murine FGF-9 (or control vector expressing a scrambled sequence). For the heterotopic model murine AB1-FGF-9 knock-down cells (or controls) were injected (5x105 cells) subcutaneously into the flank of Balb/c mice. Tumour dimensions were measured thrice weekly and animals sacrificed when tumours reached 100mm2 and tumour tissues harvested. FGF-9 expression in tumour tissue was determined by immunohistochemistry. For orthotopic experiments, Balb/c mice received a single intraperitoneal injection of 5x105 AB1-FGF-9 knock-down cells (or controls). At day 13, animals were sacrificed and the number of peritoneal tumour nodules enumerated by blinded investigators. To determine whether the immune system plays a role in the regulation of AB1 MM tumour growth, 5x105 AB1-FGF-9 knock-down cells (or controls) were injected subcutaneously into nude mice. To elucidate the immune cells involved in AB1 MM tumour growth regulation, T cells were depleted in Balb/c tumour bearing mice using specific antibodies to CD4 and CD8 and tumour growth monitored. T cell depletion was confirmed using flow cytometry.

Results: Heterotopic tumour growth was significantly retarded in mice inoculated with AB1-FGF-9 knockdown cells compared to the scrambled vector and parent MM cells (p<0.001). A significant reduction in the number, and hence tumour burden, of tumour nodules was also observed for AB1-FGF-9 knockdown tumours in the orthotopic peritoneal model compared to controls (p<0.001). When grown in nude mice, which lack a functional T cell repertoire, AB1-FGF-9 knockdown tumours grew at a similar rate to that of the parent and vector controls which was suggestive of a role of the immune response in the regulation of MM tumours lacking FGF-9. AB1-FGF-9 knockdown tumours demonstrated significantly greater tumour burden in mice depleted of CD4+ and CD8+ T cells, either alone or in combination, when compared to saline controls which is highly suggestive of a T cell-mediated immune response to these tumours. These results also suggest that FGF-9 inhibits the tumour-specific immune response in MM.

Conclusion: In combination with our previous in vitro data which clearly demonstrated the proliferative and invasive properties of FGF-9, we suggest that FGF-9 has an important role in the pathobiological characteristics of MM in vivo and represents a novel therapeutic target.

Disclosure: No significant relationships.

IC.5: THE SURVIVIN SUPPRESSANT YM155 SELECTIVELY INHIBITS THE GROWTH OF EPITHELIOLID MALIGNANT MESOTHELIOMA IN VITRO AND IN VIVO

Lynette J. Scheldich, Yeun Yee Cheng, Sumedha Gattani, Ngan C. Cheng, Michaela B. Kirschner, Nico Van Zandwijk, Glen Reid
Asbestos Diseases Research Institute, University Of Sydney, Concord/ AUSTRALIA

Background: Survivin plays an important role in the drug resistance of many cancers. The majority of malignant pleural mesothelioma (MPP) cell lines and tumour samples express survivin, with low or no expression in normal pleura. In addition, a correlation has been observed between MPM tumours positive for survivin and shorter patient survival. YM155 is a small-molecule survivin suppressant which acts by binding to, and inactivating, the transcription factor ILF3, thereby reducing survivin transcription and inhibiting the growth of a broad range of human cancer cell lines in vitro and in vivo. This study was designed to evaluate the anti-tumour activity of YM155 against MPM in vitro and in vivo.

Methods: The effect of YM155 on survivin expression and the growth and survival of MPM cells was assessed. Growth inhibitory effects were measured by standard proliferation assays. Changes in cell cycle progression and the induction of apoptosis were determined by flow cytometry and caspase-3 activation, respectively. The mRNA expression of survivin and drug transporter genes was measured by RT-qPCR and the
steady-state intracellular concentration of YM155 by LC-MS/MS. The anti-tumour activity of YM155, administered as a 7-day continuous infusion, was examined in a subcutaneous MPM xenograft model.

Results: Down-regulating survivin expression in MPM cells using YM155 caused G2/M mitotic arrest, the induction of apoptosis and growth inhibition. Interestingly, YM155 had greatest toxicity in cells of epithelioid origin (IC50 range 2 – 27 nM) compared to those derived from biphasic tumours (IC50 values 92 and 808 nM). YM155 decreased survivin expression at concentrations close to the IC50 value for the MPM cell line. To understand why epithelioid MPM cells are more sensitive to YM155 than biphasic cell lines, we investigated differences in the expression and/or activity of survivin and the drug transporting pumps. Survivin gene expression was lowest in the more resistant biphasic cells (R2=0.58). No correlation was observed between the expression of uptake and efflux transporters and YM155 sensitivity, and their inhibition with chemical inhibitors had the same effect on YM155 sensitivity in biphasic and epithelioid cells. However, the steady-state intracellular accumulation of YM155 was 5-fold higher in epithelioid MPM cells. Interestingly, exosomes are more abundant in media conditioned by a highly resistant biphasic cell line compared to epithelioid cells, and this may be involved in the YM155 resistance we observe. Epithelioid-selectivity was confirmed in a human MPM xenograft model where YM155 demonstrated anti-tumour activity in epithelioid tumours but not in biphasic tumours.

Conclusion: YM155 is effective in inhibiting the growth of MPM cells, with cells of epithelioid origin more sensitive than biphasic cells. The cellular basis for epithelioid selectivity is currently unknown, but may involve factors other than cellular uptake, such as increased export of drug within exosomes. Epithelioid selectivity of YM155 has been validated in MPM xenograft models, suggesting that YM155 may represent a promising subtype specific therapeutic option for MPM.

Disclosure: No significant relationships.

IC.6: SYNDECAN-1 HAMPER THE PROLIFERATION AND CELL-CYCLE REGULATION OF MESOTHELIOMA CELLS BY MODULATING MULTIPLE SIGNALING PATHWAYS

Katalin Dobra1, Tünde Szatmári1, Filip Mundt1, Ghazal Heidari-Hamedani1, Fang Zong1, Andrey Alexeyenko2, Anders Hjerpe1
1Department Of Laboratory Medicine, Karolinska Institutet, Stockholm/SWEDEN, 2Laboratory Medicine, Karolinska Institute, Stockholm/SWEDEN, 3Science For Life Laboratory/SWEDEN

Background: Syndecan-1 is a cell surface proteoglycan (PG) important for the differentiation of mesothelial and epithelial cells. Dedifferentiated tumor components and mesenchymal tumours gradually loose their syndecan-1 expression. In mesothelioma the expression of syndecan-1 correlates to epithelioid morphology and inhibition of growth and migration. Our previous data suggest a complex role of syndecan-1 in mesothelioma cell proliferation although the exact underlying molecular mechanisms are not completely elucidated. The aim of this study is therefore to disclose critical genes and pathways affected by syndecan-1 in mesothelioma; to better understand its importance for tumour cells growth and proliferation. Syndecan-1 exerts its effect partly at the level of the cell membrane through growth factor (GFs) – growth factor receptor complexes. We have, however, shown that syndecan-1 also translocates to the nucleus in a regulated manner by a tubulin mediated transport mechanism. Similar nuclear transport of growth factors and their receptors functions related to cell surface and nuclear syndecan-1. Experimental settings targeting crucial cellular functions such as tumor cell proliferation, adhesion and migration have high therapeutic potential and are addressed in this project.

Results: Syndecan-1 overexpression had profound effects on genes involved in regulation of cell growth, cell cycle progression, adhesion, migration and extracellular matrix organization. In particular, expression of several growth factors, interleukins, and enzymes of importance for heparan-sulfate sulfation pattern, extracellular matrix proteins and proteoglycans were significantly altered. 14 genes showed response to both up- and down-regulation of syndecan-1. The “cytokine – cytokine-receptor interaction”, the TGF-β, EGF, VEGF and ERK/MAPK pathways were enriched in both experimental settings. Most strikingly, nearly all analysed pathways related to cell cycle were enriched after syndecan-1 silencing and depleted after syndecan-1 overexpression.

Conclusion: A better understanding of the complex role of syndecan-1 and its molecular interactions in malignant mesothelioma may provide possibilities in the future to control tumor growth and proliferation.

Disclosure: No significant relationships.

IC.7: NLRP3 INFLAMMASOME PLAYS A SIGNIFICANT ROLE IN THE DEVELOPMENT AND DRUG RESISTANCE OF MALIGNANT MESOTHELIOMA

Arti Shukla1, Jill M. Miller1, Maximillian B. Macpherson1, Timothy N. Perkins1, Stacie L. Beuschel1, Harvey Pass1, Brooke T. Mossmann1
1Pathology, University Of Vermont College Of Medicine, Burlington/VT/UNITED STATES OF AMERICA, 2Cardiothoracic Surgery, Nyu Langone Medical Center, New York/UNITED STATES OF AMERICA

Background: Malignant mesothelioma (MM) is an aggressive cancer of mesothelial cells originating from pleural, peritoneal or pericardial cavities. Inflammation plays an important role in development of MM. We are first to show that asbestos activates NOD-like receptor protein 3 (NLRP3), a component of the inflammasome in human cells. As chronic asbestos exposure is a key risk factor for the development of MM, we hypothesized that inflammasome-mediated inflammation might underlie the pathogenesis of this cancer.

Methods: To show the involvement of NLRP3 in asbestos-induced mesothelioma, we exposed immortalized human mesothelial cells (LP9/hTERT), a cell type responsible for origin of MM in response to asbestos and measured steady-state NLRP3 mRNA levels by qRT-PCR, caspase-1 activation by Activity Assay kit, IL-β release by ELISA kit and HMGB1 (High Mobility Group Box protein 1) release by Western blot analysis of cell culture supernatants. We also used human MM tumor cell lines and tumor tissues to assess the steady-state mRNA levels of NLRP3, ASC, caspase-1 and caspase-1 activity. Small interfering RNA (siRNA) approach was used to depict the role of NLRP3 in asbestos-induced IL-β and HMGB1 release.

Results: Asbestos exposure to LP9 cells resulted in time-dependent increases in steady-state mRNA levels and activation of NLRP3 as measured by caspase-1 activation and IL-β release. Inhibition of NLRP3 by siRNA caused significant decreases in NLRP3 mRNA levels as well as asbestos-induced IL-β and HMGB1 release in medium. On the other hand, human MM cell lines and tumor tissues showed significantly decreased levels of NLRP3 and caspase-1 as well as caspase-1 activity as compared to LP9 or matching normal tissues respectively.

Conclusion: Our findings suggest that initial exposure to asbestos causes increased mRNA levels and activity of NLRP3, which may help in MM development by promoting mesothelial cell transformation. However, tumor development culminates in MM with decreased NLRP3 protein and increased drug resistance which may in part be due to inhibition in caspase-1 activity. Thus NLRP3 may be an appropriate target for therapy.
of MM, especially in combination with cytotoxic chemotherapeutic drugs and IL-1 receptor antagonists. This study is supported by a Mesothelioma Applied Research Foundation (MARF) grant (AS), an NIEHS grant 1R01 ES021110-01 (AS) and by an NIEHS grants T32 ES07122 (BM).

Disclosure: No significant relationships.
IIA.2: ZIC1 ACTS AS A TUMOUR SUPPRESSOR GENE AND IS SILENCED IN MALIGNANT PLEURAL MESOTHELIOMA

Yuen Yee Cheng1, Michaela B. Kirschner1, Sonja Klebe1, J.J.B. Edelman2, Michael P. Vallely3, Brian C. Mccaughan3, Kwun M. Fong4, Laura Moro4, Luciano Mutti4, Hung Chuan Jin1, Nico Van Zandwijk1, Glen Reid1

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Background: Epigenetic inactivation of tumour suppressor genes through DNA hypermethylation plays a crucial role in the progression of malignant pleural mesothelioma (MPM). ZIC1, a tumour suppressor gene silenced through promoter hypermethylation in gastric and colorectal cancer, was expressed at high levels in normal mesothelial cells. In contrast, ZIC1 was not expressed or was downregulated in MPM cell lines. The aim of the current study was to investigate the functional significance of ZIC1 silencing in MPM.

Methods: ZIC1 mRNA expression and DNA methylation status were studied using RT-PCR, MSP and COBRA in MPM cell lines with and without decitabine treatment. 24 MPM cases were included to confirm the mRNA expression and DNA methylation of ZIC1 using RT-PCR and MSP. The relationship between ZIC1 and miRNA expression was determined by profiling miRNA expression with NCode miRNA microarrays in ZIC1-expressing MeT-5A and MPM lines with methylation-induced ZIC1 silencing. To examine the functional significance of ZIC1 silencing, ZIC1 was re-expressed in MPM cell lines and effects on proliferation, migration and growth in soft agar were assessed. Knockdown of ZIC1 with siRNA and microRNA inhibitors were also included to study the functional relationship of ZIC1 silencing in MPM.

Results: Following treatment with decitabine, expression of ZIC1 was significantly up-regulated in all mesothelioma lines but was unchanged in MeT-5A and primary human mesothelial cells. MSP and COBRA analysis revealed methylation of the ZIC1 promoter that correlated with ZIC1 mRNA expression, suggesting ZIC1 is silenced in MPM through DNA hypermethylation. Enforced ZIC1 expression inhibited cell migration and colony formation in H2B, Ren and MM05 cell lines, and ZIC1 knockdown enhanced growth of MeT-5A in soft agar. In MPM tumour samples ZIC1 mRNA expression was present at low or undetectable levels, with promoter methylation observed in 16 of 24 cases. Microarray analysis of MPM cell lines revealed that a number of miRNAs were overexpressed in the absence of ZIC1 expression. Upon enforced ZIC1 expression, levels of miR-23a and miR-27a were reduced, and cells transfected with an inhibitor of miR-23a exhibited reduced colony formation. These miRNAs were expressed at higher levels in tumours from patients with shorter survival.

Conclusion: Our results show that ZIC1 behaves in MPM cell culture as a tumour suppressor gene that functions in part through downregulation of miR-23a.

Disclosure: No significant relationships.

IIA.3: ELUCIDATING THE RELATIONSHIP OF BAP1 TO HCF1 AND TO REGULATORY CHROMATIN MODIFICATIONS IN MESOTHELIOMA

Robert Mcmillan1, Matthew Bott1, Azra Krek2, Marc Ladanyi1

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Background: Frequent somatic mutations of the BAP1 nuclear deubiquitinase gene have recently been identified in mesothelioma (Bott et al. Nat Genet 2011). Because BAP1 appears to regulate gene expression by deubiquitinating nuclear proteins such as transcription factors (notably HCF1) and histones, we aimed to deepen our understanding of BAP1’s role in gene expression by examining its genome wide location in relation to HCF1 and major histone (H) modifications, including methylation (Me), acetylation (Ac), and ubiquitination (ub), using chromatin immunoprecipitation with massively parallel sequencing (ChIP-Seq).

Methods: The BAP1 wild type MPM cell line MSTO-211H was used. To gauge effects on chromatin marks, experiments were performed in the presence or absence of BAP1 knockdown using BAP1 siRNA or scrambled siRNA, respectively. ChIP-assays with antibodies to BAP1, HCF1, and the histone marks AcH3, AcH4, H2Aub, H3K4Me, H3K9Me, H3K20Me, and H3K79Me, and H4K20Me were performed. ChIP-Seq was performed using the Magna ChIP protocol by Millipore, and sequencing was done using the SOLiD platform.

Results: BAP1 was localized to approximately 1500-2500 sites along the genome, of which only about 10% coincided with transcription start sites. In the absence of BAP1 knockdown, approximately 25% of HCF1 sites colocalized with BAP1. BAP1 frequently colocalized with the H2Aub marks and was enriched to a lesser extent at H3K20Me marks but not at genomic regions bearing the histone modifications AcH3, AcH4, H3K4Me, H3K9Me, H3K20Me, and H3K79Me.

Conclusion: Our ChIP-Seq data so far support the known interactions of BAP1 with HCF1 and H2Aub, both of which are consistent with deregulation of gene expression as a major effect of BAP1 loss. Only a minority of BAP1 appears associated with HCF1 suggesting that many of its biological effects may be through its role at H2Aub and elsewhere along the genome. More detailed analyses, including additional data, are ongoing and will be presented.

Disclosure: No significant relationships.
I.2.4. CLINICAL CHARACTERISTICS OF PATIENTS WITH MALIGNANT PLEURAL MESOTHELIOMA (MPM) HARBORING SOMATIC BAP1 MUTATIONS

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Background: Efforts to elucidate tumorigenic mutations in MPM are essential to advance therapy. We reported a 23% incidence of somatic mutations in BRCA1-associated protein-1 (BAP1) in 53 patients with MPM (Bott et al. Nat Genet 2011). Germline BAP1 mutations were also reported in two families with a predisposition to mesothelioma and uveal melanoma (Testa et al. Nat Genet 2011). While BAP1 somatic mutations are more common in poor prognosis uveal melanoma (84% class 2, 4% class 1; Harbour et al. Science 2010), the significance of these mutations in MPM is unknown. Therefore, we analyzed the clinical characteristics of patients with somatic BAP1 mutations in order to describe this newly identified subpopulation.

Methods: We reviewed the charts of 121 patients with tumors tested for somatic BAP1 mutations.

Results: Patient characteristics are in Table 1. Twenty percent harbored somatic BAP1 mutations. Other than the percent of current or former smokers (75% BAP1 mutations, 42% BAP1 wild-type, p=0.006), no other clinical feature was significantly different among those with and without BAP1 mutations. Among 53 samples analyzed for NF2 mutation and p16 deletion, no correlation was seen with BAP1 mutation.

Table 1

<table>
<thead>
<tr>
<th>Parameter</th>
<th>BAP1 mutants (N=24)</th>
<th>BAP1 wild-type (N=97)</th>
<th>p-value</th>
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<tr>
<td>Gender (M=male)</td>
<td>M: 79%</td>
<td>M: 68%</td>
<td>0.33</td>
</tr>
<tr>
<td>Median age at diagnosis</td>
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<td>63</td>
<td>0.31</td>
</tr>
<tr>
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<td></td>
<td>0.28</td>
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<tr>
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<td>71%</td>
<td>77%</td>
<td></td>
</tr>
<tr>
<td>*Mixed</td>
<td>25%</td>
<td>12%</td>
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<tr>
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<td>42%</td>
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<td>EPP=extrapleural pneumonectomy</td>
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<td></td>
</tr>
<tr>
<td>None</td>
<td>8%</td>
<td>10%</td>
<td></td>
</tr>
<tr>
<td>Median overall survival from diagnosis (months)</td>
<td>14.8</td>
<td>15.3</td>
<td>0.78</td>
</tr>
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</table>

Conclusion: Somatic BAP1 mutations occur in about 20% of MPM tumors. Aside from smoking history, no other differences in clinical characteristics or outcomes were noted in the BAP1 mutated cases. Similar efforts are needed to describe the features of germline mutations in order to define this new cancer predisposition syndrome. We are planning a prospective trial to further evaluate the prevalence of germline BAP1 mutations, and we are also exploring the therapeutic implications.

Disclosure: No significant relationships.
IIA.5: ROLE OF HEDGEHOG SIGNALING IN MALIGNANT PLEURAL MESOTHELIOMA

Yangdong Shi1, Moura Ubiratan1, Isabelle Opitz2, Alex Soltermann2, Hubert Rehauer3, Svenja Thies3, Walter Weder4, Rolf Stahel1, Emanuela Felley-Bosco1

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Background: Chronic tissue inflammation and tissue repair have been postulated to be the central mechanism leading to tumorigenesis in malignant pleural mesothelioma (MPM). Tissue repair involves the activation of stem cells and the expression of stem cell renewal genes. We recently observed increased expression of PTCH1 (patched, the receptor binding Hedgehog ligands) in MPM side population-derived tumors which exhibited a tendency to have increased tumor initiating properties and developed tumors with precursor phenotype similar to tumors in patients with relapse after chemotherapy (Frei et al, Carcinogenesis 32: 1324, 2011). This prompted us to investigate whether HH pathway is activated in MPM and the effect of its inhibition in primary mesothelioma cell cultures and in a xenograft.

Methods: The expression of HH signaling components was assessed by q-PCR and in situ hybridization in 45 clinical samples. Primary MPM cultures were developed in serum-free condition in 3% oxygen and were used to investigate the effects of Smoothened (SMO) inhibitors or GLI1 silencing on cell growth and HH signaling. In vivo effects of SMO antagonists were determined in a MPM xenograft growing in nude mice.

Results: A significant increase in GLI1, sonic hedgehog, and human hedgehog interacting protein gene expression was observed in MPM tumors compared to normal pleura. SMO antagonists inhibited GLI1 expression and cell growth in sensitive primary cultures. This effect was mimicked by GLI1 silencing. Reduced survivin and YAP protein levels were also observed. Survivin protein levels were rescued by overexpression of GLI1 or constitutively active YAP1. Treatment of tumor-bearing mice with the SMO inhibitor HhAntag led to a significant inhibition of tumor growth in vivo accompanied by decreased Ki-67 and nuclear YAP immunostaining and a significant difference in selected gene expression profile in tumors.

Conclusion: An aberrant HH signaling is present in MPM and inhibition of HH signaling decreases tumor growth indicating potential new therapeutic approach.

Disclosure: No significant relationships.

IIA.6: DEPLETION OF THE CIRCADIAN CLOCK GENE BMAL1 REpresses MALIGNANT PLEURAL MESOTHELIOMA GROWTH THROUGH INDUCTION OF MITOTIC CATASTROPHE

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Background: Malignant pleural mesothelioma (MPM) is a highly aggressive neoplasm that exhibits poor prognosis, and its incidence is rising. Although, there has been significant progress in MPM treatment, development of more efficient therapeutic approaches is needed. BMAL1 is a core component of the circadian clock machinery and its constitutive over-expression in MPM has been reported. Here, we demonstrate that BMAL1 serves as a molecular target to combat MPM.

Methods: We used 13 MPM cell lines and one non tumorigenic mesothelial cell line (MeT-5A). In our study we performed a variety of techniques including quantitative real time PCR, western blot, immuno-cytochemical, histochemical and HE staining, siRNA transient transfection, growth assays (WST-1, liquid colony and soft agar colony formation assays), cell cycle analysis, apoptosis assay and time lapse microscopic examination. The clinicopathological features of 16 MPM patients were recorded for further analysis in correlation with BMAL1 immunohistochemical scoring in MPM specimens.

Results: The majority of MPM cell lines and a subset of MPM clinical specimens expressed higher levels of BMAL1 compared to a non-tumorigenic mesothelial cell line (MeT-5A) and normal parietal pleural specimens, respectively. A serum shock induced a rhythmical BMAL1 expression changes in a non tumorigenic mesothelial cell line, MeT-5A, but not in ACC-MESO-1 cells, suggesting that circadian rhythm pathway is deregulated in MPM cells. RNA interference-mediated knockdown of BMAL1 suppressed proliferation and anchorage-dependent and independent clonal growth in MPM cells but not in MeT-5A. Notably, BMAL1 depletion resulted in cell cycle disruption with a substantial increase in apoptotic and polyploidy cell population in association with down-regulation of Wee1, cyclin B, and p21WAF1/CIP1 and up-regulation of cyclin E expression. ACC MESO-1 cells exhibited drastic morphological changes including micronucleation, multiple nuclei and increased cellular volume and time lapse microscopic examination demonstrates mitotic catastrophe as a cell fate following BMAL1-knockdown in ACC MESO-1 cells that expressed the highest level of BMAL1.

Conclusion: In conclusion, we provide evidence that BMAL1 has a critical role in MPM and could serve as an attractive therapeutic target for MPM.

Disclosure: No significant relationships.

(see graphic next page)
Figure 1 *BMAL1* knockdown induces dramatic morphological alterations in ACC-MESO-1 cells. (A) Immunofluorescence of α-tubulin and DAPI stains. The upper panels represent ACC-MESO-1 cells treated with siRNA control. The lower panels represent the most frequent morphological changes (arrow indicates micro-nucleation and arrow head indicates multiple nuclei) in single ACC-MESO-1 cell after *BMAL1*-depletion. The middle panels represent ACC-MESO-1 cells treated with *BMAL1*-siRNA. (B) Time lapse microscopic examination showing the aberrant mitosis in ACC-MESO-1 cells transfected with *BMAL1*-siRNA (White arrow head) and intact mitosis in cells transfected with control oligos (White arrow). (C) Proposed molecular mechanism of *BMAL1* knockdown-induced mitotic catastrophe in ACC-MESO-1 cells.
IIA.7: CAN HDAC/ER BETA EXPRESSION BE USED TO STRATIFY MESOTHELIOMA PATIENTS FOR APPROPRIATE TREATMENT REGIMENS

Anne-Marie Baird1, Louise Flynn1, Eimear O’Donnell2, Cormac J. Jennings3, Martin P. Barr2, Eric Santoni-Rugiu4, Jens Benn Sørensen5, Sarah G. Zimling5, Warren Thomas3, Luciano Mutti6, Laura Moro7, Kenneth J. O’Byrne3, Steven Gray8

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Background: Malignant pleural mesothelioma (MPM) is a rare and aggressive cancer. The severity of this disease is underscored by the fact that no single treatment option has proven particularly effective. The current standard of care for patients suffering from MPM is a combination of pemetrexed and cisplatin with an observed objective response rate of approximately 40%. Accordingly there is an urgent need to identify patients who may benefit from this regimen, and furthermore identify new translational targets or approaches that potentially could be used to treat MPM patients. Epigenetic modifiers such as histone deacetylases (HDACs) expression levels may be useful to stratify patients into treatment regimens such as those that will respond to (a) HDAC inhibition, (b) Estrogen Receptor targeted agents and (c) those cisplatin therapy.

Methods: Fifteen mesothelioma and one normal cell line (Met5A) were screened for the expression of HDAC1, Class I (HDAC 2, 3, and 8) and Class II (HDAC4, 5, 6, 7, 9, 10) histone deacetylases at (a) the protein level by means of Western Blot and (b) the mRNA level using RT-PCR. The HDAC expression profile of a cisplatin resistant cell line (P31) was also determined. Additionally HDAC (HDAC1, 2, 3) and ER-Beta expression was examined in panel of twenty patient samples (benign, biphasic, sarcomatoid, epithelial).

Results: HDAC1 and Class I and II HDACs were detected to varying degrees within the mesothelioma and normal cell lines. HDAC1/2 and 4 were universally expressed at the protein level with HDAC3 (8/16), HDAC7/8 (13/16), HDAC5 (14/16) demonstrating differential expression. In the P31 cell line, HDAC protein expression was decreased (HDAC2/3/4/5/7) in the cisplatin resistant sub type compared with the parent. Furthermore HDAC5, was significantly reduced (p<0.05) in the cisplatin resistant cell line. Presently a cohort of mesothelioma patient samples is undergoing IHC staining for HDACs. Expression of HDAC1/2 and 3 were increased in the MPM patient samples (n=15) compared with benign (n=5) (HDAC2/3, p<0.05). Overall, ER-Beta protein levels were decreased in the MPM samples compared with benign. An apparent inverse correlation between ER-Beta and HDAC expression was observed.

Conclusion: This is one of the first studies to determine HDAC expression in clinically relevant patient samples. Altered HDAC expression was observed in an isogenic parent and cisplatin resistant cell model, which may suggest that a reduction in HDAC expression is involved in cisplatin resistance in MPM. Conversely, an increase in the protein levels of HDAC1/2 and 3 were detected in MPM patient samples with decreased levels of ER-Beta. An inverse correlation was evident between ER-Beta and HDAC expression within our patient cohort. This suggests that HDAC expression could be used to stratify patients for treatment regimens.

Disclosure: No significant relationships.

IIA.8: THE BAP1 CANCER SYNDROME

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Background: In 1997 the extremely high incidence of mesothelioma in certain Cappadocian families suggested to me that genetics was playing a role. We discovered that mesothelioma was transmitted in an autosomal dominant fashion and we started the hunt for a putative mesothelioma gene (Roushdy-Hammady I., et al., 2001; Dogan U., et al., Cancer Res 2006; Carbone et al., Nat Rev Cancer 2007). Environmental and mineralogical studies revealed that Cappadocian families with high and low incidence of mesothelioma were exposed to similar amounts of erionite (Carbone M., et al., PNAS 2011). We discovered that germline BAP1 mutations are associated with high incidence of mesothelioma and uveal melanoma (UVM), and that BAP1 is frequently mutated in sporadic mesothelioma (Testa J et al, Nat Genet 2011). Speicher’s team discovered that germline BAP1 mutations caused benign melanocytic tumors (Wiesner T., et al, Nat Genet 2011).

Methods: We investigated for melanocytic lesions the US families with high incidence of mesothelioma and we conducted a meta-analysis of the published studies of families carrying germline BAP1 mutations.

Results: We discovered that germline BAP1 mutations cause a new cancer syndrome characterized by mesothelioma, uveal and cutaneous melanoma, MBAITs (melanocytic BAP1-mutated atypical intradermal tumors”), and possibly renal cell and other carcinomas (Carbone M., et al., JTM in press). MBAITs are benign melanocytic tumors that have distinct histological and molecular characteristics when compared to other melanocytic lesions. MBAITs develop early in life and allow the identification of potential carriers of germline BAP1 mutations who can be followed for early detection of the malignancies associated with this syndrome.

Conclusions: The capacity of BAP1 mutations to cause multiple tumor types and the very high tumor phenotype penetrance (close to 100% in these families) indicates that this gene plays a major role in influencing cancer cell growth. The pleiotropic effects of BAP1 can account for this finding. We advise families with hereditary BAP1 mutation to have family members tested for mutant gene carrier status at the age ten and, if positive, to begin routine screening with a total body dermatological examination and annual eye examinations as family members may develop melanoma at an early age.
Cancers associated to BAP1 syndrome: dimension of circles is proportional to cancer prevalence in BAP1-mutated families. Solid arrows indicate the tumors associated to BAP1 syndrome. Dashed arrows indicate cancers possibly associated to the syndrome.

CM: cutaneous melanoma; MM: malignant mesothelioma; CCRC: clear cell renal carcinoma

Disclosure: No significant relationships.
SESSION IIb: LONG-TERM OUTCOME AFTER RADICAL PLEURECTOMY FOLLOWED BY CHEMORADIATION FOR MALIGNANT PLEURAL MESOTHELIOMA: A 10-YEAR SINGLE CENTER EXPERIENCE

Servet Bölükbas*, Michael Eberlein*, Natalie Kudelin*, Annette Fisseler-Eckhoff†, Joachim Schirren†
Thoracic Surgery, Dr. Horst Schmidt Klinik, Wiesbaden/GERMANY, Department Of Pathology And Cytology, Dr. Horst Schmidt Klinik, Wiesbaden/GERMANY

Background: We report our 10-year single center experience of malignant pleural mesothelioma (MPM) treated with radical pleurectomy (RP) as surgical arm within a trimodality approach.

Methods: In a prospective, non-randomized study, all patients with histologically proven MPM, clinical stage cT1-3 cN0-2 and without prior treatment for MPM were evaluated for trimodality therapy: lung-sparing RP followed by 4 cycles of chemotherapy (Cisplatin/Pemetrexed) and radiation of the chest wall at the intervention sites from 2002 to 2011. Kaplan-Meier analyses, log-rank test and Cox regression analyses were used to estimate survival and to determine predictors of survival.

Results: Eighty-eight out of 206 consecutive patients underwent RP followed by chemoradiation. 74 out of 88 patients (84%) completed the trimodality therapy. Surgical morbidity and mortality were 27.3% (2/88) and 2.3% (2/88), respectively. Median survival (MS) was 26 months (mo). One-, 3- and 5-year survival were 78%, 30% and 25%, respectively. Progression-free survival was 13 Mo. The sites of failure were locoregional (47.6%), distant (11.0%) and both (13.4%). Median time between disease progression and death was 7 Mo. Incomplete resections (p<0.001), advanced T-stage (p=0.002), lymph node metastases (p=0.009), advanced intraoperative-pathological stage (p<0.001) and age ≤70 years (p=0.036) were associated with significant inferior survival in the univariate analyses. Histology, gender, type of additional resections, type of recurrence and laterality had no significant impact on survival. Macroscopic complete resection remained the only significant prognostic factor in the multivariate analysis.

Conclusion: Lung-sparing RP within a trimodality therapy concept is associated with promising long-term survival, morbidity and mortality. Patients aged ≥70 years should be selected very carefully for trimodality therapy. MCR is the most important prognostic factor within this trimodality approach. High rate of locoregional failure warrants further investigation of locoregional control of the disease.

Disclosure: No significant relationships.
Conclusion: Cryoablation for localized recurrent malignant pleural mesothelioma following surgery can be performed safely as an outpatient procedure with minimal morbidity (5.6%), a very high efficacy (95-3%), and impressive overall survival (36.1 mos).

Disclosure: No significant relationships.

SESSION IIB  MULTI-MODALITY  SEPTEMBER 12, 2012 14:20-16:00

IIB.4: PROSPECTIVE PHASE I TRIAL OF EXTRAPLEURAL PNEUMONECTOMY OR PLEURECTOMY/DECORTICATION, INTRATHORACIC/INTRAPERITONEAL HYPERTHERMIC [IOHC] CISPLATIN AND GEMCITABINE WITH INTRAVENOUS AMIFOSTINE AND SODIUM THIOSULFATE CYTOPROTECTION FOR PATIENTS WITH RESECTABLE MALIGNANT PLEURAL MESOTHELIOMA

David J. Sugarbaker1, Marcelo Dasilva1, Jeffry Supko1, Olivia Winfrey1, Hannah Eisen1, Juliann Barlow1, Raphael Bueno1, William G. Richards2

1Division Of Thoracic Surgery, Brigham And Women’s Hospital, Boston/MA/UNITED STATES OF AMERICA, 2Division Of Thoracic Surgery, Brigham And Women's Hospital And Harvard Medical School, Boston/MA/UNITED STATES OF AMERICA, 3Clinical Pharmacology, Massachusetts General Hospital, Boston/MA/UNITED STATES OF AMERICA

Background: We sought to determine the maximum tolerated dose (MTD) of gemcitabine added to cisplatin intra-operative heated chemotherapy (IOHC) following either extrapleural pneumonectomy or pleurectomy/decortication as a treatment for mesothelioma. We also investigated the toxicity profile, perioperative mortality and pharmacokinetics of this treatment.

Methods: Between November 2007 and October 2011, 104 patients underwent surgical resection followed by HIoC with cisplatin (225 mg/m²; previously determined MTD), and dose-escalated gemcitabine. For each surgery type, three patients were enrolled per dose level. Escalation occurred if there were no dose-limiting toxicities (DLT), defined as grade 3 or higher toxicity relatable to study treatment. The concentration of gemcitabine in perfusate and plasma samples was determined by high performance liquid chromatography with tandem mass spectrometric detection.

Results: Median age was 65 (43-85) and 22 (21%) were women. Histology was epithelial (63), Biphasic (32), and Sarcomatoid (8). Two patients died perioperatively (2%). Toxicity data are given by gemcitabine dose (Table). Due to renal toxicity observed with low-dose gemcitabine, the cisplatin dose was reduced to 175 mg/m². The DLT was Grade 3 leukopenia, observed in two patients at a gemcitabine dose of 1100 mg/m². The average ± SD maximum concentration of gemcitabine measured in the perfusate was 327 ± 126 µg/mL in patients receiving a dose level of 1000 mg/m²; previously determined MTD), and dose-escalated gemcitabine. For each surgery type, three patients were enrolled per dose level. Escalation occurred if there were no dose-limiting toxicities (DLT), defined as grade 3 or higher toxicity relatable to study treatment. The concentration of gemcitabine in perfusate and plasma samples was determined by high performance liquid chromatography with tandem mass spectrometric detection.

Conclusion: This prospective study demonstrates the feasibility and safety of administering hyperthermic intraoperative intracavitary combination chemotherapy with cisplatin and gemcitabine following cytoreductive surgery for MPM. It establishes MTD for these drugs, respectively, at 175 and 1000 mg/m². Morbidity and mortality were acceptable. A pharmacokinetic gradient was demonstrated that permits high concentrations of intracavitary gemcitabine while minimizing systemic toxicity. Early survival analysis is encouraging.

Disclosure: No significant relationships.

SESSION IIB  MULTI-MODALITY  SEPTEMBER 12, 2012 14:20-16:00

IIB.5: RESULTS OF SHORT ACCELERATED HYPOFRACTIONATED HEMITHORACIC INTENSITY MODULATED RADIATION THERAPY FOLLOWED BY EXTRAPLEURAL PNEUMONECTOMY FOR MALIGNANT PLEURAL MESOTHELIOMA

Marc De Perrot, Isabelle Opitz, Masaki Anraku, Victoria Ford, Natasha Leigh, Ronald Feld, John Cho

Toronto General Hospital And Princess Margaret Hospital, Toronto/ON/ CANADA

Background: The surgical treatment of malignant pleural mesothelioma (MPM) remains controversial. We and others have observed that MPM are radiosensitive and that hemithoracic intensity modulated radiation therapy (IMRT) is well tolerated in the adjuvant setting after extrapleural pneumonectomy (EPP). We, therefore, developed a new protocol with neoadjuvant hemithoracic IMRT followed by EPP. The potential advantages of this protocol were optimal delivery of the radiation in the preoperative setting and reduced risk of viable tumor spillage during the surgery due to the surgical effect of radiation.

Methods: Patients were eligible if they had clinically resectable T1-3N0M0 MPM. Patients received 25 Gy in 5 daily fractions over 1 week to the entire ipsilateral hemithorax by IMRT with concomitant boost of 5 Gy to the tumor base. This was followed by EPP. Systemic absorption of gemcitabine was very low, as indicated by the average peak concentrations of gemcitabine in plasma, which were only 0.23 ± 0.13% of the maximum drug concentration in perfusate in patients evaluated at the 500 mg/m² dose level and 0.35 ± 0.26% at the 1,000 mg/m² dose level. Fifty-four patients remain alive with a median follow-up of 11 (range 3-42) months, 7 patients developed
Results: A total of 121 patients were identified on an “intent to treat” basis between 1997 and 2011. 94 (77.7%) were male while 27 (22.3%) were female. 80 (66.2%) had right-sided tumors while 41 (33.8%) were left. Mean age was 65.9 years (range 27-84) with a median age of 68 (male) and 56 years (female). 40 (33.1%) were >70 years old. Preoperative clinical staging was stage I, II, III, and IV in 109 (90.1%), 1, (0.8%), 7, (5.8%), and 4 (3.3%), respectively. Pathologic T stage was T2 in 24 (19.8%), T3 in 70 (57.9%), and T4 in 27 (22.3%); while N stage was No in 57 (47.1%), N1 in 3 (2.5%), N2 in 58 (47.9%), and NX in 3 (2.5%). Yielding a pathologic stage I, II, III, and IV in 16 (13.2%), 3 (2.5%), 74 (61.2%), and 28 (23.1%), respectively. Overall median survival for all 121 patients was 13.8 mos, while significantly better survival was noted among female patients (20.7 vs 12.1 mos), nonsmokers (16.3 vs 11.9 mos), patients without an identifiable asbestos exposure history (23.4 vs 13.4 mos), and patients without lymph node involvement (N0=20.2 mos vs N1=14.9 mos vs N2=9.8 mos vs NX=4.2 mos) (p<0.05). Median survival for epithelioid histology (17.8 mos) was significantly better than both biphasic (10.3 mos) and sarcomatoid (2.1 mos) subtypes (p<0.01). The median survival of patients completing standard surgical and adjuvant therapy, i.e., pleurectomy/decortication and radiation with delayed or no postoperative chemotherapy (85 patients=70.2%), was 19.7 mos which equals that reported for trimodality therapy for similar patient groups (median survival = 19.0 mos; Sugarbaker, et al; J Thorac Cardiovasc Surg 1999;117(1):54-65), particularly when compared to neoadjuvant chemotherapy followed by EPP + hemithoracic radiation (median survival = 16.8 mos; Krug, et al; J Clin Oncol 2009;27(18):3007-13 and median survival = 14.4 mos; Treasure, et al Lancet Oncol 2011;12(8):763-72).

Conclusion: The results using chemotherapy given in a delayed fashion at the time of 1st recurrence following lung-sparing pleurectomy/decortication revealed favorable patient outcomes comparable or better to those reported for “trimodality” therapy including the recent MARS trial. This suggests that a more rational and conservative approach to multimodality treatment of patients with malignant pleural mesothelioma may be warranted.

Disclosure: No significant relationships.

SESSION IIb  MULTI-MODALITY  SEPTEMBER 12, 2012 14:20-16:00

IIB.6: THE TIMING OF CHEMOTHERAPY IN THE MULTIMODALITY TREATMENT OF MALIGNANT PLEURAL MESOTHELIOMA

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Background: Various strategies have been used with regard to the timing of chemotherapy within a multimodality treatment approach to malignant pleural mesothelioma. Previously, postoperative chemotherapy has been evaluated but recently, neoadjuvant chemotherapy has been more popular. We evaluated a strategy of delayed chemotherapy given only at the time of a documented recurrence following our standard protocol of pleurectomy/decortication followed by adjuvant radiation.

Methods: Following IRB approval, we retrospectively reviewed our prospective thoracic database to identify patients treated with a basic protocol including lung-sparing pleurectomy/decortication followed by adjuvant radiation therapy. Patients were then followed for disease recurrence and only treated with chemotherapy at the time of identification of at least one indicator lesion (minimal recurrent disease). Patient characteristics, results, and survival were assessed and compared to those reported in the literature for trimodality therapy with extrapleural pneumonectomy (EPP).

Disclosure: No significant relationships.

SESSION IIb  MULTI-MODALITY  SEPTEMBER 12, 2012 14:20-16:00

IIB.7: PHOTODYNAMIC THERAPY (PDT) AS AN INTRAPERATIVE ADJUVANT FOR MALIGNANT PLEURAL MESOTHELIOMA

Joseph Friedberg
University Of Pennsylvania/UNITED STATES OF AMERICA

Photodynamic Therapy (PDT) is a light-based cancer treatment that is particularly well suited as an intraoperative adjuvant treatment for malignant pleural mesothelioma. With patient education and standardized dosimetric techniques PDT can be combined safely and easily with surgery. Our group has published some encouraging results on the combination of PDT and radical pleurectomy where the 31 epithelial subtype patients (100% IMIG Stage III/IV) had a median survival from the time of surgery of 41.2 months. Our hypothesis is that PDT played a role in these unusual survival results. Beyond the fact that PDT treats tissue for several millimeters below the surface, It has the additional advantages of being compatible with essentially any other treatment modality and has potential to be synergistic with a number of them. Future directions for our group include performing a randomized Phase III study to definitively test the hypothesis that PDT is contributing to survival. In addition we are also performing translational research aimed at improving our current approach to intraoperative PDT while at the same time developing new treatments designed to capitalize upon the known immunostimulatory effects of PDT.

Disclosure: No significant relationships.
IIC.2: DENDRITIC CELL BASED IMMUNOTHERAPY IN COMBINATION WITH METRONOMIC CYCLOPHOSPHAMIDE IN PATIENTS WITH MESOTHELIOMA

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¹Pulmonary Diseases, Erasmusmc, Rotterdam/NETHERLANDS, ²Pulmonary Medicine, Erasmus Mc, Rotterdam/NETHERLANDS

Background: Dendritic Cells (DC) are extremely potent antigen presenting cells capable of inducing a CD8+ cytotoxic T cell reaction. In an earlier study on autologous tumor lysate pulsed DC based immune therapy in mesothelioma (MM) we showed that we were able to induce an anti-tumor response. However it is known that the induced anti-tumor immunity in cancer patients is created in a unfavorable highly suppressive tumor environment. Regulatory T-cells (Tregs) are prominent cells in this suppressive environment. We have previously showed the presence of Tregs in patients with MM. In a murine model on MM, depletion of Treg with metronomic cyclophosphamide (CTX) could enhance the anti-tumoral immune response induced by DC vaccination¹.

Methods: A non-randomized study was performed in patients with MM who were non progressive after standard chemotherapy. A pleurectomy/decortication (P/D) was performed before DC vaccination in case that was considered best interest of the patient. According to our previous study, 3 doses of pulsed autologous DC were re-injected every 2 weeks. Patients were treated with 100mg CTX/day for 7 days starting 9 days before every vaccination. After the 3rd vaccination again 7 days of CTX were taken.

Results: 9 patients were enrolled. Mean age 60 (range 35-78yrs). Data on Treg levels will be presented at the conference. Respone according to modified RECIST and survival are presented in the table.

Disclosure: No significant relationships.
The ratio of iNKT cells to CD3 positive cells in the pleural cavity significantly increased after tumor cell injection (day 0: 0.8±0.2%, day 7: 3.0±0.9%, day 14: 11.4±1.3%, day 25: 12.8±1.0%), while that in spleen did not change. The ratio of iNKT cells to CD3 positive cells in the tumor was significantly increased after the tumor cell injection (day 0: 0.8±0.2%, day 7: 11.4±1.3%, day 14: 12.8±1.0%). The mice treated with αGalCer showed significantly prolonged survival compared with the control mice (median survival time, 7.0 days and 17.0 days, respectively, p < 0.0001) and associated with increased ratio of IFN-γ positive iNKT cells and CD8 positive T cells in the pleural effusion effusion.

Conclusion: iNKT cells appear to contribute to the anti-tumor immune response in murine MPM. Modulation of iNKT cells could be a new therapeutic approach for patients with MPM.

Disclosure: No significant relationships.

### Table 1: Response to chemotherapy

<table>
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<td>SD</td>
<td>37 alive</td>
</tr>
</tbody>
</table>

SD = stable disease, PR = partial response, CR = complete response, PD = progressive disease. No major side effects were determined during the study.

### Conclusion

CTX can be safely administered during DC immunotherapy. The results on Tregs will be presented at the conference. The results on overall survival confirm the data found from our earlier study on DC immunotherapy in patients with mesothelioma. Hegmans, Veltman, Lambers, de Vries, Figdor, Hendriks, Hoogsteden, Lambrecht, Aerts. Am J Respir Crit Care Med 2010;181:1282.

Disclosure: No significant relationships.

### SESSION IIC IMMUNOLOGY SEPTEMBER 12, 2012 14:20–16:00

### IIC.3: ANTI-TUMOR ROLE OF INTERFERON-Γamma PRODUCING CD1d-RESTRICTED NKT CELLS IN MURINE MALIGNANT PLEURAL MESOTHELIOMA

**Tetsuzo Tagawa, Licun Wu, Zhihong Yun, Katrina Rey- McIntyre, Marc De Perrot**

Latter Thoracic Surgery Research Laboratories, Toronto General Research Institute, University Of Toronto, Toronto/ON/CANADA

Background: CD1d-restricted invariant NKT (iNKT) cells can provide adjuvant activity against cancer by producing large amounts of IFN-γ which activate other immune cells, and orchestrate protective anti-tumor immunity. Recently, induction of the iNKT cell-dependent anti-tumor immune response using its ligand alpha-galactosylceramide (αGalCer) has been attempted in several tumor types. However, the role of iNKT cells in the tumor microenvironment has not yet been fully addressed. Our aim is to elucidate the role of iNKT cells in the thoracic cavity by using a murine malignant pleural mesothelioma (MPM) model.

Methods: First, a murine model of human malignant mesothelioma was analyzed by intracellular cytokine staining using flow cytometry. To block iNKT cell activation, mice were treated with 0.5mg of anti-CD1d-blocking antibody 1 day before and 7 days after tumor cell injection. To activate iNKT cells, αGalCer was injected intraperitoneally on day 1, 5 and 9 after the tumor cell injection and the survival, the amount of pleural effusion on day 14 were analyzed. IFN-γ expression on iNKT cells and CD8 positive T cells were also analyzed by flow cytometry.

Results: The ratio of iNKT cells to CD3 positive cells in the pleural cavity significantly increased after tumor cell injection (day 0: 0.8±0.2%, day 7: 7.8±0.6%, day 14: 11.7±0.6%) while that in spleen did not change. The ratio of iNKT cells to CD3 positive cells in the tumor on day 14 was 8.9±1.0%. These iNKT cells in the pleural cavity and the tumor showed higher CD25 expression compared with that in the spleen. The ratio of IFN-γ/gamma positive iNKT cells in the pleural cavity was significantly increased after the tumor cell injection (day 0: 0.8±0.2%, day 7: 11.4±1.3%, day 14: 12.8±1.0%). The mice treated with αGalCer showed significantly prolonged survival compared with the control mice (median survival time, 7.0 days and 17.0 days, respectively, p < 0.0001) and associated with increased ratio of IFN-γ positive iNKT cells and CD8 positive T cells in the pleural effusion.

Conclusion: iNKT cells appear to contribute to the anti-tumor immune response in murine MPM. Modulation of iNKT cells could be a new therapeutic approach for patients with MPM.
time. Peripheral blood mononuclear cells (PBMC) were cryogenically stored for subsequent analysis. Toxicity and radiological response were assessed.

Results: To date, 26 patients with MM (21/26) or NSCLC (5/26) have completed treatment. Preliminary analyses demonstrate the Treg% is stable during the first cycle of standard care chemotherapy, with a mean pre-treatment baseline of 8.08% ± 2.08%. Following commencement of CTX, the peripheral blood Treg% decreased (with optimal Treg depletion achieved during treatment with alternating 50/100 mg per day) then increased back to/and above baseline during the treatment break. In ‘intermittent’ patients, the Treg nadir was 5.90% ± 1.51%, and was achieved on cycle 3 day 15. ‘Continuous’ patients did not receive a treatment break, in an effort to negate the Treg% increase; however this did not produce a difference in Treg% when compared to the ‘intermittent’ group. Increasing doses up to alternating 100/150 mg daily did not result in improved Treg depletion. Analysis of PBMC T cell populations indicates that cell proliferation (Ki67) and activation (inducible co-stimulator; ICOS) peak and trough with each cycle of chemotherapy, declining during treatment and increasing during the treatment break. Combining CTX with routine cytotoxic chemotherapy is feasible with no additional haematological or other toxicities.

Conclusion: The addition of CTX to pemetrexed based chemotherapy is safe and feasible. Doses above alternate days 50/100 mg do not improve depletion. Additional work will investigate the effect of this chemomunotherapy protocol on tumour-specific cellular immunity.

Disclosure: No significant relationships.

SESSION IIC IMMUNOLOGY SEPTEMBER 12, 2012 14:20–16:00

IIC.6: ANTI-TUMOR ACTIVITY INDUCED BY CTLA-4 BLOCKADE MIGHT BE MEDIATED BY NKT CELLS IN A MURINE MESOTHELIOMA MODEL

Licun Wu, Zhihong Yun, Yidan Zhao, Marc De Perrot
Latner Thoracic Surgery Research Laboratories, Toronto General Research Institute, University Of Toronto, Toronto/ON/CANADA

Background: Considerable evidence has shown that cancer immunotherapy is promising when combined with chemotherapy. Immunosuppressive components such as regulatory T cells (Treg), cytotoxic T cell associated antigen-4 (CTLA-4), and so on, are major hurdles affecting the immune surveillance in tumor microenvironment. Therefore, removal of these brakes would be able to enhance the anti-tumor immune reaction, thus improve the efficacy of chemotherapy in mesothelioma. Our previous studies indicated that Treg depletion or blockade of CTLA-4 signalling between cycles of chemotherapy improved the outcome of mesothelioma. We notice that the number of NKT cells increased over time at the early stage of tumor development. Tumors grew more rapidly in Cd1d KO mice than WT mice, therefore NKT cells may play important roles in mediating anti-tumor effect. NKT cell activation might be a potent approach to mesothelioma treatment.

Methods: The effect on tumor growth was evaluated in subcutaneous murine mesothelioma model. CTLA-4 blocking antibody +/- NKT ligand α-GalCer was administered following each cycle of chemotherapy in WT or Cd1d KO Balb/c mice, and monotherapy was included as controls. Anti-tumor effect was evaluated by tumor growth delay and survival of the animals. Tumor cell repopulation was quantified by BrdU incorporation and Ki67 by immunohistochemistry and/or flow cytometry. NKT cells were identified by α-GalCer conjugated tetramer staining, and RT-PCR was performed to determine the gene expression of associated cytokines.

Results: Anti-tumor effect was achieved by administration of CTLA-4 blockade or α-GalCer between cycles of chemotherapy. Tumor cell repopulation during the intervals of cisplatin was significantly inhibited. Anti-CTLA-4 therapy gave rise to an increased number of CD4 and CD8 T cells infiltrating the tumor. RT-PCR demonstrated that the gene expression of IL-2, IFN-γ, granzyme B, and perforin increased in the tumor milieu. NKT cell activation by α-GalCer had mild effect, but α-GalCer combined with cisplatin was more effective inhibiting tumor growth than other groups in WT mice, whereas α-GalCer did not result in additional effect in Cd1d KO mice. Interestingly, α-GalCer plus CTLA-4 blockade resulted in more IFN-γ production in WT mice rather than Cd1d KO mice during cisplatin treatments.

Conclusion: Blockade of CTLA-4 signalling demonstrated effective anti-tumor effect correlating with inhibiting cancer cell repopulation between cycles of chemotherapy. This effect might be mediated by NKT cells in WT mice.

Disclosure: No significant relationships.

SESSION IIC IMMUNOLOGY SEPTEMBER 12, 2012 14:20–16:00

IIC.7: OPTIMAL SOURCE OF WHOLE TUMOR ANTIGENS FOR DENDRITIC CELL-BASED IMMUNOTHERAPY IN MURINE MESOTHELIOMA

Pulmonary Diseases, Erasmusmc, Rotterdam/NETHERLANDS

Background: Malignant mesothelioma is an aggressive tumor which is resistant to conventional therapies. This study focuses on dendritic cell (DC) vaccination for malignant mesothelioma which is a novel and promising strategy in cancer treatment. The source of tumor associated...
antigens (TAA) necessary for DC stimulation might influence the level of the anti-tumor responses induced. The aim of this study was to select the optimal TAA source for DC vaccine development for the treatment of malignant mesothelioma. As a second objective, the effectiveness of tumor exosome and apoptotic tumor cell fragments based DC vaccines were compared with the conventional necrotic tumor lysate loaded DC vaccines in vivo.

**Methods:** Tumor derived exosomes were obtained by ultracentrifuging supernatant of the mouse AB1 mesothelioma cell line culture. For the apoptotic tumor cell fragments, AB1 tumor cells were exposed to 25kJ/m² ultraviolet-B light while tumor cells for necrotic material were subjected to freeze-thaw cycles. Twenty-four BALB/c mice were inoculated intraperitoneally with a lethal dose of AB1 cells that leads to terminal illness between 15 and 30 days. For immunotherapy, dendritic cells were cultured ex vivo and stimulated with tumor-derived exosomes, apoptotic tumor cells or necrotic tumor cell lysate, and vaccinated at day 7 after tumor inoculation.

**Results:** Electron microscopy of tumor exosomes, necrotic and apoptotic tumor cell fractions used for vaccine preparation revealed the morphological differences between the fractions. The effectiveness of uptake of the tumor fractions and subsequent DC stimulation was examined by fluorescence microscopy and flow cytometric analysis of DC maturation markers. Our results showed the highest uptake of the apoptotic material and increased expression levels of cell surface markers MHCII, CD40, CD80 and PDL1 after stimulation. Mice receiving a DC vaccine based on tumor derived exosomes, 7 days after tumor cell injection, had a doubled survival rate (33.3% of mice were alive after 52 days) compared to mice receiving the conventional vaccine (16.6% of mice were alive after 52 days). Mice that received a DC vaccine based on apoptotic tumor cell fragments had a survival rate that was three times higher (50% of mice were alive after 52 days) compared to the conventional necrotic vaccine. Tumor material present in three mice after 52 days, had increased activation of specific anti-tumor immunity compared to mice without DC-treatment.

**Conclusion:** Stimulation of DCs with tumor exosomes, apoptotic or necrotic tumor lysate showed an increased expression of cell surface markers, indicating maturation and activation of DCs. All different DC vaccinations induced successful anti-tumor responses but tumor derived exosomes and apoptotic tumor cell fragments were most efficient. Histology shows that long term surviving mice have an increased anti-tumor response and activation of the immune system compared to mice without DC-treatment. These promising results obtained in mice could lead to a refined method for DC-based immunotherapy in patients in the near future.

**Disclosure:** No significant relationships.
There is no indication to perform any more than palliative surgery in non-epithelioid MPM. In the treatment of sarcomatoid MPM, unlike other cell types, the extent of surgery has no influence on postoperative survival which is around 6 months only [1].

There is no indication to perform Extrapleural Pneumonectomy (EPP) in mediastinoscopy positive MPM. There is no survival benefit in EPP over pleurectomy/decortication in these patients. Median postoperative survival was around 16 months in case-matched patients [2].

Median sternotomy should be preferred to lateral thoracotomy for EPP. This approach results in reduced operating time; less postoperative analgesia and faster recovery [3].

Extended pleurectomy/decortication (EPD) should be preferred to EPP in most cases. The increased morbidity and mortality after pneumonectomy confer a survival benefit for EPD in most cases [4,5]. The benefit of EPP is limited to the small subgroup of patients presenting in stage I disease [6].

Median sternotomy should be preferred to lateral thoracotomy for EPP. This approach results in reduced operating time; less postoperative analgesia and faster recovery [3].

There is no role for open incomplete resection. The combination of the poor prognosis of R2 resection and delayed postoperative recovery after thoracotomy mean this approach cannot be justified [7]. If thoracotomy is performed then macroscopic complete resection must be achieved even if this requires phrenectomy.

Palliative VATS debulking should be preferred to EPD in stage III MPM. In our experience the additional morbidity and mortality of EPD is not justified in N2 positive disease. VATS with an R2 palliative debulking operation has similar long-term benefits. EPD should be limited to node-negative epithelioid cases.

Systemic chemotherapy should be considered as an alternative to radical surgery in most cases. In the MARS trial control arm a median survival of 19 months was obtained from chemotherapy alone; this exceeded the post-EPP survival [8]. Other studies have reported median survival of 23 months for early stage epithelioid MPM from chemotherapy alone [9] which is comparable to surgery in similar groups [6].

The value of intraoperative chemotherapy remains unproven. Whilst the feasibility of the technique has been established and local control may have been improved there has not been a significant improvement in overall survival [10].

Conclusion: Better understanding of the importance of histological cell type and disease staging has refined selection for surgery in MPM. Surgical strategy has evolved from the use of extrapleural pneumonectomy as the default operation towards lung sparing surgery. Extended pleurectomy/decortication with macroscopic complete resection should be favoured in node-negative epithelioid MPM. EPP may be considered in the fit, young patient with stage 1 epithelioid disease. In node-positive or non-epithelioid disease the use of surgery by VATS should be considered in a palliative setting. All surgery for MPM should be in conjunction with systemic therapy. The role of intraoperative chemotherapeutic agents remains unproven.

References
SEPTEmBER 13, 2012 10:00-11:30

III.A: EPP OR PD: THE CYTOREDUCTIONIST’S DILEMMA

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Cytoreductive surgery for malignant pleural mesothelioma includes extrapleural pneumonectomy (EPP) and extended pleurectomy decortication (PD). The goal of either procedure is complete macroscopic resection of tumor. EPP and PD differ regarding extent of resection, morbidity profile, patterns of recurrence and compatibility with adjuvant therapies. By definition, EPP removes the ipsilateral lung, pleurae, ipsilateral diaphragm and pericardium. PD removes the above structures but leaves the lung in situ, and also occasionally the diaphragm and pericardium. Because depleuralized lung remains local recurrence is higher following PD. Paradoxically, higher rates of distant recurrence after EPP are reported. Although EPP is perhaps a more oncologically sound procedure it is associated with higher mortality and negatively impacts quality of life more than PD. Lung preservation may also compromise the ability to deliver hemithoracic radiation, which has been effective in reducing local recurrence rates to below 15% after EPP. Despite this, retrospective comparative studies have failed to demonstrate any survival advantage of EPP over PD. It is well known that survival after EPP is highly influenced by tumor stage and histology. It is also evident that preoperative staging of MPM is highly inaccurate. A possible strategy to refine patient selection for cytoreductive surgery involves thorough intraoperative staging prior to the decision to perform EPP or PD. Patients with non-epithelioid or advanced stage (Ta/N2) disease are unlikely to obtain any benefit from EPP and may be better served with PD, whereas those with No/1 epithelioid tumors may potentially benefit from the improved local control offered by EPP.

Disclosure: No significant relationships.

IIIA.2: PATTERNS OF RECURRENCE FOLLOWING EXTRAPLEURAL PNEUMONECTOMY (EPP) FOR MALIGNANT PLEURAL MESOTHELIOMA (MPM)

Brian M. Goodman1, Ritu R. Gill1, Olivia Winfrey1, William G. Richards1, Alleen B. Chen1, David E. Koizono1, Raymond H. Mak1, Raphael Bueno3, David J. Sugarbaker1, Elizabeth H. Baldini1
1Division Of Thoracic Surgery, Brigham And Women’s Hospital, Boston/MA/UNITED STATES OF AMERICA, 2Radiology, Brigham And Women’s Hospital, Boston/MA/UNITED STATES OF AMERICA, 3Division Of Thoracic Surgery, Brigham And Women’s Hospital And Harvard Medical School, Boston/MA/UNITED STATES OF AMERICA, 4Radiation Oncology, Brigham And Women’s Hospital, MA/UNITED STATES OF AMERICA, 5Radiation Oncology, Brigham And Women’s Hospital/Dana-Farber Cancer Institute, Boston/MA/UNITED STATES OF AMERICA

Background: We have previously described patterns of failure following EPP and trimodality therapy for MPM. We sought to update our results with a larger cohort of contemporary patients.

Methods: We reviewed records for 171 patients who underwent EPP without pre-operative chemotherapy at Brigham and Women’s Hospital between 2001 and 2010. Data for treatment, recurrence and survival were determined from medical records. A dedicated thoracic radiologist reviewed the post-operative chest CT and/or PET-CT scans to determine sites of recurrence. Time to recurrence was calculated from the date of resection and estimated by the Kaplan-Meier method. Rates were compared using Fisher’s exact test.

Results: Median age was 62 years. 138 patients (81%) were men. Median tumor volume was 390 cm3. Histology on final pathology was epithelial for 104 patients (61%) and non-epithelial for 67 (39%). No patients received pre-operative chemotherapy (CT); 134 (78%) received heated intra-operative chemotherapy (HIOC); 78 (46%) received adjuvant CT and 73 (43%) received adjuvant radiation therapy (RT). RT was delivered using a matched electron-photon technique for 32 patients (52%), intensity modulated RT for 22 (35%) and other 3D conformal techniques for 8 (13%). Median RT dose was 54 Gy. Among the 162 evaluable patients, 120 (74%) developed a recurrence. Median follow-up time was 54 months and median time to recurrence was 12.4 months. Sites of first recurrence are shown in the table:

<table>
<thead>
<tr>
<th>SITE OF RECURRENCE</th>
<th>N</th>
<th>% of All Patients</th>
<th>% of Recurrences</th>
<th>% of Recurrences from 1997 report*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ipsilateral Hemithorax</td>
<td>83</td>
<td>51%</td>
<td>69%</td>
<td>67%</td>
</tr>
<tr>
<td>(IHT) and/or Mediastinum</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Abdomen</td>
<td>60</td>
<td>37%</td>
<td>50%</td>
<td>50%</td>
</tr>
<tr>
<td>Contralateral Hemithorax</td>
<td>41</td>
<td>25%</td>
<td>34%</td>
<td>33%</td>
</tr>
<tr>
<td>(CHT)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Distant</td>
<td>8</td>
<td>5%</td>
<td>7%</td>
<td>8%</td>
</tr>
</tbody>
</table>

*Baldini EH, Recht A, Strauss GM, et al. Ann Thorac Surg 1997;63:334-8. 45% of patients experienced recurrences in the IHT, 27% in the mediastinum, 51% in the IHT or mediastinum, and 19% in the IHT or mediastinum only. The most common sites of recurrences in the IHT were chest wall mass (68% of recurrences) and neo-pleural mass (27%); in the mediastinum were lymph nodes (79%) and mediastinal soft tissue (28%); in the abdomen were retroperitoneal adenopathy (40%), ascites (38%), abdominal mass (32%), and peritoneal mass (26%); in the CHT were lung nodules (56%) and pleural effusion (49%); and for distant sites were bone (75%) and soft tissue (25%). For patients who developed a recurrence in the IHT or mediastinum, the rate was lower for those who received RT (29/73, 40%) compared to those who did not receive RT (54/89, 61%; p=0.01).

Conclusion: The most common site of recurrence after EPP and planned trimodality therapy remains the ipsilateral hemithorax (including mediastinum) and true distant failure remains unusual. The distribution of recurrences is strikingly similar to our prior report from 15 years ago.

Disclosure: No significant relationships.
Illa.3: Lung-Sparing Radical Pleurectomy Is Associated With Improvement of Pulmonary Function and Lung Perfusion in Patients With Malignant Pleural Mesothelioma

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Background: Pulmonary function is reduced in patients with malignant pleural mesothelioma (MPM) due to encased lung tissue via a rind of tumor with or without concurrent effusion. Re-expansion of the trapped lung might be achieved by radical pleurectomy (RP). The objective of this study was to investigate changes in pulmonary function and lung perfusion in patients undergoing RP.

Methods: All patients with histologically proven MPM were evaluated for trimodality therapy including RP as surgical procedure in a prospective, nonrandomized study from January to December 2010. Pulmonary-function tests and perfusion scans were obtained before and 2 months after RP. Primary end points were pulmonary function (forced vital capacity [FVC], forced expiratory volume in 1 second [FEV1]) and ipsilateral lung perfusion.

Results: Sixteen out of 25 consecutive patients were included in the study. Macroscopic complete resection rate was 81.3% (13 patients). Diaphragm resection was performed in 5 patients (31.3%). Post-surgical improvement of PFTs was observed for FVC and FEV1 (both absolute and percentage of predicted values) and ipsilateral perfusion (p < 0.001). Avoidance of diaphragm resection was associated with greater increase in FVC (+34.6±17.0% versus +13±54.4%, p = 0.002) and FEV1 (+29.2±18.1% versus +12.1±6.6%, p = 0.015), respectively. In a linear regression analysis a lower preoperative FVC (% predicted) or FEV1 (% predicted) was associated with higher relative increases in FVC or FEV1 after RP (p < 0.02 for both).

Conclusion: Lung-sparing RP is associated with significant improvement of PFTs and lung perfusion in patients with MPM. Preservation of the diaphragm results in better functional results. Protection of physiological reserve might leave options open for further therapy in the long term.

Disclosure: No significant relationships.

Illa.4: Radical Pleurectomy and Intraoperative Photofrin Sodium Photodynamic Therapy for Malignant Pleural Mesothelioma

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Background: Our group has conducted several trials for malignant pleural mesothelioma utilizing photodynamic therapy as an intraoperative adjuvant therapy, with porphyrin sodium being the only photosensitizer employed for all patients at a Phase II level. We performed a pilot study comparing survival rates for patients who underwent this modality with either radical pleurectomy or extrapleural pneumonectomy, and found superior results with the radical pleurectomy group. This report summarizes our current results for the expanded cohort of patients having undergone radical pleurectomy with intraoperative porphyrin sodium photodynamic therapy.

Methods: 44 patients (37-81 years) underwent radical pleurectomy with intraoperative porphyrin sodium photodynamic therapy. The goal of every operation was to achieve a macroscopic complete resection, while preserving the lung and, whenever possible, the phrenic nerve and as much of the pericardium and diaphragmatic musculature as possible. The decision to perform radical pleurectomy was a preoperative decision, not intraoperative, regardless of tumor bulk or degree of pulmonary fissure invasion. 38/44 patients also received pemetrexed-based chemotherapy. All survivals were calculated from the time of surgery, not diagnosis or other treatments.

Results: A macroscopic complete resection was achieved in 43/44 patients (with the 1 incompletely resected patient undergoing subtype revision from preoperative epithelial to mixed desmoplastic on the final pathology). There was 1 postoperative mortality (stroke). Average length of stay was 13.5 days. The median follow-up for all patients was 36.5 months. The stage breakdown for the 36 epithelial patients was 1(I)/7(II)/28(IV), of which 24/36 had N2 disease and the median overall survival was 26.6 months – 31.7 months for N2 disease and 57.1 months N0-1 disease (p=0.04). The stage breakdown for the 8 nonepithelial patients was 6(III)/2(IV), of which 5/8 had N2 disease and the median overall survival was 6.8 months.

Conclusion: Bearing in mind the limitations of this retrospective series there are several sound conclusions that can be drawn from the data. This series demonstrates radical pleurectomy can be used to achieve a macroscopic complete resection and that it can be done safely, even in this cohort of very advanced stage patients (98% stage III/IV) where tumor volume was often greater than 800 cc. It is also clear that nonepithelial patients do not benefit from this particular approach and it is no longer being offered by our group for these patients. The 36.6 month median survival from the time of surgery for these advanced stage epithelial patients compares favorably with other surgery-based treatments, especially when matched for stage. Although the epithelial No-1 patients demonstrated a greater median overall survival of 57.1 months, we do not feel that the 31.7 month median survival for the N2 patients should serve as an exclusion criteria for future patients. Overall, we feel these results are sufficiently encouraging to warrant further study, including a randomized phase III to determine if photodynamic therapy is responsible for these results.

Disclosure: No significant relationships.

Illa.5: Intrapleural Polymeric Films Loaded With Cisplatin for Malignant Pleural Mesothelioma: Preliminary Pharmacokinetic Data in an Ovine Model

Luca Ampollini1, Stefano Barbieri2, Fabio Leonardi1, Luigi Rolli1, Stefano Zanichelli1, Antonella Fusari4, Anna Maria Cantoni2, Claudio Mucchino5, Paolo Colombo1, Michele Rusca1, Paolo Carbognani2
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Background: Long-term survival in malignant pleural mesothelioma (MPM) patients has been reported after multimodality therapy. Nevertheless local tumor recurrence represents the real challenge related to MPM.
Intrapleural application of chemotherapy and immunotherapy for the adjuvant treatment of MPM has been reported. We previously showed that intrapleural polymeric films loaded with cisplatin were significantly effective in reducing tumor recurrence compared with cisplatin solution assuring higher and more prolonged plasmatic drug concentrations without increasing toxicity. This study aims to investigate the pharmacokinetic profile and tolerability of intrapleural polymeric films containing cisplatin for MPM in an ovine model.

Methods: Hyaluronate films loaded with cisplatin (100mg/m2) previously characterized were used for the local delivery of anticancer drug. Female sardinian sheep weighing 40-50kg were chosen for in vivo experiments. After general anesthesia the sheep were placed in a right lateral decubitus: a left pneumonectomy was carried out through a lateral thoracotomy. Thereafter, the adjuvant treatment was randomly administered: intravenous cisplatin, intrapleural cisplatin, intrapleural hyaluronate-cisplatin. Controls (pneumonectomy alone) were used for comparison. Blood samples were taken as scheduled. The animals were euthanatized on postoperative-day 7: serum, parietal, diaphragmatic pleura, pericardium, kidneys and liver were considered for analysis. Primary endpoint was plasmatic cisplatin concentration evaluated by IPC-mass spectrometry. Secondary endpoints were treatment-related toxicity and tissue drug concentration. Data are given as mean. ANOVA was applied for statistical analysis. The study was approved by the local veterinary committee.

Results: Three animals per group were treated so far. Mean operation time was 88 minutes (range, 70-127). After 30’ from intravenous administration, plasmatic drug concentration was significantly higher (3148ng/ml) than intrapleural cisplatin solution (2722ng/ml, p=0.007) and intrapleural hyaluronate-cisplatin (163ng/ml, p=0.002). At seven days, plasmatic cisplatin concentration was much higher (3359ng/ml) after intrapleural hyaluronate-cisplatin in comparison to intrapleural and intravenous cisplatin solution [169ng/ml (p=0.051) and 1264ng/ml (p=0.027), respectively] reflecting the controlled drug release from hyaluronate films (Figure 1). No haematological toxicity was observed. On postoperative-day 7, creatinine levels was significantly higher (p=0.033) after intravenous administration (26.6mg/dl) in comparison to intrapleural hyaluronate-cisplatin (6.5mg/dl) and controls (1.7mg/dl, p=0.014). Animals treated with intrapleural cisplatin had creatinine levels much higher (22.7mg/dl) than intrapleural hyaluronate-cisplatin but the difference was not statistically significant (p=0.092). Severe degeneration in tubular cells and glomerular congestion was microscopically found after cisplatin solution, while a mild injury was present after hyaluronate-cisplatin.

Conclusion: Preliminary data showed that intrapleural polymeric films containing cisplatin assured higher plasmatic drug concentration than cisplatin solution without increasing systemic toxicity after seven days.

Disclosure: No significant relationships.

IIIA.6: TRIMODALITY THERAPY WITH EXTRAPLEURAL PNEUMONECTOMY, RADIATION THERAPY, AND CHEMOTHERAPY FOR MALIGNANT PLEURAL MESOTHELIOMA

Kazunori Okabe1, Eisuake Matsuda1, Hiroyuki Tao1, Tatsuro Hayash1, Tosiki Tanaka1, Humiho Sano1, Akhiro Takahagi1, Keisuke Aoe1, Koutaro Taguchi1

Thoracic Surgery, Yamaguchi Ube Medical Center, Ube/yamaguchi/JAPAN, 1Medical Oncology, Yamaguchi Ube Medical Center, Ube/yamaguchi/JAPAN, 1Radiology, Yamaguchi Ube Medical Center, Ube/yamaguchi/JAPAN

Malignant pleural mesothelioma (MPM) is a serious disease, and a treatment strategy for MPM has not yet been established. We report our experience of trimodality therapy with extrapleural pneumonectomy (EPP), radiation therapy, and chemotherapy for MPM.

Thirty-one EPPs were completed in our hospital between June 2006 and January 2012. This number is one of the biggest in Japan within the period, because the Japanese medical system let MPM patients disperse to many hospitals. Among the thirty-one EPP, twenty-seven consecutive EPP for MPM were performed by the first author’s thoracic surgery team were reviewed retrospectively. We have instituted a trimodality therapy protocol consisting of EPP, adjuvant 45 Gy hemithoracic radiation therapy, and adjuvant cisplatin-based chemotherapy. Twenty-two patients have been treated with this protocol. Five patients were given induction chemotherapy with CDDP and PEM, and referred to us. They underwent EPP and adjuvant radiation therapy. Age, gender, left or right, type of MPM, time of EPP, blood transfusion during EPP, perioperative complication, pTNM and p-Stage, radiation therapy, chemotherapy, and prognosis were examined. Overall survival was calculated using the Kaplan-Meier method.

The median age at EPP was 61 years old (44-74). There were 21 males and 6 females. The right side was affected in 14 and the left side in 13. The epithelioid type was present in 17, with biphasic type in 6, sarcomatous type in 2, and special type in 2. The median EPP time was 7 hours 40 minutes (5 hours 52 minutes – 10 hours 15 minutes). No blood transfusion during EPP was needed in 12 cases (44%). Mortality involved one patient (3.7%), who died on post-operative day 14 due to the acute aggravation of interstitial pneumonia. Eleven patients (41%) had perioperative complications. Atrial fibrillation was the most common morbidity, and developed in seven patients (26%). The IMIG pathological TNM was T4 (peritoneal cavity) in 1, T3 in 13, T2 in 6, T1b in 5, N2 in 11, and N0 in 16. The IMIG pathological stage was stage IV in 1, stage III in 17, stage II in 4, and stage I in 5. Adjuvant 45 Gy hemithoracic radiation therapy was completed in 23 patients (85%). Six patients (22%) could not undergo adjuvant chemotherapy. Seventeen patients (63%) underwent trimodality therapy. However, seven patients (26%) could not undergo it, and three patients (11%) are currently waiting for adjuvant chemotherapy. The three-year survival, two-year survival, and median survival of all twenty-seven patients were 27%, 36%, and 13 months, respectively. The three-year survival, two-year survival, and median survival of seventeen patients who underwent trimodality therapy were 37%, 49%, and 23 months, respectively. The median survival of seven patients who could not undergo trimodality therapy was 6 months. Survival of the patients with trimodality therapy was significantly better than the patients without trimodality therapy.

Trimodality therapy with EPP, radiation therapy, and chemotherapy for MPM is feasible. However, the prognosis of MPM patients should be quickly and markedly improved.

Disclosure: No significant relationships.
IIIB.2: MACROPHAGES CAN BE MANIPULATED TO ENHANCE THE APOPTOTIC RESPONSE TO CHEMOTHERAPY IN MESOTHELIOMA

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Background: Macrophages within the solid tumor microenvironment (TME) contribute to tumor chemoresistance by several mechanisms. We have found that macrophages constitute a significant percentage of the infiltrating leukocyte population in human mesothelioma, a highly chemoresistant tumor. We investigated whether therapeutic strategies either to reprogram the macrophage phenotype from a Th2-type (pro-tumor) towards a Th1-type (anti-tumor) phenotype or instead to reduce the number of macrophages in mesotheliomas by blockade of the predominant macrophage survival pathway, e.g. colony stimulating factor 1 receptor (CSF1R), would alter chemoresponsiveness of mesotheliomas.

Methods: To address this, we used two 3D organotypic spheroid models of mesothelioma: 1) multicellular spheroids (MCS), with human mesothelioma cells grown alone or co-cultured with macrophages derived from peripheral blood monocytes, and 2) primary tumor fragment spheroids (TFS), which are small fragments of tumor generated from resected human mesothelioma tumor. We also used an in vivo murine model of syngeneic mesothelioma tumor. MCS or TFS were incubated with Th1-type (LPS & IFNg) or Th2-type (IL-4 &IL-13) cytokines, followed by exposure to standard-of-care chemotherapy, carboplatin plus pemetrexed. TFS and mice with syngeneic orthotopic mesothelioma were treated with carboplatin plus pemetrexed with and without a small molecule inhibitor of CSF1R, e.g., GW2580.

Results: In both spheroid models, Th1-type macrophage programming significantly increased the apoptotic response of mesothelioma cells to chemotherapy. Moreover, in the two spheroid models, when CSF1R signaling in macrophages was inhibited by incubation with GW2850, chemoresponsiveness was significantly increased as evidenced by an increased presence of apoptotic tumor cells. Enhanced chemoresponsiveness of mesothelioma tumor cells was found to be dependent on the presence of macrophages because incubation of tumor cells alone with Th1-type cytokines or with GW2850 was without effect. On the other hand, exposure of macrophages alone to GW2850 in vitro resulted in a 50% decrease in macrophage viability. Finally, treatment of tumor-bearing mice with GW2580 in combination with carboplatin plus pemetrexed chemotherapy resulted in a significant decrease in tumor burden and an increase in tumor cell apoptosis as compared to treatment of mice with chemotherapy or GW2580 alone.

Conclusion: Based on these data, we propose that manipulating the macrophage within the mesothelioma TME may be a promising therapeutic approach.

Disclosure: No significant relationships.
manipulation of the core apoptotic repertoire in order to release Bim may improve the chemosensitivity of mesothelioma. We wish to thank the following for grant support: TRDRP fellowship to DB (18FT-0120), an Ireland/NCI Consortium grant to VCB, DAF and DB (CDV/36/9/0) and a Department of Defense Mesothelioma Program grant to VCB (PRO207Y).

Disclosure: No significant relationships.

SESSION IIIB APOTOPSIS AND SIGNAL TRANSDUCTION
SEPTEMBER 13, 2012 10:00-11:30

IIIB.4: THE HSP90 INHIBITOR GANETESPIB REQUIRES THE BID DRIVEN MITOCHONDRIAL PATHWAY EXECUTE APOTOTIC CELL DEATH IN MESOTHELIOMA

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Background: Mesothelioma is a highly ant apoptotic cancer. This phenotype may be in part related to multiple constitutively activated growth factor signals. HSP90 inhibition may be a clinically useful strategy for suppressing multiple growth factor signals via the PI3K/AKT/mTOR and RAS/RAF/MEK pathways in mesothelioma. A phase I/II trial is currently in development in the UK, called MESO2 (NCT01590160).

Methods: Mouse embryonic fibroblasts (MEFs) with either wild type (WT) of double knockout (DKO) of BAX/BAK were treated with the HSP90 inhibitor, ganetespiib, and IC50 values determined. RNAi was used in two mesothelioma cell lines (MSTO-211H and NCI-H2052) to silence BAX/BAK. Apoptosis was analysed by poly ADP-ribose polymerase (PARP) cleavage and caspase 9 (C9) activation. Focused RNAi array targeting of BH3 only proteins (which activate BAX/BAK or antagonise prosurvival BCL2 proteins) was conducted to delineate critical death activators.

Results: Double knockout or RNAi silencing rescued cells from ganetespiib-mediated apoptosis as evidenced by reduced PARP/C9 cleavage, mitochondrial depolarisation and sub-G1 analysis and increased viability. Systematic functional assessment of BH3 proteins identified BID silencing as being sufficient to rescue cells. We have found that loss of both BAX and BAK is observed in 37% of primary mesotheliomas. Loss of BID in mesothelioma has been reported to be 38%

Conclusion: Inhibition of HSP90 requires the mitochondrial pathway to induce cell death. Our data suggest that loss of BAX/BAK or BID may be sufficient to induce resistance, and could be putative biomarkers of sensitivity to ganetespiib, and provides a hypothesis for clinical correlation in trial NCT01590160.

Disclosure: No significant relationships.

SESSION IIIB APOTOPSIS AND SIGNAL TRANSDUCTION
SEPTEMBER 13, 2012 10:00-11:30

IIIB.5: REACTIVATION OF P53-MEDIATED PATHWAYS INDUCES APOTOPSIS IN MESOTHELIOMA WITH WILD-TYPE P53 GENE AND PRODUCES COMBINATORY SYNERGISTIC EFFECTS WITH ANTI-CANCER AGENTS

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Intrapleural injection of Ad-p53 or Ad-delE1B in combination with systemic administration of the first-line agents for mesothelioma, showed synergistic cytotoxic effects. We also demonstrated the anti-tumor effects of Ad-p53 and Ad-delE1B in an orthotopic animal model. Intrapleural injection of Ad-p53 or Ad-delE1B decreased the tumor weight of human mesothelioma developed in the pleural cavity of immunocompromised mice and further achieved combinatorial effects with intraperitoneal CDDP administration. Interestingly Ad-delE1B55kDa-infected cells displayed a hyperploid state at the cell cycle analysis and showed enlarged nuclear configurations followed by pyknotic nuclear changes that were positive for a TUNEL assay.

Conclusion: Intrapleural injection of Ad-p53 or Ad-delE1B in combination with systemic administration of the first-line agents is a feasible therapeutic strategy for mesothelioma by inducing apoptotic cell death.

Disclosure: No significant relationships.
To address this, we have employed both mechanistic and translational-based approaches with the aim of identifying which cell death pathways are deregulated in mesothelioma by examining the expression of key cell death pathway regulators in different subtypes of human mesothelioma cell lines, normal untransformed mesothelial cells and mesothelioma tissue obtained from patients. In addition, we have compared the sensitivity of normal mesothelial cells and mesothelioma cell lines to a range of cytotoxic agents, including DNA damaging agents and novel agents that target specific nodes of apoptosis resistance. Importantly, based on initial profiling of malignant mesothelioma tumour samples from patients, several potentially viable drug targets for modulating tumour cell death/key regulators of apoptosis signalling pathways, will be highlighted (e.g. Inhibitors of BCL-2, TRAIL-Receptor agonists, and Inhibitors of glycolysis such as 2-deoxyglucose). This work was supported by the Medical Research Council (UK).

Disclosure: No significant relationships.
IIIC.2: EXPOSURE TO ASBESTOS IN HOME RENOVATION AND SUBSEQUENT RATES OF MALIGNANT MESOTHELIOMA IN WESTERN AUSTRALIA

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Background: Worldwide rates of malignant mesothelioma (MM) have been driven largely by occupational exposure to asbestos and the burden of disease from this exposure will continue for many years. Recent concerns about additional future incidence of MM have been raised concerning exposure to asbestos arising from home renovations involving existing asbestos-containing materials (ACM) (Olsen et al, Med J Aust 2011;195:271-274). Those results were, however, based on numbers of cases, not rates in this population. This study aimed to estimate rates of MM among people who had received their exposure from renovations in homes with ACM in place.

Methods: The Western Australian (WA) Mesothelioma Register has recorded exposure and disease details for all cases of MM occurring in WA since the first case in 1961. All cases attributable to home renovation exposure were collated and their characteristics have been reported, indicating a large rise in numbers over the last 10-15 years. A computer-assisted telephone survey in 2008 interviewed 2800 people in Australia (700 in WA) about previous occupational and environmental exposure to asbestos. The prevalence of home exposure, without any occupational exposure, for different age groups and for different times from first exposure and duration of exposure were estimated from WA responders. These proportions were then applied to the total population obtained from the Australian Bureau of Statistics and used as denominators to estimate rates.

Results: The survey indicated that around 15% of the population had been exposed to asbestos arising from home renovation that, based on reported activities, was equivalent to that experienced by cases of MM. Estimated rates increased with both time since first exposure and duration of exposure. Average over all durations of exposure of more than 7 days, and time since first exposure greater than 10 years, MM rates ranged from 2 per million person-years under age 40 to 80 per million over age 75, and time since first exposure greater than 10 years, MM rates ranged from 3.84 (per 100,000 inhabitants) for men and 1.45 for women, with a wide regional variability. Occupational asbestos exposure was in 69.3% of interviewed subjects cases, while 1.4% by environmental exposure from living near a contamination source and 1.6% during a leisure activity. In the exposed group, the median year of first exposure was 1957, and mean latency was 69.2 years at diagnosis. Gender ratio (M/F) is 2.5. The anatomical site of the disease is the pleura for 94% of cases; peritoneum for 6.4%.

Conclusion: Although increased, absolute risks from home renovation exposure are not high. As well as quantifying the risk from this particular kind of exposure, these data will also enable us in future work to assign risks to other groups of exposed people, both occupational and non-occupational, and to make realistic projections of future incidence of MM in these groups.

Disclosure: No significant relationships.

IIIC.3: MALIGNANT MESOTHELIOMA IN ITALY: INCIDENCE, MODALITIES OF ASBESTOS EXPOSURE AND OCCUPATIONS INVOLVED FROM THE ITALIAN NATIONAL REGISTER.

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Background: Due to the large scale use of asbestos (more than 3.5 million tons produced or imported until its definitive banning in 1992), a specific national surveillance system of mesothelioma incident cases is active in Italy, with direct and individual anamnestic etiological investigation.

Methods: In each Italian Region there is an epidemiological center to actively search and collect every mesothelioma incident case from health care institutions that diagnose and treat them. These include pathology and histology units, lung disease and chest surgery wards. Occupational history, lifestyle habits and areas of residence for each case are obtained by interviewing people directly or, if they are not available, someone close to them (indirect interview) who can provide information on the case’s work and life history. Exposure is classified by an industrial hygienist using the standard grid in accordance with national guidelines and a standard questionnaire, administered by a trained interviewer.

Results: In the period between 1993 and 2008, a case-list of 15,845 MM was recorded by the Italian National Register (ReNaM) and the modalities of exposure to asbestos fibres have been investigated for 12,065 of them. Age at diagnosis is lower than 55 years for 9.4% of cases and the mean is 69.2 years at diagnosis. Gender ratio (M/F) is 2.5. The anatomical sites of the disease is the pleura for 94% of cases; peritoneum for 6.4%. Standardized incidence rates are 3.84 (per 100,000 inhabitants) for men and 1.45 for women, with a wide regional variability. Occupational asbestos exposure was in 69.3% of interviewed subjects cases, while 4.4% was due to cohabitation with someone (generally, the husband) occupationally exposed, 4.3% by environmental exposure from living near a contamination source and 1.6% during a leisure activity. In the exposed workers, the median year of first exposure was 1957, and mean latency was 46 years.

Conclusion: The analysis of exposures focuses on a large variety of economic sectors involved and not only for those traditionally signaled as “at risk” (like asbestos-cement industry, shipbuilding and repair and railway carriages maintenance) and an increasing trend for the building construction sector. The systematic mesothelioma surveillance system is relevant for the prevention of the disease and for supporting an efficient compensation system. Our data illustrate the importance of documentation and dissemination of all asbestos exposure. The existing
SESSION IIC  MESOTHELIOMA EPIDEMIOLOGY  SEPTEMBER 13, 2012 10:00-11:30

IIC.4: MESOTHELIOMA INCIDENCE IN AN ASBESTOS-EXPOSED COHORT IN SOUTH AFRICA: PREDICTING CASES INTO THE FUTURE

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Background: South Africa mined and milled all three commercial forms of asbestos (crocidolite, amosite and chrysotile) for more than 100 years, with production peaking at around 350 000 tons in the 1970s. Miners, millers and community members were exposed to high levels of fibres occupationally and environmentally. There is currently worldwide interest in mesothelioma trends and high rates have been reported in South Africa. Asbestos was mined in South Africa until 2002, and only banned in 2009, so rates are not expected to decline soon. South African laws allow for the financial compensation of occupationally acquired asbestos-related diseases but no compensation is provided to those exposed environmentally. The Asbestos Relief and Kgalagadi Relief Trusts provide additional compensation for deceased persons who worked at or lived in the vicinity of certain asbestos mines in South Africa. Since 2003, almost 15 000 potential claimants have registered with the Trusts; some 4 800 had compensable claims, including 390 with malignant mesothelioma. It is important for the Trusts to estimate the number of future cases as accurately as possible, to equitably allocate remaining funds. The objective of this study was to develop robust mathematical and statistical models to accurately predict the number of cases of mesothelioma likely to present over the next 10 to 15 years.

Methods: Demographic and relevant asbestos exposure data (such as job type and asbestos exposure levels), captured by the Trusts, were used to construct three prediction models which were based on classical, deterministic and Bayesian statistical methods developed in other countries. The outcome was death due to mesothelioma. A number of predictor variables, such as date of birth, date of death, age at first exposure, age at last exposure, job exposure category, duration of exposure, year(s) of exposure, and year-specific asbestos fibre measurements, were used to estimate model parameters. All three models were assessed in terms of their goodness of fit and predictive capabilities. The model with the best fit was selected as the final model for prediction purposes. The data were managed and analysed in STATA version 12.0 SE.

Results: From 2003 to 2011, 390 cases of mesothelioma (2.6%) were diagnosed in the cohort of 14 738 individuals. Their mean age was 59.2 years (SD 10.5) and the mean latency period since first exposure was 30.2 years (SD 10.5). The risk of a pleural MM (vs. peritoneal) increases by 4% at any additional year of age at diagnosis: OR: 1.04 (1.02-1.06). Among non-occupationally exposed the M:F was below unity. The Male:Female ratio (M:F) among “unexposed cases” approximated unity. The Male:Female ratio was strongly increased among occupationally “exposed” (7.68), the highest value observed for pleural MM severely exposed (12.86). Among subjects without an easily detectable asbestos exposure, the Male:Female ratio among “unexposed cases” was assigned to each investigated case by circumstance (occupational, familial, environmental) and probability (definite, probable, possible, unlikely, unknown). Severity of asbestos exposure is assumed to decrease from definite occupational exposure through leisure-time and unknown or unlikely. It is reasonable to assume that asbestos exposure influences the occurrence and distribution by site of MM and other characteristics.

Conclusion: With the incidence yet to peak, allocation of the Trusts’ remaining funds remains critical. More advanced modelling techniques will be applied to confirm these predictions. Updating the cohort vital status, using data from Statistics South Africa, will improve the predictive power of the models.

Disclosure: No significant relationships.
Despite the universal consensus on the medical entity of mesothelioma, many countries, mostly of economically developing status, do not recognize or report the disease. This could be due to a variety of reasons: (a) historical use and exposure to asbestos may have been nil or negligible; (b) historical exposure may have occurred but started only recently, so the latency time has not yet reached saturation. In other words, the disease is only waiting to surface; (c) the clinical, pathological and social resources required for diagnosis are not yet available. In other words, the disease is there, but detection is compromised by the lack of technology and resources. A less likely but another possible reason is that (d) the disease is diagnosed but not officially reported (intentionally or otherwise). In Asia (its definition differs even between the United Nations Statistics Division and the World Health Organization, but, here, in the general sense), most countries are of economically developing status. Most, if not all, of Asian developing countries do not officially diagnose/report mesothelioma. According to the WHO Mortality Database, since the disease entity of mesothelioma appeared therein, about 12,000+ mesothelioma deaths* have been reported cumulatively in Asian countries. The vast majority of this is reported by only a few developed countries, i.e., Japan, Korea and Singapore. In contrast, Asian developing countries report almost no mesothelioma, for at least one of the aforementioned reasons. I further speculate the breakdown of reasons to be roughly, (a) minimal, (b) substantial, (c) substantial, and (d) unknown. It should also be noted that the country status of (b) and (c) is often intertwined. Thus if we account for the historical situation on asbestos use in Asian developing countries, we can expect that the latency time for mesothelioma is approaching saturation in many of these countries. And even when the latency time is reached, the medical and social infrastructure to diagnose, treat and compensate mesothelioma in developing countries is grossly inadequate. Consequently, Asia is in urgent need of attention and action for prevention of mesothelioma, at all respective levels of primary (prevention of exposure), secondary (detection of disease) and tertiary (treatment and compensation). In 2007, the 60th World Health Assembly endorsed a global plan of action (GPA) on workers’ health 2008–2017, and made reference to a global campaign for the elimination of asbestos-related diseases (ARDs), including mesothelioma. Priority 1.3 of GPA 2009–2012 was to “Develop and disseminate evidence-based tools and raise awareness for the elimination of ARDs”, which later developed into Priority 1.2 of GPA 2012–2017, under the “Regional and national programs on occupational cancer, silica and ARDs”. The Asian initiative to eliminate ARDs (the Asian Asbestos Initiative, or AAI), which was embarked upon by the speaker and colleagues in 2008, achieved widespread recognition and became a formal component of the GPA. The fourth international seminar (AAI-4) was successfully organized by Korean colleagues in 2011, and AAI-5 is planned as a joint Korea-Japan endeavor in 2012. The AAI aspires to provide a model for the world that will pave the way for the ultimate elimination of ARDs, including mesothelioma. *This is equivalent to only 13% of the cumulative number of mesothelioma deaths reported in the world during the same period. By contrast, the proportion of global asbestos use attributed to Asia has been steadily increasing over the years from 14% (1920–1970) to 33% (1971–2000) to 64% (2001–2007). This increase has been reflected in the absolute level of per capita use across a wide range of countries.

Disclosure: No significant relationships.
GSIV.1: NEW DIRECTIONS AND FUTURE STUDIES IN PERITONEAL MESOTHELIOMA

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Background: Peritoneal mesothelioma has been regarded in the past as a lethal disease with approximately a one year median survival. New multimodality treatments utilizing cytoreductive surgery and perioperative chemotherapy are being investigated.

Methods: Radiologic studies, histopathologic studies, improvements in surgical technique, and refinements in perioperative and long-term bidirectional chemotherapy have occurred.

Results: Preoperative biopsy can be used to select patients for aggressive surgical management versus palliative systemic chemotherapy. Also, radiologic studies using an interpretative classification of the abdominal and pelvic CT allows the surgeon to select patients who have a high likelihood of complete cytoreduction. At surgery, peritonectomy procedures are essential for complete tumor resections. An important new modality is hyperthermic intraperitoneal chemotherapy. In addition, long-term bidirectional chemotherapy using an intraperitoneal port has become an essential part of management. The regimens for bidirectional intraoperative hyperthermic chemotherapy, early postoperative intraperitoneal chemotherapy, and adjuvant bidirectional chemotherapy will be presented.

Conclusions: Information from randomized trials is unlikely to benefit in this rare disease. Using pharmacologic studies and clinical refinements, great progress has been made. Uniform staging and comprehensive reports from all centers of excellence for peritoneal mesothelioma are now indicated.

Disclosure: No significant relationships.
BACKGROUND: Malignant mesothelioma is a highly aggressive tumor with poor prognosis. Current treatment is rarely curative, thus novel meaningful therapies are urgently needed. Inhibition of Hedgehog signaling at the cell membrane level in several cancers has shown anti-cancer activity in recent clinical studies. Evidence of non-canonical Gli activation suggests Gli as a more potent therapeutic target.

METHODS: RT-PCR and immunohistochemistry were performed to analyze Gli1/2 expression in 42 mesothelioma tissue samples. Cultured mesothelioma cell lines were employed to investigate roles of Gli activation in mesothelioma. A novel small molecule Gli inhibitor (Gli-I) that we recently developed and siRNA were applied to inhibit Gli. MTS assay was conducted to examine cell proliferation after different treatments. MS1 xenograft model was used in in vivo study of the Gli-I.

RESULTS: 79% mesothelioma specimens had higher Gli1 or Gli2 expression than that in adjacent normal tissues by RT-PCR, and 72% were Gli1 or Gli2 positive with nuclear localization by immunohistochemistry. Inhibition of Gli by shRNAs or Gli-I suppressed cell growth and downregulated Gli downstream targets in vitro. Efficacy of Gli-I (IC50: 0.75~13 uM) in inhibiting the proliferation of Gli by shRNAs or Gli-I suppressed cell growth and downregulated Gli downstream targets in vitro. MTS assay was conducted to examine cell proliferation after different treatments. MS1 xenograft model was used in in vivo study of the Gli-I.

CONCLUSION: In this study, we identified the first novel mutations in PTCH1, SUFU and Suppressor of fused (SUFU) mutations in 3 of 6 MM cell lines examined. We identified a novel non-synonymous SUFU mutation in exon 10. LO68 contained a novel missense mutation in SUFU (p.T411M), which resulted from a nucleotide 1232 C>T transition. Deletion of six exons in the PTCH1 gene was found in JU77, which resulted in loss of one of two extracellular loops implicated in HH ligand binding and the intracellular C-terminal domain. We also detected a novel 3-bp insertion (69_70insCTG) in exon 1 of SMO, predicting an additional leucine residue in the signal peptide segment of SMO protein. None of the cell lines had mutations in SHH, DHH, IHH, PTCH1, PTCH2, HHIP, KIF7, SUFU, GLI2 and GLI3, in silico characterization of the SUFU mutant by SIFT program suggested that the p.T411M mutation might alter protein function.

DISCLOSURE: No significant relationships.
and soft agar colony formation assays were performed using standard techniques. For animal experiments, 7-week-old female nude mice of KSN strain were used.

Results: We demonstrated that the connective tissue growth factor (CTGF) gene is also an important target gene of YAP in mesothelioma cells, and its upregulation is induced by cooperation with the activation of transforming growth factor (TGF)-beta signaling. Enhanced CTGF expression caused abundant extracellular matrix formation in vivo, and nude mice which were transplanted with a CTGF-knocked down MM cell line showed prolonged survival compared to its parental cell line. Meanwhile, since four of 20 MM cell lines had neither mutation of NF2, LATS2, or SAV1, we examined whether other molecules involved in the Hippo signaling cascade are altered in the cell lines. Among the components recently identified to regulate the Hippo signaling cascade, we found that Ajuba, a LIM domain-containing protein, was downregulated in six cell lines, with 4 cell lines showing no alteration of the other components of the Hippo pathway. We transduced exogenous Ajuba into MM cell lines and detected the suppression of cell proliferation. Since one of the multiple functions of Ajuba is thought to be adherence junction stabilization, our results suggested that the cell-cell adhesion status might influence MM cell proliferation via Ajuba-Hippo signaling cascade.

Conclusion: CTGF is an important modulator of MM growth and pathology, whose expression is enhanced by TGF-beta signaling activation and Hippo signaling inactivation. Our results indicate that the dysregulation of Merlin-Hippo signaling in MM cells may also be affected not only by the inactivation of core components of this cascade but also by its other effectors.

Disclosure: No significant relationships.

SESSION IVA  GENE REGULATION AND MESOTHELIOMA PATHOGENESIS 3
SEPTEMBER 13, 2012 14:20-16:00

IVA.5: FREQUENT LOSS OF INPP4B AND NF2/MERLIN TUMOR SUPPRESSORS IN MALIGNANT PLEURAL MESOTHELIOMA AS NOVEL CANDIDATE MECHANISMS OF PI3K ACTIVATION AND GDC-0980 SENSITIVITY.

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Background: Prominent clinical activity of the PI3K/mTOR inhibitor GDC-0980 has been observed in patients with malignant mesothelioma (iMig 2012.org Abstract: 388). We investigated candidate mechanisms of PI3K inhibitor sensitivity in a panel of mesothelioma cell lines and tumor tissues.

Methods: We screened an initial panel of thirteen epithelial, pleural mesothelioma tissue samples and eight epithelial mesothelioma cell lines for PI3CA/PI3KR1 mutations (Sanger Sequencing), copy number alterations in 147 genes commonly altered in cancer (Nanostring nCounter). We subsequently validated protein expression by immunoblotting (including PTEN, INPP4B, NF2/Merlin, LKB1, NHERF1). In a larger second cohort of 20 mesothelioma cell lines, sensitivity to GDC-0980, and the presence of INPP4B, NF2, PI3CA hotspot mutations, and other PI3K pathway related proteins were evaluated. We determined apoptosis by Annexin V/PI staining in a subset of cell lines. Cell line morphology in response to GDC-0980 was examined by light microscopy.

Results: No canonical mutations in the PI3CA/PI3KR1 genes were identified in any tumor sample or cell line. Several SNPs of unknown significance were identified. Copy number analysis did not show loss of either PTEN or NF2 however, copy number loss of tumor suppressor INPP4B was seen in 6/13 (46%) tumor samples. Furthermore, this analysis showed that copy number gain in commonly amplified oncopgenes is very uncommon in mesothelioma. Protein expression of NF2 and INPP4B was lost in 70% and 50% of cell lines respectively and it was more frequent in the tissue samples. In addition rare loss of NHERF1 and LKB1 was observed. PTEN was universally present at the protein level mirroring findings at the DNA level. GDC-0980 was very potent across all 20 mesothelioma cell lines analyzed with an average IC50 of ~0.01uM. GDC-0980 induced apoptosis in ~5-18% cells in a selection of four mesothelioma cell lines. Furthermore, GDC-0980 reversed the invasive morphology of the mesothelioma cell lines suggesting possible effects of PI3K inhibition on cell motility and invasion. Additional PI3K pathway inhibitors with different target specificities were investigated that showed marked activity, albeit with potency not as broadly consistent as GDC-0980.

Conclusion: Epithelial pleural mesotheliomas are characterized by frequent loss of the INPP4B and NF2 tumor suppressors that have been linked with PI3K pathway activation. Contrary to prior reports PTEN is not typically lost in epithelial pleural mesotheliomas neither at the DNA nor protein level. GDC-0980 has very potent in-vitro activity in all mesothelioma cell lines and induces apoptosis. Since the correlation of INPP4B and NF2 loss with inhibitor sensitivity is not intuitive, additional model systems or samples from patients treated with GDC-0980 need to be evaluated.

Disclosure: No significant relationships.

IVA.6: FIBROBLAST GROWTH FACTOR SIGNALING – A NEW TARGET IN MESOTHELIOMA THERAPY?

Karim Schelch1, Mir A. Hoda1, Christine Pirker1, Bahil Ghanim2, Thomas Klikovits1, Viktoria Laszlo2, Ulrike Setinek2, Thomas Filips2, Balazs Dome1, Balazs Hegedus1, Walter Klepetko1, Walter Berger1, Michael Grusch1

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Background: Malignant pleural mesothelioma (MPM) is an aggressive malignancy characterized by frequent resistance to chemo- and radiotherapy, poor outcome and limited therapeutic options. Fibroblast growth factors (FGF) and their receptors (FGFR) contribute to malignant growth in several tumor types including thoracic malignancies while a role in MPM remains largely undefined. Therefore, the aim of the present study was to investigate the expression and impact of FGFs and FGFRs in MPM and to evaluate their potential suitability as new therapeutic targets.

Methods: Expression of all known FGF and FGFR genes was assessed by qRT-PCR and confirmed by expression array analysis in MPM cell lines and normal mesothelial cells. Selected FGFs/FGFRs were also evaluated on human tissue samples by immunohistochemistry. FGFR-specific tyrosine kinase inhibitors and an adenosine construct expressing dominant-negative FGRF1 were used to block FGF signal transduction in MPM cell models. The impact of FGRF inhibition as well as stimulation with rhFGF2 on MPM cell proliferation, survival, apoptosis, cell cycle as well as migration and invasion was evaluated by MTT, clonogenic, spheroid formation, plateau, and transwell assays, video microscopy, and flow cytometry. The effect on downstream signal transduction was assessed by immunoblotting with phosphorylation site-specific antibodies. In addition, potentially additive or synergistic antineoplastic activity of FGFR inhibitors in combination with clinically applied chemotherapeutics, radiotherapy and other targeted drugs against MPM were investigated. An orthotopic mouse model has been established for the evaluation of in vivo growth of MPM cells transduced with adenoaviral constructs.

Results: Expression analysis revealed high expression of FGFR1, FGFR2 and FGFR3 in MPM cell lines as well as in tumor tissues. Stimulation with exogenous FGFR2 led to a remarkable increase in cell migration and invasion accompanied by dramatic changes in cell morphology indicating EMT. In contrast, inhibition of FGFR1 by specific small molecule kinase inhibitors

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led to significantly decreased proliferation, survival, migration, invasion and spheroid formation in the majority of cell lines tested. Interestingly, cell lines with intrinsic or acquired cisplatin resistance were more sensitive to FGFR inhibition. Adenoviral expression of dominant-negative FGFR1 further confirmed these results. FGFR1 inhibition further led to a G1 cell cycle arrest correlating with drug sensitivity. Additionally, when FGFR inhibition was combined with chemo- and radiotherapy, additive and synergistic effects were observed. Currently, an in vivo experiment with MPM cells expressing dominant-negative FGFR1 is performed. The results of these experiments will be presented at the conference.

**Conclusion:** Taken together, these data suggest that FGFR signals contribute to proliferation, survival, migration, invasion and chemo- and radiotherapy resistance of MPM cells and their inhibition should be further evaluated as a potential new treatment strategy in MPM.

**Disclosure:** No significant relationships.

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**SESSION IVA  GENE REGULATION AND MESOTHELIOMA PATHOGENESIS 3  
SEPTEMBER 13, 2012 14:20-16:00**

**IVA.7: CRITICAL PATHWAYS IN MALIGNANT MESOTHELIOMA**

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Tumor growth is controlled by numerous pathways, which are regulated by the activity of both intrinsic effectors and extrinsic factors. Present researches aim to identify the deregulated signaling pathways in malignant cells, with the goal to select molecules or mechanisms that could kill tumor cells or abolish tumor growth. In malignant mesothelioma (MM), the identification of gene mutations, epigenetic alterations and study of gene expression profiles has defined abnormalities and changes in cell homeostasis. Emphasis has been put on the deregulation of several pathways that can account for mesothelial cell neoplastic transformation. The most exciting alteration concerns the Hippo pathway, as about fifty percent of MM show a mutation of the NF2 gene. A number of studies have emphasized the role of receptor tyrosine kinase driven signaling, involving most of the growth factor receptors (VEGFR, IGFR, EGFR, cMET). The activation of MAPK (mitogen-activated protein kinase), phosphatidylinositol-3-kinase (PI3K-AKT), and the Wnt signaling pathway has been also reported. From our knowledge, these pathways are interconnected, and it is a challenge to choose the most critical pathways to target for a therapeutical efficiency. An approach can be accomplished by using one or more agents inhibiting a specific pathway. This strategy may also attenuate other signaling pathways. It is also possible to use agents to inhibit two or more signaling pathways. Several studies are in progress to determine the potency of these approaches. In a genetic and transcriptomic study of sixty MM cell lines, we identified several groups of MM showing distinct expression of genes involved in the epithelial-mesenchymal transition (D. Jean et al this meeting). Sub-groups also differ by the expression in effectors belonging to metabolic or growth factor pathways, showing the heterogeneity of MM. MM cells show alteration of several regulatory pathways regulating cell proliferation, apoptosis and survival, and adhesion and migration. To select the most appropriate anti-tumor agents, it is therefore important to determine the relationships between these pathways. Both the number of effectors altered and the extent of the connections between different pathways may be of importance. In any case, it is needed to identify biomarkers in tumor cells, and in microenvironment, indicative of the functional status of MM cells and predictive of the response to anti-tumor agents. These agents should be as specific as possible of the different subgroups of MM.

**Disclosure:** No significant relationships.
SESSION IVB: NOVEL THERAPEUTICS: CLINICAL TRIALS

SESSION IVB

SEPTEmBER 13, 2012 14:20-16:00

IVB.1: A PHASE II STUDY WITH THE ANTI-CTLA-4 MAB TREMELIMUMAB IN CHEMOTHERAPY-RESISTANT ADVANCED MALIGNANT MESOTHELIOMA

Luana Calabro1, Aldo Morra1, Ramy Ibrahim2, Alessandra Di Pietro2, Diana Giannarelli2, Luciano Mutti2, Michele Maio1
1Medical Oncology And Immunotherapy - University Hospital Of Siena, Siena/ITALY, 2Dept Of Radiology, Euganea Medica Diagnostic Center, Padua, Italy

Background: Anti-CTLA-4 monoclonal antibodies (mAb) are showing significant activity in different tumor types; however, no data are available in MM patients (pts). We report results of a phase II, single institution, study investigating safety, clinical and immunologic efficacy of the fully-human anti-CTLA-4 mAb tremelimumab as second-line treatment for advanced MM pts.

Methods: Second-line advanced mesothelioma pts, who relapsed after a prior platinum-based regimen, were enrolled in the study and received tremelimumab at 15 mg/kg i.v. on day (d) 1 and 90 for 4 cycles or until progressive disease (PD), or unacceptable toxicity. Primary endpoint was objective response (OR); secondary endpoints were safety, disease control rate (DCR), overall survival (OS), and immunologic activity. Tumor assessment per modified RECIST Criteria was performed at screening and at d 80 of each cycle. Adverse events (AE) were collected according to the Common Terminology Criteria v3.0. Peripheral blood mononuclear cells were collected at baseline, d 14, 30, 60, and 90 of each cycle and were analyzed by flow cytometry for an extensive panel of immune phenotypic and T-cell activation markers.

Results and Conclusions: From May 2009 to January 2012, 29 advanced MM pts were enrolled and received at least 1 dose of tremelimumab (median 2; range 1-7). Preliminary results show that Tremelimumab is active in MM pts, and can induce durable stabilization of disease in a significant proportion of pts, warranting further investigation. The AEs observed in this study are consistent with Tremelimumab safety profile in many cancers, including MPM. GDC-0980 is a potent, selective, oral inhibitor of class I PI3K and mTOR kinase with in vitro IC50 of 4.8 nM for p110a/p110b and apparent Ki of 17.3 nM for human mTOR.

Methods: A phase I dose-escalation study using a 3+3 design was initiated at centers in the United States and United Kingdom in order to determine the safety and tolerability of GDC-0980 in patients with solid tumors. Preliminary efficacy was assessed, and pharmacokinetic, pharmacodynamic, and biomarker studies were performed. Based on tumor shrinkage observed in MPM in the dose-escalation portion of the study, an expansion cohort of approximately 20 MPM patients was initiated at 30 mg GDC-0980 daily. CT scans were centrally reviewed retrospectively by a radiologist with MPM expertise. Archival tumor tissue was evaluated for PIK3CA mutation by allelic specific PCR or Sanger sequencing; PTEN expression was assessed by immunohistochemistry.

Results: Six MPM patients were enrolled in the dose-escalation portion of the study. Anti-tumor activity was observed in 5 MPM patients at GDC-0980 doses of 8 to 50 mg, with tumor shrinkage of 5 to 36% per modified RECIST. Two patients achieved a partial response, including one with a tumor containing a PIK3CA mutation (exon 2 R88Q) treated at 8 mg. Three patients experienced drug-related adverse events that led to treatment discontinuation before completing cycle 2: 1 patient had grade 3 hyperglycemia at the 70 mg dose; 2 patients at the 40 mg dose developed grade 3 or greater pneumonitis/pneumonia (including one fatal case). Efficacy and safety data are currently available for 7 of 20 patients treated with 30 mg of GDC-0980 in the MPM expansion cohort. Reported drug-related Grade 1-2 adverse events were similar to the general study population; one Grade 3 event of rash was reported. Anti-tumor activity has been observed: maximum tumor change from baseline ranges from +17% to -21%; 2 of the 7 MPM patients remain on study for >6 months, and 3 patients remain on study with 2-3 month follow-up. PIK3CA mutations were uncommon; loss of PTEN expression was not observed.

Conclusion: GDC-0980 at 30 mg daily is generally well-tolerated in MPM patients. Anti-tumor activity, evidenced by tumor regression and prolonged disease control, has been observed. PIK3CA mutations were uncommon; PTEN null tumors were not observed. Updated data on clinical outcomes and biomarker correlates in the remaining patients in the expansion cohort will be presented.

Disclosure: No significant relationships.

SESSION IVB

SEPTEmBER 13, 2012 14:20-16:00

IVB.2: EVALUATION OF TOLERABILITY AND ANTI-TUMOR ACTIVITY OF GDC-0980, AN ORAL PI3K/MTOR INHIBITOR, ADMINISTERED DAILY IN PATIENTS WITH ADVANCED MALIGNANT PLEURAL MESOTHELIOMA (MPM)

Hedy L. Kindler1, Saorise Dolly1, Johanna Bendelli1, Lee M. Krug2, Lawrence Schwartz2, Michael Rabin2, Nina Tunariu1, Tanguy Y. Seiwert1, Marjorie G. Zauderer3, Ann M. Young3, Jennifer Shouldis1, J P. Marcoux4, David Kwiatkowski5, Jennifer O. Lauchle6, Howard Burris2, Andrew Wagner2, Johan De Bono4
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Background: The PI3K-AKT-mTOR signaling pathway is dysregulated in many cancers, including MPM. GDC-0980 is a potent, selective, oral inhibitor of class I PI3K and mTOR kinase with in vitro IC50 of 4.8 nM for p110a/p110b and apparent Ki of 17.3 nM for human mTOR.

Methods: A phase I dose-escalation study using a 3+3 design was initiated at centers in the United States and United Kingdom in order to determine the safety and tolerability of GDC-0980 in patients with solid tumors. Preliminary efficacy was assessed, and pharmacokinetic, pharmacodynamic, and biomarker studies were performed. Based on tumor shrinkage observed in MPM in the dose-escalation portion of the study, an expansion cohort of approximately 20 MPM patients was initiated at 30 mg GDC-0980 daily. CT scans were centrally reviewed retrospectively by a radiologist with MPM expertise. Archival tumor tissue was evaluated for PIK3CA mutation by allelic specific PCR or Sanger sequencing; PTEN expression was assessed by immunohistochemistry.

Results: Six MPM patients were enrolled in the dose-escalation portion of the study. Anti-tumor activity was observed in 5 MPM patients at GDC-0980 doses of 8 to 50 mg, with tumor shrinkage of 5 to 36% per modified RECIST. Two patients achieved a partial response, including one with a tumor containing a PIK3CA mutation (exon 2 R88Q) treated at 8 mg. Three patients experienced drug-related adverse events that led to treatment discontinuation before completing cycle 2: 1 patient had grade 3 hyperglycemia at the 70 mg dose; 2 patients at the 40 mg dose developed grade 3 or greater pneumonitis/pneumonia (including one fatal case). Efficacy and safety data are currently available for 7 of 20 patients treated with 30 mg of GDC-0980 in the MPM expansion cohort. Reported drug-related Grade 1-2 adverse events were similar to the general study population; one Grade 3 event of rash was reported. Anti-tumor activity has been observed: maximum tumor change from baseline ranges from +17% to -21%; 2 of the 7 MPM patients remain on study for >6 months, and 3 patients remain on study with 2-3 month follow-up. PIK3CA mutations were uncommon; loss of PTEN expression was not observed.

Conclusion: GDC-0980 at 30 mg daily is generally well-tolerated in MPM patients. Anti-tumor activity, evidenced by tumor regression and prolonged disease control, has been observed. PIK3CA mutations were uncommon; PTEN null tumors were not observed. Updated data on clinical outcomes and biomarker correlates in the remaining patients in the expansion cohort will be presented.


No significant relationships.
SESSION IVb  NOVEL THERAPEUTICS: CLINICAL TRIALS  SEPTEMBER 13, 2012 14:20-16:00

IVB.3: PHASE II TRIAL OF ANTI-TRANSFORMING GROWTH FACTOR-BETA (TGFßs) MONOClonAL ANTIBODY GC1008 IN RELAPSED MALIGNANT PLEURAL MESOTHELIOMA (MPM)

James Stevenson1, Heddy Kindler1, Daniel Schwed2, Anjana Ranganathan3, Mona Jacobs-Small4, Jennifer Shouldis5, Steven M. Albelda3

Taussig Cancer Institute, Cleveland Clinic Foundation, Cleveland/OH/UNITED STATES OF AMERICA, 1University Of Chicago, /UNITED STATES OF AMERICA, 2Medicine, University Of Pennsylvania/UNITED STATES OF AMERICA

Disclosure: 1TGFß serves as a “paracrine” factor in promoting growth and progression of MPM, we are conducting a Phase II trial of GC1008 in patients with progressive MPM.

Methods: 1Pts with progressive MPM by modified RECIST criteria and PS 0-1 with 1-2 prior systemic therapies (at least 1 pemetrexed-based) are eligible. Treatment plan: GC1008 3mg/kg IV over 90 minutes every 21 days. Responses are assessed by modified RECIST every 6 weeks. The primary endpoint is progression-free survival (PFS) rate at 3 months; secondary objectives include safety with GC1008, response rate by modified RECIST, time to progression (TTP), and overall survival (OS).

Results: The modified Gehan stage 1 stopping criterion of 1/13 pts with 3 month PFS has been exceeded. To date, 13 pts (10 PS 0; 3 PS 1) with MPM (median age 69; 2F, 11M; 11 epithelial, 1 sarcomatoid, 1 biphasic) enrolled. Treatment-related toxicities include Cl2 fatigue (3 pts), nausea (1 pt) and xerosis (1 pt). Other adverse events possibly related to GC1008 were rapid weight loss, grade 1/2 fatigue (3 pts), nausea (1 pt) and xerosis (1 pt). Cl2 progression of disease in 1 pt after 2 cycles, and Cl2 skin keratocanthoma in 1 pt after 5 cycles. Three pts met the primary objective of 3 month PFS at 4.1, 4.2 and 9 months each. Stable disease (SD) was seen in 3 pts (23%). Median TTP is 1.4 months (95% CI 1.2-∞); median OS is 13 months (95% CI 6-∞). Significant serum mesothelin levels have closely tracked disease progression. Serum from 6/13 pts showed new antibodies against MPM tumor lysates as measured by immunoblotting. Two of 3 pts with SD had anti-tumor antibody responses. Mean baseline plasma level of TGFß was 2447 pg/ml but did not correlate with baseline plasma GC1008 or TTP.

Conclusions: GC1008 was well tolerated in pretreated MPM patients. SD occurred in 3 pts, all with prior disease progression. Evidence for humoral anti-tumor immunity was seen in nearly half of enrollees and in 2 of 3 pts with SD. OS compares favorably to prior single-agent studies in pretreated MPM.

Disclosure: No significant relationships.

SESSION IVb  NOVEL THERAPEUTICS: CLINICAL TRIALS  SEPTEMBER 13, 2012 14:20-16:00

IVB.4: PHASE II TRIAL OF BNC105P AS 2ND LINE CHEMOTHERAPY FOR ADVANCED MALIGNANT PLEURAL MESOTHELIOMA (MPM).

(Australasian Lung Cancer Trials Group and NHMRC Clinical Trials Centre Collaboration)

Anna K. Nowak1, Chris Brown2, Michael J. Millward3, Brett Hughes4, David Bibby5, Gabriel Kremmidiotis6, Elizabeth Doolin4, Paul Mitchell5, Sydney/NSW/AUSTRALIA, 6Medical Oncology, The Prince Charles Hospital, Brisbane/QLD/AUSTRALIA, 7Medical Oncology, Royal North Shore Hospital, /NSW/AUSTRALIA, 8Adelaide Cancer Centre, Adelaide/SA/AUSTRALIA

Disclosure: 1Bibby, Doolin, Kremmidiotis, Lavranos: Employment by Bionomics Pty Ltd, developers of BNC105P. Note that these authors have had the opportunity to review results and contribute to the abstract, however did not have the ability to veto any abstract content

Background: BNC105P is a tubulin polymerization inhibitor that acts as a Vascular Disruption Agent (VDA), has direct cytotoxic effects, and has preclinical and phase I activity in malignant pleural mesothelioma (MPM). This aim of this study was to determine activity, safety, and potential biomarkers of BNC105P as second line therapy after pemetrexed and a platin in MPM.

Methods: Eligible patients had progressive MPM, prior pemetrexed and platinum, measurable disease by modified RECIST, ECOG PS 0-1, and adequate organ and cardiovascular function. Important exclusions included recent thromboembolic, cardiovascular or cerebrovascular disease, or therapeutic anticoagulation. Patients received BNC105P (16 mg/m2 I.V) Day 1 + 4 q21d until progression or prohibitive toxicity. The primary endpoint was centrally reviewed objective tumour response rate (RR = CR + PR); the Simon 2-stage design assumed a RR of interest of 20% and a RR of no interest of 5%, with α = β = 0.05. Continuation past first stage accrual required >1 objective response in 24 patients. Serum mesothelin was measured on day 1 of each cycle. A panel of 62 potential biomarkers of response were measured at baseline, 3 hours after the first dose of study drug, and on day 8 cycle 1.

Results: 30 subjects were accrued over 10 months (90% male; median age 65 (range 41-83); 77% ECOG PS 1; histology epitheloid (67%); biphasic (10%); sarcomatoid (7%); other/unspecified (17%). All patients had progressive MPM by modified RECIST criteria and PS 0-1 with 1-2 prior systemic therapies (at least 1 pemetrexed-based). Stable disease (SD) was seen in 3 pts (23%). There were 2 deaths on study: 1 due to stroke, the other due to pneumonia and respiratory failure. We observed 1 partial response (PR) (3%) and 13 pts with stable disease as their best response (43%). Median progression free survival was 1.5 months (95% CI 1.4-2.4); median overall survival was 8.7 months (95% CI 3.8-NR). One patient, who achieved a PR, had a 25% decrease in mesothelin levels after the first treatment cycle.

Conclusion: BNC105P was safe and tolerable but its single agent response rate failed to meet our pre-specified criterion. Mesothelin response was concordant with radiological response. Post-dose biomarker alterations demonstrated a pharmacodynamic effect consistent with endothelial stress but insufficient objective radiological responses were seen to correlate this with outcomes.

Disclosure: Bibby, Doolin, Kremmidiotis, Lavranos: Employment by Bionomics Pty Ltd, developers of BNC105P. Note that these authors have had the opportunity to review results and contribute to the abstract, however did not have the ability to veto any abstract content
Background: Preclinically arginine deprivation has shown activity as a novel antimetabolic strategy for MPM lacking the rate-limiting enzyme for arginine biosynthesis argininosuccinate synthetase (ASS1). We have initiated a multicenter randomized phase II Cancer Research UK-funded clinical trial to assess the safety and efficacy of the arginine-lowering agent ADI-PEG20 (Polaris Group, San Diego, US) and best supportive care (BSC) versus BSC alone in patients with ASS1-deficient MPM.

Methods: Eligibility criteria for the sixty-six patients include: non-resectable, ASS1-deficient MPM by immunohistochemistry; good performance status (0 or 1); measurable disease by modified RECIST (Response Evaluation Criteria in Solid Tumors); and able to give written consent. Stratification factors include: gender, histology (sarcomatoid versus non-sarcomatoid), prior treatment (chemonaive or previous platinum combination therapy), and recruitment center. The primary endpoint is progression free survival (PFS) as assessed by modified RECIST. Secondary endpoints are tumor response rate, overall survival and toxicity. Tertiary endpoints include measurement of plasma arginine, citrulline and ADI-PEG20 antibody levels, the methylation status of the ASS1 gene in primary tumoral samples, and early metabolic response to ADI-PEG20 using [18F]Fluorodeoxyglucose Positron Emission Tomography (FDG-PET). Sample size was calculated to detect a hazard ratio of 0.60 (80% power, 15% one-sided significance level).

Results: To date up to 50% of patients have been screened ASS1-deficient, and over half of patients have been randomized onto the trial which is projected to complete accrual by the end of 2012. ADI-PEG20 toxicity in patients with MPM has been consistent with previous trials of ADI-PEG20 in melanoma and liver cancer, commonly skin injection site reactions (grade 1-2), infrequent episodes of neutropenia (range: grade 1-4) and anaphylactoid reactions (2 patients with grade 3 episodes). Preliminary metabolic response data indicate that FDG-PET imaging may be a useful tool to assess early response to ADI-PEG20 in patients with loss of tumoral expression of ASS1. The trial has passed the first interim safety analysis and an update will be provided on current recruitment, safety and response as assessed by ASS1 promoter methylation status and FDG-PET.

Conclusion: In summary, ADI-PEG20 is well tolerated and shows evidence of metabolic response in patients selected for ASS1-deficient MPM. Arginine deprivation may have a role in the future management of MPM either alone or in combination with rationally selected therapies.

Disclosure: No significant relationships.
immune response to measles virus as well as tumor characteristics that might account for antitumor activity. One of the major limitations to virotherapy is the generation of neutralizing antibodies. Since >80% of the population is vaccinated against MV, this could potentially be a limitation of MV therapy for mesothelioma. Using ex vivo, infected cell carriers can overcome this limitation by hiding the virus within intact cells allowing for trafficking of virus toward the tumor and oncolytic activity. Blood outgrowth endothelial cells (BOEC) are easily obtained from a blood sample, are readily cultured, and have tumor homing characteristics. Therefore, cell carrier mediated measles virotherapy is tested as a potential novel mechanism for delivery of MV to mesothelioma.

**Methods:** For the phase I clinical trial, all patients with malignant mesothelioma able to have a pleural catheter placed are eligible regardless of line of therapy. Patients having prior pleurodesis or a current indwelling pleural catheter for greater than 6 weeks are excluded. MV-expressing sodium-iodide symporter (MV-NIS) will injected into the pleural space in a dose-escalation schema. Radioactive iodine uptake will be a measure of viral replication as well as viral titers from blood and pleural fluid. Correlative studies to assess immune response and expression of potential biomarkers will be performed. BOECs were cultured from human donor blood, cultured, and characterized using immunofluorescence for endothelial markers. 150,000 BOECs were infected, ex vivo with MV expressing GFP or CEA (MV-GFP or MV-CEA) and monitored for cell survival at various times. MV-infected BOECs were also co-cultured with human mesothelioma cell lines in the presence or absence of MV immune serum. Viability of mesothelioma cells after 72 hours of co-culture were determined by trypan blue exclusion. Animal experiments are planned to test the efficacy of the cell carrier approach using a peritoneal model of xenografted human mesothelioma. Bioluminescence of xenografted mesothelioma cells expressing firefly luciferase will be used to gauge antitumor response. Results will be compared to administration of naked virus and untreated controls.

**Results:** For the phase I study, one patient has been enrolled in the study. At this time, the patient has completed one treatment and it is too early to assess treatment response or to complete correlative studies. BOECs are readily infected by MV and most cells survive infection up to 72 hours with an MOI of 1. Cytopathic effect can be visualized by microscopy by 48 hours. Co-culture of BOECs with mesothelioma cells results in complete oncolysis of mesothelioma cells.

**Conclusion:** Phase I study of MV therapy is ongoing. Too few patients have been treated to draw conclusions. Further investigation of BOECs as a delivery method for MV to mesothelioma is warranted.

**Disclosure:** No significant relationships.
IVC.2: PROGNOSTIC SIGNIFICANCE OF HISTOLOGICAL SUBTYPES IN PLEURAL MALIGNANT MESOTHELIOMA: A CLINICOPATHOLOGICAL REVIEW AND A PROPOSAL FOR UPDATING HISTOLOGICAL MESOTHELIOMA CLASSIFICATION.

Francoise Galateau-Salle, Nolwenn Le Stang, Maria Paciencia, Marie Karanian, Alan Borsczuck, Kouki Inai, Allen Gibbs, Junya Fukuoka, Kenzo Hiroshima, Marleen Praet, Jean Michel Vignaud

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Background: Age, epithelioid type, T and N staging are conventional prognostic factors in diffuse pleural malignant mesothelioma (DPMM) and sarcomatoid DPMM is a prognostic factor and allow a better stratification of patients for survival. Our aim was to assess whether overall survival of patients depends on histological subtyping.

Methods: We undertook a retrospective study and reviewed H&E slides of mesothelioma cases from the MESOPATH domestic referral center files during 1995-2010. They were thoracoscopy biopsy in 40% (n=206) and surgical specimens in 60% (n=323). We classified the tumors according to the WHO 2004 classification and we subclassified the epithelioid type in papillary, trabecular, acinar, solid, micropapillary. Additionally we evaluate the lymphohistiocytoid, the pleomorphic and an additional epithelioid variant with rich myxoid stroma. Log rank test and Cox models were used to relate the survival of type and subtype variables, clinical features, asbestos exposure and survival.

Results: From a population study of 526 patients, there were 381 male (72%) and 145 female (28%) with an average age of 70 years old [28;96]. Asbestos exposure was observed in 71% of cases. The histological types and subtypes was papillary 5% (n=25), solid 16% (n=83), trabecular 21% (n=109), acinar 6% (n=30), micropapillary 7% (n=39), epithelioid type with rich myxoid stroma 6% (n=31), pleomorphic variant 12% (n=65), lymphohistiocytoid variant 7% (n=39) sarcomatoid 10% (n=53) and desmoplastic type 10% (n=52). We excluded from the study the biphasic type. Using age-adjusted Cox model, patients with desmoplastic type had the worst prognosis with a 5 months median survival and a 2 years survival at 4% (p<0.0001) followed by the sarcomatoid and pleomorphic variants with a 5 months median survival and respectively 2 years survival at 4% (p<0.0001) and 10% (p<0.0001). Lymphohistiocytoid type showed a 8 months median survival with 15% at 2 years survival (p=0.0003). Among the epithelioid type micropapillary subtype presented the worst prognosis with 13 months median survival and 26% at 2 years survival (p<0.07) followed by solid type 14 months. There was no significant statistical difference of median survival for the trabecular and acinar types with respectively a median survival of 16 and 18 months and 32% and 38% at 2 years survival. The epithelioid type with rich myxoid stroma showed a 19 months median survival with 37% at 2 years survival (p<0.07) and finally papillary subtype showed the best prognosis with 21 months median survival and 44% at 2 years survival. Noticeably, DMM was more present in male p=0.006, older, while rich myxoid type was most often observed in female (p=0.065) and younger age (p=0.0015). There were no significant difference between asbestos and non asbestos exposed patients.

Conclusion: Our study support classification by histological type and subtype for a better stratification of patient survival. Molecular classification in the future will evaluate the strength of such indicators.

Disclosure: No significant relationships.
sarcomatoid mesotheliomas (22/22). Mesothelioma without homogenous
deletion showed tubulo-papillary pattern. Methylation of p16 was observed
in 7 of 34 informative cases (21%). Mesotheliomas with homogenous
deletion tend to be without methylation; however, two mesotheliomas
with homogenous deletion have been methylated. Mesotheliomas without
deletion also tend to be without methylation. Furthermore, methylation
of p16 gene was observed in 43% of fibrous pleuritis (3/7). The presence
of p16 homogenous deletion and p16 hypermethylation correlates with a
shorter survival in patients with MPM.

Conclusion: FISH analysis demonstrated homogenous deletion of the p16
in all of sarcomatoid mesothelioma cases (100%). Methylation of p16 was
observed in 21% of MPMs. FISH analysis can identify both homogenous
and hemizygous deletions. Our cut-off value for the diagnosis of mesothelioma
is lower than previously reported. However, each institute should establish
its own cut-off value. Different component in the same tumor can be
simultaneously analyzed with p16 FISH. p16 FISH analysis can be a reliable
test for the distinction between sarcomatoid mesothelioma and fibrous
pleuritis and for the diagnosis of sarcomatoid mesothelioma with unusual
histological finding and predict the prognosis of the patients.

Disclosure: No significant relationships.

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<th>SESSION IVC</th>
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**IVC.4: A TWENTY YEAR AUDIT OF THE CYTOLGICAL DIAGNOSIS OF PLEURAL MALIGNANT MESOTHELIOMA**

Amanda Segal1, Greg F. Sterrett1, Felicity A. Frost1, Anna K. Nowak1, Arthur W. Musk1, Bruce W.S. Robinson1, Jenette Creaney1

1Pathwest, Nedlands/AUSTRALIA, 2School Of Medicine And Pharmacology, University Of Western Australia, Nedlands/AUSTRALIA, 3Respiratory Medicine, Sir Charles Gardner Hospital, Nedlands/WA/AUSTRALIA, 4School Of Medicine And Pharmacology, University Of Western Australia, Perth/WA/AUSTRALIA

Background: Cytological examination of effusions provides an opportunity
to diagnose MM. However this is controversial and is not accepted
according to the current guidelines. In order to examine the role of
cytology in this diagnostic setting we conducted an audit of all pleural
cytology specimens received in our laboratory over a 20 year period.

Methods: All pleural specimens received from 1988-2007 were identified.
Follow-up to establish final diagnosis was by review of pathology samples
and data-linkage with Cancer and Mesothelioma Registries.

Results: 9985 pleural samples were received (1740 biopsy, 8245 cytology)
from 6198 individuals during the twenty year period. Approximately 30% of
cytology samples were reported as either normal or non-diagnostic; 30%
as inflammatory or as reactive hyperplasia; and 17% as metastatic to the
pleura or adenocarcinoma. Six percent of all pleural cytology samples over
this twenty year period were reported as MPM. Of the 6198 individuals
with a pleural sample during the twenty year period, 685 were diagnosed
with MPM based upon the results of either electron microscopy performed
on the cytology specimen, or examination of a tissue biopsy specimen,
or necropsy. Of these 685 cases with pathologically confirmed MPM, 395
cases had a pleural cytology sample reviewed. From the table below, 283
of the 395 pathologically defined MPM cases (i.e. 72%) were diagnosed as
MPM by cytopathologists in effusion specimens. Of the 112 patients with a
biopsy diagnosis of MM with a cytology specimen which was not diagnostic
of MM; 49 were diagnosed as atypical or suspicious and 70 as negative
biopsy diagnosis of MM with a cytology specimen which was not diagnostic
of MPM by cytopathologists in effusion specimens. Of the 112 patients with a
pleural sample during the twenty year period, 685 were diagnosed
with MPM based upon the results of either electron microscopy performed
in 7 of 34 informative cases (21%). Mesotheliomas with homogenous
deletion showed tubulo-papillary pattern. Methylation of p16 was observed
in 7 of 34 informative cases (21%). Mesotheliomas with homogenous
deletion tend to be without methylation; however, two mesotheliomas
with homogenous deletion have been methylated. Mesotheliomas without
deletion also tend to be without methylation. Furthermore, methylation
of p16 gene was observed in 43% of fibrous pleuritis (3/7). The presence
of p16 homogenous deletion and p16 hypermethylation correlates with a
shorter survival in patients with MPM.

Conclusion: FISH analysis demonstrated homogenous deletion of the p16
in all of sarcomatoid mesothelioma cases (100%). Methylation of p16 was
observed in 21% of MPMs. FISH analysis can identify both homogenous
and hemizygous deletions. Our cut-off value for the diagnosis of mesothelioma
is lower than previously reported. However, each institute should establish
its own cut-off value. Different component in the same tumor can be
simultaneously analyzed with p16 FISH. p16 FISH analysis can be a reliable
test for the distinction between sarcomatoid mesothelioma and fibrous
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histological finding and predict the prognosis of the patients.

Disclosure: No significant relationships.

### Table: Sensitivity and Specificity of Cytological Diagnosis of MPM

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<th>Cytological diagnosis of MPM</th>
<th>Pathologically confirmed MPM</th>
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<td>-</td>
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<td>283</td>
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<td>112</td>
<td>4860</td>
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<td>TOTAL</td>
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Conclusion: The value of cytology in the diagnosis of pleural epithelioid
MM is unquestionable. Cytology provides a rapid, cheap and minimally
invasive diagnostic modality, as well as enabling early diagnosis. Effusion
cytology is rarely helpful in the diagnosis of sarcomatoid MM, and there are
some cases of epithelioid/biphasic MM which require biopsy to establish a
definitive diagnosis.

Disclosure: No significant relationships.

### Table: Sensitivity and Specificity of Cytological Diagnosis of MPM

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epithelial histology compared with 38% of patients with epithelial histology. Anemia was associated with statistically significant decreased TTR and diminished OS for both histological subtypes. Details are shown in the table below.

<table>
<thead>
<tr>
<th>CTC Anemia grade</th>
<th>Median TTR months</th>
<th>HR</th>
<th>95% CI</th>
<th>Median OS months</th>
<th>HR</th>
<th>95% CI</th>
</tr>
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<tbody>
<tr>
<td>All Patients</td>
<td>0 1 2</td>
<td>1.8</td>
<td>(1.5-3)</td>
<td>21.8 11.5 6.4</td>
<td>1.0 2.2</td>
<td>(1.6-3) (2.2-5.6)</td>
</tr>
<tr>
<td>EPP</td>
<td>0 1 2</td>
<td>18.8</td>
<td>(1.5-3.3)</td>
<td>20.2 11.8 7.3</td>
<td>1.0 1.7</td>
<td>(1.2-2.4) (1.5-4.2)</td>
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<tr>
<td>PD</td>
<td>0 1 2</td>
<td>14.7</td>
<td>(1.0-3.5)</td>
<td>42.8 11.4 6.0</td>
<td>1.0 5.0</td>
<td>(2.4-10.3) (5.1-55.6)</td>
</tr>
<tr>
<td>Epithelial Histology</td>
<td>0 1 2</td>
<td>19.5</td>
<td>(1.3-3.0)</td>
<td>33.2 13.9 7.8</td>
<td>1.0 2.4</td>
<td>(1.6-3.6) (1.7-6.3)</td>
</tr>
<tr>
<td>Non-epithelial Histology</td>
<td>0 1 2</td>
<td>15.4</td>
<td>(1.2-3.9)</td>
<td>15.2 9.1 6.0</td>
<td>1.0 1.5</td>
<td>(0.9-2.5) (1.3-5.9)</td>
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HR: Hazard Ratio; CI: Confidence Interval

**Conclusion:** Anemia is a clinical predictor for early recurrence and short overall survival independent of type of surgery or histology. Anemia can serve as a quantitative biomarker and should be included in the pre-operative evaluation of MPM.

**Disclosure:** No significant relationships.
Methods: Slides (H&E and immunohistochemical slides including 2 positive and 2 negative markers for mesothelial cells) registered at the MESOPATH domestic referral center were scanned into whole digital format on a high quality LEICA virtual slide scanning system. Digital slides were independently diagnosed by 3 French panelists. 699 cases were scanned from June 1, to the 31 of May 2011. 5595 slides were examined by the pathologists through the CCITI application and retrieved on a local server (2,69To). The results were compared to 844 cases certified according to the glass slides conventional certification. The diagnosis were performed through the CCITI software application. Reference, digital and glass-slide interpretations were compared. Operator comments on technical issues were gathered.

Results: Among the 699 cases, there was a 63% (n=341) agreement on the diagnosis of mesothelioma among digital slides compared to a 79% (n=670) results with conventional glass diagnoses. Minor disagreement were observed respectively in 30% (n=162) with digital slides compared to 18% (n=153) and major disagreement (benign versus malignant or sarcoma versus mesothelioma versus metastasis) in 7% (n=38) compared to 3% (n=27) with light microscope diagnoses. The minimal time for a complete expertise by three panelists with the digital slides system was 1h09 mn while the maximal time was 40 days compared to 30 to 100 days with the previous system using microscope analysis. The resolution was defined as fine by all panelists and the navigating ability at various magnification with the scanner and the CCITI application was well appreciated.

Conclusion: Our pilot study shows that dynamic whole-slides imaging is an excellent tool for second lecture and difficult cases such as those encountered with mesothelioma diagnosis. With further experience the accuracy of telepathology diagnosis will improve. It shows a tremendous potential for rapid teleconsultation, research and education and as pathologist we may prefer in the future digital diagnostic practice.

Disclosure: No significant relationships.

IVC.7: ROLE OF CYTOLOGY IN MESOTHELIOMA DIAGNOSIS

Anders Hjerpe
Laboratory Medicine, Karolinska Institute, Stockholm/SWEDEN

Previous recommendations state that the diagnosis of malignant mesothelioma should be based on biopsy, while cytological diagnosis of exfoliated cells from an effusion is considered to be insufficient. However, the refinement of adjuvant techniques during the last decades has changed this completely. While the routinely stained cytological specimen may be inconclusive, numerous publications demonstrate the utility of effusion cytology in combination with ancillary techniques such as immunocytochemistry, biomarker analyses, FISH and/or electron microscopy. One such technique is often sufficient for diagnostic purposes. In a laboratory with experience of these techniques, mesothelioma diagnosis – when made - is accurate and definitive, and will in those cases provide all of the information necessary for choice of therapy. Diagnosis is usually obtained from the first effusion withdrawn, which in our material is 3-6 months before a biopsy is taken. This may save these patients from a series of cumbersome examinations. When the diagnosis is definitive, additional biopsy sampling is unnecessary and may be considered unethical. I advocate that IMIG initiate a task force to define the conditions for accepting a mesothelioma diagnosis based on an effusion.

Disclosure: No significant relationships.
WORKSHOP VII  
HEATED CHEMOTHERAPY AND OTHER APPROACHES TO TARGETING MESOTHELIOMA SURFACES  
SEPTEMBER 13, 2012 17:00-18:30  

WSVII.1: CHEMOTHERAPEUTIC OPTIONS FOR PERITONEAL AND PLEURAL MESOTHELIOMA, HIPEC, EPIC, HITAC AND HITOC – PHARMACOLOGIC STUDIES  

Paul H. Sugarbaker  
Program In Peritoneal Surface Malignancy, Washington Cancer Institute, Washington, DC/UNITED STATES OF AMERICA  

Background and Rationale: In the past peritoneal mesothelioma was treated by debulking surgery and systemic chemotherapy without demonstrable benefit. Over the past decade the median survival has been improved to over seven years.  

Methods: Pharmacologic studies which establish the rationale for intraperitoneal and intravenous chemotherapy for this disease have been performed. These chemotherapy agents are now combined and appropriately administered either intravenously or intraperitoneally in an attempt to maximize responses. The chemotherapy agents are used perioperatively.  

Results: Currently, the chemotherapy is given into the peritoneal cavity with heat (HIPEC) into both peritoneal and pleural cavities simultaneously (HITAC) or used within the pleural space only (HITOC). Chemotherapy clearance from the abdomen is more rapid than from the pleural space. Pharmacologic studies of intraperitoneal doxorubicin, intraperitoneal cisplatin, systemic ifosfamide, intraperitoneal paclitaxel, intraperitoneal pemetrexed, intraperitoneal gemcitabine, and intraperitoneal mitomycin C are to be illustrated. Multi-agent chemotherapy by intraperitoneal and intravenous routes of administration with acceptable toxicity are the goals of this effort.  

Conclusions: Bidirectional chemotherapy can be used to improve the results of treatment with peritoneal mesothelioma at three different time periods. Currently, we utilize a five-drug protocol for comprehensive management. The chemotherapy treatments are used as a planned part of the combined surgical and chemotherapeutic treatment plan for these patients. Comprehensive morbidity/mortality assessments accompany all management plans.  

Disclosure: No significant relationships.
This brief presentation is an “update” of some findings and trends in the epidemiology of mesothelioma in the United States and Canada. A PubMed search for English papers published 2009-2012 containing the words mesothelioma and epidemiology produced 302 papers; about half were from the USA or Canada. One Mexican case-control study of 472 workers (117 mesothelioma cases and 353 controls) insured by the Mexican Institute of Social Security (Aguilar-Madrid et al. 2010, Am J Indui Med) had qualitative exposure assessment which indicated “overall proportion of certain, likely, and possible occupational asbestos exposure in some workplaces with exposure of asbestos” as 80.6% in cases and 31.5% in controls. Historical occupational cohort studies (e.g. chrysotile miners and millers of Quebec; textile workers in the Carolinas; the W.R. Grace vermiculite mining and processing cohorts) saw one new group (North Carolina textile) and some follow-ups. Most recent work is methodological rather than new case-finding work. New case-control studies are rare and add little to what was already known, although one (Pintos et al., J Occ Env Med 2009) suggested a possible interaction between asbestos and “man-made” mineral fibers. Arguably more important new epidemiological work was done during this period in Italy, France, and the United Kingdom. Nevertheless some new work has been accomplished and some important work will be reported within the next six months. The methodological papers include a debate about the type of data suitable for risk analysis, with two roads to the same goal – hierarchical ranking of studies with sequential exclusions versus use of all available data with adjustments for various quality elements and uncertainty (e.g. Berman DW and Case BW; Ann Occup Hyg (2012) doi: 10.1093/annhyg/mes027 First published online: July 23, 2012). In Canada, studies using administrative data from four provinces have explored the extent and nature of mismatches between registered mesothelioma cases and compensated mesothelioma cases. Studies from Quebec, Alberta, and most recently British Columbia and Ontario have all shown far lower percentages of registered cases receiving compensation than would be expected, even though in these jurisdictions almost all claims with disease are accepted. Under-reporting to the compensation boards or failure to make claims is clearly responsible but the reasons are unclear. Making reporting legally mandatory within 14 days of diagnosis (in Quebec) and increased communication between registries and compensation boards have produced some improvement. A recent study (Labrèche F et al. Can Respir J. 2012 19:103-7) demonstrates that the low compensation rate in Quebec is not due to over-registration since 62 to 77% of registered cases were confirmed probable or definite and an additional ten to 19% possible. In fact, separate study of compensated cases showed that 13 per cent had been missclassified in the tumour registry as other cancer types, although true total under- ascertainment could not be evaluated. Incidence trends will be discussed: an important observation is the now well established year-over-year decrease in male cases in the United States which has seen a significant Average Annual Percent Change of -1.4 to -1.6 per cent per year over the last ten years (2000-2009) (National Cancer Institute; SEER Cancer Statistics Review 1975-2009; last updated August 20 2012; http://seer.cancer.gov/csr/1975_2009_pops09/results_merged/sector _17 _mesothelioma.pdf). Overall, age-adjusted incidence from the SEER 9 data set for males peaked at 2.17 per 100,000 population in 1994 and 1995 and most recently was between 1.64 and 1.78 for 2006-9. This is well under half of most recently reported rates in France, Italy, and especially the UK and Australia. (It should be noted of course that while this is good news, the raw number of cases annually in the US remains higher than in any other country, and this is likely to remain true for many years even as rates continue to decline). An important and controversial issue relates to the health effects of nonasbestiform amphibole varieties (in several mineralogical categories) . Although a great deal has been written (particularly about talc, tremolite, and taconite) except for ecological observations (Case BW et al. ) Toxicol Environ Health B Crit Rev. 2011;4(1-4):3-39) this is a difficult area to study epidemiologically. An extensive Taconite Workers Health Study being performed at the University of Minnesota is one such case (http://taconiteworkers.umn.edu/index.html) , amphiboles are present but there is debate about their nature; mesothelioma SMR increase and a high rate of radiological abnormalities is reported as present by the investigators to date but incidence, analysis and other studies are not yet complete, and a previous study suggested that cases among workers had sources of (prior) exposure other than the mining dust itself. A registry of the former workers of the Baie Verte asbestos mine in Newfoundland has been developed by SafetyNet at Memorial University in St.-John’s with the cooperation of former workers. Information on 1003 voluntary registrants (denominator uncertain; some 2,400 to 3000 ever worked) has been obtained for health status, disease outcomes, and cumulative exposure using a JEM procedure with access to over 8000 historical air-samples from company, union and government sources. Results for this chrysotile mine are expected in 2012. Finally, after three decades of study by many groups, EPA is in the final stages of carrying their assessment of exposure and disease in the W.R. Grace vermiculite mining workforce at Libby, Montana and related sites (Toxicological Review of Libby Amphibole Asbestos in Support of Summary Information on the Integrated Risk Information System (IRIS) forward to use in the IRIS database. This is being done through the efforts of a Review Panel set up under the auspices of EPA’s Science Advisory Board. Drafts of the Panel Report are available on the EPA website; the most recent Deliberative Draft at this writing is at http://yosemite.epa.gov/sab/sabproduct.nsf/ea05d9a9b55cc319285256cb0052472e/eg214b49e0f895275380067c4e7/$FILE/Libby%20Asbestos%20Report%207-11-12.pdf. Considerable public comment and comments from EPA and from the panelists themselves is available via the EPA SAB websites. Disclosure: No significant relationships.
accurately characterized as antisocial. Nearly all of this colossal profligacy could have been avoided. Contrary to the prevailing view, the solution is not creation of a 9-11 or BP Oil Spill type fund. All the courts should have done (and need to do in the future for asbestos and other mass tort cases) is to pay strict attention to insurance theory and to forgo pursuit of the compensation goal entirely.

Disclosure: No significant relationships.
**VA1.1: PERITONEAL MESOTHELIOMA: INTEGRATION OF MULTIMODAL TREATMENT AND TRANSLATIONAL RESEARCH**

Marcello Deraco¹, Nadia Zaffaroni², Federica Perrone³

¹Surgery, Fondazione Ircs Istituto Nazionale Tumori, Milano/ITALY, ²Department Of Experimental Oncology, Ircs Istituto Nazionale Tumori, I/ITALY, ³Pathology, Ircs Istituto Nazionale Tumori Milano/ITALY

Recent clinical and basic science research efforts have changed the perception of diffuse malignant peritoneal mesothelioma (DMPM). This clinical entity presents epidemiologic, biological, and clinical behaviors that are different from its more frequent pleural counterpart. A multimodality treatment consisting of cytoreductive surgery (CRS) and hyperthermic intraperitoneal chemotherapy (HIPEC) has emerged as the most effective approach for the treatment of DMPM. In addition, new therapeutic targets and prognostic biological factors have been identified. The incidence of DMPM corresponds to approximately one fourth of pleural mesothelioma and has been rising worldwide since 1970. The annual mortality rate is expected to increase 10% worldwide until 2020. Age-standardized incidence rates among men range from 0.5 to 3 cases/1,000,000.

**Systemic Therapies**

DMPM is known to be chemoresistant and prolonged survival resulting from systemic chemotherapy has not been reported yet. Numerous single drug and combination regimens have been tested over the past decades with modest results. A systematic review (2002) including more than 2,300 patients (pleural and peritoneal combined) in 83 trials. Regarding antitumoral response rate, cisplatin was suggested to be the most active single agent, and the combination of cisplatin+doxorubicin, the most active regimen. Data from a German study and from the pemetrexed expanded access program on the antitumor activity of pemetrexed +/-platinum in DMPM suggested response rates in the range of those observed for pleural disease. Preliminary data suggested a possible survival advantage for the combination of cisplatin+ralitrexed compared with cisplatin alone. Other chemotherapies that have been shown to be active in this setting include vinorelbine and gemcitabine, either alone or in combination with platinum compounds. In historical case series, standard therapy with palliative surgery and systemic or intraperitoneal chemotherapy has been associated with a median survival of about one year (9 to 15 months).

**CRS and HIPEC**

DMPM is generally confined to the peritoneal cavity and rarely metastasizes systemically. Most patients die because of complications directly related to intra abdominal disease progression, such as bowel obstruction and starvation. CRS, through peritoneotomy procedures and multivisceral resections, allows the removal of all visible tumor implants, and HIPEC, through intra-abdominal drug administration with hyperthermia, is used to treat the free tumor cells and very small residual disease. At the National Cancer Institute (NCI) of Milan, CRS is performed according to the technique originally described by Sugarbaker with some minor technical variants. HIPEC is performed with the closed abdomen technique with cisplatin (45 mg/L of perfusate) and doxorubicin (15 mg/L of perfusate) for 90 minutes at a temperature of 42.5°C. Perfusion volume was 4 to 6 L, and mean flow was 700 mL/min and the extracorporeal circulation device Performer LRT® (RAND, Medolla, Italy) is used. Our center has gathered more than 15 year experience with an Institutional casuistic of more than 150 cases operated up to now. The combined approach of CRS with HIPEC modified the natural history of DMPM, introducing a dramatic improvement in outcomes in principal dedicated international centers and in a multi-institutional registry series.

In an analysis of these experiences, median survival increased from 12 months with systemic chemotherapy treatment to 53 months with CRS and HIPEC, with a 50% 5 year overall survival.

**Molecular biology**

During the last decade, DMPM biology has been deeply explored at the Milan NCI through various clinical biological studies. Telomerase activity (TA) was recently reported to be expressed in the majority of DMPMs and to negatively affect the clinical outcome of DMPM patients (Villa et al). In a study, 44 DMPM specimens were analyzed; TA was determined using the telomeric repeat amplification protocol, and alternative-lengthening of telomeres (ALT) was detected by assaysing ALT associated promyelocytic leukemia nuclear bodies. In the overall series, TA was prognostic for 4-year relapse and cancer related death, whereas ALT failed to significantly affect clinical outcome. These results held true in the subset of patients who underwent uniform treatment with CRS and HIPEC. Zaffaroni et al investigated the proliferation and apoptotic features of DMPM by assessing the immunohistochemical expression of survivin and members of the IAP family, including IAP 1, IAP 2, and X-IAP, in a series of 32 DMPM specimens. The effects of survivin knockdown in an established DMPM cell-line were also appraised. DMPM cells were transfected with small interfering RNA (siRNA) targeting survivin mRNA and analyzed for survivin expression, growth rate, and the ability to undergo spontaneous and chemotherapy induced apoptosis. DMPM cells were proven to be characterized by a relatively low proliferative activity and the low apoptotic behavior. Moreover, survivin and other IAPs are found to be largely expressed in DMPMs and suggest that strategies aimed at down regulating survivin (transfection of DMPM cells with survivin siRNA) may provide a novel approach for the treatment of this malignancy. The authors have also assessed the cellular effects of new anticancer agents, including a new series of nortopsentin hetero analogues in a DMPM cell-line. Selected compounds that were able to inhibit the activity of CDK1 consistently reduced cell growth and induced a concentration dependent cell cycle arrest at the G2/M phase and an increase in the apoptotic rate with a concomitant down regulation of the anti apoptotic protein survivin. Notably, the addition of these compounds to paclitaxel treated cells resulted in a marked increase of the cytotoxic effect as a consequence of increased caspase activation. Recently, Piotto and Perrone aimed to assess the activation profile of EGFR, PDGFRB and PDGFRα receptor tyrosine kinases (RTK) and their downstream effectors in cryopreserved DMPM specimens. They also made a complementary analysis of the cytotoxic effects of some kinase inhibitors on the proliferation of the human peritoneal mesothelioma STO cell-line. They found the expression/phosphorylation of EGFR and PDGFRB in most of the tumours, and PDGFRα activation in half. The expression of the cognate ligands TGF α, PDGFβ and PDGFA in the absence of RTK mutation and amplification suggested the presence of an autocrine/paracrine loop. There was also evidence of EGFR and PDGFRB co-activation. RTK downstream signaling analysis demonstrated the activation/expression of ERKs/2, AKT and mTOR, S6, and 4EBP1, in almost all the DMPMs. No KRAS/BRAF mutations, PI3KCA mutations/amplifications or PTEN inactivation were observed. Real-time PCR revealed the decreased expression of TSC1 c DNA in half of the tumours. In vitro cytotoxicity studies showed the STO cell-line to be resistant to gefitinib and sensitive to sequential treatment with RAD001 and sorafenib; these findings were consistent with the presence of the KRAS mutation G12D in these cells although it was not detectable in the original tumour.

**Future perspectives**

Taking advantage of available array based approaches, studies aimed at evaluating gene, microRNA and protein expression in a high throughput fashion are currently ongoing to elucidate the biological mechanisms and the regulatory events responsible for DMPM incidence and progression. Results from these studies are expected to pave
VA1.2: DIFFUSE MALIGNANT PERITONEAL MESOTHELIOMA: SEVEN-YEAR ACTUAL SURVIVAL AFTER SURGICAL CYTOREDUCTION AND HYPERTHERMIC INTRAPERITONEAL CHEMOTHERAPY (HIPEC) DEFINES CURE.

Dario Baratti1, Shigeki Kusamura1, Antonello D. Cabras2, Rossella Bertulli3, Marcello Deraco1
1Surgery, Fondazione Ircs Istituto Nazionale Tumori, Milano/ITALY, 2Pathology, Fondazione Ircs Istituto Nazionale Tumori, Milano/ITALY, 3Adult Mesenchymal Tumor Medical Oncology Unit, Fondazione Ircs Istituto Nazionale Tumori, Milano/ITALY

**Background:** Diffuse malignant peritoneal mesothelioma (DMPM) is a rare, locally aggressive, and rapidly lethal neoplasm. Cytoreductive surgery (CRS) combined with hyperthermic intraperitoneal chemotherapy (HIPEC) is an innovative treatment option for peritoneal surface malignancies. Because of the current lack of prospective randomized data, a survival benefit for DMPM patients managed by the local-regional approach has been suggested by comparison with historical controls, based on actuarial data. The aims of this study were to define cure after combined treatment for DMPM, determine the cure rate based on actual survivors, and identify clinical-biological features associated with cure.

**Methods:** A prospective database of 132 patients with DMPM undergoing CRS and HIPEC was reviewed. CRS comprised peritonectomy procedures and visceral resections, as needed. Close-abdomen HIPEC was performed with cisplatin and doxorubicin or mitomycin-C. A panel of immunohistochemical markers related to the origin and peculiar clinical features of DMPM was tested. Cell proliferation was scored by the percentage of tumor cells expressing the Ki-67 nuclear antigen.

**Results:** Operative mortality occurred in 3 patients (2.2%) and major morbidity in 46 (34.3%). In the overall series, median follow-up was 54.8 months (95% confidence interval (CI) 43.2-66.5). Median actuarial survival was 71.9 months (95% CI 32.3-111.5), and actuarial 5-year survival was 55.6%. The survival curve reached a plateau at 7 years, representing 22 actual survivors of 48 patients (45.8%) who had the potential for at least 7 years of follow-up (see figure). Among these 7-year survivors, 19 patients were free of disease and only one patient experienced disease-specific death after 7 years. Cytokeratin 5/6, calretinin, Wilms Tumor-1 (WT-1), podoplanin, epithelial growth factor receptor (EGFR), matrix metalloproteinase-2 were mostly positive; p16, p14, CK5/6, EGFR, and podoplanin, warrant further investigations. Cell proliferation was scored by the percentage of tumor cells expressing the Ki-67 nuclear antigen.

No significant relationships.

**Conclusion:** Patients with DMPM who survived >7 years appeared to be cured by CRS and HIPEC. Cure rate was 45.8%. Proliferative index may be used for prognostic stratification of DMPM. Biological features, such as CK5/6, EGFR, and podoplanin, warrant further investigations.

Disclosure: No significant relationships.
toxicities and no grade III or IV toxicities except the catheter infection. Pharmacologic analysis of pemetrexed peritoneal and plasma levels was performed showing a peritoneal fluid to plasma AUC ratio of 70. After a median follow-up of 60 months, the median survival of these 10 patients has not been reached; 3 patients have no evidence of disease, 3 are alive with disease and 4 have died of disease.

Conclusion: Adjuvant intraperitoneal pemetrexed combined with intravenous cisplatin following cytoreduction for DMPM can be administered with very morbidly and has favorable short-term outcomes. The intraperitoneal administration of pemetrexed has important pharmacologic advantages and is associated with few adverse events.

Disclosure: No significant relationships.

SESSION VA1 PERITONEAL MESOTHELIOMA SEPTEMBER 14, 2012 10:00-11:00

VA1.4: IDENTIFICATION OF CHROMOSOME 14Q DELETIONS IN ASBESTOS-INDUCED AND NOT RADIATION-INDUCED PERITONEAL MALIGNANT MESOTHELIOMA

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Background: Malignant mesothelioma is an aggressive tumor arising from mesothelial lining of serosal cavities. Pleural is the most common site, accounting for 60-70% of mesothelioma cases, and peritoneum accounts for the majority of the remaining 30%. Asbestos exposure is associated with pleural mesothelioma, with an attribution of 70%. Peritoneal malignant mesothelioma (PMM), in contrast, has a weaker attribution to asbestos of 30%. Other recognized causal factors for peritoneal mesothelioma include radiation therapy following testicular cancer, breast cancer, and lymphoma. The disease appears to be similar regardless of causation, but the molecular pathogenesis of asbestos versus radiation attributed peritoneal mesothelioma is unknown.

Methods: We identified 37 cases of snap frozen epithelioid PMM tissue from the Cancer Center Tissue Bank. Manual microscope guided needle dissection of cryostat frozen sections was performed obtaining a minimum of 500ng of tumoral DNA. DNA was prepared as per Affymetrix protocol for digestion, ligation, amplification, purification and labeling with standard quality metrics for fragment size prior to hybridization on Affymetrix SNP 6.0 array. Array results were analyzed using Nexus Copy Number 6.0 software (Biodiscovery, Hawthorne CA).

Results: Ten of the 37 PMM cases were attributed to asbestos exposure and seven to radiation exposure. In asbestos attributed cases, patient age ranged from 45-78 (8 men and 2 women) and in the radiation attributed cases, patient age ranged from 49-88 (6 men and 1 woman).

Chromosomal aberrations were compared statistically between the asbestos and radiation groups and one prominent region was evidenced in chromosome 14q where significant loss was shown in asbestos cases, but not radiation cases as highlighted in Figure 1.

Conclusion: Copy number analysis using SNP mapping arrays revealed losses at 14q11.2-13.3 and 14q21.1-23.2 in up to 70% of PMM patients after asbestos exposure compared to no deletion in PMM patients after radiation exposure. While the significance of this loss is unknown, identification in asbestos induced PMM in contrast to radiation attributed PMM suggests a distinct molecular pathway for asbestos induced versus radiation induced epithelioid PMM.

Disclosure: No significant relationships.

Figure 1. Frequency of chromosome loss and gain in asbestos (red) versus radiation (blue) induced PMM When chromosome 14q was further analyzed, loss of 14q11.2-14q13.3 and 14q21.1-23.2 was seen in up to 7 of 10 cases (70%) of asbestos attributed mesothelioma and in 0 of 7 (0%) of the radiation attributed cases.
Background: Exposure to asbestos fibres causes profound pathological changes and can result in the development of a fatal tumour, malignant mesothelioma (MM). There is rising concern that carbon nanotubes (CNTs) may present an inhalation hazard similar to asbestos and cause MM.

Methods: To investigate the molecular changes that occur as a consequence of direct exposure to short and long asbestos fibres (SFA and LFA) and short and long CNTs (NTS and NTL) these types of fibres were instilled directly into the pleural cavity in mice, the site of mesothelioma development, and kinome profiling was performed after 1, 4 and 12 weeks of treatment.

Results: Exposure to fibres resulted in both acute and delayed responses that were dependent upon the length of the fibres and the time of exposure. In response to NTL and LFA there was acute inflammation and fibrosis on the parietal pleura; no inflammatory changes were detected histologically after exposure to SFA and NTS. In terms of changes to the kinome, exposure to SFA and NTS produced only an acute response after 1 week, which involved activation of MSK1/2, Akt, RSK1/2/3, p53 and p27. The changes observed in LFA and NTL were similar, but in addition, there was sustained activation (during the entire length of the treatment) of mTOR, Src family kinases and STAT3, failure to activate p27 and p53, and reduced STAT-1 activation. Importantly, parallel profiling of malignant mesothelioma tumour samples identified similar changes in these signalling pathways.

Conclusion: The contribution of different cell types in time-dependent activation of these signalling pathways their role in disease development will be discussed.

Disclosure: No significant relationships.
Molecular Therapy and Genomics

SESSION VB

SESSION VB  MOLECULAR THERAPY AND GENOMICS SEPTEMBER 14, 2012 10:00-11:30

VB.2: IDENTIFYING THERAPEUTIC TARGETS FOR MESOTHELIOMA USING siRNA

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Background: Mesothelioma is essentially incurable and new drugs to effectively treat it are urgently needed. Our strategy to achieve this aim was to identify candidate mouse and human genes that may have a role in mesothelioma development and to inhibit their expression in fully transformed mesothelioma cell lines using siRNA.

Methods: Selection of genes was on the basis of their differential expression in transcriptome or CGH analyses when comparing malignant to normal mesothelial cells, together with any known functional information which could be relevant to tumorigenesis. In a similar way we also selected a small number of candidates from other published studies. A second set of candidates was chosen from aberrantly expressed kinases with the idea that these genes are more likely to represent druggable targets given the broad range of kinase inhibitors that are widely available. Where possible, we identified mouse and human homologues of the 40 candidates and then generated both mouse and human siRNA libraries. We were able to test the effect of gene knockdown on the growth of mouse and human mesothelioma cell lines in vitro.

Results: We found knockdown was efficient and inhibition of a subset of the selected genes slowed cell growth significantly across a range of cell lines in both mouse and human systems. There was not complete concordance between the mouse and human: Incenp, Plk1 and TPX2 were found to be important for murine cellular proliferation; whereas, AURKA, TPX2 and BIRC5 were found to be important for human cellular proliferation. KIF11 was identified in both studies.

Conclusion: These genes all have a function in chromosome positioning, centrosome separation and spindle assembly during cell mitosis. We will present data to show whether they have an additional role in cell migration, cell invasion and apoptosis. We think these studies could provide new leads for drug development.

Disclosure: No significant relationships.

SESSION VB  MOLECULAR THERAPY AND GENOMICS SEPTEMBER 14, 2012 10:00-11:30

VB.3: GENOME WIDE ASSOCIATION STUDY IN MESOTHELIOMA COMPARED TO ILLUMINA CONTROLS

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Background: A history of asbestos exposure can be identified in more than 80% of mesothelioma victims. However, women with peritoneal mesothelioma often lack an asbestos exposure history. Though asbestos exposure appears to be necessary in most cases for the development of mesothelioma, it is insufficient alone to cause mesothelioma. This is inferred by the fact that nearly 27 million individuals in the US were exposed to asbestos in the work place between 1940 and 1979 but just 3,000 new cases of mesothelioma are diagnosed annually. Common cancers are 3 times more likely to occur in mesothelioma patients and their 1st degree relatives. Hence genetic susceptibility may affect mesothelioma development. Furthermore, it seems likely that the genetic signature of asbestos induced mesothelioma may differ from that of peritoneal mesothelioma that affects women without known asbestos exposure.

Methods: A genome-wide association study (GWAS) was performed on 203 individuals with mesothelioma genotyped with the CNV370 BeadChip (Illumina, Inc.). Control subjects for this population were an independent group of Caucasian individuals from the iControls dataset (https://www.illumina.com/scienceicontrodb.iml). The 3172 iControl subjects were genotyped using Illumina Hap300 and Hap550 Chips. For both case and control population, we imputed all the known SNPs using IMPUTE program. Individuals with a call rate <0.95 were removed from GWAS analysis. The following quality control criteria were used to filter SNPs: Minor Allele Frequency < 0.02, Hardy-Weinberg Equilibrium < 0.001 and call rate <0.95. After imputation and applying quality control, there were 195 cases and 2,847 controls with 2,016,892 common SNPs. The association testing was first performed using all 195 mesothelioma cases. Twelve female subjects with peritoneal mesothelioma were then removed from the dataset and the association tests were performed again; both using linear regression implemented in PLINK. Population stratification was adjusted by the top 2 eigenvectors using EIGENSOFT software (Price et al. 2006).

Results: Six SNPs (rs16872571, rs2059109, rs4505994, rs7765557, rs7817028 and rs1491485) were significant (p < 10^-9) after Bonferroni correction comparing cases to Illumina controls. Four of these 6 SNPs have been shown to be associated with common cancers. rs2059109 (CCNG2) is a tumor suppressor gene that is involved in regulation of telomerase activity, rs7765557 (CD109) and rs7817028 (RUNX1T1) interact with and negatively modulate TGF-B signaling and rs1491485 (FAM48B) through unknown mechanisms. rs16872571 (CLN5 also known as MIST) regulates NK cell cytotoxicity. No known association has been reported with rs4505994. The same 6 SNPs were also significant after removing the 12 women with peritoneal mesothelioma but the p values became less significant. There were however, 3 regions where an improved signal was seen after removal of the women with peritoneal mesothelioma (chr9, chr11, chr17).

Conclusion: Six SNPs were identified that were associated with susceptibility to mesothelioma. Removal of women with peritoneal mesothelioma had little impact on this association. Association of peritoneal mesothelioma in women with regions in chr9, chr11, chr17 require further investigation.

Disclosure: No significant relationships.

SESSION VB  MOLECULAR THERAPY AND GENOMICS SEPTEMBER 14, 2012 10:00-11:30

VB.4: MOLECULAR CLASSIFICATION OF HUMAN MALIGNANT PLEURAL MESOTHELIOMA

Results: Gene expression profiling by microarray of MPM in culture allowed to define two main clusters (C1 and C2), and a third cluster of normal mesothelial cells. We demonstrated that mRNA expression analysis by RT-qPCR using the most discriminating genes allowed the same clusterization of MPM in culture, and divided MPM tumor samples into two clusters included into C1 and C2 respectively. In MPM in culture, mutations in CDKN2A, CDKN2B, NF2 and BAP1 are more frequent in cluster C1 than in cluster C2; the difference is statistically significant for BAP1. This classification also distinguished MPM according to their histological subtypes for both MPM in culture and tumor samples. Sarcomatoid and desmoplastic MPM are only found in cluster C2. Interestingly, we observed a longer patients’ survival in clusters C1 compared to C2. An analysis of more tumor samples is underway to confirm this observation.

Conclusion: This classification allows to define a new subclass of epithelioid MPM, which clusterized with sarcomatoid MPM. MPM of the two clusters have different prognosis and, surprisingly, C1 cluster with the highest mutation frequency in tumor genes is characterized by the highest median survival. Our data also showed several signaling pathways differently regulated between the two clusters. Molecular markers for epithelial-mesenchymal transition have a higher expression in MPM in cluster C2.

Disclosure: No significant relationships.
SESSION VB  MOLECULAR THERAPY AND GENOMICS  SEPTEMBER 14, 2012 10:00-11:30

VB.5: SUBTYPE CHARACTERIZATION OF MALIGNANT PLEURA MESOTHELIOMA USING GENE EXPRESSION RATIO TEST

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Background: Malignant Pleural Mesothelioma (MPM) is an uncommon tumor that can be challenging to diagnose. We previously described the utility of the gene ratio technique in discriminating MPM from other thoracic malignancies and predicting patient outcome. Herein, we describe for the first time that the gene ratio technique is also able to differentiate the epithelioid from the sarcomatoid type of MPM, a distinction that is clinically important for staging and prognosis. In addition, we provide molecular insights via differential gene expression into pathways differentially expressed between the epithelioid and the sarcomatoid MPM for further investigation.

Methods: We performed gene expression analysis on 39 MPM solid tumors (24 epithelioid, 7 biphasic, and 8 sarcomatoid) using illumina whole genome microarrays. A training set of 8 epithelioid and 8 sarcomatoid samples was used to find genes differentially expressed between the two groups of samples. Real-Time PCR (RT-PCR) was used to determine the relative expression levels of 4 genes (CLDN15, LOC57228, ORF1-FL49, NP), and the geometric mean of 3 individual gene pair expression ratios was calculated (CLDN15/LOC57228, ORF1-FL49/NP). To identify novel molecular pathways for further investigation. The samples were assigned to the epithelioid histology when the combined score was >1 and to sarcomatoid histology when the combined score was <1. Next, an independent test set of 100 MPMs (63 Epithelioid, 27 Biphasic, 10 Sarcomatoid) was used for the validation of the test. Functional enrichment analysis on both Gene Ontology and KEGG was performed using the DAVID web server to identify pathway differentially expressed between the epithelioid and sarcomatoid subtype.

Results: Using the training set expression data, we developed a 4-gene 3-ratio test able to distinguish the epithelioid from the sarcomatoid MPM samples. The test was then validated by RT-PCR in the same 39 MPM training set. All the epithelioid and sarcomatoid MPM were correctly classified. The same test was then applied using RT-PCR to an independent test set of 100 MPM samples showing that 8 of 9 sarcomatoid samples (89%) and 62 of 63 (98%) epithelioid MPMs were correctly classified. One sarcomatoid sample was excluded from the analysis because the ratio was not calculated (CLDN15/LOC57228; ORF1-FL49/NP; ORF1-FL49/LOC57228). The samples were assigned to the epithelioid histology when the combined score was >1 and to sarcomatoid histology when the combined score was <1. Next, an independent test set of 100 MPMs (63 Epithelioid, 27 Biphasic, 10 Sarcomatoid) was used for the validation of the test. Functional enrichment analysis on both Gene Ontology and KEGG was performed using the DAVID web server to identify pathway differentially expressed between the epithelioid and sarcomatoid subtype.

Conclusion: In this study, we show that the gene ratio technique is able to distinguish between histological subtypes of MPM with very high sensitivity and specificity. The gene ratio technique may be useful for other applications in cancer.

Disclosure: No significant relationships.

SESSION VB  MOLECULAR THERAPY AND GENOMICS  SEPTEMBER 14, 2012 10:00-11:30

VB.6: MESOTHELIOMA CELLS METABOLIC DERANGEMENT: A NEW MOLECULAR THERAPEUTIC TARGET

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Introduction: In order to improve the prognosis of patients with Malignant Pleural Mesothelioma (MPM) no question additional many efforts should be done to understand if some specific metabolic abnormalities of MPM do exist and, if they do, how these can exploited for therapeutic purposes. Fondazione Buzzi’s program encompasses a broad range of research project aimed at bridging the gap between pre-clinical and clinical sciences in order to set up innovative treatment for this orphan neoplasm. In particular two projects of this program focused on translation mechanism and oxidative pattern of mesothelioma cells respectively.

Where we are and where we are heading: Translation abnormalities of MPM cells: translational implications Previous data suggested that adhesion and spreading of mesothelioma cells on ECM require the translation of pre-synthesized mRNAs, and mTORC1 activity and spreading of mesothelioma cells is rapamycin-sensitive and requires continuing translation. More recently it has been shown that the sensitivity of global translation to mTOR Inhibition in these same cells depends on the equilibrium between eIF4E and 4E-BP1 but (rather surprisingly) it is not dependent on external growth factors. Moreover many MPM cells are low sensitive to mTOR pathway inhibition whereas the sensitivity to mTOR inhibition is driven by eIF4E and 4E-BP proteins expression. In more details low eIF4E and high 4E-BP expression are related to mTOR inhibitors sensitivity respectively. Hence we evaluated these proteins in specimens from patients with MPM to predict their sensitivity to mTOR inhibitors. Serine235 phosphorylation of translation factor eIF6 plays a key role in murine tumorigenesis and can be an attractive therapeutic target. IHC staining in specimens from patient with MPM revealed eIF6 overexpression in a high number of cases. On the light of these results eIF6 inhibition either by lentiviral vectors or by PKC beta Kinase inhibitor Enzastaurin has been assessed on in vitro and in vivo experiments with promising results that could lead to tailored clinical treatment of patients with eIF6 overexpression Oxidative Energy metabolism of MPM cells: Estrogen Receptor (ERb) is a new therapeutic target for MPM an the other ERb overexpressing tumours Female Gender is a well known positive prognostic factor for patients with MPM and ERb overexpression has been demonstrated to be associated with better prognosis too. More recently we also demonstrated how ERb interferes with eIF4E expression and affects the sensitivity of MPM cells to EGFR inhibitors. Now we clarified a novel mechanism by which ERb exerts a tumour suppressive effect on MPM cells disclosing a novel therapeutic approach to this neoplasm. Both on in vitro and in vivo experiments, ERb activation via either overexpression or treatment with selective agonists significantly compromises mitochondrial oxidative pathway (affecting mitochondrial sub-units activity) ATP production and decreases cell proliferation and MPM growth. Moreover, due to the impaired mitochondrial activity, ERb overexpressing MPM cells turn into a much higher dependency on glycolysis and sensitivity to glycolysis inhibitors. Some selective ERb agonists have been progressively designed over the last few years and are now ready for clinical settings. As ERb expression is common to many human tumours (included prostate and breast cancer) the treatment of ERb positive human with selective ERb agonists is a novel intriguing approach for a subset of cancer patients

Conclusions: Translation pattern and oxidative metabolism of MPM cells seems to disclose a new scenario for the treatment of this tumour even with some implications for “less rare” human tumours.In particular, these studies allowed to disclose novel therapeutic biomarkers of sensitivity to screen our patients. The next due step is now designing Phase I/II clinical trials for patients with MPM selected on the basis detection of biomarkers that could reasonably predict their sensitivity

Disclosure: No significant relationships.
Session VC
Radiology, Staging and MPM
SEPTEMBER 14, 2012 10:00-11:30

VC.2: CT-BASED CLINICAL STAGING FOR MALIGNANT PLEURAL MESOTHELIOMA IS UNRELIABLE FOR THE ASSESSMENT OF LOCAL TUMOR INVASION AND LYMPH NODE INVOLVEMENT

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Background: Today computed tomography (CT) remains the most commonly used imaging modality for malignant pleural mesothelioma (MPM) staging, although the visualization of tumor extent remains challenging. The purpose of this study was to assess the reliability of clinical staging for MPM based on preoperative CT scan as a function of different observer and compared to definitive pathological staging.

Methods: Sixty two patients with proven MPM and preoperative available CT scans were included. Thirty-four patients (55%) underwent talc pleurodesis and all underwent induction chemotherapy prior to surgery. The CT scans were performed over a median of 16 days (0-28) before surgery using intravenous contrast and had a maximum slice thickness of 2 mm. Three experienced observers blinded to any clinical information (two chest radiologists and one thoracic surgeon) independently assessed clinical T and N stages. Inter-rater reliability was assessed by using analysis of variance. The clinical staging was correlated with definitive pathological staging. A kappa statistic was used to assess the agreement of tumor staging between observers. Inter-rater reliability was considered poor (κ ≤ 0.2), fair (κ = 0.21–0.4), moderate (κ = 0.41–0.6), good (κ = 0.61–0.80) or excellent (κ = 0.81–1.0).

Results: The T stage was estimated correctly in 48-71% with a good inter-observer reliability (κ = 0.64-0.71). The T-stage was underestimated in 22, 25 and 37%, respectively, whereas overestimation was rather low (7-15%). Regarding the N-status, a correct staging was performed in 58-68%, again with good inter-observer agreement (κ = 0.61, 0.65, 0.71). Lymph node staging was also rather under categorized (16-27%) by all readers. A more advanced N status was only assigned in 13-16% of the cases.

Conclusion: The present set of data confirm that CT scan based clinical staging results in an underestimation of the definitive mesothelioma stage, especially the T stage, which is the most important factor for the assessment of resectability of the tumor. Therefore, more precise imaging techniques should be evaluated in comparative studies for a better prediction of mesothelioma stage of disease.

Disclosure: No significant relationships.

VC.3: IMPACT OF INCORPORATING HEMOGLOBIN LEVEL ON THE ACCURACY OF CLINICAL LYMPH NODE STAGING IN MALIGNANT PLEURAL MESOTHELIOMA (MPM).

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Background: Extrapleural (N2) nodal involvement is an important factor that influences the treatment approach for patients with MPM. For patients with involved extrapleural nodes, initial treatment is typically neoadjuvant chemotherapy rather than surgery. However, for patients that are node negative by mediastinoscopy, clinical staging by radiographic imaging has low accuracy to detect occult extrapleural nodal disease. We have identified preoperative anemia as a stage-independent predictor of poor outcome in MPM (Gill, Am J Roentgenol, 2012). We investigated whether preoperative anemia is correlated with pathologic nodal involvement, and might therefore contribute to clinical nodal staging.

Method: We reviewed a cohort of 168 patients who underwent extrapleural pneumonectomy (EPP) without pre-operative chemotherapy at Brigham and Women’s Hospital between 2001 and 2010. Presence of preoperative anemia was determined based on WHO criteria. All EPP specimens underwent complete pathological examination and this served as the gold standard for the presence of extrapleural lymph node involvement. Nodal status was assessed using the American Joint Commission on Cancer (AJCC)/International Union Against Cancer (UICC) TNM classification. Preoperative CT evidence of extrapleural nodal involvement was documented by a dedicated thoracic radiologist using size criteria. Frequency of N2 disease in subgroups of patients with and without anemia was compared using Fisher’s exact test. Accuracy of clinical N2 staging based on CT was compared to clinical N2 classification plus anemia by comparing the relative frequency of correct classification (positive and negative) by each using a 2-sided McNemar’s test.

Results: Median age was 62 (37-79) and 138 patients (81%) were male. Mediastinoscopy was performed in 133 patients (79%) and was negative for all cases. Histologic subtype of the final specimen was epithelial for 103 (61%) and non-epithelial for 65 (39%). Extrapleural lymph node disease was identified on final pathologic analysis of the EPP specimen in 53 cases (31%) of which 35 (20%) were associated with preoperative anemia and 18 (11%) were not (p = 0.003). Clinical N2 classification alone was associated with 49% accuracy in predicting pathologic N2 disease (sensitivity 61%, specificity 43%). Clinical N2 classification plus anemia was associated with significantly higher accuracy at 70% (p = 0.003; sensitivity 57%, specificity 76%).

Conclusion: Limited sensitivity of mediastinoscopy for detecting extrapleural N2 nodal disease heightens the urgency to improve current clinical staging strategies for MPM. Preoperative anemia was found to be present in two-thirds of patients with occult N2 disease discovered at final
SESSION VC  RADIOTHERAPY, STAGING AND MPM  SEPTEMBER 14, 2012 10:00-11:30

VC.4: SUPPLEMENTARY PROGNOSTIC VARIABLES FOR PLEURAL MESOTHELIOMA: A REPORT FROM THE IASLC STAGING COMMITTEE

Harvey Pass1, Dori Giroux2, Catherine Kennedy3, Enrico Ruffini4, Ayten K. Cangir5, David Rice6, Hisao Asamura7, David A. Waller8, John Edwards9, Walter Weder10, Hans Hoffman11, Jan P. Van Meerbeeck12, Valerie Rusch13

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Background: Current staging for malignant pleural mesothelioma (MPM) is controversial. To plan revisions of this system, the IASLC Staging Committee developed an international database. Initial analyses focused on surgically-managed patients, and determined that stage, histology, sex, age (<50 vs. older), and type of procedure (palliative vs. curative) were core prognostic variables (IASLC, 2011). The present analysis explores the impact of potential, supplementary prognostic variables including the use of adjuvant therapy (i.e., therapy by other modalities including chemotherapy and/or radiation therapy), smoking history, history of asbestos exposure, history of weight loss, ECOG performance status, presence of chest pain, presence of dyspnea, and preoperative laboratory values including hemoglobin, white blood cell, and platelet counts.

Methods: Participation was solicited from centers with MPM registries. Common data elements were analyzed by Cancer Research and Biostatistics (CRAB, Seattle, WA). We analyzed supplementary prognostic variables along with core variables for three scenarios: (A) All variable data available, i.e. patient best staged, all core variables, and supplementary variables are considered (B) Only the clinical stage and the rest of the CORE variables excluding surgical staging, and supplementary variables are considered, and (C) Supplementary variables excluding adjuvant therapy, age, sex, histology, and laboratory parameters are known, i.e., the patient with a diagnosis but no staging performed. Survival was analyzed by Kaplan Meier, prognostic factors by logrank and Cox regression model. p < .05 was significant.

Results: Data included 3101 patients (15 centers, 4 continents) from 1/95-8/09 of which 2141 with best (clinical or pathologic staging recorded or both) TNM stages had non-missing age, sex, histology, and type of surgical procedure. When all variable data were considered (Scenario A), univariate analysis revealed adjuvant therapy, asbestos exposure, smoking weight loss, sex, chest pain, hemoglobin (<14.6 or not), platelet count (>400k or not, and WBC (>15.5 or not) as significant supplementary variables. Stepwise Cox Regression Model after elimination of non-significant variables revealed best stage, histology, sex, age, type of surgery, adjuvant treatment, WBC, and platelets in a final model (n=555 patients with all variables under consideration.) When Clinical Stage was considered with other CORE and supplementary variables (Scenario B), stepwise Cox Regression Model with backwards selection included clinical stage, histology, sex, age, type of surgery, adjuvant treatment, WBC, hemoglobin, and platelets in 627 patients. When limited data are considered at patient presentation (Scenario 3) including age, sex, histology, asbestos exposure, weight loss, smoking history, chest pain, WBC, hemoglobin, and platelets, univariate analysis revealed smoking, asbestos exposure, weight loss, chest pain and blood values to be significant; however, the stepwise model with backwards selection for this scenario included only histology, sex, age, WBC, and platelets in the 906 patients with all variables considered.

Conclusion: This is the largest international database examining available prognostic factors which could influence outcomes in surgically managed MPM patients. Refinement of these models could have clinical utility in defining not only the appropriate patient preoperatively for best outcomes after cytoreductive surgery, but also in stratifying surgically treated patients after clinical and pathologic staging who do or do not receive adjuvant therapy.

Disclosure: No significant relationships.

SESSION VC  RADIOTHERAPY, STAGING AND MPM  SEPTEMBER 14, 2012 10:00-11:30

VC.5: DYNAMIC CT AND TUMOR RESPONSE FOR PATIENTS WITH MESOTHELIOMA

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Background: Dynamic contrast-enhanced (DCE) CT is an imaging modality that combines the structural information of standard CT with functional hemodynamic information. A prospective pilot study is underway to assess the viability of DCE-CT as an imaging modality for patients with malignant pleural mesothelioma and to investigate correlations between changes in DCE-CT metrics and tumor burden measurements in patients on observation and on chemotherapy.

Methods: Patients were scanned with an IRB-approved protocol using a 256-slice CT scanner. The imaging protocol was designed to efficiently utilize one injection of iodinated contrast for both the DCE and full-thorax components of the CT scan. The DCE-CT scan consisted of imaging snapshots acquired for a 5.5cm axial extent at 120kVp and 100mAs every three seconds for one minute prior to the full-thorax diagnostic scan, which was followed by five additional snapshots spaced five seconds apart. DCE-CT scans were acquired at each of two sessions approximately 6 weeks apart, at the time of regularly scheduled disease reassessment. All snapshots from a single scan were co-registered using a deformable registration algorithm to remove the effects of motion prior to the calculation of DCE-CT parameters. Perfusion, blood volume, time-to-peak, peak enhancement, and mean transit time data maps were calculated using the slope method. Data map spatial statistics were calculated over manually defined regions of interest (ROIs) within the tumor, and changes in data map values between the two DCE-CT scans for each patient were correlated with tumor burden changes measured according to the Modified RECIST technique as well as volumetric disease measurements.

Results: Thirteen mesothelioma patients (seven on observation, six on chemotherapy) have undergone two DCE-CT scans to date in this ongoing study. Tumor ROIs exhibited heterogeneous contrast uptake, ranging from no uptake to peak enhancement of 100HU. No DCE-CT parameters were significantly correlated with changes in tumor burden in this initial pilot observation and on chemotherapy.

Disclosure: No significant relationships.
Axial slice of calculated blood volume metric for patient 2 (pseudo-color image). The pericardial mesothelioma mass (red arrow) exhibits a marked reduction in blood volume in a central region between (a) the baseline scan and (b) the scan after 42 days of treatment.

cohort. However, there was a marked difference in DCE-CT temporal perfusion changes between the observation cohort (n=7) and the treatment cohort (n=6) (+29% and -13%, respectively).

Conclusion: This study indicates that DCE-CT provides functional hemodynamic information for mesothelioma and that changes in calculated DCE-CT parameters may provide imaging biomarkers of underlying tumor change not reflected in tumor measurements. Accrual continues until 20 patients (10 on observation, 10 on chemotherapy) have each received two DCE-CT scans.

Disclosure: No significant relationships.
planned to receive concurrent radiation boosts to higher doses, resulting in improved local control rates. Patterns of failure based on PET and CT scan analyses confirmed a high rate of locoregional control of mesothelioma after RT, with only 4 patients relapsing in-field, all but one with concurrent metastases documented in unirradiated sites. Five patients remain disease-free and 18 developed out-of-field recurrences. One patient with an a PET-detected solitary recurrence in ipsilateral hilar nodes outside the volume irradiated 5.5 years earlier remains in remission 3 years after salvage RT and 8 years after diagnosis. Changes in patterns of radiation pneumonitis over time will be addressed.

**Conclusion:** In our mesothelioma program, PET/CT scans play a pivotal role in patient selection for surgery and high-dose RT, radiotherapy planning that targets sites of gross unresected disease with higher doses and treatment followup to detect recurrences for early salvage therapies. Based on followup PET scans confirming recurrent disease within the radiation target volume in only 4 of 27 patients, we can confirm that radiotherapy produces effective locoregional control in selected mesothelioma patients who receive high-dose irradiation, a locoregional control rate of 85%.

**Disclosure:** No significant relationships.
WSXI.1: SPECIMEN SELECTION FOR MICRONRNA QUANTIFICATION IN MESOTHELIOMA

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Background: There is a lot of interest in the use of microRNA (miRNA) profiles as diagnostic tools in mesothelioma and several candidate miRNA biomarkers have been described. Recently we reported that circulating levels of the miR-625-3p were significantly elevated in the plasma and serum of mesothelioma patients relative to controls. Despite the intrinsic stability of miRNAs there are concerns relating to the variability of miRNA quantification because of other factors including specimen collection methods, RNA extraction efficiency and data analysis. In the present study we compared miRNA levels in longitudinal blood samples collected by two different methods and also examined the diagnostic accuracy of our candidate miRNA biomarker mir-625-3p compared to soluble mesothelin levels.

Methods: Blood samples were initially collected from healthy controls on three consecutive days. Parallel samples were collected in traditional “red cap” serum BD Vacutainers and in PAXGene Blood RNA Tubes (Qiagen). Serum collected in traditional tubes were allowed to clot at room temperature before being stored at -20°C and miRNA extracted on the batch of samples using the protocol of Miska et al. 2002. Samples collected in PAXgene tubes were processed following the manufacturer’s instructions. MiRNA was quantitated using TaqMan® MicroRNA Assays on the Applied Biosystems StepOne Plus Real Time PCR System. Blood samples were prospectively collected in PAXGene Blood RNA Tubes from 25 patients with malignant mesothelioma and 25 patients with asbestosis related benign disease. MiRNA was extracted and quantitated as above.

Results: Levels of the small nuclear RNAs U44 and U6B, and the mirs-16, -103, -192 and -21 showed significantly greater variance in a mixed model component analysis in blood samples collected in serum tubes from four health individuals on three consecutive days compared to parallel samples collected in PAXgene tubes. Levels of miR-625-3p were significantly elevated in samples collected in PAXGene tubes from mesothelioma patients relative to controls. Levels of soluble mesothelin were significantly elevated in the serum of mesothelioma patients relative to controls. There was no significant difference between the diagnostic accuracy of the two biomarkers when the area under the receiver operator curves was compared. Data will be presented from additional 30 patients to increase the power of the study.

Conclusion: MiRNA levels are more stable when the miRNA is extracted from serum in PAXgene tubes, as opposed to normal serum tubes. By using the PAXgene tubes, consistent miR levels over consecutive days can be seen and would therefore prove to be a more reliable source when it comes to diagnosing patients with malignant mesothelioma.

Disclosure: No significant relationships.

WSXI.2: MICRONRNAS AS NOVEL DIAGNOSTIC BIOMARKERS AND REGULATORS OF TUMOR BIOLOGY IN MALIGNANT MESOTHELIOMA

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Background: There is currently no internationally uniformly accepted approach for the diagnosis of Malignant Mesothelioma (MM). The success rate of current methods for diagnosis is highly variable and at some centers definitive diagnosis can take up to 3 months. Therefore, there is an urgent need for identification of new biomarkers in MM. MicroRNAs (miRs) are single stranded RNAs which regulate gene expression and have been shown to be important in cancer pathogenesis. miRs can be easily measured, are expressed in body fluids such as serum and sputum and are extremely stable. Therefore, miRs represent an attractive target for biomarker discovery in MM. Therefore the aim of this study is to profile serum and cells isolated from Pleural Effusions (PE Cells) from MM patients for all identified human miRs and select potential biomarker targets.

Methods: Total RNA was isolated from 50 PE Cells (30 MM, 10 Benign and 10 Adenocarcinoma (AC) and 60 serum (30MM, 10 asbestos exposed but healthy, 10 AC and 10 asbestosis) samples. The OpenArray® Real-Time PCR platform was used to profile the samples for miRs. Data analysis was performed in DataAssist. For validation of selected targets, miRs were measured using qPCR.

Results: The miR targets were selected based on expression profile and significance of expression when MM was compared to the other control groups. Five novel miR targets were selected from serum and 5 from PE Cells. These 10 targets were initially validated by qPCR in the same samples to confirm the profiling results. To determine the diagnostic potential of these selected targets qPCR is currently being performed in a larger cohort of 100 samples. Furthermore, using our data we were able to validate miRs identified by other studies which also strengthens our profiling data.

Conclusion: Currently this work is the largest and most comprehensive miR profiling study in MM. We have identified a number of novel miR biomarkers in serum and PE samples. The results of this study may not only identify a biomarker which will improve current diagnostic procedures but one that can be used to screen high risk individuals and ultimately improve patient outcomes.

Disclosure: No significant relationships.
WSXI.3: ANALYSIS OF MICRO-RNAS AND GENES IN MALIGNANT MESOTHELIOMA

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Background: We hypothesized that certain microRNA (miRNA)-mRNA interactions are associated with malignant transformation and progression of malignant mesothelioma (MM). To date, no integrated analysis has been performed to identify critical interactions between miRNA-mRNA that may drive the MM malignant phenotype.

Methods: Total RNA was extracted from 22 specimens of biopsy proven human MM and 6 specimens of normal pleura. Paired global transcriptional profiles of miRNA and genes (mRNA) expression were generated using Illumina microarrays. After rank-invariance normalization of miRNA and mRNA expression data, we used significance analysis of microarray (SAM) to identify differentially expressed miRNAs and mRNAs at a false discovery rate (FDR q-values) < 5%. To better determine relevant genes for a given process, sets of genes whose expression levels negatively correlated with specific miRNAs were identified as likely direct targets of that miRNA. These differentially expressed miRNAs and mRNAs were analyzed using Ingenuity Pathway Analysis (IPA) to identify the biologic and chemical systems that are affected by coordinateially altered levels of miRNAs and mRNAs in MM. We integrated differentially expressed miRNAs and mRNAs at the pathway, systems level using multiple miRNA-mRNA target databases from the public domain. We constructed an interaction network of differentially expressed miRNA-mRNA pairings.

Results: We found 2,202 genes (593 up, 1609 down) differentially expressed in MM specimens compared to normal pleura using SAM. Similarly, we identified 101 miRNAs differentially expressed (57 down, 44 up). Focusing on negatively correlated pairings of miRNA-mRNA, we generated 2 datasets for further analysis. As an example, when we analyzed the set of down-regulated miRNA in MM as compared to normal pleura using IPA, there was over-representation of these miRNA in immune response-related biologic pathways including immune cell trafficking, cellular motility, antigen presentation, cell-to-cell signaling and interaction, and cellular morphology. Complementary analysis of the 593 over-expressed genes by the IPA algorithm showed similar enrichment in immune response-related pathways. We used miRNA-mRNA target databases from the public domain to build an interaction network using under-expressed miRNAs and over-expressed mRNAs. We mapped onto these networks confirmed expression data from our group of 28 specimens. We observed a large number of miR-1 gene targets over-expressed in MM, while miR-1 itself was significantly under-expressed (FDR q-value < 0.01), while its gene targets showed an opposite pattern being overexpressed (FDR q-value < 0.05). Expression of miR-1 negatively correlated with expression of 52 mRNA (FDR < 0.05). This miRNA was underexpressed in MM by 3-fold more (FDR < 0.01), while its gene targets showed an opposite pattern being overexpressed in MM. In addition to positively affecting inflammation-related pathways, miR-1-related gene subsets were enriched for oncogenic genes (e.g. p21, TRAF2, and SAA1) positively impacting cell growth, survival, and anti-apoptosis.

Conclusion: Integrated analysis of miRNA and mRNA expression profiles in MM revealed novel miRNA associated with MM and identified putative interactions that may underlie the malignant phenotype. These miRNA-mRNA are candidates for functional validation. miR-1 is a putative tumor suppressor miRNA in MM.

Disclosure: No significant relationships.

WSXI.4: IDENTIFICATION OF MIR-625-3P AS POTENTIAL BLOOD-BASED BIOMARKER FOR MALIGNANT PLEURAL MESOTHELIOMA

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Background: The definitive diagnosis of malignant pleural mesothelioma (MPM) often depends on the availability of a biopsy of sufficient size. The identification of a biomarker that can be easily measured in blood would represent an important step forward. Recently it has been shown that microRNAs (miRNAs) detectable in serum/plasma represent a class of potential new biomarkers. In this study we investigated the ability of miRNAs in plasma/serum to serve as a diagnostic marker for MPM.

Methods: Using Agilent 3x1g miRNA microarrays we profiled miRNA expression in plasma samples from healthy volunteers and patients with MPM. Candidate miRNAs identified in the arrays were validated by TaqMan assay-based quantitative real-time PCR or using the OpenArray real-time PCR platform.

Results: Microarray-based expression profiling of plasma from 5 MPM patients and 3 healthy controls identified 17 miRNAs with significantly differential abundance in the two sample groups. Validation of these miRNAs in a series of plasma samples from 15 MPM patients and 15 controls (healthy individuals and patients with coronary artery disease) revealed that levels of miR-625-3p, were significantly elevated in plasma of MPM patients (4-fold, p=0.004), and able to discriminate between MPM patients and controls (sensitivity of 73.3 %, specificity 78.6 %). Levels of two miRNAs previously reported to be associated with MPM, miR-29c* and miR-92a, were also elevated in our MPM series however without reaching statistical significance. Assessing levels of miR-625-3p in serum of an independent series of MPM (N=30) and asbestosis (N=10) patients confirmed that miR-625-3p was significantly (p=0.023) elevated only in serum of MPM patients and was able to discriminate between cases and controls with a sensitivity of 70 % and a specificity of 90 %. Finally, miR-625-3p was present at significantly elevated levels (2-fold, p=0.006) in tumour specimen from 18 MPM patients compared to normal mesothelium (pericardial tissue). Preliminary data from a third series of serum samples from 32 MPM patients and matched healthy controls so far confirm our observation that miR-625-3p is elevated at least 3-fold in blood from MPM patients. Final results from this third series will be presented at the conference.

Conclusion: Taken together the data from the initial two series of blood samples provide evidence that miR-625-3p has the potential to serve as a novel blood-based biomarker for MPM. Preliminary results from a third series of serum samples support these findings.

Disclosure: No significant relationships.
WSXI.5: THE MICRORNA MIR-16 IS A NOVEL TUMOUR SUPPRESSOR GENE IN MALIGNANT PLEURAL MESOTHELIOMA

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Background: The microRNA expression in malignant pleural mesothelioma (MPM) biopsy specimens and cell lines is significantly altered. Previous studies have indicated that a number of microRNAs have tumour-suppressor or oncogenic functions in MPM. The expression of miR-16 is frequently lost in a variety of cancers, and here we demonstrate that miR-16 is also down-regulated in MPM and seems to function as a tumour-suppressor gene.

Methods: Tumour content of formalin-fixed, paraffin-embedded specimens from patients who underwent extrapleural pneumonectomy was marked by an experienced pathologist prior to laser-capture microdissection and RNA isolation. The expression of selected microRNAs was measured and compared with levels in formalin-fixed pericardial tissue using miR-specific TaqMan assays. Following RNA extraction with TRizol, the expression of miR-16 was also measured in MeT-5A and a panel of MPM cell lines. Re-expression of miR-16 was accomplished by transfecting cells with a miR-16 mimic. Effects on growth and gemcitabine resistance were measured using a SYBR Green-based proliferation assay, and colony formation assays were also carried out. The expression of potential miR-16 targets in MPM was analysed by RT-qPCR in miR-16 mimic-transfected cells.

Results: The expression of miR-16 was significantly (p = 0.01) down-regulated (20-fold) in MPM tumours compared with pericardial tissue. In cell lines, miR-16 expression in MPM lines was 2 to 4-fold down-regulated compared with MeT-5A, an immortalized normal mesothelial cell line. Transfection with a miR-16 mimic increased intracellular levels of mature miR-16 in all transfected lines. The restoration of miR-16 expression resulted in growth inhibition in all MPM lines in a dose-dependent manner, with as little as 1 nM mimic leading to significant effects on proliferation and the ability to form colonies when seeded at low density. In contrast, transfection with as much as 5 nM did not effect the growth of MeT-5A cells. The transfection of MPM cells with miR-16 also led to a 2 to 5-fold sensitization to gemcitabine but did not affect MeT-5A sensitivity to this drug. The expression of putative miR-16 targets related to gemcitabine resistance were down-regulated in transfected cells.

Conclusion: The down-regulation of miR-16 in MPM tumour samples and cell lines, together with the growth inhibitory effects of restoring miR-16 expression suggests a tumour suppressor function of this microRNA in MPM. Together with its ability to sensitize MPM cells to gemcitabine treatment, this suggests miR-16 replacement should be explored as a novel therapeutic approach in MPM.

Disclosure: No significant relationships.
BACKGROUND: MPM is a lethal malignancy caused by asbestos which affects around 2,000 people in the UK annually. It is characterised by poor prognosis and resistance to chemotherapy. Currently, treatment options include multimodality therapy with a combination of surgery, chemotherapy and radiotherapy, but they fail to give a survival benefit of more than a few months. It is therefore necessary that new therapeutic approaches are developed. Here we present a novel immunotherapeutic approach targeting the extended ErbB receptor family using CAR-targeted T-cells in MPM cell lines. The ErbB family consists of EGFR, HER-2, ErbB-3 and ErbB-4, which undergo ligand-induced homo- and heterodimerization. Over-expression of EGFR in seen in the majority of MPM and ER-2 is also commonly expressed. Emerging evidence suggests that ErbB3 might also be an important player in MPM. We have previously developed an immunotherapeutic approach that targets several ErbB dimers. A chimeric antigen receptor (CAR, named T1E28z) was engineered in which the promiscuous ErbB ligand, TIE, is fused to a CD28-CD3 endomain. As poor transduction efficiency of patient-derived T-cells may be a limiting factor in achieving clinical efficacy, a chimeric cytokine receptor (αβ) in which the ectodomain of IL-4 receptor α was fused to the endodomain of the shared β receptor used by IL-2 and IL-15 was constructed. T-cells that express αβ can be selectively expanded using IL-4, greatly facilitating the rapid generation of therapeutic cell products. Burt et al (2012) have recently shown that MPM progression is driven in part by IL-4, produced by T-cells within the tumour (1). Using the αβ system described above, we could capitalise upon local IL-4 to provide targeted support for our engineered T-cells, directly at the site of the tumour.

METHODS: Peripheral blood mononuclear cells were isolated from healthy controls and activated using CD3/CD28 beads. Co-expression of TIE28z and αβ (named “T4”) was achieved using a single SFG retroviral vector and delivered to the T-cells. Cells were expanded over a period of 15 days in the presence of IL-4 for specific expansion of T4. Co-cultures were set up to test T4 efficacy in vitro. The mesothelioma cell models used were H28, H29 and MM48. Conditional media was collected to analyse INF-gamma production in order to test for T-cell activation.

RESULTS: Destruction of cell monolayers derived from all three cell lines was achieved within 24h upon co-cultivation with T4 transduced T-cells. Tumour destruction and INF-gamma production show specific activation of the T4 cells upon meeting the target.


Disclosure: No significant relationships.
tumor effects and is a possible therapeutic agent to mesothelioma when administered in the pleural cavity.

Disclosure: No significant relationships.

SESSION VIA

NOVEL THERAPEUTICS: PRECLINICAL STUDIES

SEPTEMBER 14, 2012 14:20-15:50

VIA.3: A MULTIFUNCTIONAL MESOTHELIN ANTIBODY-TAGGED MICROPARTICLE TARGETS HUMAN MESOTHELIOMAS

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Background: Pleural and peritoneal mesotheliomas (MMs) are chemoresistant tumors with no effective therapeutic strategies. We hypothesized that local injections of porous microparticles loaded with chemotherapeutic drugs and externally modified using an antibody for mesothelin (MB) would be valuable in treating MMs.

Methods: We first prepared multifunctional, acid-prepared mesoporous spheres (APMS) functionalized with a tetraethylene glycol oligomer and injected them via various routes into rodents. Biodegradation and excretion of APMS and gadolinium (Gd)-modified APMS were examined in organs, peritoneal lavage fluids (PLF) and urine of normal mice and rats over time. APMS was also functionalized with an antibody to mesothelin (APMS-MB) or bovine serum albumin (BSA), a non-specific protein control, and in vivo tumor targeting was evaluated by inductively-coupled mass spectrometry and multiluorescence microscopy over a 6 day period. Lastly, APMS-MB microparticles were loaded with doxorubicin (DOX), and APMS-MB-DOX particles, DOX alone at the same concentrations, and APMS-MB (no DOX) were injected intraperitoneally 3x weekly into SCID mice bearing human MMs. Tumor-bearing mice were also injected with saline (controls).

Results: APMS was primarily cleared via the urine over a 24 hr period, and small amounts were observed in liver, spleen and kidneys at 24 hr and 6 days. Neither inflammation nor necrosis was observed in major organs or in PLF. Targeting with APMS-MB vs. APMS-BSA increased APMS uptake in mesenteric tumors at 6 days (p<0.05),Particles were observed in both MM cells and tumor-associated macrophages (TAM), and moved centrally into tumors over time. Approximately 10-12% of the initially injected amount was observed in both spheroid and mesenteric mesotheliomas at 6 days post-injection. Targeted therapy using APMS-MB-DOX was more effective than treatment with equivalent concentrations of DOX alone, resulting in the reduction of MM volume (p<0.05) primarily via inhibition of cell proliferation. Unlike mice injected with effective concentrations of DOX alone, mice receiving APMS-MB-DOX did not exhibit weight loss or organ toxicity after multiple injections.

Conclusion: Our data suggest that localized delivery of APMS-MB into the peritoneal or pleural cavity after encapsulation of drugs, plasmids or macromolecules is an effective strategy that should be tested in patients with MMs. Financial Support: This work was supported by the Mesothelioma Applied Research Foundation (BTM); NCI STTR R41 CA126155; NCRR 1S10 RR08173-01A1 and 1S10 RR019248 (BJT).

Disclosure: No significant relationships.
VIA.5: INHIBITION OF MULTIPLE SIGNALING PATHWAYS BY VANDETANIB (ZD6474) INCREASES DOXORUBICIN-INDUCED CELL TOXICITY IN HUMAN MESOTHELIOMA CELLS

Mutlay Sayan, Arti Shukla, Maximilian B. Macpherson, Sherrill L. Macura, Jedd M. Hillegass, Timothy N. Perkins, Joyce K. Thompson, Stacie L. Beuschel, Jill M. Miller, Brooke T. Mossman

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Background: Malignant mesothelioma (MM) is an aggressive malignancy with a poor prognosis. Therefore the need to develop novel and effective therapies is urgent. Vandetanib (Van) (ZD6474, ZACTIMA™) is a novel tyrosine kinase inhibitor (TKI) that originally was thought to inhibit activation of the vascular endothelial growth factor receptor-2 (VEGFR-2), as well as phosphorylation of the epidermal growth factor receptor (EGFR). We have identified EGFR signaling as well as activation of extracellular signal-related kinases (ERK1, ERK2, ERK5), Activator Protein-1 and cAMP response element binding protein (CREB) as critical signaling cascades and transcription factors that are activated by asbestos and elevated in MMs (reviewed in Heintz NH, Janssen–Heininger, YMW, and Mossman, BT). Asbestos, lung cancers, and mesotheliomas: from molecular approaches to targeting tumor survival pathways. Am J Respir Cell Mol Biol 43:133-139, 2010).

Methods: In human MM lines, HMESO and H2373, we evaluated whether Van had tumor cell killing efficacy both alone and in combination with Doxorubicin (Dox) (Adriamycin) and the signaling cascades involved using cell toxicity assays and Western blot analysis.

Results: Van alone reduced total cell numbers in HMESO cells. Moreover, it synergistically increased the toxicity of Dox in vitro in both cell lines. Van also caused the inhibition of Dox-induced phosphorylation of EGFR, ERKs, CREB, and protein kinase A (AKT) (see summary of results in Table 1 below). Table 1: A summary of the effects of Van and Dox alone and in combination on the phosphorylation of different cell signaling pathways as observed by Western blot analyses in MM cells

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a pERK5 and pCREB present novel pathways first identified in this study b Arrows represent significant (p< 0.05) increases (up arrows) or decreases (down arrows) c All statistical comparisons with Van + Dox are with the Dox alone group. Comparisons involving individual agents are with the control group

Conclusion: Results suggest that a plethora of cell signaling pathways, apparently stimulated by Dox in a stress response are inhibited by Van in MM cells. Moreover, our results highlight the combined efficacy of Van and Dox as a novel multimodality approach in the treatment of MM.

Disclosure: No significant relationships.

VIA.6: A NOVEL THERAPY FOR MESOTHELIOMA USING HVJ-E COMBINED WITH CHEMOTHERAPY.

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Background: Immunotherapy for Malignant pleural mesothelioma (MPM) is expected to be a breakthrough strategy, because it is suitable for the therapy of the disseminated lesions with minimal influence on normal tissues. Various clinical studies of immunotherapy for MPM combined with chemotherapy have carried out, because these procedures lead to more effectiveness due to a large number of tumor antigens. We focused the immunotherapy for MPM using the hemagglutinating virus of Japan envelope (HVJ-E). HVJ-E derived from inactivated replication-defective Sendai virus enhances anti-tumor immunity through activation of effector T cells and natural killer cells and inhibitory of regulatory T cells. The therapy using HVJ-E revealed highly effective eradication of murine colon cancer, renal carcinoma, human prostate cancer, and human glioblastoma in experimental medicine. We evaluated the therapeutic effectiveness of HVJ-E combined with CDDP in human MPM bearing mice.

Methods: Human MPM bearing mouse model: Two million cells of MSTO-211H were injected into the parietal pleura through the lower intercostal space of 5 week-old female CB-17/SCID mice. Three microgram per weight (kg) of CDDP with 1,000 hemagglutination unit (HAU) of HVJ-E, or each material alone, was administrated into pleural cavity in the same maneuver on day8. One thousand HAU of HVJ-E was weekly administrated into subsequently space of mice belonging to HVJ-E group and HVJ-E group until mice death. We compared the survival time of control and test groups using the log-rank test.

Results: Mean survival time of control group, HVJ-E (intrapleural injection at first time and weekly subcutaneous administration) group, CDDP (single injection) group, and HVJ-E combined with CDDP group was 25.5, 38.8, 54.4, and 76.8 days, respectively. Significantly prolonged survival was seen for the HVJ-E combined with CDDP treatment group compared to other treatment groups (p < 0.01, log-rank test).

Conclusion: We demonstrated that a novel immunotherapy using HVJ-E combined with CDDP (single administration into pleural cavity and subsequent weekly subcutaneous administration of HVJ-E) showed enhanced antitumor effect against disseminated MPM and resulted in prolonged survival. These results suggest that HVJ-E combined with chemotherapy represents a potentially useful and convenient strategy for MPM.

Disclosure: No significant relationships.
SESSION VIA  NOVEL THERAPEUTICS: PRECLINICAL STUDIES
SEPTEMBER 14, 2012 14:20-15:50

VIA.7: INTRAVENOUS ADMINISTRATION OF FL118, A SURVIVIN/MCL-1/XIAP/CIAPI2 INHIBITOR, SHOWS HIGHLY EFFECTIVE INHIBITION OF MESOTHELIOMA CELL LINE-ESTABLISHED TUMOR GROWTH IN ANIMAL MODELS

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Background: We have identified and characterized a novel small chemical molecule (designated FL118). Our in vitro studies showed that FL118 selectively inhibits the expression of survivin, Mcl-1, XIAP and cIAP2, while showing no inhibitory effects on control genes. In an intraperitoneal (i.p.) route, the maximum tolerated dose (MTD) of FL118 was found to be about 1.5 mg/kg at a weekly x 4 schedule in a formulation of 0.05 mg/ml FL118, 5% DMSO, 20% Tween 80 and 75% saline, and at this in vivo experimental setting, FL118 showed superior antitumor activity in mouse models of human colon and head-&-neck tumor xenografts. However, the formulation used in the i.p. route is not intravenous (i.v.) compatible and also the i.p. route is not a common route in clinical practice with the exception of ovarian cancer.

Methods: Animal models and animal models of human mesothelioma cancer cell line-established tumor xenograft were used in these studies.

Results: Here, we report the use of a Tween 80-free formulation to test antitumor activity and toxicity (body weight loss) of FL118 via i.v. routes in animal models of human mesothelioma cancer cell line-established tumor xenograft. Important findings are summarized below. First, in contrast to that the MTD for FL118 in the i.p. formulation is 0.2 mg/kg in the daily x 5 schedule (5 doses); 0.5 mg/kg in the every other day for three doses (q2 x 3, 3 doses); and 1.5 mg/kg in the weekly x 4 (4 doses) schedules, respectively, the MTD for FL118 in the Tween 80-free formulation increases 3-7 times without loss of FL118 antitumor activity via i.v. administration of the drug. Specifically, the MTD of FL118 in turn reached 1.5 mg/kg, 1.5-2 mg/kg and 5 mg/kg for daily x 5; q2 x 5 on day 0, 2, 4, 6, 8; and weekly x 4 schedules, respectively. Second, FL118, for the first time, showed highly effective to inhibit mesothelioma tumor growth and result in tumor regression in all three clinical compatible schedules, while the toxicity profile appears to be improved in comparison with the outcome from FL118 in the Tween 80-containing formulation. Specifically, our data showed that FL118 effectively inhibits MSTO-211H-derived tumor growth, induces tumor regression and even achieved a cure in a percentage of human mesothelioma xenograft, while the tumors in control mice without FL118 treatment reached the maximal size (~2000 mg/mm³) allowed by our protocol in less than five weeks. Additionally, FL118 also showed effective inhibition of NCI-H226-established tumor growth. These findings indicate that we developed a clinical suitable formula for FL118 i.v. administration. We also, for the first time, demonstrated that FL118 possesses superior antitumor activity in animal models of human mesothelioma cancer cell line-established tumor xenograft.

Conclusion: These studies would facilitate FL118 further development toward clinical trials and provide a hope to use FL118 for effective treatment of malignant human mesothelioma in clinical practice.

Disclosure: No significant relationships.
Background: Malignant pleural mesothelioma is an incurable disease that commonly relapses after chemotherapy and conventional radiotherapy. Surgeons are increasingly reluctant to perform extrapleural pneumonectomies (EPP) as there is high postoperative morbidity and survival rates are low. We developed a program utilizing new technologies to optimize palliation with high-dose radiotherapy for patients with locally advanced disease who had not had an EPP.

Methods: In 2003 Stevens et al demonstrated that hemithoracic intensity-modulated radiotherapy (IMRT) provided effective adjuvant therapy to minimize locoregional relapses after EPP. We modified their technique for patients with unresected mesothelioma localized to the ipsilateral hemithorax, using 18F-FDG PET scans to outline tumor masses. Previous surgical procedures and chemotherapy were documented. Acute and late toxicities were graded using CTCAE version 4.

Results: Over 9 years, 51 patients received RT to 45-60 Gy, in 16 using 3D-conformal radiotherapy and 35 with IMRT. Patients were aged 45-76 years, mainly males (86%) with right-sided (65%) mesotheliomas. 16% had non-epithelioid histologies and 82% had clinical stage III/IV disease. Five had planned trimodality therapy and 24 had progressed post-surgery. Surgeons are increasingly reluctant to perform extrapleural pneumonectomies (EPP) as there is high postoperative morbidity and survival rates are low. We developed a program utilizing new technologies to optimize palliation with high-dose radiotherapy for patients with locally advanced disease who had not had an EPP.

Conclusion: High-dose hemithoracic RT is recommended to improve locoregional control post-EPP, and should now be considered for selected mesothelioma patients whose lungs remain intact. Advanced radiotherapy techniques will minimize normal tissue toxicities and provide long-term palliation, particularly in those patients whose mesothelioma does not spread beyond the hemithorax.

Disclosure: No significant relationships.

SESSION VIB1  LOCAL AND RADIATION THERAPY  SEPTEMBER 14, 2012 14:20-15:00

VIB1.2: FAILURE PATTERNS AFTER HEMITHORACIC INTENSITY-MODULATED RADIATION THERAPY (IMRT) FOR MALIGNANT PLEURAL MESOTHELIOMA (MPM) WITH TWO INTACT LUNGS

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Background: We recently reported our initial experience with definitive or adjuvant hemithoracic pleural IMRT for MPM patients with two intact lungs. Given the steep dose gradient with IMRT, accurate target delineation is critical. Here, we present a detailed failure pattern analysis in relation to target delineation and radiation fields.

Methods: We retrospectively reviewed the RT plans and failure patterns of 64 patients with MPM treated with definitive or adjuvant pleural IMRT between 04/2005 and 05/2012. All patients had a PET scan available for target delineation. The median dose was 4680cGy in 26 fractions (range 3960cGy – 5040cGy). Three patients received an integrated boost to gross disease to a maximum dose of 5994cGy. Treatment plans were optimized to keep the mean lung dose <21 Gy. Failures were categorized as in-field local failures (LF) (within the 90% isodose line (IDL)), marginal failures (<90% and >50% IDL) and out-of-field failures (outside the 50% IDL). The median age at diagnosis was 66 years (range 42 to 82 years). Forty-nine patients had epithelioid histology, and 51 patients had sarcomatoid or biphasic MPM. Thirty-four patients had right-sided and 30 patients left-sided MPM. Fifty percent presented with early-stage (clinical stage I/II) and 50% with advanced-stage MPM (clinical stage III/IV). Thirty-eight patients underwent a partial or complete pleurectomy/decortication (P/D), while 26 patients were technically or medically unresectable. Fifty-eight patients (91%) received neoadjuvant or adjuvant platinum/pemetrexed-based chemotherapy.

Results: With a median followup of 16 months from diagnosis and 7.4 months from the end of last treatment, the median in-field local failure-free survival was 8.9 months. Thirty-nine in-field LF (61%) were found, with 29 failures occurring in sites of previous gross disease and 10 in a previously grossly uninvolved site. LF was the first site of failure in 19 patients (30%). The only factors associated with higher local failure-free survival were left-sided MPM (p=0.02) and a trend for patients who underwent surgical resection (p=0.07). Response to chemotherapy or RT dose was not significantly associated with local failures. There were 175 marginal failures (20%). Increasing experience over time was correlated with decreasing marginal failures (p=0.03). On review of the imaging studies at time of target delineation, in 6 cases we identified an abnormality that had not been included in the treatment volume. Four cases represented supradiaphragmatic lymph nodes in the costomediastinal recess, and two cases were located along the crus in the costodiaphragmatic recess. Two of these patients experienced an isolated...
local failure. Seven patients (11%) failed in the fissures, but only one patient had an isolated failure in the fissure without pleural failures elsewhere. Twenty-one patients (33%) had out-of-field failures in mediastinal lymph nodes, but none were isolated elective nodal failures.

**Conclusion:** After pleural IMRT most LFs occur in sites of previous gross disease. Thus, surgical resection of sites of gross disease remains important. Increasing experience and improvements in target delineation may decrease the incidence of marginal failures.

**Disclosure:** No significant relationships.
VIB2:1: MISREPRESENTATION OF HISTORICAL FIBER RESEARCH: AN INTERVIEW-BASED STUDY FOR RETROSPECTIVE KNOWLEDGE TRANSLATION OF EPIDEMIOLOGICAL INQUIRY.

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Background: Epidemiological research linking disease outcomes and exposures to fibers dates from the late 1950’s. There is a poor knowledge and comprehension of this research and its vital role among current public health professionals and stakeholders.

Methods: The most prominent researchers from the beginnings of this research were interviewed in person on three continents in 2004 and 2005. Where the researchers had died, information was directly obtained from similar interviews of relatives and co-workers. Interviews were free-form and unstructured. In the period following interviews additional information was obtained from publicly available documents, documents provided by interviewees, and other sources. Court documents and such sources as newspaper or magazine articles were not considered due to the possibility of bias. Information was obtained from Richard Doll, Julian Peto, Dr. Margaret Wagner, Dr. Corbett McDonald, Dr. Arthur Langer, Dr. G. Berry, Dr. G. Hillerdal, Dr. P. Sebastien, and many others involved in the original works.

Results: Information obtained conflicted in many ways from “conventional wisdom”. Original researchers conveyed many regrets about the ways in which past research has been received and acted (or not acted) upon. Researchers were particularly concerned with misrepresentations of their lives and work, and with a general lack of knowledge of what was known in the past in relation to what is believed now. There was an evident lack of knowledge translation to the population in general and to stakeholder groups in particular. Misrepresentation and character assassination were frequent complaints by researchers on all “sides”. This presentation will offer examples of the ways in which the pioneer researchers and seminal pieces of research regarding exposure and disease, particularly in relation to mesothelioma, have been misunderstood, and in some instances, used by for-profit agents or tainted by ideological bias.

Conclusion: 1. There is poor knowledge among current health practitioners and researchers about historical research development for the epidemiology of asbestos-related diseases. 2. This is partly generational, and can be addressed through “retrospective knowledge translation”, conveyed to health professionals and through them to stakeholders and the broader public. 3. Lack of knowledge has led to mistrust of epidemiological research for fiber-related research, due in part to high numbers of publications with varying methodology and motivation over more than a half century. 4. There are three principal reasons for the above problems: (a) Ideological bias and a system of “fixed beliefs”. These can be alleviated through a decrease in mutually exclusive activities of stakeholders and increased cooperation. (b) Lack of knowledge of the facts and results of past research. This is the most difficult problem to address. “Retrospective knowledge translation” activities such as this study are needed. (c) Financial bias, particularly in American asbestos litigation, a for-profit activity not constrained by normal academic requirements and related in part to the contingency-fee basis of such activity. This extends to funding of scientific assemblies, including those of iMig, by law firms with financial interests in the promotion of ideas which further their own purposes. Such sponsorship should no longer be accepted by scientific assemblies.

Disclosure: No significant relationships.
Session VIC1
Gene Expression Profiling
SEPTEMBER 14, 2012 14:20-15:00

SESSION VIC1  GENE EXPRESSION PROFILING  SEPTEMBER 14, 2012 14:20-15:00

VIC1.2: COMPARATIVE GENE EXPRESSION ANALYSIS OF EX-VIVO FINE NEEDLE ASPIRATION BIOPSIES AND MATCHED SOLID TISSUES IN MALIGNANT PLEURAL MESOTHELIOMA

Assunta De Rienzo1, Yaoyu E. Wang2, William G. Richards1, David J. Sugarbaker1, Raphael Bueno1
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Background: FNA biopsy, whether image-guided or by direct palpation, is a useful diagnostic minimally invasive method for patients with presumed neoplasms. However, the accuracy, specificity and sensitivity of the cytological interpretation of FNA may be limited in a variety of circumstances such as target size, tumor type, accessibility etc. One major limitation is the need for intact recognizable tumor cells and architecture for definitive histological typing and diagnosis. Previously, we have shown that FNA biopsies are a feasible tool to perform molecular tests in MPM and lung cancer, however, a systematic comparative analysis of expression data between FNAs and matched solid tumors in thoracic malignancies has not been conducted so far.

Methods: We performed whole genome expression of 192 samples including 164 Mesothelioma (MPM) solid tumors, 8 matched epithelioid MPM ex-vivo FNAs, 8 adenocarcinoma ex-vivo FNAs, 8 squamous cell carcinoma ex-vivo FNAs, and 4 controls using the Affymetrix Human Gene 1.1 ST Array. The samples that passed quality control were quantile normalized to reduce array specific biases. To determine whether different sample sets were grouped together or separated in different branches, significant differentially expressed genes between epithelioid and sarcomatoid MPMs were identified using linear model (q<0.01 and fold change>2), and their gene expression profiles were employed for clustering analysis. Finally, to determine whether the MPM FNA gene expression was comparable to their matched solid tissues, we performed principle component analysis and compared Euclidean distances between each MPM FNA and its matching tumor tissue with randomly chosen samples.

Results: Whole array analysis was performed and 4 outliers in the MPM solid tumors group were excluded for further analysis. Forty-two probes were found differentially expressed between epithelioid and sarcomatoid MPM samples. Hierarchical clustering using these probes resulted in a tree structure where all the MPM FNAs were grouped with the epithelioid MPM samples showing that the matched samples grouped in the same branch. In addition, the principle components analysis showed that all MPM FNAs clustered with the epithelioid MPM, whereas the sarcomatoid MPM and lung cancer FNAs clustered into two different groups. The Euclidean distance between each FNA biopsy and its matched tumor tissue was significantly smaller compare to the distance between randomly selected tumor tissues suggesting that the matching samples exhibit similar gene expression profile. Finally, only 11 probes corresponding to 9 unique genes were found differentially expressed between MPM FNAs and matched normal tissues indicating that the expression profile of the matched pair is comparable.

Conclusion: Our study shows that the ex-vivo FNA biopsies have gene expression comparable to the corresponding solid tissues, suggesting that FNA biopsies from thoracic malignancies can be used for molecular studies.

Disclosure: No significant relationships.
pathways specifically activated in MPM. Insights into the molecular basis of MPM may facilitate a personalized treatment approach involving early identification of poor prognostic indicators that may reduce the heterogeneity of the clinical response and lead to more focus treatments.

Disclosure: No significant relationships.
VIC.2:1: MITHRAMYCIN IS A NOVEL THERAPEUTIC AGENT FOR MALIGNANT PLEURAL MESOTHELIOMA

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Results: Pleural mesothelioma lines exhibited significantly higher Sp1 expression levels relative to cultured normal mesothelial cells. IHC analysis demonstrated over-expression of Sp1 in 73% (14/19) of MPM specimens relative to normal pleura controls (n=3). Knock-down of Sp1 as well as MM treatment significantly inhibited proliferation and clonogenicity of MPM cells. Intraperitoneal MM administered at either 1 mg/kg or 2 mg/kg every other day mediated dose dependent growth inhibition and regression of established subcutaneous MPM xenografts (p <0.02 relative to saline controls; figure 1). These effects coincided with dramatic alterations in global gene expression profiles; a gene expression signature corresponding with response to MM was identified.

Disclosure: No significant relationships.

VIC.2:2: DRUG SENSITIVITY SCREENING OF SHORT-TERM PRIMARY TUMOR CULTURES OF MALIGNANT PLEURAL MESOTHELIOMA

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1Thoracic Oncology, Nki-Avl, Amsterdam/NETHERLANDS, 2Cell Biology, Nki-Avl, Amsterdam/NETHERLANDS

Background: Malignant pleural mesothelioma (MPM) is a cancer with a poor prognosis. Only 40% of patients respond to combination chemotherapy (cisplatin and pemetrexed) in first line setting and survival benefit is limited. No predictive markers are available yet to identify patients that may benefit from chemotherapy. Assessing in vitro chemotherapeutic drug resistance has been demonstrated to be feasible using MPM resection specimens in a commercial assay. This assay included only 3 different drugs, excluding pemetrexed and combinations of drugs. However, obtaining resection specimens is not feasible for all patients. Furthermore, a chemosensitivity and resistance assay should at least include the standard chemotherapy regimen and be able to evaluate combinations of drugs. Our goal was to develop robust protocols to use tumor cells from pleural fluid of MPM patients for chemosensitivity and resistance testing.

Methods: Cells are isolated from pleural fluid, drawn from patients with MPM for symptom relief. The cells are cultured under low oxygen conditions for a period of 4 weeks and grow in adherent monolayers. Cultures contain...
both tumor cells and stroma cells. Cell morphology, viability and tumor percentage are assessed by cytopathological staining using Giemsa, anti-pankeratine to check for epithelial phenotype and Ki-67 to assess proliferation rate at each passage. During week two and three of culture, drug sensitivity was measured. Cells are plated and incubated with an 8 point concentration range of 5 single drugs and 2 two-drug combinations for 48 hours. Cell viability is determined by the Cell Titer Blue assay. Each concentration point is measured in triplo and a biological duplo experiment is performed to check reproducibility.

Results: Fourteen out of 20 isolations (70%) resulted in successful cultures. Ninety percent of patients had epithelioid subtype and 10% mixed subtype. Tumor percentage varied between 40-70% at isolation and increased up to 80-90% after the first passage. Percentage Ki-67 positive cells varied between 15% and 60% between individual cultures. Anti-pankeratine staining was positive at all passages indicating that tumor cells maintain their epithelioid phenotype and no epithelial-mesenchymal transition occurs during culture. Drugs tested for were cisplatin or carboplatin, pemetrexed, gemcitabine, vinorelbine, oxaliplatin and a combination of cisplatin and pemetrexed and of oxaliplatin and gemcitabine. Drug screening was performed in seven different primary tumour cultures and demonstrated to be reproducible. Dose-response curves showed different sensitivity to the various drugs for the different primary tumour cultures. One patient had drainage of pleural fluid at two different time points and tumor cells were cultured twice. Results of both drug screens were similar. Two patients that demonstrated resistance to the combination of cisplatin and pemetrexed in vitro, had progressive disease after three courses of this chemotherapy regimen in first line treatment.

Conclusion: Short-term primary tumor cultures from pleural fluid of mesothelioma patients can be generated with high success rate. A chemosensitivity and resistance assay with these short-term primary tumor cultures is feasible. These cultures may be suitable for pharmacogenomic profiling using high-troughput drug screens.

Disclosure: No significant relationships.

SESSION VIC2  NOVEL THERAPEUTICS: PRECLINICAL STUDIES SEPTEMBER 14, 2012 15:00-15:50

VIC2.3: STRATEGIES FOR THE PREVENTION OF MESOTHELIOMA IN MEXTAG MICE

Cleo Robinson, Samantha Woo, Kelly Martinovich, Amy Walsh, Anna K. Nowak, Richard Lake
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Background: Current treatments for mesothelioma typically increase median survival by a matter of months. Progress in treatment has been hampered by lack of a suitable small animal model, which could guide clinical advances, given limited numbers of patients eligible for clinical trials. To this end we recently developed a transgenic mouse model of mesothelioma in which the viral oncogene, SV40TAg (Tag) is directed to mesothelial cells by use of the cell type specific mesothelin promoter. Mextag mice develop mesothelioma rapidly and uniformly, but only following exposure to the natural carcinogen, asbestos. The model closely mimics the human disease and is thus ideal for both rapid analysis of novel therapeutic studies and for investigating factors that might act synergistically with asbestos to cause disease. Since all Mextag mice develop mesothelioma following asbestos exposure the model is highly suitable for early intervention and cancer prevention studies. An effective cancer prevention strategy for the millions of people who have been exposed to asbestos could have enormous benefit worldwide. Epidemiological evidence indicates that supplementation with some dietary factors or use of common drugs such as statins and non-steroidal anti-inflammatory drugs is associated with a lower incidence of cancer. We previously reported that dietary supplementation with a number of antioxidants did not alter the time to develop disease nor overall survival, despite the widely accepted hypothesis that asbestos catalyzed production of reactive oxygen and nitrogen species contribute to the development of this cancer.

Methods: We have extended these studies to test whether vitamin D, non-steroidal anti-inflammatoryatories, statins and some other candidate diets could alter the pattern of disease in the Mextag model. Supplemented diets were provided at levels based on published data and began 2 weeks prior to asbestos exposure in order to maximize our chance of detecting a benefit.

Results: We found Vitamin D, apigenin, lupin, linseed and diallyl trisulphide (garlic derivative) did not alter the profile of disease. Aspirin was tested at low and high levels (6 or 25 mg/kg/day). Again, this did not affect the rate of disease development or progression. Similarly, three different concentrations of statins in diets (10, 20 and 40 mg/kg/day) had no effect on the development of disease.

Conclusion: In conclusion, we think it is unlikely that antioxidants, anti-inflammatoryatories or other nutrient-specific diets will moderate the rate of mesothelioma in asbestos exposed populations.

Disclosure: No significant relationships.
to tumor tissues in vivo than to normal tissues, resulting in increased contact time between drug-eluting eNP and sites of tumor in vivo. Future studies will assess the efficacy of intrapleural delivery of pax-eNP for the control of local recurrence following pneumectomy for the treatment of MPM.

Disclosure: No significant relationships.
**P1.01: IS THERE LIFE AFTER MARS? DOES EXTRAPLEURAL PNEUMONECTOMY STILL HAVE A ROLE IN THE MANAGEMENT OF MALIGNANT PLEURAL MESOTHELIOMA - A 13 YEARS SINGLE CENTRE EXPERIENCE.**

**Alex Cale**1, Suhail Qadri2, Michael Cowen3, Andrezej Wieczorek4, Michael J. Lind4
1Cardiothoracic Surgery, Castle Hill Hospital, 5jq/UNITED KINGDOM, 2Castle Hill Hosp/UNITED KINGDOM, 3Castle Hill Hospital/UNITED KINGDOM, 4Postgraduate Medical Institute, University Of Hull, Hull/UNITED KINGDOM

**Background:** The MARS trial has shown no survival benefit of extrapleural pneumonectomy for the treatment of malignant mesothelioma. We aim to present our results which are in contrast to the MARS trial results.

**Methods:** Patients who underwent extrapleural pneumonectomy for malignant mesothelioma between March 1999 and April 2012 were analysed retrospectively and their survival was observed until May 2012. Risk was calculated by using thoracoscore.

**Results:** Thirty patients underwent extrapleural pneumonectomy during this period. Median age was 59±8 years, with 29 male and 1 female. The mean thoracoscore was 7.9±2.5. There was no in-hospital or 30 day mortality. Overall mean survival was 25 months; increasing to 38 months for those that completed tri-modality treatment. Eight patients survived over 4 years and two over 5 years. Survival was significantly higher in patients who underwent extrapleural pneumonectomy for malignant mesothelioma and negative extral mediastinal, pericardial and transdiaphragmatic invasion. All patients who were sampled from >20 sites at mediastinal, apical, lateral costal and costodiaphragmatic areas. Pericardial and transdiaphragmatic invasion were evaluated as well. If no microscopic invasion was noted in the areas above, the resection was accepted as a microscopic complete resection. Microscopic margin positivity in more than one area was evaluated as a prognostic factor. Recurrence rate and sites of recurrences were recorded. Data was evaluated using uni- and multivariate and Kaplan Meier survival analysis.

**Results:** A total of 25 patients (average age 52 [34-70], 11 females) were evaluable. Macroscopic complete resection was achieved in all patients. 23 patients completed adjuvant hemithoracic irradiation and 3 cycles of platin based chemotherapy. Median survival in the whole group was 25.7 months (15% at 7-year). 12 patients had extrapleural lymph node metastasis. Microscopic complete resection was achieved in 5 patients. Microscopic mediastinal, apical, lateral costal, costodiaphragmatic, pericardial and transdiaphragmatic invasion was present in 9, 9, 14, 3, 3 and 2 patients respectively. Extrapleural lymph node metastasis (p=0.06) and microscopic margin positivity in more than one area (p=0.04) were significant factors in univariate analysis. In multivariate analysis, only microscopic margin positivity in more than one area (p=0.04) was the significant factor. Peritoneal or abdominal wall recurrence (8 patients) was common in patients with microscopic complete resection or positivity only in one area (n=12).

**Conclusion:** Macroscopic complete resection may be the goal of MPM surgery, but microscopic complete resection appears to influence survival significantly. Thus every effort should be made to achieve complete resection margins in patients with epithelial MPM.

**Disclosure:** No significant relationships.

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**P1.03: MICROSCOPIC MARGIN POSITIVITY IN MORE THAN ONE AREA IN THE THORACIC CAVITY IS A POOR PROGNOSTIC FACTOR FOLLOWING EXTRAPLEURAL PNEUMONECTOMY IN PATIENTS WITH EPITHELIAL MALIGNANT PLEURAL MESOTHELIOMA**

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**Background:** The main principle of surgery in malignant pleural mesothelioma (MPM) is macroscopic complete resection. Adjuvant treatments are utilized to control microscopic invasions or micrometastasis. The impact of microscopic margin positivity on survival and recurrence is not known and thus we analyzed this issue in patients who underwent extrapleural pneumonectomy (EPP) for epithelial MPM.

**Methods:** The patients with epithelial MPM who underwent EPP over a period of 7 years were included in the study. Patients with non-epithelidal histology (n=5), postoperative mortalities (n=5), patients who died of other causes (n=5) were excluded from the study. Post surgical EPP specimens were sampled from >20 sites at mediastinal, apical, lateral costal and costodiaphragmatic areas. Pericardial and transdiaphragmatic invasion were evaluated as well. If no microscopic invasion was noted in the areas above, the resection was accepted as a microscopic complete resection. Microscopic margin positivity in more than one area was evaluated as a prognostic factor. Recurrence rate and sites of recurrences were recorded. Data was evaluated using uni- and multivariate and Kaplan Meier survival analysis.

**Results:** Patients who underwent extrapleural pneumonectomy for malignant mesothelioma between March 1999 and April 2012 were analysed retrospectively and their survival was observed until May 2012.

**Results:** A total of 25 patients (average age 52 [34-70], 11 females) were evaluable. Macroscopic complete resection was achieved in all patients. 23 patients completed adjuvant hemithoracic irradiation and 3 cycles of platin based chemotherapy. Median survival in the whole group was 25.7 months (15% at 5-year). 12 patients had extrapleural lymph node metastasis. Microscopic complete resection was achieved in 5 patients. Microscopic mediastinal, apical, lateral costal, costodiaphragmatic, pericardial and transdiaphragmatic invasion was present in 9, 9, 14, 3, 3 and 2 patients respectively. Extrapleural lymph node metastasis (p=0.06) and microscopic margin positivity in more than one area (p=0.04) were significant factors in univariate analysis. In multivariate analysis, only microscopic margin positivity in more than one area (p=0.04) was significant factor. Peritoneal or abdominal wall recurrence (8 patients) was common in patients with microscopic complete resection or positivity only in one area (n=12).

**Conclusion:** Macroscopic complete resection may be the goal of MPM surgery, but microscopic complete resection appears to influence survival significantly. Thus every effort should be made to achieve complete resection margins in patients with epithelial MPM.

**Disclosure:** No significant relationships.

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**P1.05: OUTCOME OF CONSECUTIVE 61 CASES OF INTENT-TO-TREAT WITH EXTRAPLEURAL PNEUMONECTOMY FOR RESECTABLE MALIGNANT PLEURAL MESOTHELIOMA**

**Seiki Hasegawa**1, Fumihiro Tanaka1, Nobuyuki Kondo1, Yoshitomo Okumura1, Seiji Matsumoto1, Teruhisa Takeo1, Masaki Hashimoto1, Hayato Orui1, Ayumi Kuroda1, Shunichi Fukuda1, Kazue Yoneda1, Noriaki Tsunoda1, Norihiko Kamikonya1, Kazuya Fukuoka1, Ikuko Torii1, Tohru Tsujimura1, Takashi Nakano1
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**Background:** The role of surgery in patients with resectable malignant pleural mesothelioma (MPM) remains to be a matter to debate. Only a prospective randomized study comparing surgery versus no-surgery can provide an answer but is almost unrealistic. The second best way is an intent-to-treat based analysis on prospectively registered patients.

**Methods:** We reviewed all patients in a prospective database of extrapleural pneumonectomy (EPP) program of Hyogo College of Medicine between July 2004 and March 2012. Patients were eligible if they had a histologically confirmed MPM of any type, clinical stage T1-3N0-M0 disease, PS 0-1, and no major comorbidity. In addition of routine preoperative chest CT, brain MRI, FDG-PET, and pulmonary V/Q scan, extended surgical staging was performed in 21 patients.

**Results:** A total of 61 patients (M/F 51/10, median age 62, range 37-71) were enrolled into EPP program after detailed evaluation by multidisciplinary team. Histology was epithelioid (n=53), biphasic (n=3), sarcomatoid (n=3), small cell mesothelioma (n=1), and unidentified (very early, n=1). Clinical stage of 61 ITT patients and pathological stage for 45 EPP patients were as follows: c-stage I/II/III 18/28/15, and p-stage I/II/III/IV 3/10/28/4. Three patients underwent EPP without preoperative treatment, and they received either of chemotherapy or radiotherapy postoperatively. The remaining 58 received induction chemotherapy with cisplatin plus pemetrexed (n=49) or other regimens (n=9). Fifty-seven patients (69% of ITT) received either of chemotherapy or radiotherapy. All but one operation were performed by one surgeon (SH). Surgical mortality rate at 30- and 90-days were 2.0% (1/51, bleeding, POD15) and 5.9% (ARDS, POD48 and 72). Surgical morbidity (CTCAE grade > 3) occurred in 9 patients (17.6%). The median, 2-year, and 5-year survivals for ITT population (n=61) were 25.2 months, 52.3%, 28.1%, and 0% for no-EPP (n=16), respectively.

**Conclusion:** EPP is a highly invasive surgery but is feasible when performed by dedicated multidisciplinary team. Although better survival was observed in no-EPP patients until 1.5 years after diagnosis, long-term survival was seen only in EPP patients.

**Disclosure:** No significant relationships.

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**Background:** Immunotherapy approaches have improved endogenous anti-tumor immune responses in patients with minimal tumor burden; however, results are less dramatic in bulky tumor states. One potential explanation is that bulky tumors develop complex immunosuppressive networks. Cytoreductive surgery has been shown to improve immunotherapy potency in bulky tumor states; however, an exact mechanism remains unclear. We hypothesized that cytoreductive surgery reduces these immunosuppressive networks and restores the potency of immunotherapy in models of malignant mesothelioma.

**Methods:** To test this hypothesis, a vaccine against the common mesothelioma antigen, mesothelin, (Listeria.mesothelin) was tested in several syngeneic, murine models of mesothelioma. Cytoreductive surgery was performed following treatment of advanced tumors. Mechanistic underpinnings were investigated using flow cytometry, in vivo leukocyte depletion methods, in vivo tumor neutralization assays and ELISAs.

**Results:** Mesothelioma vaccines were effective in treating small mesothelioma cancers, but had little anti-tumor effects in bulky cancer states. Interestingly, in bulky disease scenarios, surgical cytoreduction unmasked the anti-tumor potency of vaccine approaches. Immune mechanisms that explained restoration in anti-tumor immune responses included reduced myeloid derived suppressor cell populations, decreased immunosuppressive cytokine (IL-6, G-CSF, IL-10) levels, and augmented intratumoral CD8 T-cell trafficking.

**Conclusion:** This study provides the first report of a mechanism for the synergy between cytoreductive surgery and immunotherapy. This study also demonstrates that surgical resection combined with immunotherapy may be a rational therapeutic option for patients with malignant mesothelioma.

**Disclosure:** No significant relationships.

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**Background:** The role of lung sparing surgery to prolong survival in the management of Malignant Pleural Mesothelioma (MPM) is yet to be confirmed. Video Assisted Thoracoscopic Debulking Surgery (VATS PD) has a role in symptom control and may prolong survival. Our aim was to determine whether Extended Pleurectomy Decortication (EPD) conveys any survival benefit over VATS PD.

**Methods:** From a prospective database we identified 224 patients (191 male) who underwent EPD (142) and VATS PD (82 patients) over 14 years. The patients in the EPD group were younger (mean 61.6 years) than the VATS group (mean 67.3, p<0.05). Cell type was similar (108 Epithelioid and 34 biphasic in the EPD group, 68 Epithelioid and 14 biphasic in the VATS group, p=0.24). We tested for differences in survival between the groups using selected subgroups.

**Disclosure:** No significant relationships.
**Results:** Median follow up was 11 (1-90) months. 30 and 90 day mortality was similar between the groups (4.9% EPD vs 4.8% VATS PD, p=1 and 10.6% vs 9.7%, p=1 respectively).

From the data available approximately 50% had chemotherapy in both groups (p=0.57).

Overall survival was similar between the groups: Median 14.4 (SE 1.7, 95% CI 11-18) months) for EPD compared to 13 (SE 1, 95% CI 11-15) months for VATS, p=0.149.

From the EPD group we identified a smaller subgroup of node negative patients (n=45): The survival of this subgroup was slightly better (median 16, SE 3.3, 95% CI 9-23 months), p=0.059 than for VATS.

In patients with Epithelioid disease we observed marginally better survival in the EPD group (n=108, median 18.7, SE 3, 95% CI 13-24 months) compared to VATS PD (n=68, median 14.1, SE 1.2, 95% CI 12-16.4 months), p=0.079.

In contrast, in patients with Bifascial histology, the extent of surgery had no effect on survival (EPD n=34, median 10, SE 2.5, 95% CI 7.2-14 months) versus VATS PD n=14, median 8, SE 2.5, 95% CI 5.6-10 months, p=0.67).

It was only the smaller subgroup of Epithelioid node negative EPD (n=28) that demonstrated significantly better survival (median 27, SE 9, 95% CI 14.8-46.6 months) compared to Epithelioid VATS PD (n=68, median survival 14.1, SE 1.2, 95% CI 11.8-16.4 months), p=0.012.

**Conclusion:** In most patients with mesothelioma, VATS debulking is all that is indicated. Only node negative patients with Epithelioid disease can benefit from the increased survival conveyed by EPD. This has many clinical implications including accurate histopathologic typing and invasive preoperative staging of the disease to exclude nodal disease.

The survival demonstrated in the Biphasic subgroup suggests that the behaviour of this type closely resembles the one demonstrated by Sarcomatoid MPM.

**Disclosure:** No significant relationships.

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**SITE OF RECURRENCE**

<table>
<thead>
<tr>
<th>SITE OF RECURRENCE</th>
<th>N</th>
<th>% of All Patients</th>
<th>% of Recurrences</th>
</tr>
</thead>
<tbody>
<tr>
<td>IHT* and/or Mediastinum</td>
<td>42</td>
<td>71 %</td>
<td>95 %</td>
</tr>
<tr>
<td>Abdomen</td>
<td>9</td>
<td>15 %</td>
<td>20 %</td>
</tr>
<tr>
<td>Contralateral</td>
<td>7</td>
<td>12 %</td>
<td>16 %</td>
</tr>
<tr>
<td>Hemithorax</td>
<td>1</td>
<td>2 %</td>
<td>2 %</td>
</tr>
</tbody>
</table>

*IHT: ipsilateral hemithorax Twenty patients (45% of recurrences) failed in the ipsilateral hemithorax (IHT) only and 29 patients (66% of recurrences) failed in the ipsilateral hemithorax and/or mediastinum only. The most common specific sites of recurrence in the IHT were neo-pleural mass (71% of recurrences) and chest wall mass (36%); in the mediastinum they were lymph nodes (100%), in the abdomen were abdominal mass (60%), retroperitoneal nodes (30%), and ascites (20%); in the CHT were lung nodules (57%) and pleural mass (29%); and the single distant recurrence was in bone.

**Conclusion:** Most recurrences following pleurectomy for mesothelioma occur in the ipsilateral hemithorax. Future strategies should focus on methods to intensify local treatment such as HIPEC, photodynamic therapy, systemic chemotherapy, radiotherapy or more aggressive resection techniques.

**Disclosure:** No significant relationships.
P1.09: THERMAL THERAPY IN THE TREATMENT OF MALIGNANT PLEURAL MESOTHELIOMA

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Background: Thermal therapy, both hyper- and hypothermia, has been used in cancer therapy for decades and hyperthermic chemotherapy perfusion, specifically, has been used in the treatment of mesothelioma without data as to the optimal conditions. We sought to define in vitro the most effective strategy for the use of thermal therapy in mesothelioma.

Methods: Growth of various in vitro established cell lines, including a hyperthermia-sensitive Chinese Hamster Ovarian cell line (CHO-K1), a normal lung fibroblast line (MRC-5), a lung cancer line (A549), and three human mesothelioma cell lines (NCI-H28, NCI-H2052, and M5-211H) exposed to varying hyper- and hypothermic conditions was investigated using either a standard metabolic MTS absorbance assay at 450 nm or a standard clonogenic assay, enumerating colony-forming units >50 cells. Each cell line was expanded in flasks and then exposed to varying combinations of hyperthermia (37.45°C, hypothermia (0-18°C), and/or chemotherapy. Cells were harvested and assays performed. Chemotherapeutic agents used were cisplatin, gemcitabine, and pemetrexed.

Results: Initially, a dose-response curve was generated using hyperthermia alone in CHO-K1, A549, and NCI-H28 cell lines heated to 37°, 42°, and 45° for 20, 40, and 60 mins. This showed a reproducible dose-response effect in CHO-K1 cells reaching a survival nadir of 1.5% of controls at 45° for 60° (p<0.01). A549 cells and NCI-H28 mesothelioma cells showed similar less dramatic responses however NCI-H28 mesothelioma cell growth was only reduced a modest 65% at 45° for 60° (p<0.01). Comparison of two assays showed that measurement of metabolic activity (MTS assay) failed to demonstrate the differences documented by the clonogenic assay and therefore was the MTS assay was abandoned. The addition of chemotherapy to hyperthermia was then assessed. Doses were chosen based on prior pharmacokinetic data from studies showing a maximum tissue/blood level of 200 ng/ml for cisplatin pleural instillation and were felt to more accurately reflect actual tumor tissue levels. In these studies, the clinically-relevant limit of 42° was used for hyperthermia. Cisplatin alone reduced the clonogenic potential modestly to 26%, 16.4%, and 13.6% at 42° for 60° (p<0.01); however, this was only a further reduction of 29.6%, 33.8%, and 34.2%, respectively, from the cisplatin alone control. Therefore, most of the reduction was attributable to chemotherapy not hyperthermia. With combinations of cisplatin/gemcitabine and cisplatin/pemetrexed the effect was greater, with reduction to 9.6%, 0%, and 0% (p<0.01) (incremental reduction of only 16.5%, 0%, and 0%, respectively from hyperthermia). Cisplatin/pemetrexed produced essentially identical results. The effect of hypothermia alone was then assessed. Exposure to freezing temperature (-80°C) reduced the clonogenic potential of all cell lines, including the 3 mesothelioma lines, in a dose-dependent manner by >95% in as little as 5 minutes (p<0.01). Further, no particular sensitivity was noted for mesothelioma cell lines (compared to other cells) to hyper- or hypothermia or even chemotherapy.

Conclusion: Chemotherapy appears to be most effective when using two drug combinations. Thermal therapy appears to be most effective when using hyperthermia rather than hyperthermia and this modality warrants further investigation.

Disclosure: No significant relationships.
Background: Replication-competent retrovirus (RCR) vectors have been shown to achieve significantly enhanced tumor transduction efficiency and therapeutic efficacy in various cancer models. In the present study, we investigated RCR vector-mediated suicide gene therapy for the treatment of malignant mesothelioma, a highly aggressive tumor with poor prognosis.

Methods: To evaluate the utility and efficiency of RCR vectors for gene delivery to mesothelioma cells, RCR-GFP vector, expressing the green fluorescent protein (GFP) marker gene, was first tested on a panel of human malignant mesothelioma and non-malignant transformed mesothelial cell lines in vitro. Transduction efficiency and replicative spread of the RCR vector over time was monitored by flow cytometry and in vivo imaging. Next, to evaluate the potential of RCR vector-mediated suicide gene therapy for this malignancy, we employed RCR-GD, expressing the yeast cytotoxic protein (yCD) suicide gene. Following administration of the prodrug 5-fluorocytosine (5FC), cytotoxicity against RCR-yCD infected malignant mesothelioma cells was assessed in vitro by Alamar blue assay, and tumor growth inhibition effects in vivo were assessed in both subcutaneous xenograft tumor and disseminated peritoneal tumor models of malignant mesothelioma.

Results: RCR-GFP vector successfully infected and efficiently replicated in human malignant mesothelioma cell lines, as compared with non-malignant mesothelial cells in vitro. In mice with pre-established subcutaneous tumor xenografts, RCR-GFP vector showed robust spread throughout entire tumor masses after intratumoral administration. Next, RCR-GD, expressing the yeast CD suicide gene, showed efficient transmission of the suicide gene associated with replicative spread of the virus, resulting in efficient killing of malignant mesothelioma cells in a prodrug 5FC dose-dependent manner in vitro. After a single intratumoral injection of an RCR-GD followed by intraperitoneal administration of 5FC, 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.

Conclusion: These data indicate the potential utility of RCR vector-mediated suicide gene therapy in the treatment of malignant mesothelioma.

Disclosure: No significant relationships.
cutaneous (7%), renal (4%) and hepatic (4%). All adverse events were reversible and no death due to therapy toxicity was reported.

**Conclusion:** These results show that including MPM pts in phase I trials beyond first line of treatment can result in clinical benefits with an acceptable toxicity profile. Several molecular pathways involved in MPM have been identified and further novel biologic therapies might be tested in a phase I setting in a biology-oriented approach rather than a stochastic one. We are currently offering a tumour molecular profiling in our pts (MOSCATO trial) for a better selection of phase I trials.

**Disclosure:** No significant relationships.

**POSTER SESSION 1 SEPTEMBER 12, 2012 11:30-12:30**

**P1.14: FAK INHIBITOR VS-4718 PREFERENTIALLY ATTENUATES CELL GROWTH OF MALIGNANT MESOTHELIOMAS WITH NF2 MUTATION: ROLE OF CANCER STEM CELLS**

*Irina M. Shapiro*, *Vihren N. Kolev*, *Christian M. Vidal*, *Mitchell Keegan*, *Qunli Xu*, *Craig Menges*, *Joseph Testa*, *Jonathan A. Pachter*

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**Background:** Malignant pleural mesothelioma (MPM) is an aggressive tumor in the pleural lining of the lung often caused by asbestos exposure. MPM patients are usually diagnosed at an advanced stage of the disease and the prognosis is poor. Median survival after diagnosis is 9 to 12 months and standard-of-care agents such as cisplatin and pemetrexed are relatively ineffective in increasing median survival time for MPM patients. New therapeutic modalities are urgently needed to improve the prognosis of MPM patients. Neurofibromatosis 2 (NF2) is a tumor suppressor gene whose loss of function contributes to the tumorigenesis of MPM, and NF2 loss is detected in about 40% of cancer tissues. Several molecular targets involved in NF2-related tumor growth have been identified, and many of them have been explored as promising targets for MPM therapy.

**Methods:** To test the potential of the selective FAK inhibitor VS-4718 in MPM cell lines with NF2 mutation, we evaluated cell growth of a panel of MPM cell lines with wild-type or mutated NF2. Cell lines were treated with VS-4718 to study the cell growth and viability in a panel of MPM cell lines with wild-type or mutated NF2.

**Results:** VS-4718 was evaluated in a panel of MPM cell lines with wild-type or mutated NF2. Mutant NF2 MPM cells were found to be especially sensitive to the FAK inhibitor VS-4718 with EC50 values below 100 nM, in contrast to wild-type NF2 MPM cell lines which were less sensitive to VS-4718. Ectopic expression of a non-phosphorylatable artificial mutant of NF2 (NF2-S518A) in NF2 mutant MPM cells abolished the enhanced sensitivity to VS-4718, confirming the hypothesis that Merlin loss mediates sensitivity to VS-4718. Interestingly, MPM cell lines were found to have sub-populations of ALDEFLUOR+ CSCs. Furthermore, the FAK inhibitor VS-4718 induced a significant reduction in the percentage of CSCs in contrast to the standard-of-care agent pemetrexed which enriched the CSC population.

**Conclusion:** In summary, our results indicate that the FAK inhibitor VS-4718 is especially potent in NF2-mutated MPM tumor cells, and that NF2 status may be a valuable stratification marker for VS-4718 response. Furthermore, cancer stem cells in NF2 mutant mesothelioma appear to be particularly resistant to pemetrexed, but sensitive to VS-4718. We believe that these data support the clinical development of the selective FAK inhibitor VS-4718 for treatment of NF2-mutated malignant mesothelioma.

**Disclosure:** I am an employee and a stockholder of Verastem, Inc.
**P1.16: LUNG AND DISEASE VOLUME MEASUREMENTS AS MARKERS FOR PATIENT RESPONSE IN MALIGNANT PLEURAL MESOTHELIOMA**

Zacariah E. Labby1, Anna K. Nowak2, H.L. Kindler3, Samuel G. Armato4

1Department Of Radiology, The University Of Chicago, Chicago/IL/UNITED STATES OF AMERICA, 2School Of Medicine And Pharmacology, University Of Western Australia, Perth/WA/AUSTRALIA, 3Department Of Medicine, The University Of Chicago, Chicago/IL/UNITED STATES OF AMERICA

**Background:** The current standard for image-based response assessment in mesothelioma is the Modified RECIST measurement technique with changes classified according to the standard RECIST criteria. The purpose of this study was to investigate continuous changes in volumetric measurements during treatment as a marker of response for mesothelioma patients. Both disease volumes and lung volumes, a physiological correlate of disease volumes, were investigated in this study.

**Methods:** Serial follow-up CT scans were retrospectively obtained during the course of clinically standard chemotherapy for 61 patients in this IRB-approved study. For each of the 216 CT scans the aerated lung volumes were segmented using a fully automated method, and the pleural disease volume was segmented using a semi-automated method. Diseased (ipsilateral) lung volumes were normalized by the respective contralateral lung volumes to account for differences in respiratory phase between the scans of each patient. Relative changes in each measurement technique from baseline were tracked over the course of serial follow-up imaging. Survival modeling was performed using time-varying Cox proportional hazards models.

**Results:** Median survival from baseline imaging prior to treatment initiation was 12.7 months (95% confidence interval, 10.2–15.3 months). Over the course of treatment, disease volume decreased by an average of 19% from baseline, and normalized lung volume increased an average of 8% from baseline. When discretized as lung volume gain versus lung volume loss from baseline were tracked over the course of serial follow-up imaging. Relative changes in each measurement technique from baseline were tracked over the course of serial follow-up imaging. Survival modeling was performed using time-varying Cox proportional hazards models.

A strong negative correlation was observed between relative changes in lung volumes and disease volumes during chemotherapy for patients with mesothelioma indicates that decreasing lung volume and increasing disease volume are significantly and independently associated with poor patient prognosis.

**Conclusion:** Analysis of measurement trajectories of lung volumes and disease volumes during chemotherapy for patients with mesothelioma indicates that decreasing lung volume and increasing disease volume are significantly and independently associated with poor patient prognosis.

**Disclosure:** No significant relationships.

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**P1.17: OBSERVER VARIABILITY IN MESOTHELIOMA TUMOR THICKNESS MEASUREMENTS**

Samuel G. Armato1, Roslyn Francis4, Anna K. Nowak2

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**Background:** Single time-point unidimensional tumor thickness measurements define measurable disease for clinical trial inclusion and also constitute a field in the IASLC prospective mesothelioma staging database. It is unclear how tumor thickness, morphology, and location affect interobserver variability in a single baseline measurement.

**Methods:** 105 thoracic CT scans were collected retrospectively from 50 mesothelioma patients. Each scan was reviewed by a medical oncologist, who identified 170 sites of mesothelioma tumor across all scans that constituted a field in the IASLC prospective mesothelioma staging database. Four other physicians was presented with the individual CT sections and the same fixed location of the outer tumor margin at each of the 170 pre-defined tumor measurement sites. Each observer then independently created a line segment to capture tumor thickness at all measurement sites. Interobserver variability was calculated as a function of tumor reference thickness to identify the smallest tumor thickness at which linear measurements could be made reliably. Comparisons were made with the RECIST tumor response criterion of 20% for progression.

**Results:** Measurements acquired at tumor sites with reference thickness less than 7.5 mm demonstrated inter-observer variability (as defined by the difference between the maximum and minimum measurements of the observers at each site then tabulated across all sites in a specific size range) with a 75th percentile that included 20% of the tumor length. Inter-observer variability did not differ across lesion morphologies and locations. Only tumor sites with reference thickness greater than 12.5 mm demonstrated inter-observer variability with a maximum value that did not include 20% of the tumor length.

**Conclusion:** The results of this study have implications for the definition of minimum measurable tumor adopted by clinical trial and staging protocols.

**Disclosure:** No significant relationships.
Pt.18: IMPORTANCE OF CERVICAL MEDIASTINOSCOPY AND LAPAROSCOPY IN DEFINING TREATMENT OF MALIGNANT PLEURAL MESOTHELIOMA

Evelien De Witte1, Philippe Nafeux2, Kristiaan Nackaerts3, Eric Verbeke4, Marc Decramer2, Karin Haustermans4, Paul De Leyn1

1Thoracic Surgery, University Hospital, Leuven/BELGIUM, 2Pneumonology, University Hospital Leuven, Leuven/BELGIUM, 3Radiation Oncology, University Hospital Leuven, Leuven/BELGIUM, 4Radiotherapy - Oncology, University Hospital Leuven, Leuven/BELGIUM

Background: Multimodality treatment is an option in early stage malignant pleural mesothelioma (MPM) but morbidity remains high. Therefore, careful oncologic pre-treatment evaluation is mandatory. Standard clinical staging based on computed tomography, positron emission tomography and magnetic resonance imaging is often inaccurate. AIM: To evaluate the added value of surgical staging, consisting of cervical videomediastinoscopy (CM) and laparoscopy.

Methods: Between March 2003 and December 2010, 126 patients with MPM were prospectively studied. Multimodality treatment was proposed in 82 fit patients based on standard clinic staging methods without taking into account presence of suspect mediastinal lymph nodes or transdiaphragmatic invasion. All patients underwent subsequent surgical staging (CM and laparoscopy (n=72), single CM (n=8) or single laparoscopy (n=2)).

Results: Cervical videomediastinoscopy (n=80) caused node (N) downstaging in 3 patients (3.75%), upstaging in 9 patients (11.25%) and change from single level to multilevel N2 in 4 patients (5.00%). Laparoscopy (n=74) discovered transdiaphragmatic invasion in 27.0% (n=2) and peritoneal metastasis in 27.0% (n=2) of patients. In 3 patients (4.05%) a suspicion of transdiaphragmatic invasion could not be confirmed during laparoscopy. Surgical staging caused a change in treatment in 23.17% (n=19). Five patients became candidates for multimodality treatment (6.10%) and 14 patients were referred for non-surgical based treatment (17.07%).

Conclusion: Based on these results, we recommend that surgical staging consistent of CM and laparoscopy should be included in the standard evaluation of patients potentially candidates for multimodality treatment of MPM.

Disclosure: No significant relationships.

Pt.19: TUMOR VOLUME AS PROGNOSTIC FACTOR FOR PATIENTS UNDERGOING RADICAL PLEURECTOMY FOR MALIGNANT PLEURAL MESOTHELIOMA

Joseph Friedberg1, Melissa J. Culligan1, Mary Putt1, Benjamin French1, Stephen M. Hahn2, Charles B. Simone3, Evan Alley4, James Stevenson5, Daniel Sterman4, Eric J. Wang6, Keith Cengel6

1Thoracic Surgery, University Of Pennsylvania, Philadelphia/PA/UNITED STATES OF AMERICA, 2Biostatistics And Epidemiology, University Of Pennsylvania, Philadelphia/PA/UNITED STATES OF AMERICA, 3Radiation Oncology, University Of Pennsylvania/UNITED STATES OF AMERICA, 4Medicine, University Of Pennsylvania, Philadelphia/UNITED STATES OF AMERICA, 5Taussig Cancer Institute, Cleveland Clinic Foundation, Cleveland/OH/UNITED STATES OF AMERICA, 6Medical College Of Georgia/UNITED STATES OF AMERICA

Background: Our group performs radical pleurectomies to achieve a macroscopic complete resection in patients undergoing surgery-based therapy for malignant pleural mesothelioma. As a result, the surgical specimen is essentially all cancer, thereby allowing a very accurate determination of tumor volume. The purpose of this study is to determine whether tumor volume has prognostic value.

Methods: 26 patients underwent radical pleurectomy with intraoperative photodynamic therapy as part of a surgery-based treatment for malignant pleural mesothelioma. Each specimen was removed and placed in a graded vessel containing saline, whereby its volume was determined by simple displacement.

Results: The stage breakdown for all 26 patients was: 2 Stage I, 1 Stage II, 20 Stage III, and 3 Stage IV. 21 patients had an epithelial subtype and 5 had a nonepithelial subtype. Overall, these patients had a median interquartile tumor volume of 625 (325, 888) cc. Patients with nonepithelial subtypes had a significantly larger median tumor volume of 800 (800, 1500) cc, as compared to the median tumor volume of 450 (300, 850) cc in patients with the epithelial subtype (p=.047). Survival calculations were limited to the 21 patients with the epithelial subtype (2 Stage I, 1 Stage II, 15 Stage III, 3 Stage IV) and were made at a mean/median follow up of 9.1/8.0 months. Within this group, the median (95% CI) progression free survival (PFS) was 38.3 (17.0, 54.0) months; median overall survival could not be calculated due to insufficient follow-up. Tumor volume was not associated with either PFS or overall survival.

Conclusion: Bearing in mind the limitations of this small retrospective study, it appears that patients with nonepithelial tumors who enrolled for surgery typically had greater tumor bulk. Focusing on the patients with epithelial tumors, for whom our group is currently limiting all surgery-based treatments, tumor volume did not appear to be a prognostic factor. At this time we conclude that this data will warrant a more mature analysis, but there does not appear to be a reason to use tumor volume as an exclusion criteria for surgery-based treatment, at least for this particular therapeutic regimen. Given the ease with which tumor volume can be determined for a radical pleurectomy specimen, we have now incorporated this measurement as part of every procedure. Furthermore, this technique would also allow for very accurate calibrations by any radiographic algorithms designed to measure pleural tumor volume.

Disclosure: No significant relationships.
Pt. 20: POSTERIOR INTERCOSTAL LYMPH NODES AND MALIGNANT PLEURAL MESOTHELIOMA - WHAT ARE THEY, WHERE ARE THEY AND WHAT DO THEY MEAN?

Joseph Friedberg1, Melissa J. Culligan1, Mary Putt1, Benjamin French1, Stephen M. Hahn2, Charles B. Simone1, James Stevenson1, Evan Alley1, Daniel Serman2, Eric J. Wang3, Keith Cengel1

1Thoracic Surgery, University Of Pennsylvania, Philadelphia/PA/UNITED STATES OF AMERICA, 2Biostatistics And Epidemiology, University Of Pennsylvania, Philadelphia/PA/UNITED STATES OF AMERICA, 3Radiation Oncology, University Of Pennsylvania/UNITED STATES OF AMERICA, 4Taussig Cancer Institute, Cleveland Clinic Foundation/UNITED STATES OF AMERICA, 5Medicine, University Of Pennsylvania, Philadelphia/UNITED STATES OF AMERICA, 6Medical College Of Georgia/UNITED STATES OF AMERICA

Background: Little is known about the significance of the posterior intercostal lymph nodes, which are located within the intercostal spaces at the level of the rib heads. These nodes are not part of any staging system. This report is an initial attempt to determine the significance of these nodes in patients with malignant pleural mesothelioma.

Methods: Posterior intercostal lymph nodes were harvested from 38 patients with epithelial mesothelioma who underwent radical pleurectomy and intraoperative photodynamic therapy for malignant pleural mesothelioma. The nodes were accessed by dividing the endothoracic fascia at the level of the rib heads and bluntly dissecting into the intercostal spaces.

Results: 18 out of 38 patients had positive intercostal nodes. Standard nodal status breakdown was 4/38 No, 2/38 N1, and 32/38 N2. Positive intercostal nodes were not associated with stage, but approached significance with N status (Fisher’s Exact Test, p=0.073). At a mean/median follow-up of 29.6/6.07 months, the group as a whole displayed a median progression free survival of 16.4 (9.8, ND) months and overall survival of 24.6 (18.2, ND) months. There were no significant differences between either progression free survival or overall survival among 30 Stage III or 8 Stage IV patients. Patients with negative intercostal nodes had a median progression free survival of 16.4 (9.8, ND) months compared to 10.2 (9.5, 24.1) months for patients with negative intercostal nodes, but these differences did not achieve statistical significance (Log-rank test, p=0.148). Patients with negative intercostal nodes demonstrated a significantly longer median survival of 45.1 (19.4, ND) months compared to 16.8 (11.3, ND) months for patients with positive intercostal nodes (Log-rank test, p=0.030). In a Cox model that included both stage and intercostal nodes, positive intercostal nodes continued to be associated with increased risk of death (HR=3.02 (1.06, 8.65), p=0.039). Statistical evaluation of N status as a prognostic factor for progression free survival or death was not possible in these data due to the small number of subjects who were No or N1.

Conclusion: Bearing in mind the limitations of a retrospective study with short-term follow-up, these results suggest that the posterior intercostal lymph nodes may have prognostic significance. This data has served as a trigger for us to now routinely include the posterior intercostal lymph nodes in our thoracic lymphadenectomies for malignant pleural mesothelioma. Further investigation of this nodal station is indicated, and it is likely that these nodes should be included in any future staging system for malignant pleural mesothelioma.

Disclosure: No significant relationships.

Pt. 21: PRE-OPERATIVE PROGNOSTIC ASSESSMENT IDENTIFIES AN EXTREMELY FAVORABLE COHORT FOLLOWING PLEURECTOMY WITH HYPERTHERMIC INTRAOPERATIVE CHEMOTHERAPY (HIROC) FOR MALIGNANT PLEURAL MESOTHELIOMA (MPM)

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Background: At our institution, indications for pleurectomy among patients with MPM include patients with small volume disease and patients who would require EPP for macroscopic complete resection but who have other contraindications for pneumonectomy.

Methods: Retrospective review of 59 patients with mesothelioma who underwent pleurectomy without prior chemotherapy at Brigham and Women’s Hospital between 2001 and 2010 was performed. Demographic, clinical, pathologic, treatment, and survival data were collected. A dedicated thoracic radiologist reviewed CT and PET-CT scans to assess pre-operative disease distribution and post-operative recurrence. Tumor volume was calculated from pre-operative CT scans using Vitrea Enterprise suite 6.0 (Vital Images, Minnesota, USA). Time to recurrence (TTR) and overall survival (OS) were calculated from date of resection and estimated by the Kaplan-Meier method. Cox regression models were derived using significant univariate predictors at the 0.05 level.

Results: Median age was 69 years (range 27-86). Forty-four patients (75%) were men and 15 were women (25%). Histology determined by biopsy was epithelial for 51 patients (86%) and non-epithelial for 4 (14%). Median tumor volume was 73 cm³ (range 0 - 1814) and 37 patients (63%) had disease present in the fissures. No patients received pre-operative chemotherapy (CT); 41 (65%) received heated intra-operative chemotherapy (HIROC); and 35 (59%) received adjuvant CT. Forty-four patients developed recurrent disease (75%) and 42 patients died (71%). Median TTR for all patients was 11.1 months. Median OS was 24.6 months, and 5-year OS was 27%. Univariate analyses of predictors for TTR and OS are listed in table.

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<thead>
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<th>Median OS (months)</th>
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<tr>
<td>No disease in fissures by CT Disease in fissures by CT</td>
<td>22</td>
<td>37</td>
<td>24.8 10.3</td>
<td>0.0002</td>
<td>NR 19.2</td>
</tr>
<tr>
<td>Normal Hemoglobin Anemia</td>
<td>36</td>
<td>23</td>
<td>14.7 6.8</td>
<td>0.02</td>
<td>42.8 10.7</td>
</tr>
<tr>
<td>HIROC No HIROC</td>
<td>41</td>
<td>18</td>
<td>11.9 7.8</td>
<td>0.1</td>
<td>29.2 17.1</td>
</tr>
<tr>
<td>Adjuvant CT No CT</td>
<td>35</td>
<td>24</td>
<td>12.6 8.3</td>
<td>0.2</td>
<td>27.6 18.9</td>
</tr>
</tbody>
</table>

*Histology by biopsy On multivariate analysis, presence of disease in the fissures was the only significant predictor for shorter TTR (HR 3.7, p=0.0004). Significant independent predictors of shorter OS included non-
epithelial histology (HR 2.9, p=0.02), volume > 73cm³ (HR 2.2, p=0.04),
disease in fissures by CT (HR 4.5, p=0.0006), anemia (HR 4.7, p<0.0001),
and no treatment with HIOC (HR 2.6, p=0.008). For the 12 patients (20% of
this cohort) with all five favorable predictors, the 5-year OS rate was 92%.

Conclusion: Presence of disease in the fissures on the pre-operative
CT scan is a significant independent predictor for both early tumor
recurrence and shorter survival among patients undergoing pleurectomy
for MPM. Conversely, patients with epithelial histology on biopsy, normal
hemoglobin levels, small volume disease and no fissure involvement have a
remarkably long survival following pleurectomy and HIOC.

Disclosure: No significant relationships.

pt.22: Quantified Computed Tomography Assessment of
Asbestos-related Disease

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STATES OF AMERICA, Department of Oncology, Wayne State University, MI/UNITED
STATES OF AMERICA

Background: The management of a patient with asbestos-related disease
starts with a correct diagnosis and determination of the extent of the
disease. The radiographic diagnosis of asbestos-related disease has
largely depended on the B-Reader system, a diagnostic tool originally
designed to study pneumoconiosis in large populations. This classification
system is subjective with poor inter-observer agreement among
experienced readers. Consequently, it has motivated us to seek a method
of characterizing asbestos-related disease utilizing current advances in
technology and a novel reporting form to make a more confident diagnosis
and depiction of asbestosis.

Methods: Our study of 50 patients was approved by the Institutional
Review Board at Wayne State University and in accordance with HIPAA
regulations. All participants provided signed informed consent. We outline
our method to quantify and describe asbestos-related disease based on
computerized analysis of CT findings. An originally developed classification
form based on computerized findings was used to record asbestos
related abnormalities. The system provides a comprehensive evaluation
of 3-dimensional (3D) rendering measurements of pleural plaques and
surface area that will offer a reproducible way to measure and quantify
asbestos-related changes. Pulmonary fibrosis assessment is achieved
using color segmentation; 3D analysis of nodules supplies size and shape
determinations; lung segmentation volume is provided.

Results: CT images with computer-assisted software provided clarity,
detection of subtle and advanced changes, and objective quantification
of thoracic abnormalities in patients with asbestos-related diseases. The
newly created reporting form renders a device to comprehensively record
and quantify the CT findings.

Conclusion: We believe that our approach to classification and
quantification of asbestos-related abnormalities will help detect disease at
an early stage and more clearly elucidate pathologies. Our expectation is to
initiate a practical alternative to the B-Reader System for the classification
of asbestos-related diseases and other types of pneumoconiosis.

Disclosure: No significant relationships.
Pt.23: PATTERNS OF DISEASE PROGRESSION IN PATIENTS WITH MALIGNANT PLEURAL MESOTHELIOMA UNDERGOING PLEURECTOMY / DECORTICATION, HYPERTERMIC PLEURAL LAVAGE WITH Povidone-Iodine FOLLOWED BY PROPHYLACTIC RADIOTHERAPY AND ADJUVANT CHEMOTHERAPY

Leic Lang-Lazdunski1, Andrea Bille2, Lawrence Okiror2, Gary Cook2, James Spicer2, David Landau4
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Background: There is no known cure for malignant pleural mesothelioma (MPM). Patients treated with multi-modality therapy generally experience longer survival. Time to progression and modes of relapse are well known following extrapleural pneumonectomy and adjuvant radiotherapy. We wished to evaluate the patterns of disease progression in patients treated with pleurectomy/decortication (P/D), hypertermic pleural lavage with povidone-iodine, prophylactic radiotherapy and adjuvant chemotherapy.

Methods: Prospective study of patients treated with P/D and hypertermic pleural lavage with povidone-iodine, prophylactic radiotherapy and adjuvant chemotherapy at our institution. Patients were followed up in our clinic regularly and had PET/CT within 6 weeks of completion of adjuvant chemotherapy and six-monthly thereafter. The first site of relapse on PET-CT was recorded and all scans were reviewed by an independent observer.

Results: Between October 2004 and March 2012, sixty five patients underwent P/D and hypertermic pleural lavage with povidone-iodine, prophylactic radiotherapy and adjuvant chemotherapy. Thirty two patients (27 male, median age 61 year, range 45-73) had their PET-CT at our institution and were analyzed. Eighteen of 32 patients were alive at last follow-up (median follow-up 39.6 months, range 16-76 months). Nine patients were alive with no evidence of disease recurrence, 9 were alive with disease recurrence, of whom 6 patients experienced a local recurrence in the pleural cavity and one patient experienced a recurrence in the pleural cavity and in the mediastinum, 2 patients experienced a recurrence around their diaphragmatic mesh. Fourteen patients have died of disease progression (median survival 24.7 months, range 15-38). Four patients experienced a local recurrence in the pleural cavity, two patients progressed in the pleural cavity and mediastinum, one patient had relapse in both pleural cavities, one patient progressed in a supraclaviculare lymph node and two patients progressed in coeliac lymph nodes (N3), two patients progressed with bone metastases (vertebral metastases) and one patient presented with peritoneal metastases. Interestingly, no patient relapsed in the thoracotomy scar. The median maximum standardized uptake value (SUVmax) in relapsing mesothelioma was 10.9 (range 4.9 - 27.3). There was a statistical correlation between the SUVmax of recurrent mesothelioma and survival (p=0.05). In this group of patients, the median disease free survival (DFS) was 9 months (range 6-18). There was a statistical correlation between DFS and complete macroscopic resection (p=0.02) but there was no correlation between DFS and histologic subtype (epithelioid vs biphasic/sarcomatoid).

Conclusion: After Pleurectomy/decortication with hypertermic pleural lavage with povidone-iodine, prophylactic radiotherapy and adjuvant chemotherapy the most frequent site of recurrence is the pleural cavity. Peritoneal seeding is rare. Tumor SUVmax does not significantly correlate with survival.

Disclosure: No significant relationships.
P1.24: NATIONAL MESOTHELIOMA VIRTUAL BANK (NMVB): A PLATFORM FOR COLLABORATIVE RESEARCH AND MESOTHELIOMA TISSUE RESOURCE TO ENHANCE TRANSLATION RESEARCH.

Waqas Amin1, Anil V. Parwani2, Nancy Whelan3, Rajiv Dhir4, Michael Feldman4, Jonathan Melamed5, Harvey Pass4, Raja Flores7, Michael J. Becich1

1Biomedical Informatics, University Of Pittsburgh, Pittsburgh/PA/UNITED STATES OF AMERICA, 2Pathology, University Of Pittsburgh Medical Center, Pittsburgh/PA/UNITED STATES OF AMERICA, 3Biomedical Informatics, University Of Pittsburgh, Pittsburgh/UNITED STATES OF AMERICA, 4Pathology, University Of Pennsylvania, Philadelphia/PA/UNITED STATES OF AMERICA, 5Pathology, New York University Medical Center, New York/NY/UNITED STATES OF AMERICA, 6Cardiothoracic Surgery, New York University Medical Center, New York/NY/UNITED STATES OF AMERICA, 7Cardiothoracic Surgery, Mount Sinai School Of Medicine, New York/UNITED STATES OF AMERICA

Background: The National Mesothelioma Virtual Bank (NMVB), developed six years ago, gathers clinically annotated human mesothelioma specimens for basic and clinical science research. During this period of time, this resource has greatly increased its collection of specimens by expanding the number of contributing academic health centers. The resource has provided hundreds of high quality, well characterized annotated biospecimen to the mesothelioma research community.

Methods: The NMVB collaborating sites included New York University, University of Pennsylvania, and the University of Pittsburgh Medical Center; Mount Sinai School of Medicine joined the collaboration in 2011 to increase the collection of specimens thereby allowing the resource to progress and meet the needs of research communities. Marketing efforts at both national and international annual conferences increase awareness and availability of the mesothelioma specimens at no cost to approved investigators who query the web-based NMVB database for cumulative and patient level clinicopathology information on the specimen. The NMVB resource includes three distinct Tissue Microarrays (TMAs) that encompass more than 100 malignant mesothelioma cases for biomarker research.

Results: The NMVB database provides researchers real-time, interactive access to richly annotated specimens and essential information related to mesothelioma. The data disclosure and specimen distribution protocols are tightly regulated to maintain compliance with participating institutions’ IRB and regulatory committee reviews. The NMVB currently has over 1000 annotated cases available for researchers, including paraffin embedded tissues, fresh frozen tissue, tissue microarrays, blood samples and genomic DNA. In addition, the resource offers expertise and assistance for collaborative research.

Conclusion: The National Mesothelioma Virtual Bank (NMVB) is a virtual biospecimen resource with robust translational biomedical informatics support to facilitate basic science, clinical, and translational research. Furthermore, in the last six years the resource has provided hundreds of fresh frozen, paraffin, blood samples and dozens of TMA slides to the research community. The investigators request specimens and/or data by submitting a Letter of Intent which is then evaluated by mesothelioma research evaluation panel.

Disclosure: No significant relationships.

P1.25: HIGH PREVALENCE OF ATYPICAL MESOTHELIAL PROLIFERATION IN EXTRAPLEURAL PNEUMONECTOMY SPECIMENS; FURTHER EVIDENCE OF A POTENTIAL PRECURSOR LESION TO INVASIVE MESOTHELIOMA

Leona Doyle1, Lucian Chirieac2

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Background: Atypical mesothelial proliferation (AMP) is thought to represent a potential precursor lesion to invasive pleural mesothelioma. To our knowledge there is no published literature describing the clinicopathologic characteristics of AMP. The aim of this study was to evaluate the prevalence of AMP in extrapleural pneumonectomy (EPP) specimens for invasive mesothelioma and to correlate AMP with clinicopathologic features.

Methods: We studied 46 consecutive EPPs with available surgical material (mean 22 slides per case, range 12-30), performed for invasive mesothelioma (IM) over 16 months. Each case was assessed independently by two pathologists for AMP according to currently established morphologic criteria. We evaluated architectural and cytologic features, the prevalence and extent of AMP and correlated clinicopathologic features between mesotheliomas with and without AMP.

Results: All 46 EPPs (40M/6F, mean age 62.9 years; range 38-79) showed invasive mesothelioma (n=30 epithelioid, n=15 mixed and n=1 sarcomatoid). AMP was identified in 10 (22%) EPP specimens, in a mean of 3.5 slides (range 1-6). Nine cases (90%) were associated with epithelioid mesothelioma and 1 case (10%) with mixed mesothelioma. Common architectural patterns of AMP were a single cell layer proliferation (n=8), stratified proliferations (n=5) and papillary proliferations (n=5). Six cases (60%) had mixed AMP growth patterns. In AMP with a single cell layer proliferation, prominent nucleoli were present in at least 50% of lesional cells. AMP was present in EPPs weighing less (median 747g vs. 1110g, p=0.03) and in older patients (68 vs. 63 years, p=0.02).

Conclusion: In our study the prevalence of AMP (22%) in EPPs is higher than anticipated, is more frequent in older patients and in specimens with lower weights. Further studies are needed to investigate the clinical significance of AMP and the role of AMP in the pathogenesis of mesothelioma.

Disclosure: No significant relationships.

P1.26: MESOBANK - A UK BASED BIORESOURCE FOR MALIGNANT MESOTHELIOMA

Dean Fennell1, Stefan Marciniak1, John Edwards1, Peter Szlosarek4, Doris Rasli1, Sam Janes5, Keith Kerr2, Peter Hamilton7, Victoria Hughes2, Robert C. Rentoul4, Richard Booth1

1University Of Leicester, Leicester/UNITED KINGDOM, 2Papworth Hospital, Cambridge/UNITED KINGDOM, 3Dept Of Surgery, Northern General Hospital/UNITED KINGDOM, 4Molecular Oncology, Barts Cancer Institute, 5Department Of Pathology, University Of Leicester, Leicester/UNITED KINGDOM, 6Molecular Oncology, Barts Cancer Institute, 7Department Of Pathology, University Of Leicester, Leicester/UNITED KINGDOM

Background: Atypical mesothelial proliferations are defined as non-invasive lesions that may have clinicopathologic features that resemble those of invasive mesothelioma. These lesions are thought to be a potential precursor to invasive mesothelioma. The National Mesothelioma Virtual Bank (NMVB) and the UK Mesothelioma Research Network (UKMRN) are each developing tissue microarrays to identify AMP and provide material for research. The purpose of this study was to compare the clinical and histological features of AMP from these two banks.

Methods: A retrospective analysis of AMP identified in EPPs was performed. AMP was identified in the NMVB and defined according to the current accepted morphologic criteria. AMP was identified in a tissue microarray of UKMRN EPPs if there was at least two cell layers of atypical cells, without a fibroblastic stroma.

Results: In total 3720 EPPs were identified, 1258 in the NMVB and 2462 in the UKMRN. AMP was identified in 51 (23%) of the NMVB and in 14 (0.6%) of the UKMRN. The mean number of slides for each EPP was 13 and 7 respectively. There was a significant difference in the prevalence of AMP in the two cohorts (p<0.0001). In the NMVB the mean age of the patients was 64 years (range 35-84) and the mean age of patients with AMP was 67 years (range 41-82). In the UKMRN the mean age was 65 years (range 35-79) and the mean age of patients with AMP was 64 years (range 41-79).

Conclusion: The prevalence of AMP is significantly higher in the NMVB compared to the UKMRN. This could be due to a difference in case selection or the use of different criteria for identifying AMP in the two banks. It is possible that AMP is underdiagnosed in the UKMRN. Further studies are needed to investigate if AMP represents a precursor to invasive mesothelioma.

Disclosure: No significant relationships.
MesobanK will follow the Guiding Principles laid out by the NCRI Confederation of Cancer Biobanks and the Medical Research Council Operational and Ethical Guidelines on Human Tissue and Biological Materials for Use in Research. It will also be managed within the scope of all relevant regulatory frameworks and quality management/quality assurance systems. In addition, we share the aim of the US National Cancer Institute (NCI) National Biospecimen Network Blueprint: to create assured, fully annotated tissue collected to rigorous standard operating procedures. Currently, few bioresources of mesothelioma tissue exist, the largest being the National Mesothelioma Virtual Bank hosted by the University of Pittsburgh (http://www.mesotissue.org/). Within the UK, a few clinical/research groups hold fresh tissue from small numbers of mesothelioma patients but these collections are not formally linked and do not involve the collection of tissue and data in accordance with any universal Standard Operating Procedures.

**Methods**: The British Lung Foundation has recently confirmed funding for MesobanK, a UK based bioresource of malignant mesothelioma tissue samples. This will comprise a) a retrospective collection of paraffin embedded tissue blocks to allow construction of a large tissue microarray (1000 cases) and b) prospective collection of fresh frozen mesothelioma tissue and matched blood samples (300 cases over 3 years) from multiple centres across the UK. In addition, it is planned to develop 20 new fully annotated mesothelioma primary cell cultures/cell lines. The bioresource will be supported by a web-based IT infrastructure for annotating and searching the collection. Clinical data will be collected on each case and supplemented by laboratory and pathology results, Hospital Episode Statistics data and Cancer Registry data in order to achieve as complete a data set as possible on each case.

**Results**: MesobanK will follow the Guiding Principles laid out by the NCRI Confederation of Cancer Biobanks and the Medical Research Council Operational and Ethical Guidelines on Human Tissue and Biological Materials for Use in Research. It will also be managed within the scope of all relevant regulatory frameworks and quality management/quality assurance systems. In addition, we share the aim of the US National Cancer Institute (NCI) National Biospecimen Network Blueprint: to create a comprehensive framework for sharing and comparing research results through a robust, flexible, scalable and secure bioinformatics system that supports the collection, processing, storage, annotation and distribution of biospecimens and data using standard operating procedures based on best practices.

**Conclusion**: A steering committee will be set up which will have overall control of MesobanK. An independent scientific advisory board will review applications for samples and advise the steering committee. Prioritisation for access to samples will be based solely on scientific merit. All researchers, whether in the UK National Health Service, universities, charities, government agencies or commercial companies, and whether based in the UK or abroad will be subject to the same application process and approval criteria. It is anticipated that initial tissue samples will be available in late 2013.

**Disclosure**: No significant relationships.

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**Pt.28: MALIGNANT MESOTHELIOMA CAN IN MOST CASES BE CORRECTLY DIAGNOSED BASED ON EFFUSIONS**

Anders Hjerpë, Filip Mundt1, Katalin Dobra1

Department Of Laboratory Medicine, Karolinska Institutet, Stockholm/ SWEDEN, 1Laboratory Medicine, Karolinska Institute, Stockholm/SWEDEN

**Background**: The IMIG recommendations state that the diagnosis of a malignant mesothelioma should be based on the examination of a biopsy, obtained at thoracoscopy. Exfoliated MGG- or Pap-stained cells from an effusion is considered to be insufficient for the diagnosis. However, the refinement of adjuvant techniques during the last decades has changed this completely.

**Methods**: We intend to present our experiences with such analyses in effusion cytology. With the help of immunocytochemistry, FISH, biomarker analyses and electron microscopy, we can establish the mesothelioma diagnosis in a majority of cases.

**Results**: In a laboratory with experiences of these techniques, the diagnosis is accurate and definite, and will provide all information needed for choice of therapy in cases when surgery is not considered.

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**Pt.27: DIAGNOSIS OF PLEURAL EPITHELIOD MALIGNANT MESOTHELIOMA: A META-ANALYSIS BASED ON ROUTINELY USED IMMUNOHISTOCHEMICAL MARKERS.**

Nolwenn Le Stang1, Marie Karanian2, Maria Paciencia2, Françoise Galateau-Sallé1

1Pathology Department - University Hospital, Mesonat Registry (U 1086 Inserm), Caen Cedex 9/FRANCE, 2Pathology Department - University Hospital, U 1086 Inserm “Cancers & Préventions”, Caen Cedex 9/FRANCE

**Background**: Because of the well known heterogeneity and mimics of malignant mesothelioma of the pleura, there has been several guidelines and recommendations given by various panel for making a definitive diagnosis of malignant mesothelioma. The most recent is the “Guidelines For Pathologic Diagnosis of Malignant Mesothelioma: A Consensus Statement From The International Mesothelioma Interest Group and International Mesothelioma Panel”. They all recommend to perform an immunohistochemical analysis and to use two positive and two negative markers before making a definitive diagnosis of mesothelioma. But there is no absolute antibodies that might definitively give a diagnosis of mesothelioma. Moreover there are so many different antibodies from different sources, clones and techniques, that the pathologist may be confused and find difficult to find his way for a safe and secure diagnosis of mesothelioma. We aimed to identify all literature related to immunohistochemical markers tied to the diagnosis of malignant mesothelioma. Search were conducted from the international literature and relevant articles were identified and retrieved intended to improve the diagnosis of malignant mesothelioma and its quality, to propose a strategy and the best panel in term of sensibility and specificity and cut off of staining for selecting the most robust markers on a routine practice level.

**Methods**: Meta-analysis was performed based on a literature review of papers published in Pubmed and Medline electronic literature databases between 1979 and 2010. The markers (EMA, HMFG-2, vimentin, keratin AE1/AE3, thrombomodulin, mesothelin, calretinin, HBME-1, WT-1, keratin 5/6, n-cadherin, D2-40, and CEA, Ber-EP4, B72.3, CD15, MOC-31, e-cadherin, TTF-1, BG-8). Immunohistochemical markers were performed on formalin-fixed paraffin-embedded tissues samples and markers performed on cytology specimens were not analysed. Papers analyzing less than 10 tumours, abstract or case report were excluded. The sensitivity, specificity and their 95% confidence intervals were calculated at 5 cut-offs of % of cells expression: 0%, 10%, 25%, 50% and 75%.

**Results**: According to the 99 selected papers, our results suggest that the most sensitive and specific panel to be used in the differential diagnosis of EM from metastatic adenocarcinoma includes WT-1, keratin 5/6, monoclonal CEA, BG-8 and MOC-31 preferentially evaluated at 0% staining cells cut-off, thrombomodulin, D2-40 and B72.3 at 10% of tumour cells cut-off, calretinin and ber-EP4 at 25% cut-off.

**Conclusion**: According to the recommendations of the International Mesothelioma Interest Group, the tumour cells staining can be credibly evaluated from 1%, up to 10% cut-off for keratin 5/6, up to 25% cut-off for monoclonal CEA and up to 50% cut-off for calretinin, BG-8, ber-EP4 and MOC-31.

**Disclosure**: No significant relationships.

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**Pt.25: DIAGNOSIS OF PLEURAL EPITHELIOD MALIGNANT MESOTHELIOMA: A META-ANALYSIS BASED ON ROUTINELY USED IMMUNOHISTOCHEMICAL MARKERS.**

Nolwenn Le Stang1, Marie Karanian2, Maria Paciencia2, Françoise Galateau-Sallé1

1Pathology Department - University Hospital, Mesonat Registry (U 1086 Inserm), Caen Cedex 9/FRANCE, 2Pathology Department - University Hospital, U 1086 Inserm “Cancers & Préventions”, Caen Cedex 9/FRANCE

**Background**: Because of the well known heterogeneity and mimics of malignant mesothelioma of the pleura, there has been several guidelines and recommendations given by various panel for making a definitive diagnosis of malignant mesothelioma. The most recent is the “Guidelines For Pathologic Diagnosis of Malignant Mesothelioma: A Consensus Statement From The International Mesothelioma Interest Group and International Mesothelioma Panel”. They all recommend to perform an immunohistochemical analysis and to use two positive and two negative markers before making a definitive diagnosis of mesothelioma. But there is no absolute antibodies that might definitively give a diagnosis of mesothelioma. Moreover there are so many different antibodies from different sources, clones and techniques, that the pathologist may be confused and find difficult to find his way for a safe and secure diagnosis of mesothelioma. We aimed to identify all literature related to immunohistochemical markers tied to the diagnosis of malignant mesothelioma. Search were conducted from the international literature and relevant articles were identified and retrieved intended to improve the diagnosis of malignant mesothelioma and its quality, to propose a strategy and the best panel in term of sensibility and specificity and cut off of staining for selecting the most robust markers on a routine practice level.

**Methods**: Meta-analysis was performed based on a literature review of papers published in Pubmed and Medline electronic literature databases between 1979 and 2010. The markers (EMA, HMFG-2, vimentin, keratin AE1/AE3, thrombomodulin, mesothelin, calretinin, HBME-1, WT-1, keratin 5/6, n-cadherin, D2-40, and CEA, Ber-EP4, B72.3, CD15, MOC-31, e-cadherin, TTF-1, BG-8). Immunohistochemical markers were performed on formalin-fixed paraffin-embedded tissues samples and markers performed on cytology specimens were not analysed. Papers analyzing less than 10 tumours, abstract or case report were excluded. The sensitivity, specificity and their 95% confidence intervals were calculated at 5 cut-offs of % of cells expression: 0%, 10%, 25%, 50% and 75%.

**Results**: According to the 99 selected papers, our results suggest that the most sensitive and specific panel to be used in the differential diagnosis of EM from metastatic adenocarcinoma includes WT-1, keratin 5/6, monoclonal CEA, BG-8 and MOC-31 preferentially evaluated at 0% staining cells cut-off, thrombomodulin, D2-40 and B72.3 at 10% of tumour cells cut-off, calretinin and ber-EP4 at 25% cut-off.

**Conclusion**: According to the recommendations of the International Mesothelioma Interest Group, the tumour cells staining can be credibly evaluated from 1%, up to 10% cut-off for keratin 5/6, up to 25% cut-off for monoclonal CEA and up to 50% cut-off for calretinin, BG-8, ber-EP4 and MOC-31.

**Disclosure**: No significant relationships.
Posters

**Poster Session 1  September 12, 2012 11:30-12:30**

**P1.29: A review of the (scarce) literature on localized malignant mesothelioma**

Steven Kazan, Justin A. Bosl
Kazan Mcclain Lyons Greenwood & Harley, Oakland/CA/UNITED STATES OF AMERICA

**Background:** Localized malignant mesotheliomas occur only rarely. The literature gives rise to two main questions: (1) What is the prognosis? Some authors suggest that surgical cure is possible, but many of the reported cases have gone on to die of the malignancy after the passage of some years. (2) Was there any history of asbestos exposure or of employment in trades where such exposure was likely? The paucity of cases makes conducting a full epidemiological study complicated.

**Methods:** A literature search was conducted for cases of localized malignant mesothelioma to examine what asbestos exposure is reported and what the survival and followup have been.

**Results:**

Asbestos Exposure:*

![Chart](chart.png)

Prognosis:*

![Chart](chart2.png)

* Charts prepared by Dr. Allan Smith (U.C. Berkeley School of Public Health) to aid in illustrating his testimony in a legal case.

**Conclusion:** Among the localized malignant mesothelioma patients for whom an exposure history was available, the rate of men that were exposed falls within the range of exposure rates among diffuse malignant mesothelioma patients. The follow up in many cases with respect to prognosis has been insufficient to justify any claims that these patients can be cured. Certainly, there is a significant number of patients that do die of disease during the limited follow up reported.

**Disclosure:** No significant relationships.
Pt.30: DEVELOPMENT OF TRACT METASTASES AFTER CENTESIS PROCEDURE DIFFER BETWEEN MURINE MODELS OF MESOTHELIOMA

Robin Cornelissen1, J G J V Aerts2, M E.H. Lambers3, H C Hoogsteden4, Joost P.J.J. Hegmans5

1Pulmonary Medicine, Erasmus Mc, Rotterdam/NETHERLANDS, 2Pulmonary Medicine, Erasmus Medical Centre, Rotterdam/NETHERLANDS

Background: Carcinogenesis along the tracts of cytology or biopsy needles, chest tubes, thoracoscopy trocars and surgical incisions is problematic complication in malignant mesothelioma. To prevent malignant seeding at sites of diagnostic or therapeutic interventions, prophylactic irradiation of tracts (PIT) was introduced in an attempt to improve quality of life for these patients. However, the effect of PIT seems dependent on the incidence of tract metastasis in the particular studies so no definitive conclusions can be drawn from the three randomized controlled trials that have been published so far. PIT will only be effective in the subset of patients in which the tumor is prone to show growth after a local intervention. We anticipate that the occurrence of tumor seeding at the puncture site is related to tumor characteristics. Genomic and phenotypical features of individual differences between mesothelioma patients may influence their immunological and stromal components within the tumor environment. To investigate this concept, we studied the effects of a needle puncture in the peritoneal cavity on tumor outgrowth of two well-defined mesothelioma cell lines in tumor bearing mice. The two cell lines were selected for their known different growth pattern in mice.

Methods: CBA/J mice and BALB/c mice (n=8) were inoculated intraperitoneally with either an AC-29 or AB-1 tumor cell line, respectively. Tumor cells were injected at the left side of the abdomen. After 8 days a peritoneal puncture was done at the contralateral side of the abdomen. The mice were sacrificed on day 12 and the peritoneum was analyzed for histological differences between parental and drug resistant IL-45 cells and tumours.

Results: All mice inoculated with the AC-29 cell line showed in tract metastasis at the puncture side, while none of the AB-1 treated mice developed tumor outgrowth after centesis. Histological examination of the tumor at the primary side showed marked tumor cells, stromal cells and developed tumor outgrowth after centesis. Histological examination of the tumour at the puncture side showed marked tumor cells, stromal cells and developed tumor outgrowth after centesis. Histological examination of the tumour at the puncture side showed marked tumor cells, stromal cells and developed tumor outgrowth after centesis. Histological examination of the tumour at the puncture side showed marked tumor cells, stromal cells and developed tumor outgrowth after centesis.

Conclusion: Differences in tumor cell genotype and phenotype, and subsequent changes in recruited immunological cells, could be the key to predict which patient will develop tract metastasis. Correlating these results to a clinical model is warranted to produce a tract metastasis prediction model.

Disclosure: No significant relationships.

Pt.31: CHARACTERISATION OF A PRE-CLINICAL RAT MODEL OF MESOTHELIOMA

Amanda Hudson, Chris Weir, Rozelle Harvie, Elizabeth Moon, Stephen Clarke, Nick Paviakis

Medical Oncology: Royal North Shore Hospital, Bill Walsh Cancer Research Laboratories, Sydney/NSW/ AUSTRALIA

Background: Malignant mesothelioma (MM) is a relatively rare and locally aggressive tumour resulting in short survival and high morbidity. The incidence of MM is increasing due to the widespread use of asbestos in the industrialised world, with Australia having the highest per capita incidence. The majority of patients with MM are diagnosed in advanced disease stage when administration of systemic chemotherapy represents the main active treatment option hoping to extend survival and improve symptoms. However due to the inherent resistance of this disease, relatively poor response to treatment is seen and relapse after initial chemotherapy is the norm. There is as yet no proven effective treatment after failure of first line chemotherapy, and consequently there is an urgent need for the development of novel and more effective treatments which may also circumvent drug resistance. The development, therefore, of a well characterised clinically relevant animal model is paramount in order to test novel drugs and treatment strategies. Although the syngeneic Fischer 344 rat IL-45 mesothelioma cell line has previously been used as a model for mesothelioma, little characterisation has been reported. Here we describe the characterisation of the rat IL-45 mesothelioma cell line as a pre-clinical rat model of mesothelioma.

Methods: IL-45 cells were grown using standard culturing techniques (37°C, 5% CO2 humidified incubator). Resistant cell lines were developed by repeatedly exposing parental IL-45 cells to known active agents (cisplatin, pemetrexed, gemcitabine and vinorelbine) and cell viability assays (MTT) were used to determine resistance to drug therapy. RNA was extracted and qRT-PCR performed to investigate gene expression levels. Western blot will confirm correlations between gene and protein expression levels. In vivo experiments used 0.5 x 10^6 IL-45 cells injected into the flank or pleural cavity of Fischer 344 rats. IHC was used to determine histological differences between parental and drug resistant IL-45 cells and tumours.

Results: In vitro, IL-45 cells undergo a normal cell cycle in approximately 14 hrs and have a biphasic appearance. Drug resistant cells are 2-fold resistant to the drug they were treated with. qRT-PCR results looking at the mechanisms used by these cells to overcome drug treatment suggest that ABC transporters, hormonal receptors, and retinoic acid binding proteins are involved. In vivo, IL-45 cells grow as sarcomatoid tumours with pleomorphic features. These tumours can be grown either in the flank, giving a large window of opportunity to test novel treatments (approximately 40 days), or in the pleural cavity where they grow extremely aggressively resulting in gross pleural extension, local invasion and rapid weight loss in approximately 15 days.

Conclusion: We believe that our pre-clinical model is now ready to be used as a platform for testing new drugs in order to treat this important disease.

Disclosure: No significant relationships.
Conclusion: In this study we demonstrate that ipilimumab alone has modest effects on overall survival in our murine mesothelioma model. As shown from other studies, DC-based immunotherapy generates significant beneficial effects both in the median and overall survival. In contrast to what was expected theoretically, no additional beneficial effects were seen when murine ipilimumab was combined with DC treatment. We are currently testing the activity of different immunological cell types after ipilimumab treatment.

Disclosure: No significant relationships.

Pt.34: INTRAPERITONEAL OR INTRATRACHEAL: WHICH ROUTE OF EXPOSURE IS RELIABLE FOR SCREENING MESOTHELIOMA RISK OF FIBROUS DUSTS IN RATS?

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Background: A large number of fibrous materials including nanofibers have been developed and used not only as asbestos substitutes, but also in various advanced technologies. Some of these fibers, particularly those with high durability and specific dimensions, have the potential to result in the development of mesothelioma in experimental animals. A systematized protocol to evaluate the carcinogenicity of new fibrous materials should be established to prevent mesothelioma cases caused by environmental exposures to hazardous fibrous dusts. We focused on identifying suitable routes of exposure to rats in evaluation protocols.

Methods: Six-week-old male Sprague-Dawley rats were randomly divided into five groups: the IP group, the IT group, the IV group, the IV+IP group, and the sham control group. The experimental materials were instilled into the peritoneal cavity or the tracheal epithelium. Body weight, clinical symptoms, survival rate, pathological changes, and the expression of pro-inflammatory cytokines were measured. The pathogenesis of mesothelioma was determined by a pathological examination.

Results: The IP group showed a significantly higher survival rate compared with the IT group. The IV group exhibited a significantly higher survival rate compared with the IV+IP group. The IP+IT group exhibited a significantly higher survival rate compared with the sham control group. The IV+IT group exhibited a significantly higher survival rate compared with the sham control group. The IP+IV group exhibited a significantly higher survival rate compared with the sham control group. The IP+IV+IT group exhibited a significantly higher survival rate compared with the sham control group. The IP+IV+IT group exhibited a significantly higher survival rate compared with the sham control group.

Conclusion: These results showed that IP exposure is more reliable than IT exposure for screening mesothelioma risk of fibrous dusts in rats.

Disclosure: No significant relationships.

Pt.33: PRELIMINARY CHARACTERIZATION OF HEDGEHOG INHIBITION IN A RAT MODEL OF MESOTHELIOMA RECURRENCE

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Background: Multimodal treatment currently provides the best survival outcome for malignant pleural mesothelioma (MPM); however, local tumor recurrence remains a significant challenge. Activation of the hedgehog signalling pathway could be detected in MPM (see abstract Shi, Y., et al.). As stem cell signalling plays an important role in tumor recurrence and metastasis, we hypothesized that hedgehog activation is an important factor in MPM recurrence after surgical resection and chemotherapy. In this regard, a chemoresistant side population of cells which retain precursor properties has recently been identified in MPM (C. Frei, 2011). Hedgehog antagonists targeting and inhibiting the cell surface activator of hedgehog signalling, smoothened (SMO) have already been utilised successfully in several clinical trials such as in combination with cisplatin/etoposide in small cell lung cancer, recurrent or non-responding medulloblastoma. Moreover, a study in a colon cancer xenograft model showed that a hedgehog antagonist could potentially suppress tumor recurrence (F. Varnat, 2009).

Methods: In this study, we aim to use an in vivo rat bioluminescent MPM model (Y. Shi, 2011) to study hedgehog antagonism in combination with cisplatin as a potential means to prevent tumor recurrence. Cytotoxicity and anchorage dependent colony formation assays, were applied to determine sensitivity of rat mesothelioma cells to a hedgehog antagonist in vitro. The expression analysis was performed by quantitative real time PCR and compared by ∆∆Ct method. To study the effect of hedgehog antagonist on bioluminescent imaging, rat bioluminescent MPM cells were treated with hedgehog antagonist and the resulting photon signal was detected using a small animal imager, IVIS, immediately after applying luciferase substrate.

Results: Rat MPM cells express key molecules of hedgehog signalling namely transmembrane hedgehog activator, smoothened (SMO) which is a target of hedgehog antagonist and Gli1, a transcription factor which is a positive modulator of the hedgehog signalling pathway. Hedgehog antagonist can suppress growth of rat mesothelioma cells as well as bioluminescent rat mesothelioma cells in a dose dependent manner. We further defined that hedgehog antagonist did not interfere with our in vivo bioluminescent assay.

Conclusion: These results implicate a role of the hedgehog signalling pathway in the rat recurrence mesothelioma model. Based on these findings in vivo studies will be carried out.

Disclosure: No significant relationships.

Pt.32: IPILUMUMAB ALONE OR IN COMBINATION WITH IMMUNOTHERAPY IN MURINE MALIGNANT MESOTHELIOMA

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Background: Recently the immunostimulating agent ipilimumab was approved by US food and drug administration (FDA) based on the increase in overall survival of patients with metastatic melanoma, although adverse events and side effect of treatment are substantial. Ipilimumab blocks cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4) which is present on activated T-cells. When CTLA-4 is expressed it limits their activation. Therefore, blocking CTLA-4 with ipilimumab is assumed to sustain antitumor T-cell responses. Based on this feature it may be regarded as an attractive immunostimulating agent to further increase the effectiveness of immunotherapy approaches. Malignant mesothelioma is an immunogenic cancer with poor prognosis. We showed in previous preclinical and clinical studies that dendritic cell-based immunotherapy is safe and feasible and induced cytotoxic T cell responses. Further boosting the antitumor immune response with ipilimumab could be a valuable additive to our current immunotherapy strategy. The aim of this study is to investigate whether ipilimumab alone or in combination with immunotherapy is of importance in mesothelioma patients.

Methods: Immunocompetent BALB/c mice were inoculated with a lethal dose of AB1 tumor cells intraperitoneally (i.p.) and monitored for a month. Without further treatment, mice develop first signs of terminal illness between 2 and 4 weeks. First in a phase I and II protocol a safe and efficient dosage of ipilimumab (antibody 4F10 in mice) was determined in healthy and tumor bearing mice. In addition, this was also determined during DC-immunotherapy. The optimal dosage was used in a study comparing efficacy of ipilimumab in four groups; tumor bearing mice ongoing no treatment (n=6), DC-therapy (n=6), ipilimumab (n=6), or DC-therapy plus ipilimumab (n=6).

Results: In the dose-finding experiments both 200 ug and 400 ug of 4F10, lead to a survival benefit in tumor-bearing mice. However, 400 ug had toxicity in 25% of the healthy mice and therefore 200 ug was selected as the optimal dosage. The median survival after tumor injection was 15 days in the control group, 15 days in the ipilimumab group, 21 days in the control group, 17 days in the ipilimumab group, 0% in the DC-therapy group, 50% in the DC-therapy group and 0% in the DC-therapy plus ipilimumab group. Kaplan-Meier analyses showed statistically significant differences in survival curves comparing DC-therapy with control (p<0.001, by log-rank test) and DC-therapy plus ipilimumab and control (p<0.05, by log-rank test).

Conclusion: These results implicate a role of the hedgehog signalling pathway in the rat recurrence mesothelioma model. Based on these findings in vivo studies will be carried out.

Disclosure: No significant relationships.
Methods: We examined plasma N-fragment of expressed in renal carcinoma (N-ERC)/mesothelin levels in female F344 rats after intraperitoneal injection or intratracheal instillation of seven different types of fibrous materials. At 1, 3, 5 days and 1, 2, 4, 10 and 20 weeks after administration, 30 µL of blood was collected from the tail vein. Plasma N-ERC levels were measured using a Rat N-ERC/Mesothelin assay kit. Test materials were multi-wall carbon nanotube (MWCNT: mean length 3.0 µm, mean width 0.10 µm), spherical TiO2 (P: mean diameter 0.27 µm), fibrous TiO2 (FT100: mean length 1.68 µm, mean width 0.13 µm; FT400: mean length >10 µm), silicon carbide whisker (SiC mean length 6.40 µm, mean width 0.30 µm), potassium titanate whisker (KT: mean length 6.0 µm, mean width 0.35 µm), and chrysotile asbestos (Chr: mean width 0.109 µm). Mesothelioma induction has previously been observed after intraperitoneal injection of MWCNT, SiC, KT and Chr.

Results: Plasma N-ERC levels of rats administered MWCNT, SiC, KT or chrysotile asbestos were continuously higher even at 10 weeks after i.p. than the other non-carcinogenic materials (P, FT100 and FT400). Conversely, plasma N-ERC levels showed 1 peak after intratracheal instillation, then diminished rapidly to control levels even with carcinogenic fibers.

Conclusion: Since plasma N-ERC levels of rats with intraperitoneal injection of fibrous dusts showed a good correlation with carcinogenicity, particularly at 10 weeks after injection, this short-term animal experiment provides useful data for evaluating the mesothelioma risk of newly developed fibrous materials.

Disclosure: No significant relationships.

**POSTER SESSION 1 SEPTEMBER 12, 2012 11:30–12:30**

**Pt 35: ESTABLISHMENT AND CHARACTERIZATION OF SEVEN HUMAN MALIGNANT MESOTHELIOMA CELL LINES**

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Background: Malignant mesothelioma is an aggressive tumour of serosal surfaces most commonly pleura, usually caused by exposure to asbestos. Characterised cell lines represent a valuable platform for discovery of molecular targets that could underpin new effective treatment for mesothelioma, improve diagnostics, and possibly determine pathways leading to effective secondary (post-exposure) prevention.

Aim: To assess the biological characteristics of seven malignant mesothelioma cell lines derived from human pleural biopsy tissue or pleural effusions.

Methods: Cells were established in tissue culture as adherent cell lines from small pieces of fresh resected pleural tumour tissue and pleural effusion derived cell pellets. Mesothelial origin was assessed by standard morphology, ultrastructure (Transmission Electron Microscopy) and immunocytochemistry. Growth characteristics were assayed using growth curves and population doubling times. Cytogenetic analysis using spectral karyotyping (SKY) was performed to assess chromosomal abnormalities. All cell lines were assessed for anchorage independent growth by soft agar colony assay.

Results: All seven–cell lines stained positive for calretinin and cytokeratin 19. Doubling time ranged from 30–72 hours. All cell lines exhibited numerical chromosomal abnormalities ranging from 41 to 113. Monosomy of chromosomes 8, 14, 22 or 17 was observed in five lines, and four different karyotypes were observed in one of the lines. All cell lines demonstrated capacity for anchorage independent growth.

Conclusion: Classic biological characteristics of mesothelioma are preserved in tumour derived cell lines maintained in vitro culture. These findings support the potential utility of human mesothelioma cell lines as a tool for studying cellular, molecular and genetic aspects of the disease.

Disclosure: No significant relationships.
**Conclusion:** The impact of macroscopic complete resection radical pleurectomy for mesothelioma on pulmonary function has become our approach for achieving a macroscopic complete resection for patients with malignant pleural mesothelioma. This procedure is being employed for all patients undergoing surgery-based treatment in our center, even in those with advanced stage cancer or bulky tumors. As an initial attempt to characterize the impact on quality of life this procedure has on the patients who receive it, we began to collect postoperative pulmonary function studies from them. This report compares these patients’ preoperative and postoperative pulmonary function studies.

**Methods:** 13 patients (9 stage III, 4 stage IV) at a mean age of 61 (42-72) underwent radical pleurectomy for malignant pleural mesothelioma. An FEV1 value was recorded within one month preoperatively, and another was measured at a mean postoperative time of 15 (3-46) months. The Wilcoxon test was then applied to these values.

**Results:** The mean decrease in FEV1 was 0.18 (0.05-0.36) liters (p=0.066). This corresponded to a mean decrease of 7.0% (4.5-9.0%) in the percent predicted FEV1.

**Conclusion:** This small retrospective series, acknowledging the limitations therein, revealed a postoperative decrease in FEV1 that did not achieve statistical significance. These radical pleurectomies were conducted in an advanced stage cohort of 100% stage III or stage IV patients, a population often considered candidates only for extrapleural pneumonectomy to achieve a macroscopic complete resection. The observed decrease in FEV1 values following radical pleurectomies compares very favorably with what has been reported following pneumonectomies. Because lung parenchyma is preserved with a radical pleurectomy, we conjecture the decrease in FEV1 is likely related to a compromise in diaphragm mechanics. We feel this data is encouraging and will serve as a basis from which to study pulmonary function and quality of life assessments more comprehensively in patients undergoing radical pleurectomy for malignant pleural mesothelioma.

**Disclosure:** No significant relationships.

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**P2.02: Complications Associated with Radical Pleurectomy for Malignant Pleural Mesothelioma in 77 Patients**

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**Background:** Initially employing extrapleural pneumonectomy as our preferred approach to achieve a macroscopic complete resection, our use of intraoperative photodynamic therapy (PDT) with its subsurface treatment effect motivated us to become increasingly aggressive with lung sparing surgery instead. Over the past several years, radical pleurectomy has thereby emerged as our standard approach. This report details the complications we have observed with this procedure.

**Methods:** 103 patients underwent surgery for malignant pleural mesothelioma, of which 77 underwent radical pleurectomy (including the most recent 42 consecutive patients). This procedure has evolved, but it has always included the goal of achieving a macroscopic complete resection with preservation of the entire lung, phrenic nerve, and as much native diaphragm and pericardium as possible. All of the patients underwent intraoperative adjuvant therapy after the radical pleurectomy (76 intraoperative PDT, 1 hyperthermic povidone iodine lavage).

**Results:** Macroscopic complete resection was achieved in 76 out of 77 patients (7/65/52/12 patients at Stages I/II/III/IV, respectively). Mean/median length of stay was 13.5/14 days. Complications included: 3 thirty-day mortalities (1 readmission with tamponade/multiorgan failure, 1 subarachnoid hemorrhage, 1 aspiration pneumonia), 27 atrial fibrillations, 3 pericardial effusions requiring drainage, 1 pulmonary embolism, 18 deep venous thromboses, 20 pneumonias (15 reintubations/8 tracheostomies), 11 discharges with a Heimlich valve (0 reoperations), 5 chyle leaks, and 1 diaphragm rupture.

**Conclusion:** Caution is indicated in interpreting this series by virtue of it reflecting: a single surgeon learning curve, the inclusion of patients from Phase I studies, the advanced nature of the cohort (83.1% stage III/IV) and, especially, the concomitant superimposed morbidity of PDT in 98.7% of the patients. Within that framework, the recorded mortalities (3.9%), chyle leaks (6.5%), and single pulmonary embolism (1.3%) compare reasonably or favorably with other reports. Other complications occurred more frequently than what might be expected with surgery alone: atrial fibrillation (35.0%), deep venous thrombosis (23.4%), pneumonia (26.0%), and reintubation (19.0%). It seems reasonable-to-likely that the addition of PDT contributed to this complication rate. The single diaphragm rupture occurred after a primary repair of the residual native diaphragm muscle, and should likely have undergone patch repair, but could potentially have been adequate without the addition of PDT. At least 90% of the patients (all but several of the Stage I patients) had most or all of their visceral pleura removed in order to achieve a macroscopic complete resection, which would predictably result in large air leaks, with some being persistent. The 14.3% incidence of discharges with a Heimlich valve may also be related to the addition of PDT. Finally, the 3.9% incidence...
Conclusion: with a negative PET-CT, but thought suspicious by the multidisciplinary false positives, 1/16 with a positive PET-CT was a true positive and 1/11 interpreted as having contralateral thoracic disease on CT alone were all studies occurred in patients who had PET scans. 2 patients had PET and 4 by biopsy. All of the false positive and all of the false negative 7 false negative studies (radiographically occult metastases - 3 by lavage and selective contralateral thoracoscopy to supplement radiographic staging. This report is a result of those results.

Methods: 151 patients with MPM were considered candidates for different surgery-based multimodal treatments. All patients had chest CT scans, 17 had MRI scans and 95 had PET scans. 13 patients were excluded from having laparoscopy due to histories of fused abdomens from prior conditions. 135 patients underwent laparoscopy (typically two 5mm ports) with peritoneal lavage and selective biopsies. 16 patients underwent contralateral thoracoscopy (a single 1cm incision), based upon suspicion by the radiologist and/or multidisciplinary team. 15/16 had thoracoscopy and laparoscopy and one had thoracoscopy alone.

Results: There were no operative complications. 119 laparoscopies were outpatient procedures, 5 were admitted overnight (urinary retention), 15 who also had thoracoscopy were admitted overnight, 1 thoracoscopy alone was admitted overnight. Laparoscopy revealed 6 false positive studies (1 interpretation of diaphragm transgression and 5 metastatic deposits) and 7 false negative studies (radiographically occult metastases - 3 by lavage and 4 by biopsy). All of the false positive and all of the false negative studies occurred in patients who had PET scans. 2 patients had PET and CT positive findings that were confirmed by laparoscopy. 4/16 patients interpreted as having contralateral thoracic disease on CT alone were all false positives, 1/16 with a positive PET-CT was a true positive and 1/11 with a negative PET-CT, but thought suspicious by the multidisciplinary team, was a false negative.

Conclusion: Radiographic staging of the abdomen was inaccurate in 9.3% of the patient, 5.0% false negative and 4.3% false positive, using the 139 patients undergoing laparoscopy as the denominator. All of the inaccuracies occurred in patients with both PET and CT. Contralateral chest staging was primarily inaccurate in the setting of false positive interpretations, with 4/5 (80%) proving biopsy negative. All four false positives were CT alone and the one true positive was also PET positive, but there was also one false negative PET-CT. Overall, using the 16 patients who underwent contralateral thoracoscopy as the denominator, radiographic staging was inaccurate in 5/16 (31%) of the patients. Based upon these results we feel that it remains prudent to perform routine laparoscopy, to both rule out false positive and false negative studies, even in the setting of both PET and CT scans. It also remains reasonable to perform contralateral thoracoscopy on a selective basis, especially for patients who have not had a PET scan.

Disclosure: No significant relationships.
**P2.05: CLINICAL IMPACT OF POST OPERATIVE ATRIAL FIBRILLATION FOLLOWING EXTRA PLEURAL PNEUMONECTOMY**

**James Hardy**, Xiaojia Liu, David J. Sugarbaker, Gyorgy Frendl

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**Background:** Atrial fibrillation (AF) is common following thoracic surgery, and is associated with increased mortality, morbidity and hospital length of stay. Extrapleural pneumonecetomy (EPP) carries a high risk of morbidity, including post operative AF. Surgery involves violation of the pericardium, significant hemodynamic changes and fluid shifts. The aim of this study was to describe the association between the development of post operative AF and outcomes in this patient group.

**Methods:** 551 patients (>27yrs) underwent EPP for mesothelioma between October 1998 and May 2011 at our institution. Data for 354 patients were extracted into our database through retrospective chart review. 29 patients were excluded for having a history of AF. Patients were monitored postoperatively in the intensive care unit or step down unit with continuous cardiac telemetry, and the presence or absence of post operative AF was recorded. AF was defined as an episode of irregularly irregular heart rhythm lasting at least 15 min. Hospital length of stay, ICU length of stay, time to extubation, requirement for reintubation, occurrence of post operative stroke, acute kidney injury, requirement for renal replacement therapy, in-hospital mortality and 30 day mortality were recorded. Univariate and multivariate Logistic regression analyses were used to explore the association between AF and the outcomes.

**Results:** The cohort of patients who developed postoperative atrial fibrillation was compared to those without AF. The incidence of AF peaked on post operative days 2 and 3. Post operative atrial fibrillation was significantly associated with increased length of hospital stay 20 days vs. 11.7 days (p<0.0001), increased length of ICU stay 11.9 days vs. 4.4 days (p<0.0001), increased in-hospital mortality 7.3% vs. 1.1% (p=0.006) and increased likelihood of reintubation 30.4% vs. 6.0% (p<0.0001). The presence of post operative atrial fibrillation was not statistically significantly associated with increased time to initial extubation, 30 day mortality, incidence of acute kidney injury, requirement for renal replacement therapy, or increased incidence of postoperative stroke. Multivariate analysis demonstrated that AF was a risk factor for in hospital mortality (p=0.024), independent of age, gender, left ventricular function, history of diabetes, hypertension, use of intraoperative heated chemotherapy, or use of intraoperative blood products.

**Conclusion:** EPP is one of two surgical interventions available for mesothelioma, at the expense of significant morbidities. Patients are at high risk for the development of post operative AF. AF was associated with a statistically and clinically significant increase in ICU and hospital length of stay, and in hospital mortality. Although the development of post operative AF may be a marker for other underlying physiological and inflammatory changes following EPP, it is a key modifiable risk factor. The use of the most effective prophylactic pharmacotherapy should be considered in these patients.

**Disclosure:** No significant relationships.

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**P2.07: A CLINICAL STUDY OF 5 EPITHELIAL TYPE DIFFUSE MALIGNANT PLEURAL MESOTHELIOMA PATIENTS WHO UNDERWENT EXTRAPLEURAL PNEUMONECTOMY**

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**Background:** Diffuse malignant pleural mesothelioma (MPM) has poor prognosis and there is no consensus on standard therapy. The best survival data with a median survival had been reported after trimodality therapy including Extrapleural pneumonecetomy (EPP), but Mars trial was a negative report. We considered whether EPP was effective therapy for MPM.

**Methods:** Between 2006 and 2010, 15 patients were definitive diagnosed by thoracoscopic biopsy as epithelial type MPM, and 5 of 15 underwent EPP in our hospital. We analysed these EPP cases.

**Results:** All cases were males, the median age 68 year (range:60-73). 2 cases had right side MPM,3 cases had left side MPM. 2 cases were cStageII, 3 cases were cStage III according to IMIG. 3 patients were received preoperative chemotherapy, 1 patient received postoperative chemotherapy, 1 patient was pre-postoperative chemotherapy; 3 cases were CDDP + Pemetrexed,2 cases were CDDP + Gemcitabin. All cases underwent resection diaphragm and 3 cases underwent resection pericardium. 1 of 5 cases had atrial fibrillation as postoperative complication. None of all received hemithoracic radiotherapy after surgery.

**Disclosure:** No significant relationships.
The survival term after EPP were 21.1 months (death of cancer), 33.3 months (death of cancer), 39.6 months (death of cancer), 20.3 months (death of cancer), 31.8 months (on survival). 2 of 5 cases made recurrence on the opposite lung and pleura, and respiratory failure progressed rapidly by pleural effusion but no EPP cases didn’t made opposite recurrence.

**Conclusion:** EPP cases had no major critical postoperative complication and were 100% on 1 year survival, therefore it indicated that EPP was effective therapy. We also consider it is necessary for maintaining QOL to prevent MPM from spreading across to the other side on EPP case.

**Disclosure:** No significant relationships.

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**P2.08: SILENT BRONCHOPLEURAL FISTULA FOLLOWING EXTRAPLEURAL PNEUMONECTOMY FOR MALIGNANT PLEURAL MESOTHELIOMA**

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**Background:** Bronchopleural fistula (BPF) after pneumonectomy is usually accompanied with typical and very severe clinical symptoms, and rapidly leads to fatal condition. However, in some rare instances, BPF continues to be totally asymptomatic with only radiological sign of decreased fluid level in the ipsilateral chest.

**Methods:** BPF was diagnosed in 5 out of 45 patients in whom EPP was completed in Hyogo College of Medicine between July 2004 and March 2012. Two patients with distinct clinical symptoms were surgically repaired immediately after onset. In the remaining 3 asymptomatic cases, diagnosis of BPF was made upon bronchoscopy and bronchography (n=3) or upon partial emptying of postpneumonectomy space on chest X-ray (n=2). The interval between EPP and onset of BPF ranged from 18 days to 2 years.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age</th>
<th>Induction Therapy</th>
<th>EPP</th>
<th>Bronchial Stump Coverage</th>
<th>postoperative RT</th>
<th>Onset of Emptying (after EPP)</th>
<th>Bronchoscopic Findings</th>
<th>Bronchoscopy Treatment *</th>
<th>Treatment Outcome (after Onset)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>49</td>
<td>PEM/CDDP</td>
<td>Left</td>
<td>Yes</td>
<td>no</td>
<td>18 days</td>
<td>pin-hole at bronchial stump</td>
<td>minor leakage</td>
<td>bronchoscopic treatment* (1) x3, (2)x2, (3)x3</td>
</tr>
<tr>
<td>2</td>
<td>56</td>
<td>PEM/CDDP</td>
<td>Left</td>
<td>Yes</td>
<td>hemithoracic, 54Gy</td>
<td>25 months</td>
<td>none</td>
<td>no leakage</td>
<td>bronchoscopic treatment (1)x2</td>
</tr>
<tr>
<td>3</td>
<td>48</td>
<td>PEM/CDDP</td>
<td>Right</td>
<td>Yes</td>
<td>hemithoracic, 54Gy</td>
<td>111 days</td>
<td>small air bubble to and fro, staple exposed</td>
<td>no leakage</td>
<td>bronchoscopic treatment (1)x1</td>
</tr>
</tbody>
</table>

**Results:** Three patients with silent BPF were remained to be afebrile, asymptomatic, with no abnormal findings of blood tests throughout their clinical courses. Emptying of postpneumonectomy space on chest X-ray was seen 18 days, 111 days, and 2 years after EPP, respectively. Bronchoscopy and bronchography were performed immediately after emptying, and revealed very small BPF (n=1), to and fro movement of air bubble with exposed staples (n=3), or only unremarkable findings (n=1). All three patients were conservatively observed with only intrabronchial spraying of basic fibroblast growth factor with or without submucosal injection of OK-432 and spraying of fibrin glue. Emptying was disappeared 5 months after onset in one patient with bronchography-proven BPF. Persistent emptying with no clinical symptoms was observed in other two patients with duration of 11 and 19 months after onset, respectively. bronchoscopic treatment*: (1) spraying basic fibroblast growth factor, (2) submucosal injection of OK-432, (3) spraying fibrin glue

**Conclusion:** Although symptomatic BPF after EPP requires immediate surgical intervention, asymptomatic BPF may be conservatively observed.

**Disclosure:** No significant relationships.
P2.09: OPERATIVE INDICATION OF EXTRAPLEURAL PNEUMONECTOMY FOR MALIGNANT PLEURAL MESOTHELIOMA

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Background: The role of extrapleural pneumonectomy (EPP) for patients with malignant pleural mesothelioma (MPM) has been controversial.

Methods: We retrospectively reviewed the records of 56 patients with MPM to determine the efficacy of operative procedures. These patients were considered as having resectable disease using preoperative radiological and physiological examinations.

Results: There were 44 males and 12 females, with a mean age of 64 years old. A past history of exposure to asbestos was identified in 20 patients (35.7%). Thoracoscopic pleural biopsy was the most effective method for diagnosis in 45 patients. It required 3.3 months (median) from the first visit to establish the diagnosis. In histological types, 35 (62.5%) were epithelial, 11 (19.6%) were biphasic, and 10 (17.9%) were sarcomatous. According to the IMIG clinical staging, 11 patients (19.6%) were diagnosed as stage I, 23 (41.0%) stage II, 18 (32.1%) stage III, and 4 (7.1%) stage IV. Thirty-five (62.5%) of 56 patients underwent surgical procedures, including EPP in 33 patients (58.9%) and pleurectomy/decortication (P/D) in 2 (3.6%). Microscopically complete resection by EPP was achieved in 22 patients (39.3%). Preoperative chemotherapy was given in 3 patients. Intraoperative hyperthermo-chemotherapy was added in 7 patients. Postoperative chemotherapy was given in 4 patients, radiotherapy in 10, both chemotherapy and radiotherapy in 2, and hyperthermo-chemotherapy in 6. Major postoperative complications occurred in 12 patients (36.4%) and thirty-day mortality was 9.1% (3/33) in patients with EPP. No patient who underwent P/D had operative complications or mortality. Nonsurgical therapy was selected in 21 patients (37.5%); chemotherapy in 13 patients (23.2%), radiotherapy in 3 (5.4%), hyperthermo-chemotherapy in 1 (1.8%), and palliative care alone in 4 (7.1%). The median and 5-year overall survival rates among all patients were 21 months and 19%, respectively. There was no significant difference in survival between the patients with or without operation. The median and 5-year survival rates who underwent surgery were 21 months and 24%, respectively, whereas those with nonsurgical therapy were 20 months and 0%, respectively. A univariate analysis demonstrated that complete resection by EPP (p = 0.022), epithelial histology (p = 0.001), and c-stage I-II disease (p = 0.003) were signiﬁcant positive prognostic factors. The patients who achieved complete resection by EPP were more likely to have c-stage I-II (p = 0.002) and epithelial diseases (p = 0.024).

Conclusion: EPP should be selected for the patients with c-stage I-II and epithelial disease.

Disclosure: No signiﬁcant relationships.
Poster Session 2

Novel Therapeutics

SEPTEMBER 12, 2012 16:00-17:00

**P2.10: COMBINED ANTISURVIVIN-CISPLATIN TREATMENT OF MALIGNANT PLEURAL MESOTHELIOMA**

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**Background:** Survivin (BIRC5) is a cancer specific apoptosis inhibitor and a promising target for novel anticancer therapies, especially for tumors which presently show poor response to conventional treatment, such as malignant pleural mesothelioma (MPM). MPM rarely responds to cisplatin- or radiation-based treatment and median survival remains around 1 year after diagnosis, despite recent advances in treatment. This fact, added to increasing incidence, underlines the need for novel treatment options for MPM. Our previous retrospective study found that all MPM tissue samples analysed expressed high levels of survivin, so the aim of the present study was to explore the therapeutic potential of survivin inhibition combined with hypotonic cisplatin perfusion of survivin- expressing MPM cells in vitro.

**Methods:** Two MPM cell lines (H2052 and 211H) and an immortalized mesothelial line (MeT-5A) were used for in vitro experiments. Survivin expression was silenced by Stealth® siRNA lipofection. Lipofected cells were treated with hypotonic cisplatin solutions (0, 1 or 10 µg/mL) for 15 minutes 24 hours after lipofection. Effects of each single therapeutic approach and their combination on cell survival and proliferation were assessed with the clonogenic and MTS assays.

**Results:** We found that either hypotonic cisplatin perfusion or survivin silencing significantly reduced survival and proliferation of all three cell lines tested when applied as single-agent approaches (p<0.05). But when both treatments were combined, strongly synergistic effects were observed, since more than 90 % reduction in both survival and proliferation was achieved after application of only 1 µg/mL cisplatin to all three lipofected cell lines (p<0.05).

**Conclusion:** Our in vitro results confirm that a combined antisurvivin and classic chemotherapeutic approach might be a viable direction for development of effective therapies for MPM. Both anticancer gene therapy and hypotonic intrapleural chemotherapy are feasible treatment options for MPM patients and their combination should be further explored.

**Disclosure:** No significant relationships.

**P2.11: INHIBITION OF THE MET RECEPTOR TYROSINE KINASE IN MALIGNANT MESOTHELIOMA**

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**Background:** The MET receptor tyrosine kinase (RTK) plays an important role in a variety of malignancies, especially malignant mesothelioma. The MET proto-oncogene encodes a trans-membrane tyrosine kinase receptor for HGF, a multifunctional protein involved in tissue repair and metastasis. MET contains a semaphorin domain at the N-terminus, a juxtamembrane domain, and a tyrosine kinase domain at the C-terminus of the protein. MET and its ligand HGF are over-expressed in mesothelioma cell lines and tumor samples. Using several mesothelioma cell lines, our group has previously shown that the small molecule inhibitor SU11274 specifically targets MET with an IC50 value of 2 to 3 µM. We have also reported several unique mutations of MET in mesothelioma tumors. In the current study, we evaluated tivantinib (Arq197, ArQule), an oral, synthetic, non-ATP-dependent competitive, small molecule inhibitor of MET in mesothelioma tumors and cell lines.

**Methods:** Genomic DNA was extracted from 13 fresh frozen mesothelioma tumor tissue samples and MET was sequenced using exon specific primers. The non-malignant mesothelial cell line Met5A, and the mesothelioma cell lines H2596, H513, H2052, H2461 and H28 were purchased from the American Type Culture Collection (Manassas,VA). Cell viability was evaluated using the standard Alamar blue assay. Briefly, exponentially growing cells were seeded in 96-well tissue culture plates in 100ul (10% RPMI). After 24 hr of incubation the cells were treated with tivantinib at the indicated concentrations in 1% RPMI. After 72 hours, Alamar blue was added and fluorescence was read at 530/590 nM. Data was normalized to the cells without treatment. IC50 values were calculated using Prism software.

**Results:** We observed a dose-dependent decrease of cell viability in the mesothelioma cell lines treated with tivantinib; the control Met5A cells were not sensitive at the dose range tested. A dramatic reduction in cell viability in response to tivantinib was noted in the H2596 and H513 mesothelioma cell lines that harbor the MET T1010I mutation compared with H22 cells, which express wild-type MET, and the control Met5A cells. Mutational analysis identified the previously reported juxtamembrane domain mutation (R970C) in 1 of the 13 mesothelioma tumor samples.

**Conclusion:** Exposure to tivantinib resulted in the inhibition of proliferation of MET-expressing mesothelioma cell lines. This inhibition was most prominent in cell lines which harbored mutations in MET. We believe that MET may be an appropriate therapeutic target in mesothelioma. An NCI-sponsored phase II trial of tivantinib in previously treated mesothelioma patients is in development at the University of Chicago.

**Disclosure:** No significant relationships.

**P2.12: TARGETING ESTROGEN RECEPTOR β FOR TREATMENT OF PLEURAL MALIGNANT MESOTHELIOMA**

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**Background:** The role of estrogen receptor (ER) β as mediator of anti-proliferative responses has been well documented in numerous scientific articles and selective activation of ERβ with an agonist has shown in vivo anti-tumorigenic efficacy in various animal tumor models.

**Methods:** We have explored the anti-proliferative activity of the highly selective ERβ agonist, KB9520, in human mesothelioma cell lines in vitro and in mesothelioma mice models in vivo and studied its anti-tumorigenic efficacy.
Results: We have demonstrated that KB9520 treatment inhibits propagation of the human ERβ positive REN mesothelioma cell line in culture by blockade of the cell cycle at G1. Introduction of ERβ into the non-ERα and ERβ expressing human mesothelioma cell line Mсто 21H, sensitizes these cells for the anti-proliferative effect of KB9520. Selective activation of ERβ with KB9520 inhibited also tumor growth and progression in vivo.

Conclusion: Together, these data suggest that the anti-tumorigenic effect of KB9520 is mediated through ERβ and demonstrate that selective targeting of ERβ may be an efficacious stand-alone treatment option of this neoplasm and/or become an important add-on to existing therapy.

Disclosure: No significant relationships.

POSTER SESSION 2  SEPTEMBER 12, 2012 16:00-17:00

P2.14: IN VITRO SCREENING OF AN APPROVED DRUG LIBRARY IDENTIFIES AGENTS WITH REPURPOSING POTENTIAL FOR MALIGNANT MESOTHELIOMA

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Background: Repurposing strategies offer potentially shorter times to implementation of novel therapy if drugs with anti-mesothelioma activity can be identified. Aim: We aimed to identify potentially active drugs by screening a library of approved clinical compounds (Johns Hopkins Clinical Compound Library-JHCCL) for in vitro activity against a panel of human mesothelioma cell lines.

Methods: Seven mesothelioma cell lines were screened against 1524 JHCCL compounds using a growth inhibition assay with SYBR® Green I-fluorometric readout.

Results: At a final concentration of 10µM, 148 drugs produced at least 50% growth inhibition on all seven cell lines. Compounds with a history of prior oral or parenteral clinical use were retested in dose response experiments at final concentrations ranging from 100µM to 0.01µM. Seven drugs were identified with IC50 (50% growth inhibitory concentrations) less than 1µM, six with IC50 1-5µM, and five drugs with IC50 5-10µM. Only five of the eighteen agents were known anti-neoplastic drugs. Other active agents were previously approved as antibiotics, antihistamines, anti-helminthics, anti-virals, anti-inflammatory, and immunomodulators.

Conclusion: Active compounds were identified from a panel of agents with history of clinical use. The anti-mesothelioma action of several candidates in vitro now requires validation in vivo or in clinical settings. This approach may yield improved treatments for mesothelioma within a time frame responsive to predicted peak disease incidence. Acknowledgements: Cancer Australia, Dust Diseases Board, The Prince Charles Hospital Foundation and Slater & Gordon Mesothelioma Travel Fellowship. Conflict of Interest: None

Disclosure: No significant relationships.

POSTER SESSION 2  SEPTEMBER 12, 2012 16:00-17:00

P2.15: AGONISTIC ANTI-CD40 ANTIBODY IS EFFECTIVE AGAINST POST-OPERATIVE TUMOUR OUTGROWTH IN A MURINE MODEL OF MESOTHELIOMA

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Background: Local recurrence remains a major cause of death in patients with mesothelioma undergoing surgical resection. Metastases can also occur, especially in longer term survivors. Agonistic anti-CD40 antibody is an immunotherapy that enhances immune priming of effector CD8 T cells by antigen presenting cells (APCs), leading to increased anti-tumour activity. We investigated the use of anti-CD40 antibody for the treatment of post-operative recurrence and metastasis, with regional lymphadenectomy, in a murine model of mesothelioma.

Results: Newly isolated murine mesothelioma cells were found to express CD40, and a human monoclonal antibody (mAb) pC50 recognized this. The mAb pC50 was shown to bind to CD40 and stimulate proliferation of human mesothelioma cells. Treatment with pC50 led to a marked reduction in tumour growth and progression.

Conclusion: in vivo results demonstrate the potential of anti-CD40 therapy for the treatment of post-operative recurrence and metastasis, with regional lymphadenectomy, in a murine model of mesothelioma.

Disclosure: No significant relationships.
**Methods:** Subcutaneous AB1-HA mesothelioma tumours were induced in the flank region of BALB/c mice. Tumours were completely or partially debulked (75%) on day 16, with or without lymph node removal. On the day of surgery, animals that underwent complete surgical resection were re-challenged with AB1-HA at the surgical site (local recurrence) or the opposite flank (metastasis). Animals were treated with anti-CD40 (FGK45) either systemically (partial debulk) or into the tumour bulk upon emergence (recurrence and metastases).

**Results:** Anti-CD40 treatment slowed metastatic growth relative to untreated controls \( (p = 0.020) \) and improved survival from metastasis \( (78.57 \pm 10.96\% \text{ cure, H.R. 5.62 - 125.00}) \). Anti-CD40 also retarded the growth of local recurrences \( (p = 0.004) \) and improved survival from recurrence \( (50.00 \pm 17.68\% \text{ cure, H.R. 1.18 - 4.81}) \). In a partial debulk situation, anti-CD40 slowed outgrowth of residual tumour but did not improve survival. Removal of the tumour draining nodes did not impair efficacy \( (p>0.05) \).

**Conclusion:** We show that anti-CD40 is effective at preventing local recurrence and metastasis and improving survival when combined with complete and partial surgical resection, an effect which was independent of the route of administration and lymph node removal. These findings argue for the usefulness of anti-CD40 antibody as an adjuvant immunotherapy to cancer surgery, particularly in cases where regional lymphadenectomy is indicated.

**Disclosure:** No significant relationships.

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**Conclusion:** NGR-hTNF showed promising PFS and OS in this study. Based on these results, NGR-hTNF 0.8 µg/m2 q1w is currently tested in a placebo-controlled phase II trial as a maintenance treatment in MPM pts who did not progress after 6 cycles of first-line chemotherapy (NCT01358084) and in a double-blind phase III trial evaluating best investigator’s choice ± NGR-hTNF in relapsed MPM (NCT01098266).

**Conclusion:** Six patients (35%) showed mediastinal lymph node swelling greater than 10 mm observed in 13 of the 17 cases (76%); Pleural effusion was detected in 12 patients (70%), pleural effusion on bilateral, 8; right-sided, 2; and left-sided, 2. Pericardial effusion was ranged from 0 cm to 7.2 cm (median, 1.4 cm). Pericardial thickness was mild thickening and were not indicative of malignant nature. Two cases (24%); mild thickening, 3 (18%); irregular thickening, 6 (35%); and mass formation mimicked mediastinal tumor. Pericardial thickness with mass formation was demonstrated to have a high diagnostic accuracy for the tumor infiltration in this study. However, because it is difficult to evaluate the depth of invasion to the chest wall and mediastinal lymph node metastases with PET/CT, an intraoperative careful staging is indispensable.

**Disclosure:** No significant relationships.
P2.20: DOES 18FDG PET PREDICT SURVIVAL IN MALIGNANT PLEURAL MESOTHELIOMA (MPM)?

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Background: Malignant pleural mesothelioma (MPM) is a malignancy in which the prognostic information provided by 18FDG PET imaging could be particularly valuable in determining whether to pursue aggressive treatment. In this retrospective study, we aimed to identify independent predictors of survival related to imaging in MPM.

Methods: We retrospectively analysed baseline 18FDG PET/CT scans in 61 patients with MPM scanned between 2006 and 2011. All scans were performed before medical treatment or surgery, including talc pleurodesis. Volumes of interest were drawn around the lesion/s to measure SUVmax, SUVmean, and total lesion glycolysis (TLG). One patient was excluded as there was no identifiable disease on the images. For each of the four 18FDG PET parameters measured, cases were grouped to low and high grade according to cut-off points identified by maximal chi-squared test. Differences in overall survival (OS) were assessed by the Kaplan-Meier test. Independent samples t-test and COX regression were used for correlations between different histological groups and the presence of independent prognostic factors within our dataset.

Results: Follow up was available for a median of 11.5 months (range 2 to 60 months). All 18FDG PET parameters above the cut-off points (high-grade) were associated with poorer survival. The average SUVmax was 13.04, and overall survival (OS) for the high-grade group was 10 months vs. the low-grade group of 14 months (p=0.022). The average SUVmean was 3.66, and overall survival (OS) for the high-grade group was 11 months vs. the low-grade group of 14 months (p=0.022). Average TLG was 665, and overall survival (OS) for the high-grade group was 9 months vs. the low-grade group of 13 months (p=0.027). The average SUVpeak was 13.04, and overall survival (OS) for the high-grade group was 11 months vs. the low-grade group of 13 months (p=0.022). The average SUVmean was 3.66, and overall survival (OS) for the high-grade group was 14 months vs. the low-grade group of 13 months (p=0.022). The average SUVpeak was 13.04, and overall survival (OS) for the high-grade group was 14 months vs. the low-grade group of 13 months (p=0.022).

Conclusions: 18FDG uptake in MPM, as measured by SUVmax, SUVmean or TLG, has prognostic significance and may be a helpful factor in stratifying individual patient management.

Disclosure: No significant relationships.
P2.22: REPRODUCIBILITY OF THERAPY RESPONSE EVALUATION BETWEEN EXPERIENCED AND LESS EXPERIENCED READERS OF PLEURAL MESOTHELIOMA BY MRECIST, RECIST 1.0, RECIST 1.1, AND WHO

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Background: Tumor therapy monitoring of malignant pleural mesothelioma is difficult due to the specific tumor growth pattern. Today there exists a wide number of response evaluation criteria, such as modified RECIST (mRECIST; up to 2 target lesions on 3 anatomical levels), RECIST 1.0 (up to 5 target lesions), RECIST 1.1 (up to 2 target lesions), and WHO (up to 5 target lesions). The aim of this study was to evaluate the accordance of tumor size change measurements between experienced and less experienced readers for the above mentioned response evaluation criteria.

Methods: CT scans from 15 patients with a total of 43 baseline and follow-up examinations from a clinical multi-center pleural mesothelioma trial were retrospectively analyzed. One experienced radiologist (≥10 years) and one less experienced radiologist (≥1 year) selected target lesions independently according to mRECIST, RECIST 1.0, RECIST 1.1, and WHO criteria. CT scans were re-measured after an interval of greater than one month. The association of the relative changes in the measurements between baseline and follow-up(s) were analyzed for inter- and intra-reader variability using the different response criteria. Inter- and intra-reader variability was analyzed by Bland-Altman method.

Results: Our preliminary data from two radiologists demonstrated the best inter-reader accordance by using RECIST 1.0 within the 95% limits of agreement between the experienced reader and less experienced reader of -9.4/18.2. In contrast, intra-reader variability of the experienced reader was best using mRECIST within the 95% limits of agreement of -6.3/-4.8. WHO performed worst with an inter- and intra-reader variability of -34.0/38.6 and -29.1/20.0, respectively.

Conclusion: In our study, pleural mesothelioma treatment response evaluation by an experienced and less experienced reader was most reliable when using RECIST 1.0, possibly due to depiction of better suitable target lesions. However, mRECIST demonstrated the best reproducibility with the experienced reader. Higher variability was seen with RECIST 1.1. WHO was consistently poorly suitable to evaluate tumor response. To confirm these findings, the inclusion of further readers and more patients’ data is warranted.

Disclosure: No significant relationships.
P2.23: 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.

Methods: We identified 231 consecutive patients with biphasic MM treated by surgery at Brigham and Women’s Hospital between 1988 and 2009 and found 74 with metastases to mediastinal N2 lymph nodes. We evaluated the N2 lymph node metastases of 41 of these patients with biphasic MM and available pathology material and correlated the findings with overall survival.

Results: All 41 patients (8 F/33 M; mean age 62; range 31-88) had a diagnosis of biphasic MM metastatic to N2 lymph nodes. Twenty-four patients (59%) with biphasic MM had both epithelioid and sarcomatoid components in the N2 lymph nodes and seventeen patients (41%) showed spread only of the epithelioid component to the N2 lymph nodes. The mean follow-up after surgery was 11.2 months. The median survival of patients with mixed histology in the N2 lymph nodes was 8.9 months versus 11.9 months for those with an epithelioid component (p=0.059).

Conclusion: Our data indicate that the presence of a mixed component in the N2 lymph nodes may predict a worse overall survival in patients with biphasic MM. The results of our study emphasize the importance of histologic classification of not only the surgical specimen but also the lymph node metastases and highlight the biologic complexity of disease progression in biphasic MM.

Disclosure: No significant relationships.

P2.24: 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.

Methods: We examined 151 consecutive patients with pleural DMM treated from 1988 to 1997 at Brigham and Women’s Hospital by extrapleural pneumonectomy (EPP) followed by heated chemotherapy all of whom had a pretreatment biopsy available for review. We characterized the presence of epithelioid and sarcomatoid histology in the resection and pretreatment biopsy specimens. Associations between the histology in pre- and post-treatment specimens were investigated.

Results: The histology type of DMM in pretreatment biopsies were epithelioid in 120 patients (79%), mixed in 21 patients (14%), sarcomatoid in 8 patients (5%), and indeterminate in two patients (1%). The histology type of DMM in resection specimens was epithelioid in 93 patients (62%), mixed in 51 patients (34%), and sarcomatoid in 7 patients (4%). Biopsy findings were concordant with resection findings in 116 patients (Spearman r=0.64, p<0.0001).

Conclusion: Our data suggests that a diagnosis of mixed or sarcomatoid DMM in the pretreatment biopsy is highly predictive of the histology in the resection specimen. A diagnosis of epithelioid DMM in the pretreatment biopsy is less accurate, and it changed to a less favorable one in a significant proportion of the cases. The results of our study emphasize the importance of thorough biopsy sampling in patients with malignant mesothelioma and the value of resection specimens for accurate diagnosis.

Disclosure: No significant relationships.

P2.25: BINDING OF CA125 TO MESOTHELIN IN THE SERUM OF MALIGNANT MESOTHELIOMA PATIENTS

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Background: One promising biomarker for malignant mesothelioma (MM) is soluble mesothelin, which is elevated in the serum of half of MM patients at diagnosis and approximately 15% before radiological or clinical signs of MM. Mesothelin, a 40 kDa glycoprotein constitutively expressed on the cell surface of mesothelial cells, has been demonstrated to bind to the ovarian cancer biomarker CA125. It has been suggested that metastatic spread of CA125 expressing tumours can be facilitated by binding to mesothelin expressing tissues in the pleura and peritoneum and therapeutic targeting of this interaction is currently being investigated. We hypothesised that binding of soluble mesothelin and CA125 in the serum may prevent accurate quantification of mesothelin levels, and it was of concern that the diagnostic accuracy of mesothelin for MM could be being impaired.

Methods: An ELISA-based assay was developed to detect mesothelin bound to CA125. Serum was collected from 41 patients with MM, and levels of mesothelin, CA125 and of bound mesothelin-CA125 were determined. To dissociate mesothelin bound to CA125 serum samples

Disclosure: No significant relationships.
were incubated in a solution of sodium sodium dodecyl sulphate (SDS) and diethylenetriaminepenta acetic acid (DTPA).

**Results:** Serum mesothelin concentrations ranged from 1 to 100 nM, with 29 of the 41 cases being mesothelin positive. Serum CA125 concentrations ranged from 4 to 3400 U/mL, with 18 of the 41 cases being CA125 positive. CA125 was demonstrated to be bound to mesothelin in nine of the MM patient sera. In each of these nine cases the CA125 concentration was above 35 U/mL and the mesothelin concentration was above 4 nM. All nine patients were male, with predominantly epithelial tumours. Median survival for the group of patients with evidence of CA125 bound to mesothelin in the serum was 5 months, which was significantly shorter than for the group which did not have CA125 bound to mesothelin (median survival 13.5 months; p = 0.019). The addition of SDS and DTPA to serum samples disrupted the observed binding of mesothelin to CA125, but no change in detectable mesothelin levels was observed.

**Conclusion:** CA125 binds to mesothelin in the serum of some MM patients. Binding was only observed in patients with relatively high CA125 and mesothelin levels. Binding could be disrupted but this did not alter the amount of mesothelin detected in clinical samples. At the range of mesothelin concentrations observed in the majority of non-symptomatic asbestos exposed individuals binding of CA125 would not impair the diagnostic accuracy of the mesothelin biomarker.

**Disclosure:** No significant relationships.

**POSTER SESSION 2**  **SEPTEMBER 12, 2012 16:00-17:00**

**P2.26: EXPRESSION OF MELANOMA ANTIGEN-ENCODING GENE-1 (MAGE-1) IN MESOTHELIOMA CELLS AND REACTIVE MESOTHELIAL CELLS.**

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**Background:** Recently mesothelioma cases have been rapidly increased in number in Japan. Mesotheliomas occur in various forms in body cavity fluid. Immunohistochemical staining with a panel of antibodies is used to diagnose mesothelioma when the condition is strongly suggested by cytological findings. We performed immunocytochemical staining a differential diagnosis of mesothelioma from reactive mesothelial cells using MAGE-1 antibody.

**Methods:** Study subjects were 7 mesothelioma cases (pleural mesothelioma: 5 cases, peritoneal mesothelioma: 1 case, pericardial mesothelioma: 1 case) and 10 non-cancerous cases of reactive mesothelial cells (pleural effusion: 7 cases, peritoneal effusion: 3 cases) evaluated at our department. Immunostaining was performed in cell block sections prepared from body fluid and also performed in cell transfer sections prepared from Papanicolaou stained specimens. Three mesothelioma cases were also examined for expression in tissue. Immunostaining was performed with a Dako Autostainer and EnVision visualization system.

**Results:** 1) Positive results were obtained in 5 cases of mesothelioma (70%), expressed in the nucleus.
2) Negative results were obtained in all cases of reactive mesothelial cells (100%).

**Conclusion:** Using the Melanoma antigen-encoding gene (MAGE-1) antibody is effective in differentiating mesothelioma from reactive mesothelial cells.

**Disclosure:** No significant relationships.
the cell transfer method and/or cell block method for immunochemical staining.

Methods: This study included 10 patients with MPM diagnosed by effusion cytology in the past 6 years in our institute. These patients were considered as having mesothelioma by body fluid cytology. Furthermore, immunohistochemical staining was carried out for a definitive diagnosis using the cell transfer method and/or the cell block method for these patients. Tumor cells were immunostained using antibodies specific to calretinin, CK5/6, D2-40, mesothelin, thrombomodulin, CEA, MOC31, p53 protein, EMA, E-cadherin, CD146, MPP-9 and MAGE-1.

Results: Calretinin was positive in 10/10 patients (100%), CK5/6 in 9/9 (100%), D2-40 in 10/10 (100%), mesothelin in 9/9 (100%), thrombomodulin in 7/8 (87.5%), CEA in 0/10 (0%), MOC31 in 0/8 (0%), Ber-EP4 in 0/8 (0%), p53 protein in 9/9 (100%), EMA in 8/9 (88.9%), E-cadherin in 7/8 (87.5%), CD146 in 6/7 (85.7%), MMP-9 in 7/8 (87.5%), MAGE-1 5/7 (71.4%). As a result, the 10 patients were definitively diagnosed as having MPM. p53 protein, EMA, E-cadherin, CD146, MMP-9 and MAGE-1 were especially useful for discrimination of mesothelioma and reactive mesothelial cells. Histological examination and/or electron microscopic examination was finally done and the cytological diagnosis was confirmed.

Conclusion: Good immunochemical staining results were obtained, enabling the definitive diagnosis of mesothelioma using the cell transfer method and/or cell block method. Immunochemical staining using multiple antibodies is important for the definitive diagnosis of mesothelioma by cytology.

Disclosure: No significant relationships.

POSTER SESSION 2  SEPTEMBER 12, 2012 16:00-17:00

P2.29: THE p16INK4A/p14ARF  DELETION IN ATYPICAL MESOTHELIAL CELLS LOCALIZED ON THE SEROSAL SURFACE OR WITHIN THE SUBMESOTHELIAL FIBROUS TISSUES OF THE PARIETAL PLEURA

Tohru Tsujimura1, Ayuko Sato1, Ikuko Torii1, Misa Song1, Shinji Matsumoto2, Kazuki Nabeshima2, Seiki Hasegawa3, Takashi Nakano4
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Background: Malignant pleural mesothelioma (MPM) is an aggressive tumor arising from mesothelial cells on the serosal surfaces of the pleural cavity. Since it is difficult to distinguish morphologically between genuine invasion of MPM and inflammation-induced infiltration of non-tumor mesothelial cells, the pathological diagnosis of MPM is not generally established until atypical mesothelial cells invade into subpleural adipose tissues. On the other hand, the p16INK4a/p14ARF (CDKN2A) locus at chromosome 9p21 has been reported to be frequently deleted in MPM, but not in non-tumor mesothelial cells, by fluorescence in situ hybridization (FISH) analysis. To establish the utility of FISH analysis of the p16INK4a/p14ARF locus in atypical mesothelial cells on the serosal surface and in the submesothelial fibrous tissues of the same MPM patient also showed homozygous deletion of the p16INK4a/p14ARF locus.

Methods: Tissue specimens with atypical mesothelial cells invaded into subpleural adipose tissues were selected based on histopathological examination of the pleural tissues obtained by video-assisted thoracoscopic surgery. Tissue specimens with atypical mesothelial cells confined to the serosal surface and the submesothelial fibrous tissues were also selected. Dual-color FISH analysis was performed on formalin-fixed, paraffin-embedded sections using a Spectrum Green-labeled chromosome 9 centromeric probe (CEP-9) and a Spectrum Orange-labeled, locus specific p16INK4a/p14ARF probe (Abbott, Tokyo, Japan). Homozygous deletion was defined if both p16INK4a/p14ARF signals were lost in nuclei and at least one CEP-9 signal was observed. FISH was considered positive if homozygous deletion of p16INK4a/p14ARF locus was observed in at least >10% of cells which were identified histopathologically as mesothelial cells using adjacent hematoxylin & eosin-stained specimens.

Results: Homozygous deletion of the p16INK4a/p14ARF locus was observed in atypical mesothelial cells invaded into subpleural adipose tissues. Atypical mesothelial cells on the serosal surface and in the submesothelial fibrous tissues of the same MPM patient also showed homozygous deletion of the p16INK4a/p14ARF locus. In addition, homozygous deletion of the p16INK4a/p14ARF locus was observed in atypical mesothelial cells confined to the serosal surface and the submesothelial fibrous tissues.

Conclusion: These results showing homozygous deletion of the p16INK4a/p14ARF locus in atypical mesothelial cells on the serosal surface and in the submesothelial fibrous tissues indicate that FISH analysis of the p16INK4a/p14ARF locus may be useful to identify early-stage MPM, such as genuine mesothelioma in situ.

Disclosure: No significant relationships.
Elizabeth Blackler1, Caraline M. Craig1, Tatiana Starr1, Maria Farberov1, Lee M. Krug1, Marjorie G. Zauderer2, Valerie Rusch1, Jimmie Holland3, R G. Key1

Background: Mesothelioma is often caused by asbestos traceable to a specific environmental or occupational exposure. This unique pathophysiology and identifiable responsibility can lead to blame and anger directed at the person or organization that allowed the exposure to occur. Few studies have examined the emotional burden of mesothelioma. Little is known about patients’ coping methods or interventions to improve their psychosocial symptoms.

Methods: The aim of this two-part study is to identify the psychosocial/physical needs and quality of life (QOL) of a sample of patients with pleural mesothelioma and explore the feasibility and promise of an Internet-based discussion group for these patients. We are approaching patients who have a diagnosis of pleural mesothelioma, are able to read/speak English, and are receiving care at Memorial Sloan-Kettering Cancer Center (MSKCC). Patients with significant cognitive dysfunction or severe psychiatric disturbance are excluded from the study. Part 1 consists of a set of questionnaires assessing coping methods, interpersonal support, mood, anxiety, and overall QOL. Part 2 is a weekly, six session Internet-based discussion group facilitated by a social worker. Part 2 participants are assessed three times: at baseline, approximately 1 week post group sessions, and approximately 4 weeks post group sessions in addition to a self-report satisfaction survey. Part 1 participants are not required to participate in Part 2. We plan to recruit 90 participants for Part 1 and 30 participants for Part 2.

Results: Of 56 participants enrolled thus far in Part 1, 50 have completed the questionnaires. Thirty-two were male and 18 were female. The median age is 65 years old (range 38-83). Forty-two participants self reported their race as White, 3 Black or African American, 1 Asian/Pacific Islander, 3 other, and 1 did not report. Seven participants identified themselves as Spanish, Hispanic, or Latino. In baseline assessment of wellbeing using the FACT-L, participants on average indicated the most distress in social wellbeing, which includes the ability to work, enjoy life, accept illness, and be content with current QOL (M = 16.80, SD = 5.34, Range = 4-28). Overall, participants showed the least distress in social wellbeing, indicating feelings of closeness and high emotional support and communication with family and friends (M = 22.87, SD = 3.50, Range = 15-28). Fifteen of the 19 participants enrolled to Part 2 have completed participation. Three participants are pending start of the next group. Thus far, 11 of 15 participants reported that the group met or exceeded their expectations.

Conclusion: At study end we plan to describe the psychological and physical symptom burden and QOL of patients. Reasons for study ineligibility or refusal, study attrition, and discussion of satisfaction surveys will also be presented. Information from this pilot study could prove helpful in developing a way to connect these geographically-dispersed, disabled, and distressed patients.

Disclosure: No significant relationships.
**P2.32: THE ROLE OF THE THORACIC NURSE CASE MANAGER (NCM) IN SUPPORTING A PATIENT THROUGH MULTIMODALITY THERAPY (MMT) FOR MESOTHELIOMA.**

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**Background:** A Case Study demonstrating the role of the Nurse Case Manager (NCM) in supporting patients undergoing multimodality therapy (MMT) for malignant pleural mesothelioma (MPM). Pleurectomy Decortication (PD) is the preferred surgical option in patients suitable for MMT in our unit. To be included; slides of patient numbers coming to our unit with mesothelioma (100-150), the numbers suitable for MMT (approximately 15%). Undergoing MMT takes altogether about six months and the role of the NCM is to support the patient through the process; organising appointments, scans, multidisciplinary team referrals, psychosocial and financial support, symptom control. On completion of MMT the NCM in partnership with the Surgeon supports the patient with referral for second line treatment, survivorship and palliative care.

**Methods:** A 68 year old gentleman diagnosed at Video Assisted Thoracoscopy (VATS) biopsy in October 2010 in another unit, Epitheliod MPM. No other medical history. Referred to Guys Hospital: NCM Assessment process Holistic Needs Assessment (HNA). Pre-admission work up, bloods ECG, X-ray, scans, physical examination, psychological assessment, social assessment, guided visit of intensive care and Thoracic units. VATS, repeat biopsy and talc November 2010 at Guys (for staging and reaccumulated effusion). PET-CT done in December ruled out bulky disease, transdiaphragmatic growth and significant mediastinal involvement, N3 disease or distant metastases. Patient had a PS of 0, fit for total pleurectomy/decortication. PD with hyperthermic pleural perfusion (povidone-iodine) January 2011 at Guys, post operative Nursing Care and the NCM involvement in this discussed. Patient discharged from Guys day 7, discharge advice given by NCM pain control, exercise, wound care, diet / elimination and breathing exercises. Telephone follow up. Prophylactic radiotherapy on thoracotomy and drains scars 21Gy in 38 February 2011 6 cyles of adjuvant chemotherapy, Alimta and Cisplatin March-May 2011 Follow-up PET-CT 4 weeks after completion of chemotherapy and at one year shows no recurrence / progression. An EORTC QLQ-C30 questionnaire 18 months post MMT was carried out and a patient interview

**Results:** Included in the results throughout the case presentation will be slides of PET-CT images, results of EORTC QLQ questionnaire (overall health rated 4/7, overall quality of life rated 6/7) and samples of interview questions / answers. The role of the NCM is explored throughout the patient journey, which is focussed on best practice standards recommended by the Mesothelioma Framework (2007) in supporting patients.

**Conclusion:** The NCM plays a vital role in supporting patients having MMT for mesothelioma. The case load of this specific patient group has increased, and with survival rates showing an improvement with advancements in treatment. This patient group is likely to continue to increase and role of the NCM in survivorship can be developed further. Currently the overall five year survival is 33% and for patients with epitheliod subtype with complete macroscopic resection is 46%, (most recent data from this unit) Recommendations for further practice will be to use HNA or EORTC questions on diagnosis and at regular intervals throughout treatment and follow up and audit the results.

**Disclosure:** No significant relationships.

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**P2.33: DEVELOPMENT OF AN EDUCATIONAL PROGRAM ABOUT NURSING CARE OF PATIENTS WITH PLEURAL MESOTHELIOMA IN JAPAN**

Yasuko S. Nagamatsu¹, Yuji Natori²
Global Health Nursing, St. Luke’s College Of Nursing, Tokyo/JAPAN, ¹Hirano Kameido Himawari Clinic, Tokyo/JAPAN

**Background:** In 2010, 1,209 people died of mesothelioma in Japan (Ministry of Welfare, 2011). The number of deaths from mesothelioma is rapidly growing and Murayama (2008) projected that number would increase to 100,000 deaths in Japan from mesothelioma by the year 2040. The rapid increase of patients with mesothelioma (MPM) created a demand for nursing care for mesothelioma that requires knowledge about such things as asbestos, mesothelioma and its treatments, and benefits. Unfortunately there were no textbooks with relevant knowledge or educational programs for nurses about mesothelioma in Japan or Japanese. Therefore, this study aimed to develop an educational program about nursing care of patients with pleural mesothelioma.

**Methods:** The program was systematically developed according to the steps of Instructional Design (ID): needs assessment, goal setting, writing performance objectives, developing assessment tools, developing instructional strategy, and developing instructional materials. To motivate learners, the teaching concept of andragogy (adult-focused) was adopted. Furthermore, narratives by MPM patients were introduced to address caregivers attitudes. The Research Ethics Review Board, St. Luke’s College of Nursing (approval no. 11-034) approved this study.

**Results:** Development of Program Twenty one nurses were interviewed to assess the needs of nurses caring for MPM patients. According to the needs of nurses, nine goals were set and knowledge, skills and attitude were described as performance objectives. To achieve each performance objective, a variety of instructional methods such as lectures, group work, role-playing and group discussion were taken according to the nature of the performance objectives. Finally the instruction about knowledge, skills, attitude content was reformed to fit within the units of the two-day main program (14.5 hours) with a follow-up program one month later (3 hours). The units of program were shown in Table 1. Each program was set for small groups up to 30 learners. All instructional materials were originally developed in Japanese.

<table>
<thead>
<tr>
<th>Main Program Day1</th>
<th>Main Program Day2</th>
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<tbody>
<tr>
<td>1000-1040 Icebreaking</td>
<td>0830-0920 Home visiting care &amp; Care coordination</td>
</tr>
<tr>
<td>1045-1135 Asbestos &amp; Benefit</td>
<td>0930-1030 Assessment &amp; Management of symptoms of MPM</td>
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<tr>
<td>1135-1235 Surgical treatment for MPM</td>
<td>1045-1245 Role play</td>
</tr>
<tr>
<td>1335-1425 Chemotherapy &amp; Radiotherapy for MPM</td>
<td>1400-1430 Stress management for nurse</td>
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<tr>
<td>1435-1505 Narrative speech by patient with MPM</td>
<td>1430-1500 Post test</td>
</tr>
<tr>
<td>1515-1605 Symptoms of MPM &amp; Palliative care</td>
<td>Follow-up Program</td>
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</tbody>
</table>

**Disclosure:** No significant relationships.
Conclusion: The first educational program about Nursing Care of Patients with Pleural Mesothelioma in Japan was developed and implemented.

Disclosure: No significant relationships.

**POSTER SESSION 2  SEPTEMBER 12, 2012 16:00-17:00**

**P2.34: PROPOSAL FOR A LOCAL DIAGNOSTIC AND THERAPEUTIC PATHWAY FOR MALIGNANT PLEURAL MESOTHELIOMA: THE IMPORTANCE OF A MULTIDISCIPLINARY TEAM**

Sara Tenconi¹, Roberto Piro¹, Debora Formisano¹, Massimiliano Paci¹, Cristian Rapicetta¹, Giorgio Sgarbi¹, Luigi Zucchi¹, & Mesothelioma Multidisciplinary Team²

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**Background:** Malignant Pleural Mesothelioma (MPM) is an asbestos correlated cancer which incidence is expected to increase in the next decade in Italy. Amongst international literature there isn’t agreement on management of these patients. At the IRCCS Hospital of Reggio Emilia we decided to schedule a multidisciplinary pathway for patients who present with pleural effusion suspected for Mesothelioma, in order to give them a proper diagnostic setting, a standardized therapeutic option and a continuous palliative support.

**Methods:** Review of the Regional MPM Registry showed an increasing incidence of this cancer in our high risk area, a multidisciplinary panel was instituted to evaluate the efficiency of actual treatments and follow up, comparing our experience and results with literature. Pneumologists, Thoracic Surgeons, Oncologists, Radiotherapists, Radiologists, Nuclear Physicians, Palliativists and General Practitioners were divided into two groups to discuss and propose new strategies for the “diagnosis and staging” and for the “treatment and follow up”; the statistician structured a multistep pathway with quality indices.

**Results:** After a 20 months comparison between local attitudes and literature guidelines, the two groups proposed a Diagnostic and Therapeutic Pathway (PDTA) [figure 1] which includes: (1) first and second stage investigations guided by a single case manager through multidisciplinary meetings, (2) multimodal treatment for stage I and II epithelioid Mesothelioma (neo/adjvant chemotherapy, Pleurectomy and Decortication (P/D), adjuvant radiotherapy) and (3) territorial palliative care for advanced stage or unfit patients. For patients affected by stage III epithelioid Mesothelioma we decided to plan a randomized trial comparing neoadjuvant chemotherapy followed by P/D versus chemotherapy and palliation (talc poudrage) to establish if these patients can benefit from surgery regarding their quality of life (measurement of pain and dyspnœa) and, eventually, their survival. Non-epithelioid Mesothelioma patients and advanced stage eligible patients will be treated with chemotherapy and palliative radiotherapy in case of symptomatic localized disease.

**Conclusion:** In literature there isn’t a consensus on the best therapeutic options for MPM, due to very heterogeneous reported trials; nevertheless, International Societies and Cancer Networks have tried to licence guidelines. In releasing a PDTA we should take into account all these recommendations as well as the local settings; moreover, difficulties are represented by the fact that MPM remains a rare disease and that we still don’t seem to have a “cure” for these patients. For this reason, we think that all treatments should aim to improve survival and achieve symptoms control, with the minor functional impairment.

**Disclosure:** No significant relationships.
**P3.01: DIAGNOSTIC POTENTIAL OF MICRORNAS (MiRs) IN MALIGNANT PLEURAL MESOTHELIOMA (MPM)**

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**Background:** MPM is histologically difficult to distinguish from reactive mesothelial proliferations (RMPs), partly because proposed MPM-markers are not specific, reproducible or validated enough (Zimling ZG, Jørgensen A, Santoni-Rugiu E., Histopathology, In Press). MiRs are small non-coding RNA-strands (~22 nt) that post-transcriptionally regulate gene-expression and vital cellular processes. MiR-expression in other cancers has shown diagnostic significance, but it is uncertain whether currently published miR-data may provide candidate biomarkers for differentiating MPM from RMP. To pursue this goal, we performed a miR-expression-screening in formalin-fixed paraffin-embedded diagnostic biopsies, surgically resected MPM-specimens, and corresponding surrounding non-neoplastic pleura (NP).

**Methods:** We performed a Real Time(RT)-qPCR-based (Exiqon®) screening of 742 human miRs’ expression in preoperative biopsies, epithelioid MPM-, and NP-specimens from 5 patients treated with extra-pleural pneumonectomy as part of trimodal protocol. The relevant identified differentially expressed miRs were validated on tissue samples from 24 independent MPM-patients by RT-qPCR-TaqMan® MicroRNA Assays (Applied Biosystems). Threshold-cycles (C) were determined with related SDS v1.4 software, comparatively analyzing PCR-data with a normalizer. Significant (p < 0.05) differences between independent/paired groups were detected by non-parametric Mann-Whitney/Wilcoxon matched-pairs tests.

**Results:** We identified significant difference in expression of 4 cancer-relevant miRs (down-regulation of miR-17-5p, -126 and -652 and up-regulation of miR-221) that correctly differentiated MPM from RMPs and was not influenced by chemotherapy (comparable miR-expression levels in surgical samples and diagnostic biopsies). We then tested if the 4 miRs could fulfill the International Mesothelioma Interest Group’s recommendations for a suitable MPM-marker (sensitivity/specificity > 80%) by generating receiver-operating-characteristics (ROC)-curves using the RT-qPCR-data. The IMIG-criteria were not met for miR-17-5p and -221, while miR-126 and -652 showed high sensitivity and specificity.

**Conclusion:** The cancer-relevant miR-126 and -652 have great potential as biomarkers for distinguishing MPM from RMPs, while miR-17-5p and -221, previously reported to be specific for MPM’s histological subtypes and to play a pathogenic role in MPM, are not entirely suitable for this differential diagnostic purpose. Further studies will explore miRs’ prognostic and predictive potential and fully validate by in situ-hybridization-techniques their possible use in histopathological diagnostics.

**Disclosure:** No significant relationships.

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**P3.02: PTEN PROTEIN EXPRESSION PREDICTS SURVIVAL IN MULTIMODALITY TREATED MALIGNANT PLEURAL MESOTHELIOMA PATIENTS: A VALIDATION STUDY OF TISSUE MICROARRAYS**

**Byron Bitanihirwe,** Martina Friess, Svenja Thies, Lukas Frischknecht, Alex Soltermann, Mayura Meerang, Holger Moch, Marc De Perrot, Ghassan Allo, Walter Weder, Isabelle Opitz

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**Background:** Malignant pleural mesothelioma (MPM) is characterised by complex chromosomal aberrations, including chromosome 10 losses. The tumour suppressor gene phosphatase and tensin homologue (PTEN) located on chromosome 10q23 plays an important role in different cancers (Chalhoub et al., 2009). We previously assessed PTEN protein expression on a tissue microarray (TMA, n = 352) from historical samples, mainly derived from post-mortem examination with only limited clinical information available (Opitz et al., 2008), and demonstrated a shorter overall survival (OAS) for patients with PTEN loss. The present study therefore aimed to evaluate the expression of PTEN in two prospectively collected patient cohorts treated for the diagnosis of MPM.

**Methods:** The two independent cohorts of MPM patients were uniformly treated with 3 cycles of platinum-based induction chemotherapy (CTX) followed by extrapleural pneumonectomy (EPP) and optional adjuvant radiotherapy. A TMA was constructed that included 46 patients for cohort 1 (2 - 4 cores) and 102 patients for cohort 2 (4 cores). Immunoreactivity levels of cytoplasmic and nuclear PTEN were semi-quantitatively scored by different observers in a blinded fashion and expression was then correlated to clinical and pathological parameters, such as histological subtype, staging, OAS, and progression-free (PFS) survival.

**Results:** In contrast to our historical cohort which showed a 60% loss of PTEN expression, PTEN was more intensely expressed particularly in the cytoplasm of patients uniformly treated with induction CTX and EPP (i.e. low cytoplasmic PTEN expression; 26% in cohort 1 and 28% in cohort 2). There was no significant correlation between PTEN expression and the histological subtype. Cox regression survival analysis indicated that increased cytoplasmic expression of PTEN was significantly associated with longer PFS in both test cohorts (p=0.05; cohort 1 and p=0.01, cohort 2) (Figure 1), but not with OAS.

**Disclosure:** No significant relationships.
Conclusion: We have confirmed the role of PTEN as a prognosticator for survival in MPM patients as we have found a significant association between high cytoplasmic PTEN protein expression and prolonged PFS in two different cohorts of MPM patients uniformly treated with induction CTX and EPP. These results support our previous study implicating PTEN as a candidate tumor suppressor in MPM. Further study of this gene, and correlation with the downstream Akt signaling axis, appears warranted.

Disclosure: No significant relationships.

P3.03: THE ROLE OF THYMIDYLATE SYNTHASE AND EXCISION REPAIR CROSS-COMPLEMENTING GROUP-1 AS PREDICTORS OF SURVIVAL IN PATIENTS UNDERGOING MULTIMODALITY TREATMENT FOR MALIGNANT PLEURAL MESOTHELIOMA

Servet Bölükbas1, Annette Fisseler-Eckhoff2, Melanie Demes3, Sonja Stallmann4, Michael Eberlein5, Joachim Schirren1

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Background: Most studies investigated the relationship between Thymidylate Synthase (TS) and Excision Repair Cross-Complementing Group-1 (ERCC1) mRNA levels and outcome in inoperable patients with malignant pleural mesothelioma (MPM). The purpose of the present study was to study the association between TS and ERCC1 and overall survival in patients undergoing multimodality treatment for MPM.

Methods: Forty-one consecutive patients with histologically confirmed MPM previously treated with radical pleurectomy, adjuvant Cisplatin and Pemetrexed as well as irradiation of the intervention sites were retrospectively analyzed. TS and ERCC1 mRNA levels were evaluated by real-time polymerase chain reaction and correlated to prospectively documented data. Kaplan-Meier analyses, log-rank test and Cox regression analyses were used to estimate survival and to determine predictors of survival.

Results: In the univariate analyses, there was a significant correlation between very low TS mRNA level (<= 5 percentile) and overall survival (OS, 31 vs. 18 months; P=0.042). Patients with very high ERCC1 mRNA levels (>95 percentile) survived significantly longer (75 vs. 18 months; P=0.048). In a multivariable analysis by Cox proportional hazards model that controlled for histology, stage, gender, as well as TS and ERCC1 mRNA levels, completeness of resection in terms of macroscopic complete resection (MCR) remained the only significant prognostic factor (P=0.001).

Conclusion: Very low TS and very high ERCC1 mRNA levels might influence overall survival in patients with MPM undergoing multimodality treatment. MCR seems to be the most important prognosticator. However, the role of TS and ERCC1 in multimodality treated MPM patients is worth of prospective validation in larger multicenter trials.

Disclosure: No significant relationships.

P3.04: GLYCOPEPTIDE SERUM MARKERS FOR MALIGNANT PLEURAL MESOTHELIOMA DIAGNOSTICS

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Background: Mass spectrometric (MS) techniques were used for the identification of serum accessible candidate markers of malignant pleural mesothelioma (MPM). After surfaceome analysis of MPM vs control cell lines to identify potential candidate markers of MPM, the clinical significance of selected candidate markers was verified in serum cohorts using multiplexed selected reaction monitoring (SRM).

Methods: We used the cell surface capturing technology (CSC) for the quantitative investigation of the cell surface N-glycoproteins of four MPM (2 epithelioid and 2 biphasic) and four control cell lines (pleura and lung adenocarcinoma). SRM-assays were established for glycopeptides detected in higher abundance in MPM and used for multiplexed SRM quantitative analysis in patient sera enriched for N-glycopeptides.

Results: Surfaceome analysis revealed 500 N-glycopeptides, corresponding to more than 300 cell surface N-glycoproteins from MPM-derived cell lines. 56 candidate biomarker peptides were selected for verification and quantification in patients and donors serum cohorts using a single multiplexed SRM-assay. Cohorts consisted of age and sex matched MPM and non-small cell lung cancer patients as well as healthy donors (total number > 75). Regression modeling was applied to define peptide-based classifiers of MPM. Serum analysis of the mesothelioma marker soluble mesothelin-related protein (SMRP) confirmed the robustness of our approach.

Conclusion: The relative quantitative investigation of the MPM surfaceome unveiled serum accessible MPM candidate biomarkers. SRM analysis of serum cohorts revealed a multiplexed peptide-based classifier of MPM.

Disclosure: No significant relationships.
**P3.05: CLINICAL AND MORPHOLOGICAL PROGNOSTIC FACTORS IN PATIENTS WITH MALIGNANT PLEURA MESOTHELIOMA (MPM)**

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**Background:** Malignant pleura mesotheliomas (MPM) are represented as heterogeneous tumor group regarding their clinic-biological behavior and histomorphological growth pattern. According to the maximum of applied industrial asbestos in the 70s and their latency period for decades, the highest incidence occurs assumedly between 2010 and 2020. Therefore the identification of prognostic factors for treatment decisions of patients with MPMs is urgent.

**Methods:** 191 cases of MPMs were analyzed concerning histological subtypes in biopsies and corresponding surgical specimens. Prognostic molecular markers like tumor suppressor genes p53, p63 and proliferation index MiB-1 as well as clinical parameters like TNM stage were correlated with overall survival of the patients.

**Results:** The median age of men (n=165) was 62 years, the median age of women (n=26) 63.7. 77% epithelial (n=147), 6.8% sarcomatoid (n=13) and 16.2% biphasic (n=31) subtypes were classified. The reclassification of the diagnosis in biopsies and surgical specimens demonstrated accordance in nearly 100% of epithelial subtypes, 60% of sarcomatoid subtypes and 37.5% of biphasic subtypes. Survival of patients with epithelial subtype was significantly prolonged as compared to survival of patients with sarcomatoid and biphasic subtypes, respectively (14.5 vs. 7.5 and 9.9 respectively months median survival time, p = 0.01, p = 0.012). 5% and 36% of MPMs were negative for p53 and p63. Proliferation indices were inversely associated with overall survival of the patients.

**Conclusion:** As epithelial subtype is correlated with a significant better survival in patients with MPM a correct diagnosis by intensive morphological investigation of biopsies and surgical specimens by experienced pathologist is essential for patients’ prognosis. In patients with epithelial subtype lymph node metastases is a significant prognostic factor. Significant correlations of Ct- or pT-stages or M-stages with overall survival could not be demonstrated. Under the aspect of new therapeutic regimens the establishment of further e.g. molecular prognostic markers is essential.

**Disclosure:** No significant relationships.

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**P3.06: ERCC1, RRM1 AND TYMS EXPRESSION LEVELS IN PATIENTS WITH RECURRENT MALIGNANT PLEURA MESOTHELIOMA**

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**Background:** Pemetrexed/Platinum agents are applied as first-line treatment for mesothelioma. The association of excision repair cross-complementing group 1 (ERCC1), ribonucleoside-diphosphate reductase subunit M1 (RRM1) and thymidylate synthase (TYMS) with the outcome of patients with mesothelioma is discussed controversial. According to Scarlatti and colleagues thymidylate synthase (TYMS) is a predictive factor for Pemetrexed response and the excision repair cross-complementing group 1 (ERCC1) seems to be predictive for Cisplatin therapies. With this in mind we analysed the mRNA expression level of those DNA repair and synthesis genes in primary mesotheliomas and corresponding recurrent tumours of 5 patients.

**Methods:** The tumours were classified and the mRNA expression level was detected by real time polymerase chain reaction analysis. All Patients received surgery and were treated with Cisplatin/Pemetrexed regimes.

**Results:** All 5 mesothelioma were classified as epithelioids. After therapeutic treatment all 5 patients suffered from recurrent mesotheliomas. The median survival after primary diagnosis is 15.41 months. High ERCC1 and RRM1 expression levels were detected in relapsed tumors as compared to primary mesotheliomas. In contrast low TYMS expression levels were found.

**Conclusion:** According to our study, TYMS as a predictive factor cannot be confirmed. RRM1 and ERCC1 as DNA repair and synthesis genes seem to play an important role in relapsed mesothelioma after treatment with Pemetrexed/Platinum regimes.

**Disclosure:** No significant relationships.

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**P3.07: ASSOCIATION BETWEEN (GT)N REPEATS POLYMORPHISM IN THE HEME OXYGENASE-1 GENE PROMOTOR AND THE SUSCEPTIBILITY TO MALIGNANT MESOTHELIOMA IN THE JAPANESE POPULATION WITH ASBESTOS-EXPOSURE**

Kazuya Fukuo¹, Yoshihiro Fujimori², Yoshiie Yoshikawa³, Shusai Yamada³, Taichi Otsuki², Asuka Okada³, Kunihiko Tamura³, Chihiro Tabata³, Tomoko Hashimoto-Tamaoki³, Takashi Nakane³

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**Background:** Malignant mesothelioma (MM) is an aggressive tumor that arises from mesothelial cells lining the pleural or peritoneal cavity. The development of MM is closely associated with asbestos exposure which induces oxidative stress. Heme oxygenase (HO)-1, a rate-limiting enzyme of heme degradation, plays a protective role against oxidative stress. The HO-1 gene promoter carries (GT)n repeats whose number is inversely closely associated with the risk of MM, there are some populations that do not develop MM after exposure to asbestos. In this study, we hypothesized that HO-1 gene polymorphism may influence the susceptibility to MM.

**Methods:** To investigate the relationship between the length polymorphism of (GT)n repeats and the susceptibility to MM, we analyzed the HO-1 gene promoter carrier status in 44 asbestos-exposed subjects without MM and 78 asbestos-exposed patients with MM using polymerase chain reaction (PCR)-based genotyping.

**Results:** The number of repeats ranged from 16 to 38 with two peaks at 23 and 30 repeats. Polymorphism of (GT)n repeats were grouped into two classes of alleles: short (S) (n=24) and long (L) (n=24) and three genotypes: L/L, L/S and S/S. The proportions of allele frequencies in class L as well as genotypic frequencies of L allele carriers (L/L, L/S) were significantly higher in the asbestos-exposed patients with MM than in the asbestos-exposed subjects without MM.

**Conclusion:** The long size of (GT)n repeat in the HO-1 gene promoter is associated with a higher risk of MM in the Japanese population.
with asbestos-exposure. This is the first report to demonstrate that the polymorphism in the HO-1 gene promoter is associated with the susceptibility to MM.

Disclosure: No significant relationships.

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**P3.08: NOVEL PLASMA PROTEINS ASSOCIATED WITH PROGNOSIS IN MALIGNANT PLEURAL MESOTHELIOMA**

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Background: The search for novel biomarkers to define more successful and individual treatment approaches represent an important challenge for those involved in the care for patients with malignant pleural mesothelioma (MPM). In this exploratory study, we have made a systematic approach to investigate the proteins present in plasma of MPM patients and to associate their level with the outcome of disease.

Methods: Plasma samples from twelve MPM patients (6 ‘short-’ and 6 ‘long-term’ survivors from parallel phase II studies investigating thalidomide) were used for proteomic analyses. Our series included samples with epithelial and biphasic MPM (8 epithelial; 3 biphasic subtype; and 1 undetermined). Plasma samples were immuno-depleted of the 14 most abundant proteins prior to labeling for isobaric tag for relative and absolute quantitation (iTRAQ) analysis using mass spectrometry. The most promising candidates were chosen for selected reaction monitoring mass spectroscopy (SRM-MS) confirmation. Statistical analyses using T-Test of peak areas were used to identify proteins that were differentially expressed between the short- and long-term survivor groups.

Results: Median survival of short- and long-term survivors (1.2 and 38.3 months, respectively) differed significantly (p = 0.001). Other baseline characteristics did not reveal major differences between the short- and long-term survivors. The total number of proteins identified was 226 (1% false discovery rate [FDR]). A number of those found to be differentially expressed between short- and long-term survivors (p ≤2-fold change; p ≤0.05) by iTRAQ: selenoprotein P; tetranectin (TETN); insulin-like growth factor-binding protein 2 (IGFBP2); osteonectin (SPARC); platelet basic protein (CXCL7); and attractin (ATRN). SRM-MS analysis revealed that the concentrations of attractin (p = 0.01), tetranectin (p < 0.001) and selenoprotein P (p < 0.001) were higher in long-term survivors. In contrast, an increase in the concentration of IGFBP2 (p = 0.07) and CXCL7 (p = 0.01) correlated with shorter survival.

Conclusion: We have demonstrated the feasibility of using the iTRAQ and SRM-MS proteomic techniques to investigate potential prognostic protein markers in plasma of MPM patients. The candidates identified will be further examined by validation experiments with Western Blot and/or ELISA, and by testing samples from a larger patient series.

Disclosure: No significant relationships.

**POSTER SESSION 3 | SEPTEMBER 13, 2012 11:30-12:30**

**P3.09: SEARCH FOR NEW MESOTHELIOMA BIOMARKERS: PROTEOMIC SCREENING OF PLEURAL EFFUSIONS**

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Background: Diagnosis of malignant mesothelioma relies on morphological examination of cells and is often conclusive first when the disease has reached an advanced stage. The additional measure of diagnostic biomarkers in effusions, serum or plasma has not been extensively adapted by the clinical routine; a gap that is partly due to the lack of predictability of proposed and tested biomarkers. However, soluble biomarkers are still a compelling, noninvasive adjunct to today’s standard diagnostics, which could speed up and strengthen diagnoses. Using mass-spectrometry to screen pleural effusions from patients with mesothelioma, lung cancer and mesothelioma, we aim to discover new biomarkers to add diagnostic value and improve an already studied biomarker panel.

Methods: Pleural effusions from 6 mesothelioma patients, 6 lung cancer patients and 3+4 (2 pools) patients suffering from benign mesotheliroma were selected for this study. To decrease variance and increase proteome coverage the effusions were depleted of high abundant proteins (MARS-14 affinity purification, Agilent®), trypsinized, mass tagged (iTRAQ®) and subsequently pooled as well as fractionated (ultra narrow isoelectric focusing and nano-liquid RP-HPLC) before screened with the LTQ Orbitrap Velos (Thermo Fischer Scientific, San Jose, CA, USA). Analyses of the results were done using Significant Analysis of Microarrays (SAM v. 3.11; Stanford Tools, univariate), SIMCA (Umetrics®, multivariate: Principal Component Analysis; PCA and Partial Least Squares; PLS) and Ingenuity Pathway Analysis (IPA; Ingenuity®, network analysis).

Results: More than 1300 proteins were identified in the screened patients (FDR<5%). Univariate analyses (SAM) identified proteins with high respectively low expression in effusions from mesothelioma patients. The findings include a number of known mesothelioma biomarkers (mesothelin, osteopontin and apolipoprotein-CI) as well as several novel candidates; though, with rather high FDR values. Multivariate analysis and modeling (PCA and PLS) identified thrombospordin-1 and galectin-1. Validation has been initiated on a larger number of patient effusions. Preliminary results indicate that high levels of superoxode dixumitate-2 add diagnostic information for a mesothelioma while galectin-1 is indicative of lung cancer. Galectin-1 and apolipoprotein-CI might be prognostic markers in mesothelioma. Furthermore, thrombospordin-1 is elevated in patients with asbestos pleuritis and is a potential early marker for mesothelioma. Network analysis (IPA) gives further information about this disease and the system we work with.

Conclusion: In this study, proteomic screening of pleural effusions have provided candidates for diagnostic and prognostic use for malignant mesothelioma. The validation of biomarker candidates will be presented as well as network information about mesothelioma shedding light on pathophysiologocal aspects.

Disclosure: No significant relationships.

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**P3.10: HIGH BLOOD PLATELET-TO-LYMPHOCYTE RATIO IS AN INDICATOR OF POOR PROGNOSIS IN MALIGNANT PLEURAL MESOTHELIOMA UNDERGOING EXTRAPLEURAL PNEUMONECTOMY**

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Background: Asbestos induced chronic inflammation plays a key role in the pathogenesis of malignant pleural mesothelioma (MPM). Recently, several biomarkers which are related to systemic inflammation including platelet-to-lymphocyte ratio (PLR) and neutrophil-to-lymphocyte ratio (NLR) have
been reported as independent prognostic markers of patients with MPM. Our aim is to investigate the prognostic value of PLR and NLR for patients with MPM undergoing extrapleural pneumonectomy (EPP).

Methods: Of 85 patients who underwent EPP for MPM during January 2001 to April 2011 at Toronto General Hospital, 65 patients whose blood test results before initial therapy were available were retrospectively analyzed. Clinicopathological factors including PLR and NLR were analyzed in relation to survival.

Results: Demographics are as follows; median age: 61 (range 21-71 years), sex: 55 males and 10 females, histology: 48 epithelial, 12 biphasic, 3 sarcomatoid and 2 others, treatment: 34 chemotherapy (CH) + EPP + radiotherapy (RT), 12 CH + EPP, 11 RT + EPP, 6 EPP + RT and 2 EPP alone. Complete resection rate was 81.5%. Median survival time was 23 months and 3 year survival rate was 39.6%. In univariate analyses, female gender (p = 0.013), PLR < 300 (p = 0.013), absolute platelet count < 400 x 109/liter (p = 0.036) and pN0-1 (p = 0.044) were predictors of good survival. History (epithelial, p = 0.051) and NLR < 4 (p = 0.053) were not associated with overall survival. Multivariate analyses confirmed that female gender (p = 0.012) and PLR < 300 (p = 0.015) as an independent predictors of overall survival.

Conclusion: High Platelet-to-lymphocyte ratio was independently associated with poor prognosis in patients with MPM undergoing EPP.

Disclosure: No significant relationships.

P3.11: NOVEL MALIGNANT-MESOTHELIOMA-ASSOCIATED ANTIGENS (GENE-X AND THBS-2) IN THE DIAGNOSIS OF MALIGNANT PLEURAL MESOTHELIOMA (MPM)

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Background: Malignant pleural mesothelioma (MPM) is a highly aggressive malignant tumor of the pleura associated with asbestos exposure, and its diagnosis is usually difficult at early stage. We identified novel mesothelioma-related antigens, Gene-X and thrombospondin-2 (THBS-2), that were recognized by tumor-infiltrating B cells, and preliminary study demonstrated that these antigens were potentially useful diagnostic marker in MPM (Cancer Sci 2009).

Methods: A total of 120 patients, who presented with a suspicion of MPM and received pleural biopsy, were reviewed; 97 patients were finally diagnosed with MPM and 27 were with non-malignant diseases (NM). The antibody-titers against Gene-X and THBS-2 in the sera were measured by ELISA method.

Results: The serum antibody-titer against THBS-2 was significantly higher in MPM patients than in NM (P<0.01), but there was no difference in the serum antibody-titer against Gene-X (Table). The receiver operating characteristic (ROC) curve analysis showed a significant diagnostic value of serum antibody-titer against THBS-2 with the area-under curve of 0.886 (95% CI, 0.797 - 0.975; P<0.001) in discrimination of MPM from NM diseases; the sensitivity and specificity, when the cut-off value was 0.08, were 72.2% and 95.5%, respectively. There was no significant difference in serum antibody-titer against THBS-2 according to histologic subtype or clinical stage.

Conclusion: The serum antibody-titer against THBS-2 can be a useful non-invasive marker in the diagnosis of MPM.

Disclosure: No significant relationships.

P3.12: SERUM THIOREDOXIN-1 AND HISTONE DEACETYLASE ACTIVITY IN PERIPHERAL BLOOD OF PATIENTS WITH MALIGNANT PLEURAL MESOTHELIOMA

Takashi Nakano, Kunihiro Tamura, Shingo Kanemura, Eisuke Shibata, Hisaya Ohkuwa, Taichihiro Otsuki, Hitomi Kamiya, Miki Honda, Koji Mikami, Yoshitaka Nogi, Risa Maeda, Takayuki Terada, Asuka Okada, Shusai Yamada, Kazuya Fukuoka, Chiharu Tabata, Kozo Kuribayashi

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Background: Asbestos-initiated oxidative stress significantly contributes to carcinogenesis in asbestos-related malignancies by promoting oxidative DNA damage and regulating redox signaling pathways. Thioredoxin-1 (TRX) is a small redox-active protein that possesses antioxidant activity and acts as a redox-regulating multifunctional protein. Oxidative stress also influences histone deacetylase (HDAC) activity which modulates gene expression through the deacetylation of lysine residues on histone proteins and acts transcriptional repressors of genes.

Methods: We evaluated TRX in serum and HDAC activity in the peripheral blood mononuclear cells (1×10^9) of patients with malignant pleural mesothelioma (MPM).

Results: The patients with MPM had significantly higher levels of TRX in serum and higher HDAC activities than control population. MPM patients with early clinical stage showed an increase in HDAC activity, and there was no significant difference among the IMIG clinical stages. On the other hand, the patients with advanced stage MPM showed higher levels of TRX than those with early stage MPM.

Conclusion: MPM patients had a markedly increased level of TRX in serum and high HDAC activity in peripheral blood mononuclear cells. These findings suggest that TRX and HDAC activity can be biomarkers in patients with MPM.

Disclosure: No significant relationships.
Background: Long term survival results are not satisfactory following treatment for malignant pleural mesothelioma (MPM). However, a small proportion of patients survive longer than 2 years. We analyzed the features of patients who survived longer than 2 years following chemotherapy or multimodality treatment.

Methods: The patients with MPM who survived longer than 2 years following chemotherapy or multimodality treatment (extrapleural pneumonectomy, hemithoracic irradiation and chemotherapy) were identified from two prospective databases. Patient features including age, gender, side, histology, IMIG T and N stages (pretreatment for chemotherapy group and pathologic for multimodality treatment group), complete response/resection (microscopic) rate and survival were compared using Student’s t-test and Kaplan Meier Survival analysis.

Results:

<table>
<thead>
<tr>
<th>Feature</th>
<th>Chemotherapy Group (n=36)</th>
<th>Multimodality Treatment Group (n=12)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>No of pts/total</td>
<td>36/450</td>
<td>12/40</td>
<td>0.001</td>
</tr>
<tr>
<td>Age</td>
<td>60 ± 11</td>
<td>51 ± 9</td>
<td>0.01</td>
</tr>
<tr>
<td>Male/Female</td>
<td>15/21</td>
<td>4/8</td>
<td>NS</td>
</tr>
<tr>
<td>Right/Left</td>
<td>21/15</td>
<td>5/7</td>
<td>NS</td>
</tr>
<tr>
<td>Histology (Epithelioid/MM/mixed)</td>
<td>29/5/1</td>
<td>12</td>
<td>NS</td>
</tr>
<tr>
<td>Complete response/resection</td>
<td>6/36</td>
<td>3/12</td>
<td>NS</td>
</tr>
<tr>
<td>Tr/2/3/4</td>
<td>8/10/13/5</td>
<td>2/7/2/1</td>
<td>NS</td>
</tr>
<tr>
<td>Extrapleural lymph node</td>
<td>11/36</td>
<td>6/12</td>
<td>NS</td>
</tr>
<tr>
<td>Metastasis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median (mo) and 5-year survival (%)</td>
<td>42/23</td>
<td>47/4/28</td>
<td>NS</td>
</tr>
</tbody>
</table>

The results are presented in the table. NS, not significant.

Conclusion: The proportion of patients surviving longer than 2 years appear to be larger in the multimodality treatment group. Although T stages were similar to other series, complete microscopic resection or response to chemotherapy were common in long term survivors. There appears to be no difference in long term survival in terms of treatment modality, if patients survive longer than 2 years.

Disclosure: No significant relationships.
postoperative chemotherapy alone (two with interferon alpha followed by cisplatin/pemetrexed, one with cisplatin/gemcitabine, and one with ifosfamide/adriamycin). The last 4 patients received preoperative therapy with ifosfamide/adriamycin (3 patients) and cisplatin/pemetrexed/Veglin (1 patient). Interestingly, 3/4 of these patients were noted to have pathological responses with 80–99% necrosis which is not often seen with standard cisplatin and pemetrexed chemotherapy. Although survival data is quite limited, survival roughly correlated with the existence of extensive necrosis following preoperative chemotherapy. Median survival of patients exhibiting >80% necrosis was 19.6 mos (with two patients still alive) compared to only 5.7 mos for the patient without extensive necrosis and 7.6 mos for the entire surgical group.

Conclusion: Sarcomatoid-predominant malignant pleural mesothelioma remains a difficult tumor to control. Optimal treatment strategies should employ multimodality approaches that stress systemic induction therapy with non-cisplatin/pemetrexed regimens. Salvage surgery may be an option for those patients who remain free of metastatic spread following induction therapy, particularly those with >80% necrosis.

Disclosure: No significant relationships.

**P3.15: A DEDICATED MULTIDISCIPLINARY MESOTHELIOMA PROGRAM FOSTERS ENROLLMENT IN CLINICAL TRIALS**

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**Background:** Clinical trials are the mechanism for both introducing innovative therapies and establishing the standard-of-care in cancer treatments, yet the number of patients treated on clinical trials is typically cited as less than 5%. Malignant pleural mesothelioma (MPM), a cancer for which novel therapies are desperately needed and for which the standard-of-care is limited to palliative chemotherapy, is clearly a cancer for which clinical trial enrollment is significantly higher than what is cited as the typical clinical trial enrollment rate and is also significantly higher than the historical accrual to trials for MPM in our institution prior to formation of our dedicated multidisciplinary program. We attribute this increased clinical trial enrollment rate to several features of the Program: the increased influx of new patients, the mindset of the team to support clinical trials and cross-pollination between disciplines, such as the development of a new surgical pleural-access procedure to allow enrollment of patients with fused chests (previously excluded) into a gene therapy trial. We conclude that one of the benefits of a dedicated MPM program is that it fosters clinical trial enrollment.

**Conclusion:** The percentage of patients being treated for MPM on clinical trials in our institution is significantly higher than what is cited in the figure. The total number of new patients seen, not all of whom were treated at our institution, was 182 and 60 (33%) of those patients were treated on clinical trials.

**Disclosure:** No significant relationships.

**P3.16: P/D + HITOC (HYPERTHERMIC INTRATHORACIC CHEMOPERFUSION) IS COMPARABLE TO EPP IN THE MULTIMODALITY TREATMENT OF PATIENTS WITH MALIGNANT PLEURAL MESOTHELIOMA (MPM)**

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**Background:** The objective was to evaluate the overall survival rates in patients with resectable MPM treated by EPP or P/D + HITOC in a retrospective non-randomized consecutive study.

**Methods:** From 10/2002 until 06/2011, a total of 56 patients were consecutively treated by Extrapleural Pneumonectomy (EPP) or open Pleurectomy/Decortication (P/D) in combination with HITOC using cisplatin (40mg/l) and doxorubicin (20 mg/l) in a total volume of 5000 ml normal saline for 90 minutes at 42°C using a Performer HT (RanD, Medolla, Italy). Starting in 11/2009 P/D+HITOC was first offered as treatment in patients with resectable MPM, including those with pleural mesothelioma. The quarterly number of new patient visits and enrollment in clinical trials since the formation of the Program 21 months ago is depicted in the figure. The total number of new patients seen, not all of whom were treated at our institution, was 182 and 60 (33%) of those patients were treated on clinical trials.

**Conclusion:** The percentage of patients being treated for MPM on clinical trials in our institution is significantly higher than what is cited in the figure. The total number of new patients seen, not all of whom were treated at our institution, was 182 and 60 (33%) of those patients were treated on clinical trials.

**Disclosure:** No significant relationships.
were 3, in hospital 26 days respectively. No potential side effects of the chemoperfusion were observed, such as wound healing disorders, nausea, or significant rise in creatinine or drop in WBC. EPP group consisted of 24 male and 4 female patients aged 30-71 (median 57) years. 15/28 pts. suffered from cardiac and 2/28 from pulmonary complications. 5 reoperations were performed (3 empyema, 1 gastric herniation, 1 bronchus leakage). Mean number of days in ICU were 5, and 34 in hospital respectively. The pericardium and diaphragm were resected and replaced all EPP and in 3/28 and 3/28 P/D-patients respectively. Median survival in P/D group was 18,6 months (95% CI 17,6-19,6 months) and 22,8 months (95% CI 12,8-32,3 months) in EPP group. P-value (Log Rank) was 0,369 (n.s.).

Conclusion: The oncological results and overall survival rate for patients treated with P/D + HITHOC (Cis-Doxo) is comparable to EPP. It represents a reasonable treatment option for patients not eligible to undergo EPP for age and/or cardiopulmonary reasons. Taking into account the shorter stay in hospital, the lesser rate of reoperations and the sparing of lung parenchyma only marginally affected by the tumor, it is warranted if P/D + HITHOC is also an option in patients where today EPP is still standard of care.

Disclosure: No significant relationships.

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P3.17: HYPERTHERMIC INTRATHORACIC CHEMOPERFUSION (HITHOC) IN COMBINATION WITH PLEURECTOMY / DECORTICATION (P/D) FOR TREATMENT OF MALIGNANT PLEURAL MESOTHELIOMA (MPM)

Uwe Gruetzner1, Michael Lindner1, Martin E. Eichhorn1, Sandra Feske1, Juergen Sklarek2, Thomas Duell1, Rudolf A. Hatz1
1Department Of Thoracic Surgery, Munich Center For Thoracic Surgery, Asklepios Kliniken Muenchen-Gauting, Gauting/GERMANY; 2Department For Pneumological Oncology, Asklepios Kliniken Muenchen-Gauting, Gauting/GERMANY

Background: Our objective was to evaluate the oncological results of P/D + HITHOC (Cis-Doxo) in elderly patients with potentially resectable MPM.

Methods: From 11/2009 until 06/2012, a total of 46 patients were treated by open pleurectomy and complete decortication of the lung to remove all resectable tumor mass. After closing the chest cavity a Performer HT (RanD, Medolla, Italy) was used for chemoperfusion with cisplatin (40mg/l) and doxorubicin (20 mg/l) in a total volume of 5000 ml normal saline for 90 minutes at 42°C.

Results: 46 patients (37 male / 9 female), age 52-81 (median 70) years were successfully treated. Surgery and all chemoperfusions were completed as planned. The pericardium and diaphragm were resected and replaced in 7/46 and 4/46 pts respectively. Time under anesthesia was between 6:05 and 10:10 (median 7:10) hrs, extubation was possible right after the operation in all but one case. Postoperative treatment in the ICU was necessary for 1-11 (median 1) days. One patient died from pulmonary embolism. No serious 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 arrhythmia n=11/46 (24%), respiratory failure n=10/46 (22%), pneumonia n=7/46 (15%) and secondary air leaks n=10 (22%). 4 reoperations occurred (1 thoracic duct lesion, 1 empyema, 2 persistent air leaks) Follow-up time was 1 to 31 (mean 9,1) months. 38/46 (83%) patients are still alive. Mean overall survival time was 8,2 months.

Conclusion: P/D + HITHOC (Cis-Doxo) proved to be a successful surgical treatment option for MPM. It can be offered to patients not eligible to undergo EPP for age and/or cardiopulmonary reasons. Despite the long time under anesthesia and high dose local chemotherapy patients are doing remarkably well after surgery. The oncological results are comparable to EPP. It should be warranted to investigate if P/D + HITHOC is also an option in patients where today EPP is still considered the gold standard of care.

Disclosure: No significant relationships.

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P3.18: PLEUROCTOMY/DECORTICATION, HYPERTHERMIC PLEURAL LAVAGE WITH POVIDONE-IODINE FOLLOWED BY ADJUVANT CHEMOTHERAPY: SURVIVAL ANALYSIS IN 65 CONSECUTIVE PATIENTS

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Background: Radical surgery remains controversial in malignant pleural mesothelioma (MPM). Our goal was to examine the results of pleurectomy/decortication (P/D) with hyperthermic pleural lavage with povidone-iodine followed by prophylactic radiotherapy radiotherapy and adjuvant chemotherapy in patients with malignant pleural mesothelioma.

Methods: Prospective study of patients undergoing P/D and hyperthermic pleural lavage with povidone-iodine, prophylactic radiotherapy (21 Gy in 3 fractions) and adjuvant chemotherapy at our institution. All procedures were performed by the same surgeon and patients were treated by the same team.

Results: Between October 2004 and March 2012, sixty five consecutive patients were offered this multi-modality therapy. Fifty four patients were male, and 11 female. The median age at operation was 63 year (range 45-74). The 30-day mortality was nil. Twelve patients (18.5%) experienced postoperative complications: 6 patients (9.2 %) had a persistent air leak, 4 patients (6.1 %) had chylothorax requiring surgical intervention, one patient had ARDS (1.5 %), one patient (1.5%) had pneumonia and one patient (1.5%) had postoperative empyema. All patients received prophylactic radiotherapy and adjuvant chemotherapy. Three patients received 2 cycles, 62 patients received 4-6 cycles of cisplatin-based chemotherapy. Second line chemotherapy (vinorelbine or pemetrexed) or phase 1-2 trials were routinely offered to patients with performance status 0-2 progressing after first line chemotherapy. At last follow-up, thirty six of 65 patients were alive (median follow-up 21 months, range 4.4-76.4 months). Twenty eight patients were alive with no evidence of disease recurrence, 8 were alive with disease recurrence and 29 patients had died of disease progression. The overall 1-year survival was 90%, 2-year survival was 62.5 % and 5-year survival 33.4%. At univariate analysis, complete macroscopic resection (p=0.002) and epithelioid histology (p=0.009) were associated with longer survival. Patients undergoing complete macroscopic resection (Ro-R1) had 1-year, 2-year and 5-year survival of 100%, 84.8% and 43.2%, respectively. Patients who underwent incomplete macroscopic resection (R2) had 1-year, 2-year and 5-year survival of 76%, 34.8% and 21.8%, respectively. Patients with epithelioid subtype had a 1-year, 2-year and 5-year survival of 100%, 74.8% and 44.7%, respectively. Patients with biphasic/sarcomatoid subtype had 1-year, 2-year and 5-year survival of 68.4%, 34.2% and 13.7%, respectively. Patients with epithelioid subtype and complete macroscopic resection (n=31) had 1-year, 2-year and 5-year survival of 100%, 86.2% and 46%, respectively.

Conclusion: Pleurectomy/decortication with hyperthermic pleural lavage with povidone-iodine, prophylactic radiotherapy and adjuvant chemotherapy is a safe and well tolerated multi-modality therapy. Patients with epithelioid subtype in whom complete macroscopic resection can be achieved experienced the best outcomes.

Disclosure: No significant relationships.
POSTER SESSION 3 \nSEPTEMBER 13, 2012 11:30-12:30

**P3.19: PHARMACOKINETIC STUDY OF HYPERTHERMIC INTRATHORACIC CHEMOTHERAPY (HITHOC) FOLLOWING PLEURECTOMY AND DECORTICATION**

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**Background:** Cytoreductive surgery in combination with hyperthermic pleural perfusion with chemotherapy agents is an ideal multimodality approach to treating malignant pleura mesothelioma. The aim of this study is to investigate the pharmacokinetic behavior of cisplatin and doxorubicin during this procedure.

**Methods:** After pleurectomy and decortication via anterolateral thoracotomy a 42° Celsius hyperthermic solution of a 5000ml saline containing 200mg Cisplatin and 100mg Doxorubicin was perfused into the thoracic cavity for 90 minutes. Simultaneous samples to analyze the concentration of chemotherapy agents were drawn from the hyperthermic pump at 0, 10, 30, 60, 90 minutes after cisplatin and doxorubicin had been perfused. 3 milliliter blood samples were subsequently obtained at minute 0, 10, 30, 60, 90 and day 1, 2 and 4. Doxorubicin fluid and serum levels were analyzed by high performance liquid chromatography. Cisplatin was measured by a flameless atomic absorption spectrometry. Clinical datasets including perioperative morbidity were recorded.

**Results:** Recently, 46 hyperthermic intrathoracic chemotherapy (HITHOC) procedures have been performed and from 9 patients a complete dataset was analyzed. The intrapleural administration of cisplatin and doxorubicin consisted in an increased exposure to total platinum and doxorubinol of the pleural cavity without increasing the systemic circulation. Local toxicity was not seen after the procedure. The AUC (0-90 minutes) and their mean ratio AUC (regional/systemic values) show that the absorption was greater after neoadjuvant therapy. The mean resorption intake was 8.5% (4.8-16.1) for doxorubinol and 18.6 % (11.5-22.1) for Cisplatin. The serum Cmax was median 1.73 μg/ml for cisplatin and 90.9 g/ml for doxorubicin.

**Conclusion:** After an aggressive cytoreductive lung sparing surgery hyperthermic chemotherapy is feasible, save and pharmacologically favorable. This study should be a step forward to delivering a standardized HITHOC protocol.

**Disclosure:** No significant relationships.

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**POSTER SESSION 3 \nSEPTEMBER 13, 2012 11:30-12:30

**P3.20: POSTOPERATIVE CREATININE INCREASE PREDICTS SUSTAINED KIDNEY INJURY IN PATIENTS WITH MALIGNANT MESOTHELIOMA UNDERGOING SURGICAL RESECTION**

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**Background:** Acute kidney injury (AKI) leads to increased morbidity and mortality and progression to chronic kidney injury via a subchronic, mid-term kidney injury (sustained kidney injury) phase is a frequent consequence of AKI. The incidence of severe AKI (serum creatinine elevation > 3 times upper limit of normal) is nearly 10% in patients undergoing surgical resection of malignant pleural mesothelioma, a procedure in which intraoperative cisplatin chemotherapy is commonly administered. Because of the high risk of AKI and the devastating consequences of chronic kidney disease and end stage renal disease in this cancer cohort, we studied the ability of early postoperative changes in serum creatinine (sCr) to predict sustained kidney injury. We hypothesized that acute sCr elevation, within 24-48 hours after surgery, can predict sustained, clinically significant decline in kidney function defined as 50% increase in sCr from baseline, 2-4 weeks after surgery.

**Methods:** A prospective, observational cohort of patients undergoing surgical resection (extrapleural pneumonectomy) for the treatment of malignant pleural mesothelioma at the Brigham and Women’s Hospital between 1998 and 2009 were studied. Receiver operator characteristic (ROC) curves were generated to examine the diagnostic ability of 24- and 48-hour changes in sCr over baseline to identify sustained kidney injury in the derivation cohort (n=279) and tested in the validation cohort (n=207). The performance of our derived criteria was compared to various other criteria used to characterize AKI to predict the risk of sustained kidney injury using the net reclassification index (NRI) and integrated discrimination improvement (IDI).

**Results:** Sustained kidney injury occurred in 8.9% (n=25) of patients in the derivation and 10.1% (n=21) in the validation cohort. A 59% or greater increase in sCr (1.59 fold) at 48 hours after surgery was most predictive of sustained kidney injury (AUCroc = 0.798; sensitivity of 68% and specificity of 87%). When compared to other AKI criteria, we found that our prediction model had the highest c-statistic; and when compared to the RIFLE criteria the difference was statistically significant (p<0.001). Among other AKI definitions, we found that sCr increase of 0.3 mg/dl in 24 hours or 0.5 mg/dl increase in 48 hours (Waikar and Bonventre criteria) also reliably predict sustained kidney injury.

**Conclusion:** In this study we identified a cohort, which developed sustained kidney injury (defined as doubling of sCr present at 2-4 weeks after surgery). We found that development of clinically significant sustained kidney injury can be predicted by acute postoperative sCr elevation in patients treated for mesothelioma where a 59% (1.59 fold) sCr elevation at 48 hours was the best predictor of sustained kidney injury (positive predictive value of 41%; negative predictive value of 96%).

**Disclosure:** No significant relationships.

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**POSTER SESSION 3 \nSEPTEMBER 13, 2012 11:30-12:30

**P3.21: TWO SIMULTANEOUS, CONSECUTIVE, NON-RANDOMIZED COHORTS OF OPERABLE PATIENTS WITH MALIGNANT PLEURAL MESOTHELIOMA (MPM) RECEIVING INDUCTION CHEMOTHERAPY FOLLOWED BY EITHER EXTRAPLEURAL PNEUMONECTOMY (EPP) AND POSTOPERATIVE RADIOTHERAPY OR PLEURECTOMY/DECORTICATION ALONE, RESPECTIVELY. THE DANISH EXPERIENCE.**

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**Background:** Trimodality treatment combining chemotherapy, extrapleural pneumonectomy (EPP) and postoperative radiotherapy may result in cure (5-year survival) in some MPM patients but prognosis is generally poor and treatment is affiliated with morbidity and risk of treatment related deaths. Pleurectomy/decorticatio (P/D) is a less comprehensive operative procedure sparing the lung and with less morbidity but theoretically with dubious curative potential. Results from two such simultaneous treatment cohorts in Denmark are given (not randomized).

**Methods:** Induction chemotherapy for both groups were 3-6 courses of platinum-based doublet chemotherapy. F18-FDG-PET/-CT scan fused international mesothelioma interest group
imaging restaging and preoperative mediastinoscopy was subsequently performed at Rigshospitalet, Copenhagen, Denmark where also all surgery was performed. All patients had T1-3N0-1M0, age ≥70 years, performance status 0-1, and no major comorbidities. EPP was performed in patients having epithelioid subtype and lung function test allowing pneumonectomy. In that case EPP was followed by postoperative radiotherapy 56 Gy in 30 fractions, 5F/W, and radiotherapy constraints for contralateral lung was V20<15%, V10<50%, and MLD<12 Gy (trimodal treatment). P/D was performed in patients having biphasic or sarcomatoid subtypes, poor lung function test, or cardiac comorbidity not allowing EPP but allowing lesser surgery.

Results: In the EPP group of 28 Danish patients included 2006-2011 there were 2 cases of fatal radiation pneumonitis (7%) and no 30-days postoperative deaths. Median postoperative hospital stay was 15 days (range 8-29 days). The P/D group of 34 patients likewise had no postoperative fatalities and median postoperative hospital stay was 7 days (range 3-27 days). Median survival rates for EPP and P/D groups were 31.9 and 28.4 months, respectively. 5-years survival rates were 16% and 27%, respectively.

Conclusion: EPP is a safe procedure when performed in high-volume institution but affiliated with longer postoperative hospital stay than P/D. Especially postoperative radiotherapy remains a high-risk part of the trimodality treatment which should be further explored. Cure seems possible in about 1/6 of the patients. P/D is a safe procedure without treatment related deaths and the 5-year survival in about ¼ of patients may suggest that cure may be possible with this treatment when preceded by induction chemotherapy. The relative benefit of these two treatment options (trimodal treatment with EPP, or P/D) cannot be settled from these results which were offered somewhat different patient populations. However, a randomized trial might be considered.

Disclosure: No significant relationships.

P3.22: A RANDOMISED TRIAL OF EXTENDED PLEURECTOMY AND DECORTICATION (‘ePD’) WITH PEMETREXED AND PLATINUM CHEMOTHERAPY VERSUS PEMETREXED AND PLATINUM ALONE IN PATIENTS WITH MALIGNANT PLEURAL MESOTHELIOMA (‘MARS-2’).

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Background: The role of radical surgery in malignant pleural mesothelioma (MPM) is not clearly defined. There has been only one randomised trial of surgery (‘MARS-1’), which showed no benefit for extra-pleural pneumonectomy (EPP) compared to the non-surgical control arm. MARS-1 recruited more slowly than anticipated and did not progress to the larger scale trial originally planned. Extended pleurectomy and decortication (ePD) is a less extensive operation that does not mandate pneumonectomy, with probable lower morbidity and mortality than EPP. More patients with MPM may be suitable for ePD than were for EPP, enabling more rapid recruitment and randomisation.

Methods: The initial phase of the trial with be a randomised feasibility study. The primary endpoint is the ability to randomise 50 patients in 24 months. Secondary endpoints are: survival from time of randomisation and quality-of-life (assessed by the QLQ 30 and LC-13 modules). If fewer than 30% of suitable patients accept randomisation, it is unlikely that the larger study would start. The treatment plan is as follows: patients with MPM will be registered on MARS-2 and will receive 2 cycles of Pemetrexed-Platinum chemotherapy [cisplatin or carboplatin are acceptable]; patients who do not progress on CT scan will then be randomised to: (1) up to 4 further cycles of Pemetrexed-Platinum chemotherapy; or (2) ePD followed by up to 4 further cycles of post-operative Pemetrexed-Platinum chemotherapy. Major inclusion criteria are: histological confirmation of MPM, and tumour confined to one hemithorax. Major exclusion criteria are: refusal to give informed consent; refusal to accept randomisation; disease extent deemed non-resectable; inadequate pulmonary, cardiac, hepatic or renal reserve.

Results: This is the first time the final trial plan for MARS-2 has been presented at an international meeting. The trial is now under submission with Cancer Research UK (CRUK) for resource approval. The full trial protocol will be discussed in detail.

Conclusion: There is a pressing need for better data on the benefits of surgery in MPM. Audits of thoracic surgical centres in the UK show that over 100 MPM patients undergo a pneumonectomy every year. This trial aims to demonstrate whether this type of operation can offer survival and/or quality-of-life benefits to patients with MPM. The trial plan is in submission for resources. If funded and successful MARS-2 will be the largest and most detailed surgical trial in MPM.

Disclosure: No significant relationships.
**P3.23: IMPACT OF THREE DIFFERENT SURGICAL PROCEDURES FOR MALIGNANT PLEURAL MESOTHELIOMA ON IMMUNOLOGIC RESPONSES**

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**Background:** Intentional surgical radical procedures for malignant pleural mesothelioma (MPM) often achieve disappointing results. Furthermore, surgical stress may have detrimental effects on immune response. We comparatively evaluated changes of immunologic response by assessing lymphocyte subsets and cytokines in patients with MPM after three different operations.

**Methods:** Between October 2004 and May 2012, 30 patients with epithelial MPM underwent biopsy-pleurodesis in videoassisted thoracoscopy (n=15), open pleurectomy-decortication (n=8) and extrapleural pneumonectomy (n=7). Total lymphocyte count and percentage changes of lymphocyte subsets including natural-killer cell and cytokines (i.e interleukin-6 and -10) were evaluated by two-way analysis of variance test for repeated measures pre and postoperative days 1, 7 and 14. The Mann-Whitney test was performed at each time point only for significant parameters at between-group analysis of variance.

**Results:** Preoperative data were relatively homogeneous in all groups. Both the pleurectomy-decortication and extrapleural pneumonectomy groups disclosed a significantly lower median (interquartile range) proportion of natural-killer cells as compared with the biopsy-pleurodesis group on postoperative day 1 [5%(3-8%) and 4%(3-5%) Vs 12%(8-14%), p<0.003] and day 7 [7%(4-10%) and 3%(2-5%) Vs 11%(8-21%), p<0.02]. Total lymphocyte count significantly decreased only in the extrapleural pneumonectomy group (p<0.0001). No difference was found in the remaining lymphocyte subsets. Interleukin-6 and -10 were significantly increased p<0.008; day 14: p<0.05 and p<0.02) and extrapleural pneumonectomy after pleurectomy-decortication (postoperative day 7: p<0.03 and p<0.008; day 14: p<0.05 and p<0.02) and extrapleural pneumonectomy (p<0.00001). No difference was found in the remaining lymphocyte subsets. Interleukin-6 and -10 were significantly increased after pleurectomy-decortication (postoperative day 7: p<0.03 and p<0.008; day 14: p<0.05 and p<0.02) and extrapleural pneumonectomy (day 7:p<0.01 and p<0.003, day 14:p<0.04 and p<0.01).

**Conclusion:** Biopsy-pleurodesis resulted in a lesser impact on postoperative immunologic response than more aggressive procedures and this should be considered in choosing therapeutic strategy.

**Disclosure:** No significant relationships.

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**P3.24: COMBINING PRIME-BOOST ANTI-TUMOUR VACCINATION WITH DEBULLING SURGERY FOR THE TREATMENT OF MALIGNANT MESOTHELIOMA**

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**Background:** Malignant mesothelioma is a highly aggressive cancer with a very poor prognosis. Debulking surgery is often used as the principal therapy but is seldom curative. Adjuvant chemotherapy or radiotherapy can be used as to target residual disease, but these too are unlikely to be curative. There is renewed interest in the use of immunotherapy to treat mesothelioma as new modalities have been developed and immune targeting therapies have been found to be useful in other cancers. Recent work from our laboratory and others, has demonstrated that specific immunotherapies can engage the immune system with tumour. These therapies are typically more effective when used in conjunction with standard treatment protocols such as surgery or chemotherapy. Here we describe the development of a prime boost (P/B) anti-tumour vaccination protocol that when combined with debulking surgery and removal of regulatory T cells improved survival outcome in a mouse model of mesothelioma.

**Methods:** Using our established mouse model of mesothelioma, AB1-HA tumour bearing mice received influenza A PR8 (prime) and rMVAHA (boost) anti-tumour vaccinations, either before (neoadjuvant) or after (adjuvant) 75% debulking surgery. Tumour growth was monitored and immunological parameters assessed by multicolour FACS.

**Results:** Neoadjuvant P/B vaccination alone or in combination with 75% debulking surgery induced a significant increase in splenic tumourspecific CD8 T cells as well as significant increases in the proportion, activation and proliferation status of peripheral CD8 T cells relative to other treatment groups. However, a significant delay in tumour growth was only observed when neoadjuvant P/B vaccination was combined with debulking surgery; although this was not sufficient to control tumour outgrowth and cure the animals. Targeted depletion of CD8 and CD4 T cells demonstrated that CD8 T cells were essential for the delay in tumour growth caused by the combination treatment. Interestingly, depletion of CD4 T cells during P/B vaccination enhanced the survival outcome of surgery + P/B vaccination with 60% of these mice remaining tumour free for > 60 days post-surgery. Immunological memory was confirmed as tumour specific CD8 T cells were detected in the LN and spleens of surviving mice >60 days post tumour rechallenge.

**Conclusion:** Anti-tumour P/B vaccination induced tumourspecific immunity resulting in delayed tumour growth when combined with debulking surgery. Depletion of CD4 T cells during neoadjuvant P/B vaccination enhanced the therapeutic efficacy leading to cures in 60% of treated mice, suggesting that vaccine induced antitumour immunity is restrained, probably by CD4+ regulatory T cells. Based on these findings we are investigating whether combining novel immunotherapies with conventional treatments in the absence of immunological restrainers may generate effective therapy for mesothelioma.

**Disclosure:** No significant relationships.
**Background:** Plasmacytoid dendritic cells (pDC) are antigen-presenting cells specialized in antiviral response. The measles virus vaccine (MV) was recently proposed as an antitumor agent to target and specifically kill tumor cells without infecting healthy cells. Here, we investigated in vitro the effects of MV-infected tumor cells on phenotype and functions of human pDC.

**Methods:** Mesothelioma Meso13 cell line, melanoma M18 and pulmonary adenocarcinoma A549 cell line were cultured in the presence of Live-attenuated Schwarz strain Measles Virus (MV vaccine) or irradiated by UV-B to induce cell death (apoptosis). Monocytes (Mo-DC) and plasmacytoid DC (pDC) were purified from Peripheral Blood Mononuclear Cells (PBMCs) of healthy donors by counter-flow centrifugal elutriation at a purity of 95%. Mo-DC, were generated in vitro after 6 days of culture in RPMI containing 2% human albumin, 1000 U/ml rhGM-CSF and 200 U/ml rhIL-4. pDC were maintained in culture with 20ng/ml of rhIL-3 and activated in vitro with a TLR-7 agonist R848 (5ug/ml). Immature Mo-DC or pDC were cultured in the presence of MV-infected or UV-irradiated tumor cells (1 DC : 1 tumor cell). Phenotype of pDC and Mo-DC was determined by immunofluorescence followed by flow cytometry. After 18h, Mo- or pDC were co-cultured with the HLA-A*0201/NYESO-1(156-165) specific CD8+ T-cell clone, M117.167, for 6h in presence of Brefeldin-A. As a positive control, we used Mo-DC or pDC pulsed 1h with 0.1 or 1µM of NYESO-1 (156-165) peptides and washed. IFN-γ production by the T cell clone was analyzed by flow cytometry with a gate on CD8+ T cells using mAb specific for IFN-γ.

**Results:** We studied maturation, cytokine production and tumor-antigen cross-presentation by pDC, exposed either to the virus alone, or MV-infected or UV-irradiated tumor cells. We found that MV-infected cells induced pDC maturation with a strong production of IFN-α, whereas UV-irradiated tumor cells were unable to activate pDC. This production of IFN-α was triggered by the interaction of MV ssRNA with TLR7. We also observed that MV-infected tumor cells were phagocytosed by pDC. Interestingly, we observed cross-presentation of the tumor antigen NYESO-1 to a specific CD8+ T-cell clone, when pDC were cocultured with MV-infected tumor cells, whereas pDC were unable to cross-present NYESO-1 after co-culture with UV-irradiated tumor cells.

**Conclusion:** Altogether, our results suggest that the use of MV in antitumor virotherapy induces immunogenic tumor cell death, allowing pDC to spontaneously mature, to produce high amount of IFN-α, and to cross-present tumor antigen representing therefore a way to recruit cytotoxic T cells in the anti-tumor immune response. Thus the measles virus vaccine could be used as an immune strategy to treat mesothelioma patients, inducing an activation of specific cytotoxic T cell populations after activation by the antigen presenting cells: monocyte and plasmacytoid dendritic cells.

**Disclosure:** No significant relationships.

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**Discussion:**

**Background:** Dyspnoe is a common characteristic of patients with malignant pleural mesothelioma and thoracentesis is indicated to relieve symptoms. The procedure is relatively easy and the obtained pleural fluid might reflect the tumor micro-environment. The effusion consists of varying amounts of tumor cells, stromal cells, and numerous types of immune cells. These cellular populations and protein content of the effusion could correlate with the aggressiveness of a tumor. Immune cells can possess either immune stimulatory capacity (eg CD8 -cells and NK-cells) or immune suppressive capacity (eg tumor-associated macrophages or regulatory T-cells). The balance between these stimuli may determine tumor progression. Therefore, the proteomic and immunological characteristics of pleural fluid are subject of this study.

**Methods:** Pleural fluid of 140 mesothelioma patients was collected. For each sample the total cellular amount was determined. Cytospins were prepared and cell pellets were stored for later analysis. Immunohistochemical staining for tumor cells, stromal cells and immune cells was performed on the cytospins. Cytokine profiles will be determined. Clinical data and survival data are currently being collected and will be compared to the immunological data.

**Results:** Preliminary data show distinct differences in immune cell composition of pleural fluid of mesothelioma patients. The clinical significance of these data will be determined in the months to come.

**Conclusion:** We are currently undertaking a study to investigate the cellular composition of pleural fluid and its potential prognostic value in patients with mesothelioma.

**Disclosure:** No significant relationships.
P3.27: INTERNATIONAL SURVEY OF RADIATION TOXICITY FOLLOWING HEMITHORACIC INTENSITY-MODULATE RADIOTherapy IN PATIENTS WITH MALIGNANT PLEURAL MESOTHELIOMA

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Background: Hemithoracic radiotherapy plays an important role in inducing locoregional relapse in malignant pleural mesothelioma patients following extrapleural pneumonectomy (EPP). Reports of patients treated from 2003-2007 using intensity-modulated radiotherapy (IMRT) in North America and Europe found significant toxicities from pneumonitis, including radiation-related fatalities that raised concerns for the safety of IMRT. Following publication of more conservative constraints reports of decreased toxicity have been published. However, since each institutional series presented results with small numbers, we sought to create an international database of IMRT toxicity for mesothelioma.

Methods: Institutions with experience in treating mesothelioma patients with IMRT were surveyed to provide toxicity data on patients with malignant pleural mesothelioma who received high-dose hemithoracic radiotherapy between January 2009 and December 2011. The incidence of CTCAE version 4 grade 3, 4 and 5 radiation pneumonitis was recorded together with demographic data, radiation lung dosimetry, timing of chemotherapy and significant comorbidities.

Results: Data was collected from 14 institutions in the USA, Canada, France, Italy, Germany, Switzerland, Israel and Australia. All have used intensity-modulated radiotherapy to doses of 45-54 Gy in post-pneumonectomy cases. In addition, data was collected on hemithoracic IMRT to patients with both lungs intact, after pleurectomy/decortication (P/D). The radiation tolerance of the normal lungs is the main limitation encountered in planning these complex radiation treatments that often require many optimization rounds and long optimization times.

Conclusion: With ongoing technological improvements in radiotherapy planning and equipment and tighter dose constraints, the incidence of serious radiation pneumonitis following high-dose hemithoracic IMRT has fallen in all institutions for patients treated after 2008. Further studies of selected non-EPP patients whose mesotheliomas are localized within one hemithorax should be undertaken.

Disclosure: No significant relationships.

P3.28: PRESCRIPTION DOSE PREDICTION MODEL FOR HEMITHORACIC INTENSITY-MODULATED RADIATION THERAPY IN MESOTHELIOMA PATIENTS WITH TWO INTACT LUNGS

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Background: Intensity-modulated radiation therapy (IMRT) to the entire hemithoracic pleura is a promising new strategy for patients with unresectable malignant pleural mesothelioma (MPM) or after pleurectomy/decortication (P/D). The radiation tolerance of the normal lungs is the main limitation encountered in planning these complex radiation treatments that often require many optimization rounds and long optimization times. Therefore, the prescription dose goal of 5040cGy in 28 fractions is only achieved in approximately 33% of patients. Here we sought to develop a model that would predict the maximum safe radiation dose prescription for each patient.

Methods: We reviewed the radiation treatment plans of 56 MPM patients with two intact lungs who were treated with definitive or adjuvant IMRT after P/D. All patients were treated with coplanar 6MV beams using 6-9 beam angles approximately equispaced over approximately 220 degrees to encompass the ipsilateral lung. The median dose was $4680\text{cGy}$ in 28 fractions (range 3960cGy – 5040cGy). Treatment plans were optimized to keep the mean lung dose (MLD) $<21\text{ Gy}$. Treatment planning parameters including bilateral lung volumes, planning target volume (PTV), ipsilateral normal lung volume, mean total and individual lung doses, and prescription dose were recorded. The correlation between contralateral/ipsilateral lung volume ratio (CIVR), PTV/total lung volume ratio (PLVR) and prescription dose were analyzed.

Results: The median ipsilateral, contralateral and total mean lung doses were 3941cGy, 534cGy and 2044cGy. The mean ipsilateral, contralateral and total lung volumes were 1208cc, 1677cc and 2911cc, respectively. The median CIVR was 1.33. The only parameter in this study that correlated with achieving a higher prescription dose was CIVR ($p = 0.005$).

Conclusion: With ongoing technological improvements in radiotherapy planning and equipment and tighter dose constraints, the incidence of serious radiation pneumonitis following high-dose hemithoracic IMRT has fallen in all institutions for patients treated after 2008. Further studies of selected non-EPP patients whose mesotheliomas are localized within one hemithorax should be undertaken.

Disclosure: No significant relationships.
Conclusion: A higher ratio of contralateral/ipsilateral lung volume is an important treatment planning parameter associated with achieving higher radiation prescription dose. Further investigation is needed to determine if other factors are significantly associated with a higher prescription dose.

Disclosure: No significant relationships.

P3.29: HELICAL TOMOTHERAPY IN THE ADJUVANT TREATMENT OF RESECTED MALIGNANT PLEURAL MESOTHELIOMA: CLINICAL OUTCOME AND TOXICITY.

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Background: Malignant Pleural Mesothelioma (MPM) is an aggressive tumour with poor prognosis. The incidence of local and distant recurrences after surgery remains high. Although a multimodality approach seems promising in reducing the incidence of local relapse, it is still limited by the high rate of toxicity. Our study aims to evaluate the outcome of patients with MPM treated by adjuvant Helical Tomotherapy (HT).

Methods: Between June 2007 and May 2011, 29 MPM patients received adjuvant radiotherapy by HT. The median age was 63 years (34-72) and the sex ratio (M/F) was 2.2. Twenty four patients had an epithelioid type MPM. Stage 2 and 3 diseases were observed in 12 and 17 patients respectively. Extra pleural pneumonectomy was performed in 25 patients. Thirteen patients received Platinum agent/antifolate regime in the neoadjuvant setting. The median dose in the cavity of pneumonectomy was 50 Gy at 2 Gy/fraction. Event free survival (EFS) and overall survival (OS) were calculated, adverse events were assessed by the RTOG toxicity grading scale.

Results: Median follow up was 2.3 years. The most encountered acute side effect was pulmonary grade 1-2 toxicity in 19 patients and grade 1-2 upper gastro-intestinal tract toxicity and/or dysphagia in 15 patients. Grade 3 pneumonitis and dysphagia were observed in 1 and 2 patients respectively. 2 cases of Grade 5 pneumonitis were suspected before 6 months following RT. Late Grade 3-4 side effects were found in 4 patients. 19 patients had locoregional or distant relapse, 2 patients died of Grade 5 adverse events and 3 patients died from other causes. The median Event Free Survival (EFS) and Overall Survival (OS) were 1 and 1.6 y respectively. Fifty percent of patients were event-free at 1 year and 22 % at 2 years. The overall survival at 1 and 2 years were 65% and 38% respectively.

Conclusion: In the era of technological advances, HT offers a quite well tolerated technique in treating MPM after EPP. The results are promising but survival rates remain low for MPM patients completing multimodality treatment.

Disclosure: No significant relationships.

P3.30: COMPARISON OF TWO DIFFERENT METHODS TO DELIVER PLEURAL INTENSITY MODULATED RADIATION THERAPY IN MALIGNANT PLEURAL MESOTHELIOMA

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Background: The treatment of malignant pleural mesothelioma after plurectomy/decortication with radiation therapy remains a challenge due to the risk of pulmonary and cardiac toxicity. Recently, our group and others have investigated the use of pleural intensity modulated radiation therapy (IMRT) to treat patients with two intact lungs safely. IMRT can be delivered either by static field or via arc therapy. This study compares these two techniques to evaluate their potential radiation dosimetric advantage.

Methods: We compared plans for 4 left-sided and 3 right-sided cases. Plans used clinically approved planning target volumes (PTVs) and critical organ contours. Static IMRT plans employed 7-8 6 MV photon beam directions over a 215º range centered on the ipsilateral lung. Arc therapy plans used 4 full arcs with avoidance sectors to better spare the contralateral structures. Prescription dose per fraction was 180 cGy. The prescription dose ranged from 4500 cGy to 5040 cGy so as to keep the normal tissue complication probability (NTCP) of the combined lungs to ≤ 25%, our typical threshold. Planning objectives were: Lyman model NTCP for both lungs < 25%; contralateral lung, mean dose < 8 Gy; heart, V30 Gy < 50%; mean < 30 Gy; each kidney, V18 Gy < 33%; liver _ not _ GTV, mean < 30 Gy; V30 Gy < 50%; stomach _ not _ PTV, mean < 30 Gy; cord maximum < 45 Gy; bowel maximum < 55 Gy; D05 < 45 Gy; PTV D95 ≥ 94%, V95 ≥ 94%, D05 ≤ 115%. Dose calculation was done with the AAA algorithm. Contra lateral lung V5 Gy, ipsilateral lung V30 Gy, V40 Gy and mean dose were also noted.

Results: Arc therapy lowered the average total lung NTCP from 16.9% to 13% (p=0.03). The heart V30 decreased from 33.5% to 27.7% (p=0.016) with arc therapy and the mean stomach dose decreased from 17 Gy to 14.7 Gy (p=0.03). The number of beams used and monitor units (a measure of linear accelerator output) also decreased significantly with the use of arc therapy.

Conclusion: In this comparison of IMRT delivery either via static beams or arc therapy, we found a significant advantage in the use arc therapy to lower dose to the heart and lungs. Additionally, the use of arc therapy decreases the number of treatment beams and monitor units which decreases treatment time and increases patient comfort and compliance.

Disclosure: No significant relationships.
P4.01: CD26 OVEREXPRESSION IS ASSOCIATED WITH PROLONGED SURVIVAL AND ENHANCED CHEMOSENSITIVITY IN MALIGNANT PLEURAL MESOTHELIOMA

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Background: Malignant pleural mesothelioma (MPM) is an aggressive and therapy-resistant neoplasm arising from the pleural mesothelial cells. There are no established indicators to predict responsiveness to chemotherapeutic treatment for MPM.

Methods: Our present study involving 79 MPM patients demonstrated that 73.4% of MPM expressed CD26 on cell membrane.

Results: The majority of epithelioid and biphasic type of MPM expressed CD26 on the membrane, whereas sarcomatoid type demonstrated a lack of CD26 surface expression. Although sarcomatoid type was associated with poor prognosis (p<0.0001), no significant relationship between CD26 expression and survival was observed (p=0.1384). On the other hand, the response rate to chemotherapy was marginally associated with CD26 expression (P=0.053), with higher level of CD26 expression more likely to be linked to better response to chemotherapy. Moreover, CD26 expression was a significant factor associated with improved survival in chemotherapy patients (MST, 18.6 vs 10.7 months, P=0.0083). Furthermore, CD26 expression was significantly associated with better prognosis in patients with non-pemetrexed (PEM) regimens (MST, 14.2 vs 7.4 months, P=0.0442), while there was no significant association between CD26 expression and survival time for patients with PEM regimens (P=0.1225). Our in vitro and microarray studies showed that mesothelioma cells expressing high CD26 display high proliferative activity, and CD26 expression is closely linked to cell proliferation, cell cycle regulation, apoptosis, and chemotherapy resistance.

Conclusion: Our results strongly suggest that CD26 is a clinically significant biomarker for predicting response to chemotherapy for MPM.

Disclosure: No significant relationships.

P4.02: HIGH DEFINITION CIRCULATING TUMOR CELLS (HD-CTC) IN MESOTHELIOMA.

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Background: Circulating tumor cells (CTCs) are emerging as a valuable tool for monitoring cancer patient prognosis and response to treatment in some solid tumors. Commercially available CTC assay identifies CTCs via EPCAM dependent immunomagnetic-enrichment, which may not detect certain subsets of cells. We report a cohort of mesothelioma patients in whom CTCs were measured using a cytometric, enrichment free immunofluorescent protocol and correlated with outcomes.

Methods: Blood samples for CTC analysis were collected from 9 mesothelioma patients at baseline, 3 weeks, 3 months, 6 months, 9 months, and 1 year. HD-CTCs were identified via immunofluorescence and cytometric, enrichment free analysis. We also reviewed primary tumor specimens and correlated cytomorphologic appearance of the HD-CTCs to their corresponding primary tumor.

Results: A total of 9 consecutive chemotherapy naïve mesothelioma patients were included in the analysis. All had non-sarcomatoid histology. Median follow up was 18.8 months (range 4.6 to 24.4). Median survival is 19.6 months (range 4.6 to 24.3). At some point during the disease course CTCs were identified in 6 of 8 (67%) patients (range 0-566 CTC/ml, mean 17 CTC/ml). (see table) At the time of the diagnosis, CTCs were present in 5 out of 9 patients but only 1 of those patients had distant metastases. Three of 9 patients were alive at the time of data analysis. Using CTC/ml cut off of 2 CTCs/ml, patients can be divided into ‘remain favorable’ (baseline draw <2, last draw <2), ‘convert to favorable’ (baseline draw >2, last draw <2), remain unfavorable (baseline draw >2, last draw >2), and convert to unfavorable (baseline draw >2, last draw >2). Median survival is 604 days for ‘remain favorable’/’convert to favorable’ group and 475 days for the ‘remain unfavorable’/’convert to unfavorable’ group. Presence of CTCs at the time of diagnosis was not predictive of survival.
Conclusion: This small case series shows that CTCs can be effectively enumerated in mesothelioma patients. CTC presence in mesothelioma patients does not signify presence of metastatic disease or predict development of metastases. There appears to be a separation in outcomes based on CTC kinetics. However, due to the small sample size further studies are warranted to further clarify this association.

Disclosure: Epic Sciences

P4.03: EXPRESSION OF WILMS´TUMOUR GENE (WT1) IS ASSOCIATED WITH SURVIVAL IN MALIGNANT PLEURAL MESOTHELIOMA: RETROSPECTIVE ANALYSIS IN A SINGLE CENTER SERIES

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Background: Calretinin and Wilms´tumour gene (WT1) are mesothelial markers routinely used to confirm the diagnosis of malignant pleural mesothelioma (MPM). Recently, calretinin score assessed by immunohistochemistry (IHC) was implicated with poor prognosis in MPM. We investigated the prognostic value of calretinin and WT1 expression in predicting survival in a series of patients (p) diagnosed of MPM in our institution.

Methods: Fifty two patients diagnosed of MPM in Vall d’Hebron University Hospital between November 2002 and September 2011 were retrospectively reviewed. Potential prognostic factors analyzed were age, performance status (PS), neutrophil to lymphocyte ratio (NLR), clinical stage, histology, calretinin and WT1 expression, and chemotherapy treatment (CT). Calretinin and WT1 were stained for IHC analysis in formalin-fixed, paraffin-embedded sections and were considered positive cases with >1% of tumor cells stained. Survival data were calculated by the Kaplan-Meier method and Cox regression was used to evaluate the prognostic value of the variables.

Results: Patient’s characteristics: median age 68 years (31-88 years), males 75.5%, PS 1: 67.3%, asbestos exposure 53.1%, clinical stage III: 55.1%, epithelial subtype 71.4% and NLR>5 in 44.9% of all patients. Calretinin IHC expression was available in 47 p and was positive in 41 p (87.2%). WT-1 IHC expression was available in 32 p and was positive in 25 p (78.1%). All patients were considered initially unresectable and 71.4% received CT in 1st line and 34.7% in 2nd line. We found a significant association of calretinin expression with epithelial histology (p=0.030) and PS 1 (p=0.05) and no association with clinical stage (p=0.23). WT1 expression was associated with epithelial histology (p=0.016), but no association with PS and clinical stage (p=0.05) was detected. After a median follow up of 9.2 months the median survival (OS) was 15.2 months for the entire cohort. We found a significant increase in OS in patients with epithelial subtype (24.5 vs 5.0 months in epithelial vs no-epithelial, p<0.001), PS1 (14.7 m vs 2.2 months in PS 1 vs PS 2, p=0.036), NLR ≤5 (26.5 vs 13.4 months, p=0.025) and patients who received 2nd line CT (8.9 and 26.4 months for p second line, p=0.05). In the IHC markers analysis we found a significant increase in OS for p with WT1 positive expression (16.4 m vs 2.3 m, p=0.013), but no differences for calretinin expression (16.6 m vs 5.0 months, p=0.37). In the multivariate analysis epithelial histology and WT1 remained as significant prognostic factors for survival (HR 22.8; 95%CI, 1.7-523, p=0.004 and HR 9.5; 95%CI 1.7-52.3, p=0.010 respectively).

Conclusion: In our series of 52 MPM patients, epithelial histology, PS, NLR, second line CT and WT1 expression are significant prognostic factors for survival. The role of WT1 assessment is worth of prospective validation in future studies on MPM.

Disclosure: No significant relationships.

P4.04: SERUM CA-125 IS ASSOCIATED WITH WORSE SURVIVAL IN MALIGNANT PLEURAL MESOTHELIOMA: RETROSPECTIVE ANALYSIS IN A SINGLE CENTER SERIES

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Background: Malignant pleural mesothelioma (MPM) is an aggressive and difficult to treat tumor. Serum tumor markers have been used in mesothelioma as diagnostic tools complementary to pathological findings. However, the prognostic value of serum tumor markers in MPM has not been evaluated. We investigated the overall survival (OS) according to serum tumor markers in a series of patients diagnosed of MPM in our institution.

Methods: Fifty two patients diagnosed of MPM in Vall d’Hebron University Hospital between November 2002 and September 2011 were retrospectively reviewed. Baseline characteristics analyzed were age, performance status (PS), neutrophil to lymphocyte ratio (NLR), clinical stage, histology, and chemotherapy treatment (CT). Serum tumor markers analyzed were CEA, CYFRA21-1, CA-125, enolase and Ca15.3. All tumor markers were performed during the study of suspected malignancy. Survival data were calculated by the Kaplan–Meier method and Cox regression was used to evaluate the prognostic value of the variables.

Results: Patient’s characteristics: median age 68 years (31-88 years), males 75.5%, PS 1: 67.3%, asbestos exposure 53.1%, clinical stage III: 55.1%, epithelial subtype 71.4% and NLR>5 in 44.9% of all patients. CEA was available in 35 p; CYFRA21-1 in 24 p; CA-125 in 31 p; enolase in 21 p, and Ca15.3 in 16 p. We did not found association for any tumor marker with histology, stage, gender, smoking history, pleural effusion or asbestos exposure. We found frequent elevation above the upper limit of the serum tumor markers at diagnosis in MPM. CEA was elevated in 5.8% p; CYFRA21-1 in 62.5% p; CEA-125 in 51.6% p; enolase in 19% p and Ca15.3 in 62.5% of patients. After a median follow up of 9.2 months the median survival was 15.2 months for the entire cohort. We found a significant increase in OS in patients with epithelial subtype (24.5 vs 5.0 months in epithelial vs no-epithelial, p<0.001), PS1 (14.7 m vs 2.2 months in PS 1 vs PS 2, p=0.036), NLR ≤5 (26.5 vs 13.4 months, p=0.025) and patients who received 2nd line CT (8.9 and 26.4 months for p second line, p=0.05). In the analysis of tumor markers we found that CA-125 was the only tumor marker significantly associated with OS. CA-125 ≤500 ng/ml was associated with worse survival (16.6 m vs 2.3 m, p=0.013), but no differences for CYFRA21-1 expression (16.6 m vs 5.0 months, p=0.37). In the multivariate analysis epithelial histology and WT1 remained as significant prognostic factors for survival (HR 22.8; 95%CI, 1.7-523, p=0.004 and HR 9.5; 95%CI 1.7-52.3, p=0.010 respectively).

Conclusion: In our series of 52 MPM patients, epithelial histology, PS, NLR, second line CT and WT1 expression are significant prognostic factors for survival. The role of WT1 assessment is worth of prospective validation in future studies on MPM.

Disclosure: No significant relationships.
associated with survival. Patients with CA-125 elevated had a median survival of 2.1 months vs 18.9 months for patients with CA-125 in normal value (p<0.04). No differences in survival were detected with the CEA, CYFRA21-1, enolase and CA15-3 (p>0.05). In the multivariate analysis only epithelial histology remained as significant prognostic factors for survival (HR 7.05; 95%CI, 1.93-25.73, p=0.003).

Conclusion: In our series of 52 MPM patients, epithelial histology, PS, NLR and second line CT are significant prognostic factor for survival. We found that basal level of CA-125 is a significant prognostic factor for survival in mesothelioma.

Disclosure: No significant relationships.

POSTER SESSION 4  SEPTEMBER 13, 2012 16:00-17:00

P4.05: PLEURAL HYALURONIC ACID IS A USEFUL BIOMARKER FOR MALIGNANT MESOTHELIOMA

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Background: The use of biomarkers in pleural effusions has not been clinically accepted. Whilst good discrimination between malignant and benign effusions has been demonstrated using various biomarkers, their ability to assign phenotypes to particular malignancies has been limited. Hyaluronic acid (HA) is a glycoaminoglycan previously shown to be elevated in malignant mesothelioma (MM) effusions and may be useful for discriminating between MM and other pleural metastatic cancers. In this study we compared HA and mesothelin in serum and pleural effusions to investigated the ability to distinguish MM from lung adenocarcinoma metastatic to the pleura.

Methods: Serum and pleural effuse was collected from 92 MM patients, 24 lung cancer (LC) patients and 43 patients with benign pleural effusions. HA was measured using the HA Binding assay (Corgenix Inc). Mesothelin was measured using the MESOMARK™ assay (Fujirebio). A pleural effusate was defined as positive when HA concentrations were above the mean of the benign control group by two standard deviations, i.e. above 50 mg/ml. The previously defined threshold for mesothelin in effusions of 20 nM was chosen to dichotomise samples.

Results: There was no significant difference in the serum HA level between the groups. HA concentrations were approximately 1000-fold higher in the effusate than serum of MM patients. In pleural effusions, HA concentrations were significantly higher in MM compared to lung cancer (p<0.001) and of adenocarcinoma (p<0.01) [median (interquartile range)=33.8 nM (15.8-64.15) versus 3.4 nM (1.2-7.7) and 2.5 nM (0-7.1), respectively, P<0.0001]. Likewise, SLPI concentration was higher in MM than in ADCA and BPE [228.2 ng/mL (13.6-409.8) versus 101.5 ng/mL (72.9-154.5), (P<0.001) and 91.1 ng/mL (68.15-181.1) (P<0.05). ROC curves analysis revealed that SMRP (AUC=0.9059), CCL2 (AUC=0.7912), Galectin-3 (AUC=0.7584) and SLPI (AUC=0.7219) were potential interesting biomarkers to differentiate MM patients from patients with BPE or ADCA. Interestingly, we showed that combination of SMRP, CCL2 and Galectin-3 greatly improved MPM diagnosis (AUC=0.9680).

Conclusion: The measurement of SLPI, CCL2 and Galectin-3 levels in pleural fluids allowed the diagnosis of MM with good sensitivity and specificity. However, SMRP remained the best single soluble marker available. The combination of SMRP, CCL2 and Galectin-3 evaluation in pleural fluid appears as a promising panel of biomarkers for a reliable diagnosis of MPM.

Disclosure: No significant relationships.
biomarkers for better treatment stratification are needed. The main aim of this study was to evaluate the prognostic and predictive relevance of pretreatment serum C-reactive protein (CRP) in malignant pleural mesothelioma (MPM) patients.

**Methods:** Clinical data were retrospectively collected from 115 patients with histologically proven MPM. Patients with evidence for infectious disease were excluded. The association between CRP levels and survival was analyzed using Kaplan-Meier method and Cox models adjusted for clinicopathological factors.

**Results:** Median pretreatment CRP of all patients was 1.19 mg/dl (range: 0.00–22.62 mg/dl). Patients with elevated CRP (≥1mg/dl, n=62, 53.9%) had a significantly shorter overall survival (OS) compared to those with normal CRP (HR 2.81, 95% CI 1.82–4.33; p<0.001). In multivariate analyses, elevated CRP was confirmed as an independent prognosticator in MPM (HR 2.07, 95% CI 1.23–3.46; p=0.001). A significant interaction between CRP and treatment modality (p<0.001) was observed. Among patients with normal CRP, radical tumor resection within multimodality protocols (MMP) was associated with prolonged OS when compared to protocols without surgery (HR 7.26, 95% CI 3.40–15.49; p<0.001). Among patients with elevated CRP, no survival benefit was achieved by radical surgery within MMP (HR 0.91, 95% CI 0.53–1.58; p=0.74).

**Conclusion:** Our results suggest that multimodality regimens including radical resection increase survival selectively in MPM patients with normal pretreatment serum CRP levels.

**Disclosure:** No significant relationships.

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**P4.08: PERIPHERAL T CELL ACTIVITY PREDICTS SURVIVAL IN PATIENTS WITH MALIGNANT MESOTHELIOMA AND NON–SMALL CELL LUNG CANCER**

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**Background:** The importance of generating an anti-tumor immune response is manifest in the way that the balance of intratumoral T cell subsets reflects clinical outcome. Tumor-infiltration by CD8+ T cells is associated with improved and reduced survival respectively in many cancer types. However, little is known of the prognostic value of immunological parameters measured in peripheral blood and treatment modality (p<0.001) was observed. Among patients with normal CRP, radical tumor resection within multimodality protocols (MMP) was associated with prolonged OS when compared to protocols without surgery (HR 7.26, 95% CI 3.40–15.49; p<0.001). Among patients with elevated CRP, no survival benefit was achieved by radical surgery within MMP (HR 0.91, 95% CI 0.53–1.58; p=0.74).

**Conclusion:** Our results suggest that multimodality regimens including radical resection increase survival selectively in MPM patients with normal pretreatment serum CRP levels.

**Disclosure:** No significant relationships.

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**P4.09: PDGF-D/PDGF-ββ RECEPTOR-REGULATED CHEMOTAXIS OF MALIGNANT MESOTHELIOMA CELLS**

Asuka Okada1, Takahiro Yaguchi1, Hitomi Kamiya1, Miki Honda1, Eriko Masachika1, Hisaya Okuwa1, Taichihiro Ot osuki1, Koji Mikami1, Yoshitaka Nogi2, Risa Maeda2, Takayuki Terada2, Noriko Hirayama1, Shusai Yamada3, Kunihiro Tamura1, Chiharu Tabata1, Kazuya Fukouka1, Tomoyuki Nishizaki1, Takashi Nakano3
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**Methods:** After the knock-down strategy was designed to gain further insight into the PDGF-ββ receptor signals underlying the chemotaxis.

**Disclosure:** No significant relationships.
Results: The median age of the MPE and benign effusions subjects was 69 years and 74 years respectively. Male subjects comprised 53% of the MPE group compared to 75% in the benign effusion group. Sixty-three percent of MPE subjects were current or former smokers compared to 75% of subjects with benign effusions. The most common aetiologies of MPE were lung cancer (n=27), mesothelioma (n=23) and breast cancer (n=7) while the most common aetiologies for benign effusions were inflammatory pleuritis (n=11), asbestos related effusions (n=6) and parapneumonic effusions (n=5). The median absolute telomere length measured in pleural fluid cfDNA was not significantly different in subjects with malignant pleural effusions compared to subjects with benign effusions (9.7kb per diploid genome vs. 8.5kb per diploid genome, \( p=0.488 \)). There were no significant differences in the median telomere lengths in subjects with malignant pleural mesothelioma (10.3kb vs. 8.5kb, \( p=0.385 \)), lung cancer (8.5kb vs. 8.5kb, \( p=0.390 \)) or other cancers (4.7kb vs. 8.5kb, \( p=0.384 \)) compared to those with benign pleural effusions. When we categorized the pleural fluid cfDNA absolute telomere length into quartiles based on the telomere length distribution of the controls, with the first (shortest) quartile being used as the reference category, the age-adjusted OR for MPE was essentially equal in the 2nd (0.92, 95% CI 0.83-1.02; \( p=0.10 \)), 3rd (1.01; 95% CI 0.95-1.07, \( p=0.76 \)) and 4th quartiles (1.01, 95% CI 0.95-1.07, \( p=0.76 \)). In the study cohort, covariates were examined for relationships with absolute telomere length. There was no difference in pleural fluid cfDNA absolute telomere length for age <60 years compared to age > 60 years (8.0kb vs. 9.7kb, \( p=0.187 \)), females compared to males (9.5kb vs. 9.9kb, \( p=0.548 \)), cytology positive compared to cytology negative (8.9kb vs. 9.3kb, \( p=0.924 \)). There was a trend for shorter telomeres in smokers compared with non-smokers, however the difference did not reach statistical significance (9.0kb vs. 12.3kb, \( p=0.076 \)).

Conclusion: In this study we found that it was technically possible to measure absolute telomere length in pleural fluid cfDNA. However the pleural fluid cfDNA is most likely a mixture of various types of white blood cells, mesothelial and tumour cells. Further study is required to determine the absolute telomere length in the individual cells and this may be possible by initially identifying and sorting the individual cell groups using flow cytometry.

Disclosure: No significant relationships.

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**P4.11: SOLUBLE MESOTHELIN RELATED PROTEIN AND OSTEOPONTIN IN SCREENING FOR MALIGNANT PLEURAL MESOTHELIOMA**

Katrina Rey-Mcintyre1, Masaki Anraku1, Licun Wu1, Tetsuzo Tagawa1, Zhihong Yun1, Brenda O’Sullivan1, Zhuo Chen2, Geoffrey Liu2, Demetris Patsios2, Lu Chen2, Wei Xu2, Ming Tsao2, Marc De Perrot2

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Background: We established a screening program for asbestos exposed individuals using low-dose computed tomography. Incorporating biomarkers could provide more effective screening. We determined the diagnostic performance of osteopontin combined with soluble mesothelin related protein using an algorithm and determined the variance of the algorithm values over several years.

Methods: Plasma was obtained from 67 patients with malignant pleural mesothelioma and 278 asbestos exposed controls from our screening program.

Results: Using an algorithm of both biomarkers, values were significantly higher in patients with malignant pleural mesothelioma compared to asbestos exposed controls in a test group and in a validation group. Using receiver operating characteristic curves, an area under curve of 0.88 was produced for the test group and 0.83 for the validation group. A total of 114 out of 118 individuals tested in the screening program had an algorithm value below threshold. This algorithm remained consistent in a narrow range in 38 out of 42 individuals with at least 3 visits over a minimum 2 year period.

Conclusion: An algorithm combining both biomarkers remained consistent over time in a clinical screening setting. Considering the rapid progression of mesothelioma, our early screening program using low dose computed tomography can be refined with the use of tumour biomarkers.

Disclosure: No significant relationships.
P4.12: EXTRAPLEURAL PNEUMONECTOMY IN EPITELIOID MALIGNANT PLEURAL MESOTHELIOMA: IS IT OBSELETE OR IS THERE A NEED FOR A LARGER RANDOMIZED TRIAL?

Onur Ermerak1, Adamu Issaka1, Barkin Eldem1, Hakan Ozelper1, Zeynep Bilgi2, Fulden Yumuk2, Hale Basak Ozkok3, Muzaffer Metintas3, Volkan Kara1, Hasan F. Batirel1

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Background: The unfavorable survival figures in the only randomized trial in malignant pleural mesothelioma (MPM) have sparked discussions of unneccessity for the application of extrapleural pneumonectomy (EPP). However, this conclusion could prevent a subgroup of patients who would benefit from EPP and multimodality treatment. We analyzed our data in patients with epitheloid MPM.

Methods: Patients who underwent EPP for the treatment of epitheloid MPM in two hospitals between 2002-2011 were included in the study. EPP technique included en bloc resection of ipsilateral pleura, lung, pericardium and diaphragm. Patients were referred for adjuvant hemithoracic radiation and platinum based chemotherapy. All demographic and patient data was recorded in a prospective database and statistical analysis was performed using Kaplan Meier survival curves and uni- and multivariate analysis for determination of prognostic factors.

Results: 40 patients underwent EPP during this period. 35 had epitheloid tumors (Average age 54 ± 8, 14 females). In-hospital mortality was 12.5% (6% at University hospital). T stages (IMIG staging) were 1 (n=2), 2 (n=25), 3 (n=7), 4 (n=1). 14 had extrapleural lymph node metastasis and 9 also had intraparenchymal lymph node metastasis. 25 patients (66%) completed adjuvant chemoradiation. Overall median survival was 18,1 months (alive, GEM alone periods; 9.6, 15.6 months, separately). 3 patients those were performed GEM alone (over 6 months, include second line) shows long prognosis without benefit from EPP and multimodality treatment. Of the 8 chemotherapy patients, 3 were performed cisplatin(CDDP)+Pemtrexed, 2 were performed CDDP+Gemcitabine(GEM), 2 were performed GEM+Vinorelbine, and 1 was performed GEM alone. In chemotherapy group, 3 are alive (2 were received EPP, monitoring periods; 11.5, 22.3, 33.7 months) 5 were dead (OS; 18.1, 23.4, 28.3, 31.7, 34.6 months). In non-chemotherapy group, all were dead (OS; 3.8, 4.8, 6.6, 7.9 months). 3 patients those were performed GEM alone (over 6 months, include second line) shows long prognosis without losing their Quality of Life (QOL). Their OS and monitoring time were 23.4 months (dead, GEM alone periods; 14 months), and 22.3, 33.7 months (alive, GEM alone periods; 9.6, 15.6 months, separately).

Conclusion: This analysis provides active treatments bring better prognosis even for the elderly MPM patients. It was thought that GEM alone chemotherapy may keep their better QOL.

Disclosure: No significant relationships.

P4.13: A CLINICAL STUDY OF 12 DIFFUSE MALIGNANT PLEURAL MESOTHELIOMA IN 70 YEARS OR OLDER

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Background: Diffuse malignant pleural mesothelioma (MPM) is a highly aggressive tumor with very poor prognosis, but optimal treatment has not been defined yet. Asbestos exposure is the main factor involved in pathogenesis of MPM. Because of the long incubation time, it is expected the number of older patients will increase. We consider about chemotherapy for elderly MPM patients.

Methods: Between 2000 and 2011, 24 MPM patients were definitive diagnosed by thoracoscopic biopsy in our hospital, and we analyzed 12 cases over 70 years when diagnosed.

Results: Of the 12 patients, 10 were men and 2 were women with the mean age 77.3 years old (7 were in 70’s, 5 were in 80’s). 8 were epithelial type, 2 were sarcomatoid type, and 2 were biphasic type in histology. 2 were Stagell, 8 were Stagell, I was StageII, and 1 was StageIV according to IMIG. 8 patients were treated with chemotherapy (6 epithelial, 2 biphasic). Of the 8, 2 were underwent extrapleural pneumonectomy (EPP). 4 patients were treated symptomatic therapy. Of the 8 chemotherapy patients, 3 were performed cisplatin(CDDP)+Pemtrexed, 2 were performed CDDP+Gemcitabine(GEM), 2 were performed GEM+Vinorelbine, and 1 was performed GEM alone. In chemotherapy group, 3 are alive (2 were received EPP, monitoring periods; 11.5, 22.3, 33.7 months) 5 were dead (OS; 18.1, 23.4, 28.3, 31.7, 34.6 months). In non-chemotherapy group, all were dead (OS; 3.8, 4.8, 6.6, 7.9 months). 3 patients those were performed GEM alone (over 6 months, include second line) shows long prognosis without losing their Quality of Life (QOL). Their OS and monitoring time were 23.4 months (dead, GEM alone periods; 14 months), and 22.3, 33.7 months (alive, GEM alone periods; 9.6, 15.6 months, separately).

Conclusion: This analysis provides active treatments bring better prognosis even for the elderly MPM patients. It was thought that GEM alone chemotherapy may keep their better QOL.

Disclosure: No significant relationships.

P4.14: EORTC RANDOMIZED PHASE II STUDY OF EXTENDED PLEURECTOMY/DECORTICATION (E-PD) PRECEDED OR FOLLOWED BY CHEMOTHERAPY IN PATIENTS WITH EARLY STAGE MALIGNANT PLEURAL MESOTHELIOMA (MPM)

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Background: Extra-pleural pneumonectomy (EPP) is the most commonly used surgical procedure in MPM, but a recent randomized feasibility study suggested no benefit and a possible harm (Treasure 2011). Uncontrolled series suggest that lung sparing surgical approaches such as e-PD might result in equal to better outcome (Lang-Lazdunski 2011; Flores, 2008) and
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P4.16: CLINICAL AND PATHOLOGICAL FEATURES OF FIVE-YEAR SURVIVORS OF MALIGNANT PLEURAL MESOTHELIOMA

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Background: The incidence of malignant pleural mesothelioma (MPM) in Japan is predicted to increase over the next few decades. The prognosis of MPM is poor, with the median survival reported to be approximately 9-17 months. A lot of prognostic factors are known for MPM, but still prediction of disease-related future remains unclear. The aim of this study was to evaluate clinical and pathological features of five-year survivors of MPM.

Methods: We retrospectively obtained clinical data from the medical records of 467 patients who were diagnosed with MPM in Hyogo College of Medicine Hospital. Overall survival outcome was analyzed in 347 patients with MPM. Kaplan-Meier analysis was used to estimate the cumulative survival probability.

Results: Overall median survival time (MST) of 347 patients with MPM was 15.8 months. Survival rates for one and two year were 59.5% and 30.0%, respectively. Fourteen patients (4.0%) survived at least 5 years following initial treatments, including 4 remaining alive. Of the 5-year survivors, 7 were female, 6 had left-side disease, and the mean age was 61.0 years (range 35-74). Eleven had epithelioid tumors and remaining 3 unclassified ones. Six had clinical stage I disease. All patients received systemic chemotherapy. Five underwent extrapleural pneumonectomy (EPP), and 2 pleurectomy/decortication (P/D). One received adjuvant radiotherapy. Seven received multimodality treatment, while remaining 7 received chemotherapy alone. The longest survival of 93 months was achieved in one female patient receiving induction chemotherapy followed by EPP.

Conclusion: The actual five-year survival was 4.0% in 347 patients with MPM. Epithelioid histologic subtype is identified as a favorable prognostic feature. Chemotherapy alone as well as surgery-based multimodality treatment might be associated with long-term survival in patients with MPM.

Disclosure: No significant relationships.

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P4.15: PERI-OPERATIVE OUTCOME OF EXTRAPLEURAL PNEUMONECTOMY AFTER CHEMOTHERAPY FOR MALIGNANT PLEURAL MESOTHELIOMA IN 189 PATIENTS FROM TWO INSTITUTIONS

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Background: Reports in the literature suggest high morbidity and mortality for pleuropneumonectomy (EPP) after induction chemotherapy for mesothelioma patients. Therefore we analysed the peri-operative outcome of this patient cohort in a bi-institutional setting.

Methods: From 1999 to 2011, 189 MPM patients completed EPP after platinum-based induction chemotherapy at 2 institutions for thoracic surgery. The median age of both patient cohorts with more than 80% male patients and the histological subtype (mostly epithelioid) as well as the stage of disease (predominantly pT3) were comparable between both cohorts. Peri-operative mortality and major morbidity (pneumonectomy (PE), bleeding, ARDS, empyema, bronchopleural fistula (BPF), chylothorax, patch failure, others) were analysed.

Results: Overall morbidity- and mortality rates for both institutions are summarized in the following table.

<table>
<thead>
<tr>
<th>Outcome</th>
<th>EPP after Ind Ctx (n)</th>
<th>30d mortality</th>
<th>major morbidity</th>
<th>empyema / (+ BPF)</th>
<th>chylothorax</th>
<th>patch failure</th>
<th>bleeding</th>
<th>pulmonary embolism</th>
<th>ARDS</th>
<th>esophageal perforation</th>
<th>cardiac arrest</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>189</td>
<td>10 (5%)</td>
<td>63 (33%)</td>
<td>28 (15%) / (18 (9%))</td>
<td>11 (6%)</td>
<td>8 (4%)</td>
<td>7 (4%)</td>
<td>5 (3%)</td>
<td>3 (2%)</td>
<td>1 (1%)</td>
<td>1 (1%)</td>
</tr>
</tbody>
</table>

The rate of major morbidity was significantly higher in mesothelioma of the right hemithorax compared to the left side (Fisher’s Exact Test: 0.006) as BPF was only observed on the right side. The 30 day mortality did not differ significantly in right-sided compared to left-sided EPP. The overall survival (from diagnosis) of patients treated with chemotherapy and EPP was in both centres closed to 2 years.

Conclusion: Peri-operative morbidity and mortality after induction CTX and EPP for MPM patients can be maintained at a reasonable level at high volume centres with long-term experience. Surgical morbidity but not mortality is increased after right-sided EPP.

Disclosure: No significant relationships.

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P4.10: EXPANDED EFFORTS TO MEASURING PATIENT OUTCOMES IN CANCER IN A CANADIAN HOSPITAL NETWORK

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Background: The use of the National Cancer Institute of Canada (NCIC)-CTC and the European Organization for Research and Treatment of Cancer (EORTC) QLQ-C30 in the context of a cancer network is being expanded to provide more consistent and reliable data to evaluate patient outcomes.

Methods: The NCIC-CTC and the EORTC QLQ-C30 questionnaires are administered by the clinical staff at each hospital as part of routine care. The network has been improved with the use of a central reporting system, allowing accuracy and reliability of data. The data are validated and analyzed to identify key areas for research.

Results: The network has demonstrated significant improvements in the area of patient outcomes. A high level of adherence to the questionnaires has been achieved, with over 90% of patients completing the questionnaires by 4 weeks post-diagnosis. The data collected through the network has been used to identify areas for improvement in patient care and to inform research initiatives.

Disclosure: No significant relationships.
P4.17: PHARMACOKINETIC ANALYSIS OF CISPLATIN DURING HYPERThERMIC INTRATHoracic CHEMOTHERAPY PERFUSION (HITHOC) AFTER PLEUrectomy AND DECORTICATION FOR TREATMENT OF PLEURAL MALIGNANCIES

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Background: Cisplatin is a major drug for the treatment of pleural malignancies. Objectives of this study were to assess the pharmacokinetics and toxicity of intrapleural administered cisplatin during hyperthermic intrathoracic chemotherapy perfusion (HITHOC) following pleurectomy/decortication (P/D) in patients with malignant pleural mesothelioma or advanced thymoma with pleural spread (Masaoka stage IVa).

Methods: Pharmacokinetic analysis (ICP-MS) on 10 patients who received intrapleural cisplatin with a dosage of 100 mg/m² (group 1: n = 5) or 150 mg/m² (group 2: n = 5) at 42°C perfusate temperature. Simultaneous pleural fluid perfusion (perfusionate) and serum samples were collected at the beginning and every 15 minutes during one hour of HITHOC. Subsequent serum samples were collected at the end of the operation, 6, 12 and 24 hours postoperative.

Results: There were no severe local or systemic chemotherapy related complications observed. In both groups mean cisplatin levels in the perfusate slightly decreased during the HITHOC procedure. The mean area under the curve ratios (AUCperfusionate:AUCserum) of cisplatin were nearly similar between both groups. The mean AUC of cisplatin in the perfusate was approximately 57 times (group 1) and 54 times (group 2) greater than detected in the serum during one hour of chemotherapy perfusion. The mean peak of cisplatin in the serum was reached after one hour of chemotherapy perfusion and after that continuously decreased within the first postoperative day. Finally, the area under the curve of cisplatin in the serum did not significantly differ (p= 0.18) between both groups up to 24 hours after perfusion was started.

Conclusion: Cytoreductive surgery (P/D) in combination with hyperthermic intrathoracic chemotherapy perfusion (HITHOC) with cisplatin is safe and feasible with the advantage of high local cisplatin concentrations which might increase its efficacy but with respectable serum levels. This indicates a pharmacological advantage for this type of administration. But still there is no consensus about the optimal dosage of cisplatin and further studies are needed.

Disclosure: No significant relationships.

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P4.18: THE WEST PART OF JAPAN PROGNOSTIC FACTORS IN MALIGNANT PLEURAL MASOTHELIOMA WAS MORE USEFUL THAN THE EUROPEAN ORGANIZATION FOR RESEARCH AND TREATMENT OF CANCER (EORTC) PROGNOSTIC SCORING SYSTEMS IN A SMALL SCALE HOSPITAL IN JAPAN.

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Background: The European Organization for Research and Treatment of Cancer (EORTC) prognostic scoring systems has been available for malignant pleural mesothelioma (MPM) patients. Recently prognostic factors in patients in West Part of Japan has been published. In this study, patients of 70 years and above, non-epithelioid type, poor performance status, high white blood cell count, high C - reactive protein level were negatively associated with survival. We have assessed these factors in our series in a small scale hospital, Saitama Prefecture near Tokyo in Japan.

Methods: From 2005 to 2011, ten patients were diagnosed with MPM and treated in our hospital. Four of ten underwent extra-pleural pneumonectomy (EPP) and one of ten underwent tumorectomy because of pleural dissemination. After resection, they received chemotherapy. Other four were treated with chemotherapy. Only one patients was impossible to treat because of poor performance status. Age, performance status (ECOG), white blood cell count and C-reactive protein level of each patients were investigated before histological confirmation. Three risk groups, (high, moderate and low risk group) were defined by the presence of following factors: old age ( 70< ), non-epithelioid type, poor performance status ( 0< ECOG ), high white blood cell count (9000 < WBC) and high C - reactive protein level ( 4 < CRP ).

Results: Eight of ten died within three years from the diagnosis. Two were still alive but both of them suffered relapse. Risk groups were correlated with survival (below).

Conclusion: In Japan, the West Part of Japan’s prognostic factors is possible to be simple and easy method in MPM patients.

Disclosure: No significant relationships.

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P4.19: NOVEL URINARY BIOMARKERS FOR THE EARLY DETECTION OF KIDNEY INJURY FOLLOWING CYToreductive SURGERY AND INTRACAVITARY CISPLATIN LAVAGE FOR MASOTHELIOMA

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Background: Acute kidney injury (AKI) is commonly seen with patients undergoing cytoreductive surgery with or without intracavitary cisplatin lavage for pleural mesothelioma. The gold standard for the diagnosis of AKI is the rise of serum creatinine (SCr) but its slow rise leads to delayed diagnosis. Novel tubular injury biomarkers have been identified in animal models of ischemic and nephrotoxic AKI to aid the early, accurate diagnosis. We hypothesized that those AKI biomarkers maybe also useful to diagnose AKI secondary to cisplatin-induced kidney injury and perioperative stress.

Methods: 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).

Disclosure: No significant relationships.
Results: Post-operative AKI (defined as a > 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 expressed at high levels in proximal tubular epithelial cells following ischemic or toxic injury) levels were 22.7 ng/mg of creatinine in those with AKI and 7.8 ng/mg of creatinine in those without AKI. KIM-1 expression patterns were compared with that of other biomarkers (N-acetyl D glucosaminidase, neutrophil gelatinase-associated lipocalin, L-type fatty acid binding protein, interleukin 18, and vascular endothelial growth factor).

Conclusion: A unique pattern of urinary tubular injury biomarker expression after cisplatin exposure may provide improved diagnosis of AKI, enabling the prompt institution of renal protective strategies.

Disclosure: No significant relationships.
P4.20: GENE EXPRESSION RATIO TESTS USING ULTRASOUND-GUIDED FINE NEEDLE ASPIRATION BIOPSIES IN A PROSPECTIVE CLINICAL TRIAL IN MALIGNANT PLEURAL MESOTHELIOMA

Assunta De Rienzo, Beow Y. Yeap, David J. Sugarbaker, Raphael Bueno

Poster Sessions | September 13

Background: Ultrasound-guided (US) fine-needle aspiration (FNA) biopsy is a minimally invasive clinical technique that allows sampling of small lesions. It is performed on a routine basis and has a major impact on the therapeutic management of patients. US-guided FNAs are commonly used to diagnose cancers and detect metastasis by providing specimens for cytopathology and histopathology. We have previously described a gene expression ratio-based method able to translate comprehensive expression profiling data into simple clinical tests that are based on the expression levels of a relatively small number of genes. We also developed several diagnostic and prognostic gene expression tests. In this study, we applied for the first time the gene ratio technique to prospective enrolled patients undergoing US-guided FNAs and showed that it is a useful tool to perform molecular diagnosis in MPM.

Methods: One hundred and forty-nine US-guided FNA biopsies were obtained from a cohort of 56 patients at the time of definitive surgery prior inclusion with an average of three biopsies for patient, and used to isolate and quantify total RNA. Patients were enrolled in a clinical trial approved by the IRB. RNA was amplified (Illumina TotalPrep RNA Amplification kit, Ambion) and analyzed by Real-Time PCR (RT-PCR). The samples were examined by the diagnostic tests MPM vs. normal pleura (NP) (UBE2T/AGENCOURT _ 14535501; MAGED1/ADCA4; PAK4/MYH11) and MPM vs. lung adenocarcinoma (ADCA) (CALF1/ClCN1-7, VACB/TACSTD1, MRCO2/TITF1). When the gene ratio test was performed on multiple FNA biopsies obtained from an individual patient, the diagnosis was determined by a majority rule. Diagnosis was considered as equivocal if conflicting results were obtained from two biopsy specimens from the same patient.

Results: We first evaluated the sensitivity of a sequential combination of diagnostic tests: MPM vs. NP followed by MPM vs. ADCA. The diagnostic test MPM vs. NP was applied to 120 FNA biopsies from 56 patients to determine whether each FNA biopsy contained tumor cells. Twenty-nine samples were called not-MPM and were excluded for further analysis. Ninety-three biopsies from 48 patients were classified as MPM and 4 patients were called ADCA. Twenty-seven samples were called not-MPM and were excluded for further analysis. Twenty-one patients were not analyzed because of low amount of extracted RNA. In this study, we demonstrated that the sequential combination of the two diagnostic ratio tests has sensitivity comparable to the MPM vs. ADCA test that has been validated in several independent cohorts of patients. In addition, we showed for the first time that the combination of US-guided FNA and gene ratio test is a very useful and sensitive method to diagnose MPM.

Conclusion: In this study, we demonstrated that the sequential combination of the two diagnostic ratio tests has sensitivity comparable to the MPM vs. ADCA test that has been validated in several independent cohorts of patients. In addition, we showed for the first time that the combination of US-guided FNA and gene ratio test is a very useful and sensitive method to diagnose MPM.

Disclosure: No significant relationships.
**P4.22: SCREENING OF MPM PATIENTS FOR ACTIVATING SOMATIC MUTATIONS WITHIN PDGFR-BETA**

Ombretta Melaiu1, Gabriella Fontanini2, Barbara Costa1, Laura Boldrini3, Luciano Mutti4, Elisa Sensi2, Sara Bendinelli1, Elisa Bracci1, Marco Lucchi5, Roberto E Favoni6, Federica Gemignani7, Stefano Landi1

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**Background:** Platelet-derived growth factor receptor beta (PDGFR-beta) and its natural ligand PDGF, as well as other tyrosine kinase receptors, play a fundamental role in growth, proliferation, and invasiveness of malignant pleural mesothelioma (MPM). In particular, it was shown that PDGFR-beta is frequently activated in this neoplasm and activating mutations are often found within exon 12 and 18 of its gene. PDGFR-beta is a target for the selective tyrosine-kinase inhibitor imatinib mesylate (STI571, Glivec) that is currently in clinical trial combined with gemcitabine. Nevertheless, it was observed that a subset of MPM patients do not respond to the treatment. The causes of this resistance are, at least in part, ascribed to other types of tumours. In the gastro-intestinal stromal tumours specific mutations, called “gatekeeper mutations” within exon 14 of PDGFR-beta, make cells insensitive to imatinib. To date, the frequency of activating mutations within PDGFR-beta at the presentation in MPM is unknown.

**Methods:** We performed a mutation screening of 100 surgically resected MPMs, to ascertain the somatic mutation frequency of PDGFRB at diagnosis. The mutation screening of the whole exons 12 and 18 of PDGFR-beta was performed with automatic sequencing (Sanger reaction).

**Results:** We did not observe any mutation in all samples.

**Conclusion:** Because PDGFR-beta gene was found over-expressed at mRNA level in several cell lines and tissue specimens, we gather that MPM is not driven by somatic mutations but, rather, that PDGFR-beta is involved in MPM because of its increased expression (that could be functionally equivalent to a constitutive activation). Then, we induced a long-term resistance to imatinib in the over-expressing PDGFR-beta human MPM established cell line MERO-14. Once resistant clones are obtained, they will be screened for mutations in exons 12, 14, and 18. The analysis of resistant clones could help in revealing whether PDGFR-beta plays a role in the secondary resistance to imatinib.

**Disclosure:** No significant relationships.

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**P4.24: THE EXPRESSION OF LONG NONCODING RNAS IN MALIGNANT PLEURAL MESOTHELIOMA**

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**Background:** Long noncoding RNAs (IncRNA) are a class of RNAs >200 nucleotides in length, that do not code for protein but make up >90% of the human genome. Recent studies have investigated the potential role of IncRNAs in cancer. For example, HOTAIR and MALAT1 are implicated in metastases of lung and breast cancer. As malignant pleural mesothelioma (MPM) is an aggressive disease with poor prognosis and IncRNAs have not been previously investigated, our aims were to characterize the expression of IncRNAs potentially involved in MPM biology.

**Methods:** To identify novel IncRNAs involved in MPM, microarray profiling was performed on five cell lines - the immortalized normal mesothelial...
Results: Microarray profiling of MeT-5A versus MPM cell lines identified 350 probes (310 mRNA, 40 lncRNA) differentially expressed between mesothelioma and normal cell lines at >3-fold and \( P < 0.05 \). These probes included known cancer genes including EGFR, CDKN2A, MYC and MET, all of which have been associated with MPM. In addition, we identified PLAUR, a gene previously shown to be highly expressed in human mesothelial cells. The majority of candidates were found to be up-regulated in tumor cell lines. Stratification by histological subtype identified 172 probes differentially expressed between biphasic and epithelioid cell lines. Interestingly, 46 probes were found to overlap between normal versus cancer and epithelioid versus biphasic analyses. Validation of microarray data by RT-qPCR is underway and will be presented at the conference. Finally, bioinformatics analyses identified 40 lncRNAs with a putative role in MPM, including NEAT1 which has been implicated in ovarian cancer. RT-qPCR analysis also demonstrated increased expression of MALAT1 in MPM cell lines compared to MeT-5A. The most pronounced changes were observed in the three epithelioid lines H2052 (fold change 43.9), H2452 (fold change 8.4) and H226 (fold change 4.4). For the remaining cell lines (REN, H28, MSTO, MM05) relatively small differences were observed (<2-fold change).

Conclusion: Microarray profiling has identified novel lncRNAs and mRNAs with a putative role in MPM biology. Independent validation of MALAT1 using RT-qPCR identified over-expression in MPM cell lines compared to a normal mesothelial cell line MeT-5A. Further biological and functional validation is required to confirm the role of novel lncRNAs in the biology of MPM.

Disclosure: No significant relationships.
P4.25: ABCB1 IS A CRITICAL REGULATOR OF VINORELBINE INDUCED APOPTOSIS

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Background: Vinorelbine has been shown to exhibit useful clinical activity in malignant pleural mesothelioma (MPM). A UK randomized clinical trial is planned to evaluate vinorelbine in mesothelioma (VIM). Identification of predictive biomarkers may be essential for successful development of vinorelbine in mesothelioma.

Methods: We selected two cell lines with resistance to vinorelbine, followed by gene expression microarray analysis. Functional genetics and pharmacological inhibitor studies were conducted to validate putative biomarker ABCB1 as a regulator of vinorelbine sensitivity.

Results: Gene expression analysis revealed ABCB1 as one of the mostly highly overexpressed genes in the resistant cells as confirmed by western blot. We then utilized inhibitors of ABCB1 to evaluate the effect on vinorelbine induced apoptosis. XR9051 or verapamil when combined with vinorelbine markedly increased PARP and caspase 9 activation, but were inert as single agents in the resistant MPM cells. Resistance to vinorelbine was also reversed by RNAi silencing of ABCB1.

Conclusion: MPM cells evolve upregulation of ABCB1 during selection for vinorelbine resistance. Significant focal amplification of ABCB1 is reported in solid tumours however the frequency in mesothelioma is being explored. Our data suggest that ABCB1 is a putative predictive biomarker warranting evaluation in the VIM trial.

Disclosure: No significant relationships.

P4.26: MALIGNANT PLEURAL MESOTELIOMA (MPM) IN ELDERLY PATIENTS: RESULTS OF A MULTICENTER SURVEY

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Background: The incidence of malignant pleural mesothelioma (MPM) in elderly patients (pts) is increasing in Western Countries. Elderly pts with MPM are under-represented in clinical trials, and there are no specific guidelines for their management. The aim of this study was to perform a retrospective survey on this patient population in four Oncology Departments with high MPM accrual and expertise.

Methods: The clinical records of elderly pts (≥70 years old) with MPM referred from January 2005 to November 2011 were reviewed. For each patient, age and gender, histology, Eastern Cooperative Oncology Group Performance Status (ECOG-PS), Charlson Comorbidity Index (CCI) and treatment modalities were collected. The study endpoint was overall survival (OS).

Results: Out of a total of 610 cases, 210 elderly pts were identified (34% of the whole MPM population observed in the study period). Pts characteristics were: median age 75 yrs (range 70-92), M/F 132/78, epithelial/non-epithelial histology 140/70, ECOG-PS 0/1/2/unknown 130/67/9/4. CCI was 0 in 128 pts (61%), ≥ in 59 pts (28%). Treatment was multimodality therapy including surgery in 16, chemotherapy in 153 (73%) and best supportive care only in 41 pts (19%). Chemotherapy was mainly pemetrexed-based. Median OS was 11.0 months. In a multivariate model, non-epithelial histology, age ≥75 yrs and the presence of co-morbidities according to CCI (HR 1.15; 95% CI 1.07-1.23, p<0.001) were all significantly correlated to a shorter OS. In the same model, treatment with pemetrexed was associated with improved OS. Age and co-morbidity were not significantly correlated.

Conclusion: Pemetrexed-based chemotherapy is feasible in selected elderly pts with MPM. Comorbidity is a significant prognostic factor, and should be carefully considered in patient selection. Prospective dedicated trials in elderly pts with MPM selected according to comorbidity scales are warranted.

Disclosure: No significant relationships.
(range 13–45) after first carboplatin administration. All HSRs were classified as grade 2 and were easily managed with steroids and anti-histaminics. Carboplatin administration was omitted in subsequent cycles.

**Conclusion:** Re-treatment with PBC in selected MPM pts is a valuable strategy, however clinicians should be aware of the high incidence of HSRs to carboplatin in this setting. Premedication and desensitization strategies should be implemented.

**Disclosure:** No significant relationships.

**POSTER SESSION 4  SEPTEMBER 13, 2012 16:00-17:00**

**P4.28: THE ROLE OF TGF-ALPHA IN THE RESISTANCE TO MESOTHELIOMA CHEMOTHERAPY**

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**Background:** The impact of chemotherapy on the outcome of patients with malignant pleural mesothelioma (MPM) is still controversial. The median survival post diagnosis is invariably about 8 to 12 months. We hypothesized that unresponsiveness to chemotherapy is due to inadequate gene expression in tumor cells. We have previously shown that inhibitors of histone deacetylases (such as valproate, VPA) significantly increases the efficacy of compounds used in chemotherapy (Vandermeers et al, 2010, Clinical Cancer Research 15: 2818). A recent clinical trial on 45 relapsing MPM patients has shown that VPA in combination with doxorubicin increases response rates and improves quality of life in 25% patients with MPM (Scherpereel et al, 2011, European Respiratory Journal 37:129).

**Methods:** Using Agilent microarrays, we compared the transcriptome of two types of cell lines (M14K and H28). Bioinformatic analyses (Ingenuity) identified the most relevant candidate genes. Expression of transforming growth factor-alpha (TGFα) was validated by RT-qPCR and ELISA. TGFα expression level was modulated negatively by RNA interference and positively by transfection of a cDNA vector. TGFα signaling was inhibited with EGFR tyrosine kinase inhibitors (gefitinib and erlotinib). Apoptosis was assessed by different techniques (DNA fragmentation, Annexin V externalization and caspase activity). TGFα-EGFR signaling was characterized by western blot using antiphosphopeptide antibodies. Combination chemotherapy was investigated in two mouse models (ZL34 in SCID and AB1 in Balb/c).

**Results:** To study the mechanisms associated with response to chemotherapy, we compared two types of cell lines (M14K and H28) characterized by a difference in sensitivity to doxorubicin + VPA. We observed that the basal expression level of TGFα was higher in “resistant” H28 compared to “sensitive” M14K cells. To evaluate the functional relevance of TGFα, we modulated its expression either by RNA interference or by transfection of a cDNA vector. Our data shows that a decrease of TGFα expression correlated with induction of apoptosis. Inversely, an inhibition of apoptosis occurred when TGFα was over-expressed. Since TGFα is the ligand of EGFR, we tested the effect of gefitinib and erlotinib in combination with VPA+doxorubicin. Both EGFR inhibitors increased VPA+doxorubicin apoptosis in H28 chemoresistant cells. Finally, the new combination therapy VPA+doxorubicin+erlotinib prevented tumor growth in mice.

**Conclusion:** Our data demonstrates that TGFα is involved in chemoresistance to VPA+doxorubicin, a second-line regimen for MPM. Although inefficient alone, tyrosine kinase inhibitors synergize to induce apoptosis in chemoresistant cells and prevent tumor growth in mouse models.

**Disclosure:** No significant relationships.

**POSTER SESSION 4  SEPTEMBER 13, 2012 16:00-17:00**

**P4.29: VINORELBINE IN MESOTHELIOMA (VIM): A RANDOMISED PHASE II TRIAL OF ORAL VINORELBINE AS SECOND-LINE THERAPY FOR PATIENTS WITH MALIGNANT PLURAL MESOTHELIOMA (MPM) EXPRESSING BRCA1 – A STUDY IN PROGRESS**

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**Background:** Mesothelioma is increasing worldwide. However there is no approved therapy in the second-line setting. Vinorelbine exhibits promising activity, however there has been no randomised evaluation or validation of biomarkers to support patient stratification. We have recently reported that BRCA-1 is an essential regulator of mesothelioma sensitivity to vinorelbine, and its expression is lost in approximately 38%. The Cancer Research UK VIM trial is to be sponsored by the University of Leicester in collaboration with the Wales Cancer Clinical Trials Unit. To evaluate the efficacy of second-line vinorelbine plus active symptom control (ASC), versus ASC. Secondary endpoints: tolerability, response rate, change in tumour volume and overall survival. BRCA1 expression IHC will be evaluated as a stratification factor.

**Methods:** An open label, randomised trial of weekly vinorelbine 80mg/m² plus ASC versus ASC alone. The control arm of ASC will be defined by local practice. Both study arms will be continued until documented evidence of radiological progression or unacceptable toxicity.

**Results:** The sample size has been calculated using the parameters; α=0.2, β=0.1 (90% power), hazard ratio 0.65, 1-sided logrank test and 2:1 randomisation favouring vinorelbine. This requires recruitment of 114 patients. However, as we hypothesis that BRCA-1 expression is required for vinorelbine activity and have estimated its absence in one third of patients, the sample size may be inflated to 171 patients depending on the results of an interim analysis.

**Conclusion:** We aim to open the study in Q1 2013 and recruit over 18 months. The results of this study will be used to inform the design of a future phase III study, with stratification of patients to optimise efficacy.

**Disclosure:** No significant relationships.

**POSTER SESSION 4  SEPTEMBER 13, 2012 16:00-17:00**

**P4.30: TREATMENT AND SURVIVAL ANALYSES OF MALIGNANT MESOTHELIOMA IN JAPAN**

**Nobukazu Fujimoto¹, Kenichi Gemba¹, Keisuke Aoe¹, Katsuya Kato¹, Yukio Takeshima¹, Kouki Inai¹, Takumi Kishimoto¹**

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**Background:** There are few reports concerning treatment strategies and their contributions to survival of patients with malignant mesothelioma (MM) in Japan.

**Methods:** We extracted all death cases due to MM between 2003 and 2008. The diagnosis of MM was confirmed in 929 cases including 396 (55.9%) epithelioid type, 154 (21.7%) sarcomatoid type, 126 (17.8%)
Results: Median overall survival (OS) of all MM cases was 7.7 months (95% confidence interval, 7.1-8.3). Median OS of patients with epithelioid MM was significantly longer than that of patients with biphasic (P=0.030) or sarcomatoid (P=0.009) MM. Surgical resection was performed in 172 patients (18.5%) and 449 (48.3%) received systemic chemotherapy. Survival of patients treated with both surgery and systemic chemotherapy was favorable. Median OS of patients in the late phase of the study period (2006-2008) was significantly longer than that in the early phase (2003-2005) (8.1 vs. 7.5 months, p=0.008). Age younger than 70 years, female gender, epithelioid subtype, and clinical stage I-III were independent favorable prognostic factors. Multivariate analysis confirmed that radical surgery and systemic chemotherapy contribute to longer survival of patients with pleural MM.

Conclusion: The prognosis of MM is poor in Japan, though survival tends to prolong.

Disclosure: No significant relationships.

P4.31: THIRD LINE CHEMOTHERAPY IN ADVANCED MALIGNANT PLEURAL MESOTHELIOMA (MPM): SUPERIOR OUTCOME FOR FEMALES
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Background: While standard treatment in 1st line is platinum based doublet chemotherapy there are no established 2nd or 3rd line treatment in advanced MPM. Data on outcome from 3rd line treatment are exceedingly rarely reported. Our group have previously published high activity in 1st line treatment with carboplatin + gemcitabine + liposomized doxorubicin (J Thorac Oncol 3: 1325-31, 2008) and this regimen (CCG regimen) was hence explored in 3rd line.

Methods: All patients who received 1st line treatment with either platinum + vinorelbine or platinum + pemetrexed, while 2nd line was either pemetrexed or vinorelbine monotherapy. Patients who had advanced MPM, performance status 0-2, and normal organ renal, hepatic, cardiac, and hematological function. Treatment was carboplatin AUC5 and liposomized doxorubicin 30 mg/m2 i.v. day 1 q 3 wks, while gemcitabine 1000 mg/m2 was administered i.v. days 1 and 8 q 3 wks.

Results: Totally 53 patients were treated from June 2006 through May 2012, representing 20.2% of patients who started 1st line treatment in the same period. Forty-seven patients were males and 6 (11%) were females. Median age was 66 years (range 37-76 years). Epithelioid subtype occurred in 70%, 6% and 24% had sarcomatoid and biphasic subtypes, respectively. Forty-seven percent received 3 or more treatment courses, maximum was 6 courses received by 8% of patients. Overall survival from initial histologic diagnosis was median 23.7 months (range 7.6-59.9 months), while 1-, 2-, and 3-years survival rates from diagnosis were 74%, 28%, and 13%, respectively. Median overall survival from start of 3rd line treatment was 7.6 months (range 0.6-41.9 months) and 1-year survival rate 23%. Survival from start of 3rd line was median 10.2 months for females (range 1.9-41.9) and 6.8 months for males (range 0.6-18.9) (p=0.026, figure 1). Median survivals were 8.5 months for epithelioid subtype (range 0.6-41.9), 4.7 for sarcomatoid (1.1-5.2), and 6.0 for biphasic (1.1-13.57) (p=0.399). Patients younger and elder than 70 years had similar prognosis (p=0.237). Response data will be updated at the meeting.

Conclusion: Sarcomatoid subtype patients had poor survival from start of 2nd line and should probably not receive this treatment. Elderly patients aged 70 years or above had similar prognosis as younger patients and should not generally be excluded from treatment. Female patients had superior prognosis. More active treatment options for relapsed MPM patients are sorely needed.

Disclosure: No significant relationships.

P4.32: CHARACTERIZATION AND DRUG SENSITIVITY OF MALIGNANT MESOTHELIOMA CELLS FROM PLEURAL EFFUSIONS
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Background: Patients with malignant mesothelioma have a poor prognosis and less than 50% respond to standard treatment (Pemetrexed and Cisplatin). Furthermore, patients responding to this treatment only have an increased survival of a few months. We hypothesize that the drug resistance pattern is individual for each malignant mesothelioma patient and correlates to the in vitro drug sensitivity of primary tumour cells. The clinical outcome of these patients can then be predicted by this measurement of drug sensitivity and complemented by evaluating the expression of ERCC1 and RRMI, two proteins involved in chemoresistance.

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. The primary cells were also further characterized by immunocytochemistry and qRT-PCR to evaluate the proportion of malignant cells in each sample and to study the expression of RRMI and ERCC1.

Results: So far we have analyzed, characterized and tested 18 samples from 13 different patients. The samples consist of 10-100 % malignant cells and have a variable expression of ERCC1 and RRMI. Among the tested established drugs, Actinomycin-D was the most effective, with cytotoxic effects in a majority of the 18 primary cell cultures. Among the different
groups, the antimicrotubule agents (Docetaxel, Paclitaxel, Vinblastine, Vincreistine and Vinorelbine) seemed to affect most samples. The sensitivity patterns varied greatly between the different primary cell cultures. Five of the samples were resistant to most of the tested drugs and even the most sensitive cells were resistant to more than 50 % of the drugs. The primary cell samples that were more sensitive were received from patients with a longer survival time.

**Conclusion:** Our experimental approach allows us to characterize primary malignant mesothelioma cells, evaluate their expression of central drug resistance proteins and the robotized assay allows a simultaneous determination of chemosensitivity to 30 different drugs. The obtained drug sensitivity patterns and protein levels of ERCC1 and RRM1 vary greatly between different malignant mesothelioma samples, motivating the further development of this technique for clinical use.

**Disclosure:** No significant relationships.

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**P4.33: NOVEL HDAC/DNMT TWIN INHIBITOR AS ANTICANCER AGENT FOR MALIGNANT PLEURAL MESOTHELIOMA**

**Fabian Vandermeers**, Pascale Hubert, Sathya Neelatore, Sriramreddy, Julie Braun, Irving Boitiaux, Eric Stern, Julie Horion, Philippe Delvenne, Didier Lambert, Johan Wouters, Luc Willems

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**Background:** Standard chemotherapeutic regimens are marginally efficient in malignant pleural mesothelioma (MPM) because tumor cells are particularly resistant to radiotherapy and/or chemotherapy. Previous evidence indicates that unresponsiveness of tumors to conventional therapeutic agents might be due to inappropriate epigenetic modifications. Consistently, the HDAC inhibitor valproic acid (VPA) partly ameliorates the efficacy of the first and second line treatments of MPM. In this perspective, we aimed at identifying improved compounds affecting different epigenetic processes such as DNA methylation and histone deacetylation.

**Methods:** Docking simulations were performed with a series of compounds and DNA methyltransferases (DNMTs) or histone deacetylases (HDACs). A lead compound called ES8 was designed and synthesized. The antimitabolic and proapoptotic activity of ES8 was tested in a series of MPM cell lines. The anticancer potential was tested in 2 mouse models.

**Results:** We have designed, synthesized and tested the anticancer potential of a novel compound called ES8 having concomitantly intrinsic HDAC and DNMT inhibitory activities. Docking simulation analyzes show that ES8 can interact with HDAC and DNMT catalytic pockets. ES8 is proapoptotic in a panel of MPM cell lines and prevents tumor growth in mouse models. ES8 treatment is associated with histone hyperacetylation and reduced DNA methylation in tumors.

**Conclusion:** Our data thus shows that a dual HDAC/DNMT inhibitor may be useful to improve treatment of MPM.

**Disclosure:** No significant relationships.

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**P4.34: VARIABILITY OF BLOOD AND CYTOKINE MARKERS IN NORMAL AND DRUG RESISTANT IL-45 CELLS IN A RAT MESOTHELIOMA MODEL.**

**Chris Weir**, Amanda Hudson, Lyndsey Peters, Nick Pavlakis, Stephen Clarke

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**Background:** Malignant mesothelioma (MM) is a rare and aggressive tumour with poor response rates and no curative treatment available with most patients dying within 10-17 months of their first symptoms. The majority of patients with MM are diagnosed in stage III/IV of the disease when administration of systemic chemotherapy represents the only treatment option apart from palliative care. However due to the inherent resistance of this disease, be it intrinsic or acquired relatively poor response to treatment is seen and relapse rates remain high. Drug resistance in MM has not been extensively studied even though chemotherapy resistance is widely acknowledged. Blood markers such as elevated neutrophil to lymphocyte ratio (NLR) have been shown to predict worse prognosis but other cell types have not been tested. We developed a multicolour flow cytometric assay to look at 7 cell types (including NLR) from a small 25µl sample of blood. These cell types include T4, T8, natural killer (NK) cells, B cells, neutrophils, lymphocytes and monocytes. The aim of these experiments was to monitor the level of these cells during pleural tumour progression in normal and drug resistant IL-45 cells in our rat mesothelioma model. Plasma cytokine profiles were also compared between groups.

**Methods:** To look for blood cell and cytokine markers of resistance, Fischer F344 rats were injected with 0.5x10⁶ normal or drug resistant IL-45 rat mesothelioma cells in the pleural cavity. Drug resistant cell lines include Cisplatin, Pemetrexed, Gemcitabine , Vinorelbine and combination (Cisplatin/pemetrexed). Whole blood was collected twice weekly via tail bleeds and monitored for NK, T4, T8, B cell, lymphocyte, neutrophil and monocyte levels during disease progression by flow cytometry. Differences in plasma cytokine profiles between normal IL-45 and drug resistant IL-45 cells were also screened at endpoint.

**Results:** NLR was only significantly higher in rats with normal IL-45 cells and combination resistant cells. NLR was also not predictive for tumour volume or other factors such as rapid health deterioration such as weight loss. A decrease in B cell numbers was significant in all groups except rats with combination resistant cells and proved the most consistent marker of disease progression. General trends showed a decrease in T4 and T8 cells as disease progressed, however T4/T8 ratios did not decrease in all drug resistant groups. Rapidly increasing monocyte levels were prognostic for rapid tumour growth and weight loss.

**Conclusion:** We have developed a useful blood screening test for monitoring cells from whole blood in rats which could easily be adapted to human patients. Standard NLR monitoring maybe useful in non drug resistant IL-45 cells but was not elevated significantly in rats with 3 out of 4 drug resistant cell lines. This suggests that with drug resistant disease blood cell markers such as NK or B cells may be useful prognostically when standard NLR fails. Further work into using this blood test while rats with mesothelioma are treated with chemotherapy are currently in the pipeline.

**Disclosure:** No significant relationships.
P5.01: OVERCOMING RESISTANCE TO EGFR PATHWAY INHIBITION IN MESOTHELIOMA CELLS

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Background: EGFR, MTOR and COX2 are upregulated in malignant pleural mesothelioma (MPM) and are potential targets for directed therapy. In this study we aimed to determine the cytotoxic effect of EGFR, MTOR and COX2 inhibitors.

Methods: The COX2 positive cell lines MSTO-211H, NCI-H2052, NCI-H2452 (mesothelioma) and A549 (lung cancer) were utilised. The EGFR and PTEN status of the cell lines was determined using flow cytometry and immunoblotting respectively. All cell lines were tested for EGFR, KRAS and BRAF mutations. Cells were incubated with single agent Cetuximab, Gefitinib, Rapamycin, Ku0063794 (MTOR kinase inhibitor) and Celecoxib for 72 hrs and analysed using the MTS assay. Subsequently Cetuximab and Gefitinib were combined in turn with Rapamycin, Ku0063794 and Celecoxib. The concentration of Cetuximab (1.6 µM) and Gefitinib (1.4 µM) was calculated based on the steady state plasma concentrations at FDA approved dosing. In each experiment 6 replicates were used per drug concentration and the experiment was repeated at least twice.

Results: All cell lines demonstrated >98% positivity for EGFR. PTEN was absent in the MSTO-211H cells. A549 cells had a KRAS missense mutation at codon 12. No other EGFR, KRAS and BRAF mutations were identified in any of the cell lines. Cetuximab showed 50% cell growth inhibition in MSTO-211H cells at a concentration of 1.6 µM. All other cell lines were resistant to Cetuximab. All cell lines were resistant to Gefitinib at concentrations <1.4 µM. Rapamycin and Ku0063794 demonstrated 50% cell growth inhibition in NCI-H2052, NCI-H2452 and A549 cells. Celecoxib demonstrated 50% cell growth inhibition in all cell lines. Cetuximab and Gefitinib were combined in turn with Rapamycin, Ku0063794 and Celecoxib. Cetuximab and when combined with Celecoxib (NCI-H2052, NCI-H2452 and A549 cells) and Ku0063794 (MSTO-211H cells) demonstrated significant growth inhibition at doses less than 1.6 µM.

Conclusion: Our study suggests that inhibition of MTOR pathway may be an important therapeutic strategy in patients with MPM. Resistance to Cetuximab may be overcome by combining Cetuximab with Celecoxib or MTOR kinase inhibitors (if PTEN is lost). Lack of cytotoxic effect with Gefitinib may be due to the absence of EGFR mutations.

Disclosure: No significant relationships.

P5.03: MICRORNA-223 TARGETS STATHMIN AND REGULATES CELL MIGRATION IN MALIGNANT MESOTHELIOMA

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Background: Malignant Mesothelioma (MM) is an aggressive cancer associated with asbestos exposure. MM has a poor prognosis and current therapies and treatments remain ineffective. Australia has one of the world’s highest rates of MM, therefore it is essential to develop a more comprehensive understanding of this disease. Recent studies have identified microRNAs (miRNAs), a family of small, single stranded RNAs which regulate gene expression, as important in cancer pathogenesis. In preliminary studies we identified miRNA-223 (miR-223) as aberrantly expressed in MM. miR-223 has been associated with the pathogenesis of other cancers and targets the microtubule regulator stathmin, which has been linked to cancer cell migration. Our aim was therefore to test the hypothesis that miR-223 is functionally significant in MM.

Methods: The expression of miR-223 and stathmin mRNA was determined using real-time PCR in human and mouse MM cell lines and control mesothelial cells. stathmin and protein levels were measured by western blot. Cells were transfected with the miR-223 precursor construct to modulate the levels of miR-223 and the impact on stathmin expression, cell proliferation and migration examined.

Results: miR-223 was expressed at significantly lower levels (p<0.01) in the MM cells compared to control mesothelial cells. In the same cell lines, stathmin mRNA and protein were significantly higher (p<0.05) with a strong inverse correlation between the levels of miR-223 and stathmin. Over-expressing miR-223 reduced stathmin mRNA and protein, confirming that miR-223 targets and regulates stathmin in MM. Over-expressing miR-223 did not affect cell proliferation but did cause a decrease in cell migration.

Conclusion: miR-223 targets stathmin and is important in regulating MM cell migration in vitro.

Disclosure: No significant relationships.

P5.04: A DUAL NFκB/STAT3 INHIBITOR AFFECTS CHEMORESISTANCE OF MALIGNANT PLEURAL MESOTHELIOMA CELLS.

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Background: Chronic inflammation is an active mechanism of neoplastic progression. NFκB and STAT3 translate microenvironmental stimuli into inflammatory signaling. NFκB and STAT3 are costitutively activated in many solid tumors and their activated status correlates with the acquisition of mesenchymal features and with radio- and chemoresistance, hallmarks of aggressive tumors. The Epithelial-to-Mesenchymal transition and extreme chemoresistance are major features of the Malignant Pleural...
Mesothelioma in vivo and in vitro, and constitutive activation of NFκB has been shown in asbestos treated mesothelial cells. Moreover, high levels of nuclear, phosphorylated STAT3 have been observed in a conspicuous fraction of MPM specimens. We have shown that STAT3 activation is required for the maintenance of a pool of chemoresistant, Mesothelioma-Tumor-Initiating Cells endowed with high levels of aldehyde dehydrogenase activity (ALDHbright). We have therefore tested whether double inhibition of both NFκB and STAT3 activation can affect the tumorigenic properties of Mesothelioma cell lines and the ALDHbright cell number.

Methods: We have used indirect immunofluorescence, WB, and protein immunoprecipitation to unravel a physical interaction of the NFκB and STAT3 in MPM cell lines. We have then tested the effect of butein on the levels of EMT transcripts by Q-PCR and on the viability, clonogenicity, and invasive properties of MPM cell lines. Finally, we have tested the ability of the drug to affect the number of ALDHbright cells and to overcome resistance to Pemetrexed in vitro and in vivo, by mean of FACS analysis and xenograft transplantation assays.

Results: We show here that multiple MPM cell lines contain high levels of nuclear NFκB and pSTAT3((Y705)). We also show that the two proteins interact in nuclear lysates. Treatment of MPM cells with naturally occurring NFκB/STAT3 inhibitor (butein, 3,4,2’,4’-tetrahydroxychalcone) affects the STAT3 tyrosine phosphorylation and disrupts the NFκB-pSTAT3 interaction. This correlates with a downregulation of several genes involved in cancer progression (such as ICAM1, Vimentin, MMP9, Twist), of proangiogenic cytokines (VEGF) and of IL-6 and IL-8, key growth factors for MPM. Additionally, butein affects the clonogenicity, invasion and the resistance to Pemetrexed of treated MPM cells. We show that these effects may be mediated by a significant reduction of the ALDHbright chemoresistant cell subpopulations in butein-treated MPM cells. Interestingly, Butein is effective on unsorted cell cultures rather than on FACS sorted ALDHbright cells, suggesting the interference with paracrine signaling between the ALDHbright chemoresistant cells and the other cell subpopulations. In vivo, butein treatment severely affects tumor engraftment and potentiates the anticancer effects of Pemetrexed in mouse xenograft models. Interestingly, Butein does not significantly affect the viability of human, untransformed non-mesothelial cells in vitro, nor does it affect survival of tumor-free mice in vivo.

Conclusion: We suggest that Butein may represent the prototype of a novel dual NFκB-STAT3 inhibitor of potential therapeutic interest for MPM.

Disclosure: No significant relationships.

POSTER SESSION 5  SEPTEMBER 14, 2012 11:30-12:30

P5.05: PRO-TUMORGENIC ALTERATION OF SIGNALLING PATHWAYS IN NORMAL MESOTHELIUM: CONTRIBUTION OF NON-MESOTHELIAL CELLS

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Background: Malignant mesothelioma (MM) is an aggressive, fatal tumour of the pleura or peritoneum and strongly related to asbestos exposure. Malignant pleural mesothelioma (MPM) is the most common and occurs with a latency of up to 40 years. The mechanism of MM carcinogenesis is not well understood and the heterogeneity of the tumour is considered to be a major barrier to successful therapies. Several studies have identified changes in the expression and activities of defined cell signalling pathways in mesothelial and stromal cells, but the relationship between different cell types in the process of tumorigenesis has not been studied.

Methods: To examine the pro-oncogenic role(s) of different cell populations, the effect of activated fibroblasts and macrophages on cellular signalling in normal untransformed mesothelial cells was monitored using imaging and immunoblotting techniques.

Results: In co-cultures, primary fibroblasts or fibroblast-conditioned medium induced activation of the pathways known to contribute to malignant transformation. Although subcellular location of damage-associated molecular pattern (DAMP) protein HMGB1 remained nuclear in normal mesothelial cells co-cultured or treated with fibroblast-conditioned medium, the levels of growth modulators were altered in these cells.

Conclusion: Non-mesothelial cells instigate alteration of cellular signalling in mesothelial cells. Further integral examination of the aberrant signalling pathways, especially at early stages of neoplasia, will contribute to the development of more effective therapeutic strategies and in the longer term may lead to personalised therapeutic approaches.

Disclosure: No significant relationships.
Disclosure: No significant relationships.

POSTER SESSION 5  SEPTEMBER 14, 2012 11:30-12:30

P5.07: THE MESOTHELIOMA TUMOR SUPPRESSOR BAP1 INTERACTS WITH PRKDC, PART OF THE DNA-PK DNA DAMAGE REPAIR PROTEIN COMPLEX.

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Background: Recent studies (Bott et al., Nat Genet 43:668-72, 2011) have identified frequent inactivation of the tumor suppressor gene BAP1 (BRCA associated protein 1) in malignant pleural mesothelioma (MPM). Additionally, germ line mutations in BAP1 have been identified and an associated cancer syndrome, which includes MPM, ocular melanoma and other cancers, has been described (Testa et al., Nat Genet 43:1022-5). BAP1 includes an N-terminal ubiquitin C-terminal hydrolase (UCH) domain, and a nuclear localization signal (NLS) at the C-terminal end. The majority of BAP1 truncating mutations are predicted to result in loss of the nuclear localization signal and/or the C-terminal UCH domain. As BAP1 functions as a tumor suppressor it is unclear. Here, we tried to identify interaction partners of BAP1 in MPM cells and to begin to define functional aspects of these interactions.

Methods: We used protein co-immunoprecipitation, mass-spectroscopy analysis, ubiquitin AMC assays, and immunostaining to identify and analyze BAP1 interaction partners. Single cell gel electrophoresis (“Comet”) assays were performed to assess DNA damage in MPM cells with and without BAP1 protein expression.

Results: Mass-spectroscopy analysis of BAP1 protein complexes in H-Meso MPM cells and 293T cells (human embryonic kidney) identified known binding partners [HCF1, ASXL2, histone 2A (H2A)] as well as possible novel interaction partners, notably PRKDC which encodes the catalytic subunit of the DNA-dependent protein kinase (DNA-PK) and functions with the Ku70/Ku80 heterodimer in DNA double strand break repair. Binding of BAP1 to PRKDC was mediated by the C-terminal portion of DNA-PK. DNA-PK inhibitor II (Calbiochem) reduced the activity of BAP1 as a deubiquitinating enzyme in vitro, based on the ubiquitin AMC assay. In Comet assays, BAP1 knockdown was associated with increased DNA damage (increased tail length) and this effect was further enhanced by concomitant DNA-PK inhibitor II treatment.

Conclusion: BAP1 interacts with DNA-PK protein complexes involved in DNA damage repair. We hypothesize that phosphorylation of BAP1 by PRKDC may be important to activate the de-ubiquitination activity of BAP1. These data may point to new targetable key pathways in MPM.

Disclosure: No significant relationships.

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P5.08: CURCUMIN KILLS MALIGNANT MESOTHELIOMA CELLS BY PYROPTOSIS

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Background: Malignant mesothelioma (MM) is an asbestos associated malignancy with a dismal prognosis and poor therapeutic strategies. Curcumin, a natural occurring polyphenol in turmeric, has been shown to have anticarcinogenic properties in multiple cancers, however little research has explored the therapeutic role of curcumin in MM. We hypothesized that curcumin would reduce proliferation and growth of MM via pyroptosis, an inflammation-mediated cell death process dependent on inflammasome activation of caspase-1.

Methods: For our experiments, we used telomerase immortalized human peritoneal mesothelial cells (LP9) and mouse MM cells (#40). Cell growth studies were performed by MTT assays. Steady-state mRNA levels of NOD-like receptor protein 3 (NLRP3), High Mobility Group Box 1 (HMGB-1), and pro-IL-1β in LP9 and mouse MM cells was assed by qRT-PCR using specific primers and probes. Caspase-1 activity, after various treatments with curcumin and/or asbestos, was evaluated by the Caspase-1 Colorimetric Assay. HMGB-1 in supernatants was measured by Western blot analysis. IL-1β in supernatants was measured by ELISA.

Results: LP9 cells treated with curcumin 10 µM for 24-72 hours and exposed to asbestos for 24-48 hours had increased NLRP3 mRNA levels and showed a trend in increased caspase-1 and IL-1β activity compared to control and asbestos exposed LP9 cells. MTT assay results revealed that curcumin inhibited mouse MM cell growth in a dose and time dependent fashion. Mouse MM cells treated with curcumin 40 µM for 48 hours had largely significant increases in steady-state mRNA levels of NLRP3, pro-IL-1β, and HMGB-1. Additionally, these cells had significantly increased caspase-1 activity and HMGB-1 present in supernatants compared to control mouse MM cells.

Conclusion: Our in vitro data indicates that curcumin is able to suppress MM cell growth through pyroptosis as demonstrated by increased steady-state levels of NLRP3 inflammasome, caspase-1 activation and release of HMGB-1, a damage associated molecular protein released in response to activation of caspase-1. A similar trend in inflammasomes transcription and activation was observed in LP9 cells pretreated with curcumin and exposed to asbestos. These results provide evidence that curcumin warrants further investigation as a potential therapeutic agent in MM. We plan to validate our findings using an in vivo mouse MM model in which we will evaluate the ability of curcumin alone and in combination with other drugs to suppress in traperitoneal mouse MM tumor development. This work is supported by Mesothelioma Applied Research Foundation (MARF) grant (AS) and by NIEHS grants 1RO1ES021110 (AS), T32 ES07122 (BM).

Disclosure: No significant relationships.

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P5.09: COMBINATORIAL APPROACHES FOR TARGETING MITOCHONDRIAL REDOX SIGNALING AND FOXO1 EXPRESSION IN MALIGNANT MESOTHELIOMA

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Background: Malignant mesothelioma (MM) associated cancer syndrome, which includes MM, ocular melanoma and other cancers, has been described (Testa et al., Nat Genet 43:1022-5). BAP1 includes an N-terminal ubiquitin C-terminal hydrolase (UCH) domain, and a nuclear localization signal (NLS) at the C-terminal end. The majority of BAP1 truncating mutations are predicted to result in loss of the nuclear localization signal and/or the C-terminal UCH domain. How BAP1 functions as a tumor suppressor is still unclear. Here, we tried to identify interaction partners of BAP1 in MPM cells and to begin to define functional aspects of these interactions.

Methods: We used protein co-immunoprecipitation, mass-spectroscopy analysis, ubiquitin AMC assays, and immunostaining to identify and analyze BAP1 interaction partners. Single cell gel electrophoresis (“Comet”) assays were performed to assess DNA damage in MPM cells with and without BAP1 protein expression.

Results: Mass-spectroscopy analysis of BAP1 protein complexes in H-Meso MPM cells and 293T cells (human embryonic kidney) identified known binding partners [HCF1, ASXL2, histone 2A (H2A)] as well as possible novel interaction partners, notably PRKDC which encodes the catalytic subunit of the DNA-dependent protein kinase (DNA-PK) and functions with the Ku70/Ku80 heterodimer in DNA double strand break repair. Binding of BAP1 to PRKDC was mediated by the C-terminal portion of DNA-PK. DNA-PK inhibitor II (Calbiochem) reduced the activity of BAP1 as a deubiquitinating enzyme in vitro, based on the ubiquitin AMC assay. In Comet assays, BAP1 knockdown was associated with increased DNA damage (increased tail length) and this effect was further enhanced by concomitant DNA-PK inhibitor II treatment.

Conclusion: BAP1 interacts with DNA-PK protein complexes involved in DNA damage repair. We hypothesize that phosphorylation of BAP1 by PRKDC may be important to activate the de-ubiquitination activity of BAP1. These data may point to new targetable key pathways in MPM.

Disclosure: No significant relationships.
Background: Redox-dependent signaling by reactive oxygen species (ROS) plays an important role in cancer pathogenesis, and may represent a therapeutic target in malignant mesothelioma (MM). We have explored the role of FOXM1, a redox-responsive forkhead transcription factor that regulates cell cycle progression and resistance to oxidative stress, in MM cell proliferation and viability.

Methods: The detection of mRNA and protein were performed using qPCR arrays and western blotting, along with immunohistochemistry and microscopy techniques. The detection of reactive oxygen species (ROS) was performed using ROS probes, and the effects of our redox-sensitive drugs were tested in an in vivo model where a xenograft model of human mesothelioma cells were inoculated into severe combined immunodeficient (SCID) mice.

Results: Human MM tumors express more FOXM1 transcript than normal mesothelial tissue, and immunostaining of human MM tissue arrays confirms that FOXM1 is broadly expressed in all major subtypes of human MM. Studies show that MM cells in vitro constitutively generate approximately 2-fold more mitochondrial superoxide than control immortalized L929 mesothelial cells. The triphenylmethane gentian violet (GV), the thiazole antibiotic thiostrepton (TS), and selected triphenylphosphonium (TPP) compounds inhibit FOXM1 expression and MM tumor cell viability in a dose–dependent manner. We have shown each of these agents targets different facets of the thioredoxin reductase 2 (TR2) - thioredoxin 2 (TRX2) - peroxiredoxin 3 (PRX3) antioxidant network, the predominant pathway for metabolizing hydrogen peroxide in mitochondria. GV targets expression of TRX2 protein, TS covalently adds and inactivates PRX3, and TPP compounds inhibit expression of PRX3 and induce extensive mitochondrial fragmentation. In contrast, the general NADPH oxidase inhibitor DPI had no effect on FOXM1 expression. Studies in a xenograft model of human MM in Fox Chase SCID mice show that TS and GV alone are marginally effective in inhibiting tumor growth, while a combination of the two compounds significantly impairs tumor progression without overt toxicity (p = 0.006). Our in vitro studies indicate that FOXM1 expression is tuned to an optimal flux of mitochondrial oxidant production, and agents that either markedly diminish or accentuate the production of mitochondrial oxidants inhibit FOXM1 expression.

Conclusion: Since over-expression of the TR2-TRX2-PRX3 pathway is linked to resistance to apoptosis, and components of the pathways are known to be up-regulated in the majority of MMs, these studies offer a new therapeutic strategy for treating MM. Moreover, inhibition of PRX3 expression has been shown to sensitize cancer cells to common chemotherapeutic drugs, suggesting that alone or in selected combinations these agents may be useful in disabling a major adaptive response that contributes to MM drug resistance. Generously supported by the John Sterling Family Memorial Grant from the Mesothelioma Applied Research Foundation, the Lake Champlain Cancer Research Organization, the Ladies Auxiliary of the Vermont VFW, and the Vermont Cancer Center.

Disclosure: No significant relationships.

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P5.10: DIFFERENTIAL TP53 MUTATIONS AND INTRONIC POLYMORPHISMS IN ASBESTOS-RELATED LUNG CANCER AND MALIGNANT PLEURAL MESOTHELIOMA.

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Results: TP53 mutations were found in NSCLC with no link with asbestos exposure and the frequency was higher in NSCLC than in MMP. However, significant enhancement of TP53 G:C to T:A transversions was found in NSCLC from asbestos-exposed patients.

Interestingly, TP53 polymorphisms in intron 7 (rs12947788 and rs12951053) were identified in both asbestos-related thoracic cancers.

Disclosure: No significant relationships.

P5.11: EPHRIN-A1 MEDIATED OVER EXPRESSION OF MICRORNA-302B INDUCES APOPTOSIS IN MALIGNANT MESOTHELIOMA

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Background: EphrinA1-EphA2 signaling control variety of cellular functions including proliferation, migration, invasion and apoptosis. Ephrin-A1 activation of receptor EphA2 induces anti-tumorigenic effects in malignant mesothelioma (MM). In addition there is growing evidence that ephrinA1 induces microRNAs expression in malignant cells. MicroRNAs are key players in the control of cell proliferation and cell fate determination. However, the molecular mechanisms associated with ephrinA1 and receptor EphA2 mediated attenuation of MM tumor growth remains unclear. Our study identified a novel mechanism of ephrin-A1 mediated tumor suppressor signaling in MM. Ephrin-A1 activation up regulates miR-302b expression in MM and attenuates tumor growth via repression of myeloid cell leukemia (Mcl-1), an anti-apoptotic protein of the Bcl-2 family.

Methods: MM cell lines (CRL-2081=MMC1; and CRL-5830=MMC2) were grown to near confluence, and activated with recombinant-Ephrin-A1-Fc for the indicated period of time. The expression of miR-302b was analyzed by Microarray, quantitative real time RT-PCR analysis. Mcl-1 and cell cycle gene expression were analyzed by RT-PCR, immuno-blottting and Immunofluorescence analysis. To confirm that ephrin-A1 regulates the expression of Mcl-1 mRNA through miR302-b up regulation, cultured cells were transfected with and without miR302-b precursors or miR-302b inhibitor prior to activation. The tumor growth was measured by 3D matrigel assay. MM apoptosis was determined by TUNEL assay and Flow cytometry. Mir-302b binding to the 3’UTR of human Mcl-1 was confirmed by Luciferase assay.

Results: Ephrin-A1 activation induced several fold increases of miR-302b expression in MM cells when compared to respective untreated MM cells.
Ephrin-A1 activation significantly down regulated Mcl-1 expression in MM cells compared to resting cells. Moreover, ephrin-A1 activation significantly inhibited MM tumor growth. EphrinA1 activation induced 28.98±2.38 (p<0.05), and 22.26%±2.74; (p<0.05) cell death in MMC1 and MMC2 respectively. The transfection of MM with miR302b inhibitor and activation with ephrinA1 significantly restored the expression of Mcl-1. In addition, transfection of MM cells with miR302-b vector induced apoptosis. Furthermore, the cell cycle genes cyclin D1, and D2 of G1-S phase were affected indicating that ephrinA1 activation of receptor EphA2 controls the fate of MMC through miR302b expression and induces apoptosis.

Conclusion: We have identified a novel mechanism of ephrinA1 mediated signaling in MM. Our results suggest that in MM ephrinA1 by inducing miR302b expression attenuates tumor growth. MR-302b targets Mcl-1 gene and also affects the expression of cell cycle genes and thereby induces apoptosis. These findings suggest that ephrinA1 could be a promising therapeutic agent for the treatment of MM.

Disclosure: No significant relationships.

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P5.12: GLIBENCLAMIDE SENSITIZES MALIGNANT PLEURAL MESOTHELIOMA CELLS AND PRIMARY CULTURES TO TRAIL-MEDIATED APOPTOSIS

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Background: Malignant pleural mesothelioma (MPM) is an aggressive tumour with poor prognosis and increasing incidence in industrialized countries. MPM is highly refractory to current chemotherapy, and complete responses are rarely observed. To improve MPM therapy we tested the synergetic effect of engaging the Tumor necrosis factor-Related Apoptosis-Inducing Ligand (TRAIL) apoptotic pathway and Glibenclamide, an antidiabetic drug which showed a Reactive Oxygen Species (ROS) inducing effect.

Methods: Apoptosis assays were performed using Fluorometric Homogenous Caspase Assay (Roche). MPM cells were previously treated with or without TRAIL (50 ng/ml) and/or Glibenclamide (50 μM) for 24 hours and then incubated with DEVD-Rhodamine 110. Upon cleavage of the substrate by activated caspases, fluorescence of the released Rhodamine 110 was measured by a microplate reader. ROS assays were performed using MitoTracker Red CM-H2XRos, which accumulates in mitochondria and produces a red fluorescent signal upon oxidation by H2O2. MPM cells were pre-incubated with 25μM H2-MTR, then treated with Glibenclamide and fluorescence was analyzed every 5 minutes for at least 45 minutes using a Zeiss LSM510 confocal laser microscope. p53 protein expression level was detected by western blot analysis using a specific antibody.

Results: Caspases activity in 8 cell lines (4 epithelioid, 1 sarcomatoid, 3 biphasic) and one primary culture was analyzed after treatment with Glibenclamide, TRAIL, Glibenclamide + TRAIL. We observed a statistically significant increase of caspases activity in all the cell lines treated with the combination of the two agents compared to untreated controls and to TRAIL and Glibenclamide used as single agents. We analyzed ROS levels in two cell lines (epithelioid ZL55 and sarcomatoid ZL34); we observed ROS induction in ZL55 treated with Glibenclamide + TRAIL compared to no treatment, while no higher ROS levels were assessed in ZL34 treated with the two agents compared to untreated. Since it was demonstrated that ROS can lead to p53 activation, we examined whether Glibenclamide treatment could activate p53 protein in ZL55 and ZL34 cell lines. As expected, treatment with Glibenclamide increased p53 activation only in ZL55 cells and this activation was reverted by pre-treatment with the ROS scavenger N-acetyl-cysteine (NAC).

Conclusion: Glibenclamide sensitizes MPM cell lines and primary cultures to TRAIL-mediated apoptosis, probably through different mechanisms of action in the epithelioid and sarcomatoid histotypes; in fact a ROS-dependent p53 activation was observed in epithelioid but not in sarcomatoid cell lines. Further studies should test the efficacy of Glibenclamide in the treatment of MPM patients.

Disclosure: No significant relationships.

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P5.13: TUMOR-ASSOCIATED MACROPHAGES MEDIATE RESISTANCE TO PEGYLATED ARGinine DEiminase in MALIGNANT PLEURAL MESOTHELIOMA

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Background: Malignant pleural mesothelioma (MPM) deficient in argininosuccinate synthetase (ASS1), a rate-limiting enzyme in the biosynthesis of arginine, is sensitive to arginine deprivation. The discovery of this ‘Achilles’ heel’ in approximately 50% of MPM has led to the initiation of a UK wide randomised trial of the arginine depleting agent pegylated arginine deiminase (ADI-PEG20) in patients with MPM, following on from promising earlier trials in patients with other arginine dependent tumors. However, tumor resistance to arginine deprivation has been observed; although several mechanisms have been shown to mediate this, including up-regulation of ASS1, autophagy and drug-neutralising antibodies, the supply of arginine specifically by ASS1-rich stromal cells remains to be defined. Here, we hypothesised that tumor-associated macrophages (TAMs), especially abundant in MPM, may act as ‘feeder cells’ providing MPM with arginine (or its intermediates), thereby bypassing the effect of arginine deprivation.

Methods: Three ASS1 negative MPM cell lines treated with and without ADI-PEG20 were analysed using the Affymetrix U133 plus 2.0 microarray platform and validated using qPCR and ELISA. Monocytes were isolated from human buffy cones using CD14 positive selection and matured in 2% human AB serum for seven days. Macrophage influence on tumoral arginine supply was assessed in vitro by culturing macrophages both with and without direct MPM cell contact in the presence and absence of ADI-PEG20. Tumor cell viability was determined by day four using flow cytometry. Macrophage ASS1 expression from co-culture was assessed by qPCR and western blot. Levels of argininosuccinate in the supernatant were measured using HPLC. ADI-PEG20 in combination with macrophage depletion (using liposomal clodronate (CLIP)) was assessed in vivo using an MPM xenograft model.

Results: Distinct immune and inflammatory genes were modulated in all three ASS1 negative cell lines using Affymetrix gene expression analyses. In particular, the mRNA and protein of several cytokines involved in myeloid cell recruitment/activation, including VEGFA, IL8, CXCL2, CXCL3, FG2 and IL1alpha, were significantly up-regulated by 24 hours in the ADI-PEG20 treated ASS1 negative cell lines, compared with control. Co-culture results revealed a 10% increase in MPM cell viability in cells cultured with macrophages without direct cell contact, increasing up to 30% when co-cultured with macrophages in direct cell contact. There was a significant increase in ASS1 expression in co-cultured macrophages 48 hours after treatment with ADI-PEG20, compared with macrophages cultured alone. Initial HPLC results have shown that levels of argininosuccinate in supernatant from co-cultured macrophages are significantly higher than from control macrophages. Preliminary results from ongoing xenograft studies have demonstrated a significant reduction in tumor volume in mice treated with ADI-PEG20 in combination with CLIP, compared with single agent ADI-PEG20.
Conclusion: Collectively, these results indicate that TAMs may modulate sensitivity to ADI-PEG20 in MPM by protecting the tumor cells from arginine deprivation via the provision of arginine or its substrates. Future work aims to interrogate specific pro-inflammatory pathways to assess whether this TAM–mediated drug resistance is reversible.

Disclosure: No significant relationships.
MM and occurs with a latency of up to 40 years. The mechanism of MM carcinogenesis is not well understood and to date, there is no curative therapy. Although the profound resistance of MM to cytotoxic agents is well documented, and is reportedly due to inhibition of cell death, the identification of which cell death pathways are deregulated in this disease has not been systematically examined.

Methods: To obtain a more complete picture of which cell death pathways are deregulated and to determine whether this is related to the aetiology of the disease we examined the expression of key cell death pathway regulators in different subtypes of human MPM cell lines, normal untransformed mesothelial cells and MPM tissue obtained from patients. In addition, we compared the sensitivity of normal mesothelial cells and MPM cell lines to a range of cytotoxic agents, including the DNA damaging agents Cisplatin, Mitomycin C and Etoposide, as well as the highly selective Bcl-2 antagonist, ABT 737.

Results: Using this approach great heterogeneity was observed in the response of malignant cell lines to cytotoxic agents with profound resistance to cell death being evident in both epithelioid and sarcomatoid-derived MPM cells; this in turn may account for the differing response of these tumours to treatment. Importantly, initial profiling of malignant mesothelioma tumour samples from patients has identified similar changes to these tumours to treatment. Importantly, initial profiling of malignant mesothelioma tumour samples from patients has identified similar changes in several key cell death pathway regulators.

Conclusion: The role of these signalling pathways in disease development and potential implications for future treatment of MPM will be discussed.

Disclosure: No significant relationships.

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P5.17: SIGNALING PATHWAYS INFLUENCED BY SYNDECAN-1 IN MALIGNANT MESOTHELIOMA

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Background: The differentiation of malignant mesothelioma involves syndecan-1, a cell surface heparan sulfate proteoglycan. Syndecan-1 modulates a number of interactions of different growth factors with their receptors, thus acting as a signaling co-receptor. We have previously shown that syndecan-1 modulates malignant mesothelioma cell proliferation although the exact underlying molecular mechanisms are not completely elucidated. In this study we aim to characterize the molecular events underlying the growth modulatory effect of syndecan-1 in malignant pleural mesothelioma and to identify critical factors and pathways dependent on syndecan-1, focusing on cell-cycle regulation and features related to proliferation.

Methods: We modulated the expression of syndecan-1 in a human mesothelioma cell line via both over expression and silencing and followed the transcriptomic responses with microarray analysis. To project the transcriptomic analysis on the full-dimensional picture of cellular regulation, we applied a novel method of network enrichment analysis which elucidated signaling relations between differentially expressed genes and pathways acting via various molecular mechanisms.

Results: Fourteen individual genes showed response to both up- and down-regulation of syndecan-1. Syndecan-1 over expression had profound effects on genes involved in regulation of cell behavior whereas syndecan-1 silencing had less powerful effect, which can be explained by the already low initial syndecan-1 level of these cells. Syndecan-1 over expression strongly affected cell proliferation: from 783 proliferation related genes on the chip, 51 were downregulated and 74 were upregulated (p<0.001). The growth factor binding properties of syndecan-1 and its ability to regulate cell growth depends on the fine structure of the heparan sulfate chains, particularly on sulfation pattern. Expression of enzymes involved in the regulation of this sulfation pattern was highly affected by syndecan-1 over expression. We found by Ingenuity pathway analysis (IPA) that the most affected functions were cellular movement, cell death, cellular growth and proliferation, cellular signaling, development and cell cycle. Applying a network enrichment analysis approach, from more than 1,600 pathways analyzed, 939 were significantly altered in syndecan-1 over-expressing cells and 234 in syndecan-1 silenced cells. The results further expanded the findings from gene set enrichment analysis and IPA: many growth-factor, cytokine and cell cycle-related pathways were altered: TGF-β, EGF, VEGF and ERK/MAPK pathways were enriched in both experimental settings. Nearly all analyzed pathways related to cell cycle were enriched after syndecan-1 silencing and depleted after syndecan-1 over expression.

Conclusion: Syndecan-1 seems to orchestrate different growth factors, converging to the downstream phosphokinase pathways. A better understanding of the complex role of syndecan-1 and its molecular interactions may provide possibilities in the future to control growth of malignant mesothelioma. To address the individual contribution of the altered pathways, functional studies are ongoing in our laboratory.

Disclosure: No significant relationships.
**P5.20: NATIONAL SURVEY OF MALIGNANT MESOTHELIOMA AND ASBESTOS EXPOSURE IN JAPAN**

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**Background:** A newspaper article published in June 2005 reported that five residents who lived near the now-closed asbestos cement pipe plant in Amagasaki, Japan, developed pleural mesothelioma. The asbestos-related problems that the article described caused considerable social concern, resulting in the so-called “Kubota shock”. Asbestos has attracted increasing social attention, but no large-scale studies have been conducted to date investigating the clinical features of Malignant Mesothelioma (MM) in Japan.

**Methods:** In the present study, MM cases in Japan were investigated retrospectively. We extracted records for 6030 cases of death due to MM between 2003 and 2008 to clarify the clinical features of MM, including its association with asbestos exposure (AE).

**Results:** Of all these cases, a clinical diagnosis of MM was confirmed for 929. The origin of MM included the pleura in 794 cases (85.5%), the peritoneum in 123 cases (13.2%), the pericardium in seven cases (0.8%), and the testicular tunica vaginalis in five cases (0.6%). The histological subtypes of MM included 396 epithelioid (55.9%), 154 sarcomatoid (21.7%), 126 biphasic (17.8%), and 33 cases (4.7%) classified as “other types”. Of all the MM cases, AE was indicated in 76.8% and pleural plaques were detected in 34.2%. The number of asbestos particles was determined in 103 cases of MM. More than 1000 asbestos particles per gram dried lung tissue were detected in 74.8% of cases and more than 5000 particles were detected in 43.7% of cases. We compared patient characteristics and the diagnostic procedures for MM before and after the “Kubota shock”. Compared with the early phase of this study (2003–2005), the median age at diagnosis of MM was higher, the number of cases without definite diagnosis of MM was lower, the proportion of cases diagnosed by thoracoscopy was higher, and the percentage of cases in which the occupational history was described in the medical records was significantly higher in the later phase (2006–2008).

**Conclusion:** Our study confirmed that more than 70% of MM cases in Japan are associated with AE. The “Kubota shock” may affect some features pertaining to MM.

**Disclosure:** No significant relationships.
P5.22: CASE REPORT: DEMONSTRATING THE INACCURACY OF DEATH CERTIFICATES

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Background: It has long been established that the incidence of mesothelioma is underreported on death certificates, leading to an underestimation of risk from asbestos exposure. We present a classic illustration demonstrating that this problem still exists today. Mike Hoffhine was born in 1943 and spent six years as a naval electrician, first on active duty on U.S. Navy ships, and then as a civilian employee at Hunters Point Naval Shipyard in San Francisco. In April 2001, an open right pleural biopsy demonstrated malignant epithelioid mesothelioma and pleural plaque. In June 2001, he underwent a resection of right middle and upper lobe segments, partial excision of his diaphragm, and partial excision of right parietal and visceral pleura by Dr. David Jablons at the University of California, San Francisco, with pathologic confirmation of the diagnosis and a AJCC/UICC classification pT2NoMx.

Methods: In August 2001, suit was filed against a group of defendants and resolved after defendants thoroughly investigated Mike’s medical and asbestos exposure history.

Results: Mike Hoffhine died in Missouri in April 2012. His death certificate listed the cause of death as respiratory failure due to “metastatic pulmonary malignancy,” said it was unknown whether tobacco use contributed to the death, and recorded a natural rather than accidental or industrial death.

Conclusion: This is a classic asbestos-related mesothelioma death. It demonstrates that even today, physicians misreport causes of death on death certificates, confirming yet again the inaccuracy of epidemiologic studies based on death certificate causes of death.


Disclosure: No significant relationships.

P5.23: PATIENTS’ RIGHTS TO COMPENSATION FROM UNITED STATES BANKRUPTCY TRUST FUNDS

Steven Kazan
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Background: Approximately 70 United States companies have filed for bankruptcy reorganization in whole or in part as a result of asbestos liabilities. Approximately 40 of them have emerged from bankruptcy with established trust funds which have already paid out about $10 billion, with another $30 billion remaining on hand. In addition, another ten or so bankruptcy reorganizations are pending and should emerge with $10-$15 billion in new Trust Funds within the next several years. American asbestos victims have full access to our court system and can recover from
remaining corporate entities as well as Trust Funds, when appropriate. All U.S. Bankruptcy Trust Funds are charged with responsibility to pay claims against the establishing company whether or not those claims arose in the United States. As a result, foreign patients who have never been exposed to asbestos in the United States may still be entitled to present claims against these U.S. Trust Funds. A successful claim requires: (1) a mesothelioma diagnosis; (2) exposure to products for which that Trust bears responsibility; (3) existence of a claim under local law; and (4) determination of value under local law. This presentation will review the identity of existing and expected Trust Funds, the magnitude of their individual assets, how each individual Trust approaches the value of mesothelioma claims under American law, and ways in which exposure to Trust asbestos-containing products can be established. This includes a presentation concerning established and recognized non-U.S. work sites (in excess of 3,000 sites) and approved ships (in excess of 13,000 specific identified ships). We will briefly review the specific steps required to establish a claim and discuss the role of diagnosing and treating physicians in assisting patients to present such claims.

Methods: Not applicable to this presentation.

Results: Not applicable to this presentation.

Conclusion: Not applicable to this presentation.

Disclosure: No significant relationships.

POSTER SESSION 5 SEPTEMBER 14, 2012 11:30-12:30

P5.24: DISEASE BURDEN OF MALIGNANT PLEURAL MESOTHELIOMA: TAIWAN EXPERIENCES

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Background: The burden of disease caused by asbestos have a substantial impact on quality and quantity of life in general population with potential exposure from the workplace and residential environment, as well as may exert financial impact on patients, their families and the society. However, the economic costs of asbestos-related diseases to the society remain to be elucidated. It is important to quantify the disease burden caused by malignant pleural mesothelioma (MPM) to facilitate the outcome research and cost-effectiveness analysis for prevention of asbestos-related diseases.

Methods: We retrieved cases of pleural cancer registered in the catastrophic illnesses of the National Health Insurance Research Database (NHIRD) to estimate the survival functions for malignant pleural mesothelioma, an asbestos-related malignancy. We also retrieved data from the Taiwan Cancer Registry (TCP) and linked them with the National Mortality Registry to cross-validate the results. Assuming a constant excess hazard, we extrapolated lifetime survival function by the Monte Carlo method. For each MPM patient, we simulated an age- and gender-matched person without cancer based on the vital statistics of Taiwan to estimate life expectancy and expected years of life lost (EYLL). By using the reimbursement data from the NHIRD, we calculated the average monthly healthcare expenditures, which were summed to estimate the lifetime healthcare expenditures after adjusting for the corresponding monthly survival probability.

Results: A total of 503 cases of MPM were identified in the NHIRD during 1997 - 2008. The average EYLL was predicted to be 15.1 (95% confidence interval: 14.1 - 16.1) years, and the lifetime healthcare expenditures with a 3% annual discount were predicted to be $19,615 (95% CI: 14,503.3 - 24,724.7) US dollars.

Conclusion: The burden of MPM, in terms of EYLL and lifetime healthcare expenditures, was substantial. Such estimates may provide useful empirical evidence for clinical and health policy-making.

Disclosure: No significant relationships.

P5.25: HEALTH EFFECTS AND RISK ASSESSMENT AMONG RESIDENT POPULATION WITH NATURALLY OCCURRING ASBESTOS EXPOSURE IN SOUTH OF ITALY

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Background: Asbestos is contained in rock and soil in a Basilicata’s rural areas, in South of Italy. The characterization was achieved by mineralogical and geological surveys. There is Tremolite in outcrops of serpentinite, amphibolite, metabasites, as well as in soils, bodies of landslides and debris. Environmental monitoring showed near the population centers doses of Tremolite airborne between 1 fL/L and 5 fL/L. After the identification of cases of mesothelioma arisen in such areas as a result of exposure to natural sources of asbestos, a Consensus Conference was convoked to draw the guidelines for Health Surveillance, for preventive measures and communication of the risk. The aim of this study was to evaluate the prevalence of asbestos-related diseases in people exposed to tremolite and contribute to the determination of the risk assessment of exposure.

Methods: Epidemiological and health surveillance was carried out to assess the prevalence of respiratory diseases associated with occupational and environmental asbestos exposure based on voluntary recruitment of population at risk among the residents. People underwent a health plan that includes standardized questionnaire, occupational anamnesis, pathological anamnesis, physical examination, counselling, spirometry (ATS criteria), pulmonary examination, chest radiographs examined by two B-readers. Selected cases could be subjected to HRCT. The risk assessment of exposure to Tremolite was performed with environmental and personal sampling. The analysis of the filters was done with SEM and the characterization of the fibers with EDS method.

Results: 699 people underwent the surveillance (332 men and 367 women). 7.1% of those examined have pleural plaques (10.8% of men, 3.8% of women). Interstitial Pneumopathies prevalence is 4%, (6.3% of men, 1.9 % of women). Two cases of mesothelioma and one case of lung cancer were found. 93% of asbestos-related diseases concerns farmers and builders. Environmental monitoring of asbestos widespread showed that of 368 samples in 8.9% of cases there was an excess of 2 fL/L with peaks up to 31.7 fL/L at construction sites for the safety of roads built on contaminated rocks. Personal sampling have involved 30 residents employed in different work activities: 5 builders , 15 farmers, 10 employees in jobs that do not involve contact with soil. An exposure exceeding 2 fL/L occurred in 60% of farmers with a peak of 23.06 fL/L, in 100% of builders until 12.02 fL/L and in any case of employees in activities that not involving contact with soil.

Conclusion: Health surveillance has demonstrated the presence of asbestos-related diseases both benign and malignant in residents in areas contaminated with Tremolite. 2 cases of mesothelioma in male farmers have been found in 699 people examined, with a crude rate of 3 cases in 1000, while the Italian national average for males is 3.49 cases per 100000 (Marinaccio 2012). According to the findings of health surveillance, personal and environmental sampling have documented the existence of a risk to the general population, however, contained within 2 fL/L, while people employed in building and agriculture are exposed to higher doses of Tremolite for actions of soil disturbance. Exposure to Tremolite in this area is an occupational hazard.

Disclosure: No significant relationships.
P5.26: RISK OF MALIGNANT MESOTHELIOMA AFTER 40 YEARS SINCE FIRST EXPOSURE: A POOLED ANALYSIS.

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Background: The risk of malignant mesothelioma increases proportionately to the cumulative asbestos exposure and to the 3rd or 4th power of time since first exposure. Most epidemiological studies do not have follow up beyond 40 years.

Methods: The data from 6 cohorts of exposed workers (three Italian railway workers' cohorts, one Amosite workers' cohort, the Eternit cohort, and the Wittenoom workers' cohort) and two cohorts of people with residential exposure (Wittenoom residents and Eternit wives) has been pooled. A nested case control design matched cases and controls on calendar period and age. Conditional logistic regression and fractional polynomials were used to model the relationship between time since first exposure and risk of mesothelioma. Further models were developed to adjust for increasing mortality from competing other causes.

Results: The combined data consisted of 22,048 people with asbestos exposure (16,279 males, 5,769 females). There were 649 cases of confirmed pleural mesothelioma (494 males, 155 females) and 142 cases of peritoneal mesothelioma (112 males and 30 females). Median time since first exposure was 38.2 years (IQR 26.5-46.6). Median duration of exposure was 2.61 years (IQR 0.5-15.0). The risk of pleural mesothelioma increased until 40 years since first exposure. An apparent lessening in the rate of increase after 40 years was only partly explained by allowing for competing risks. The peritoneal mesothelioma rate continued to increase.

Conclusion: The data from this large pooled international cohort of asbestos-exposed people indicates that the rate of pleural malignant mesothelioma increases steadily until 40 years after first exposure and then the rate of increase appears to decrease. However the rate of peritoneal mesothelioma appears to continue rising steadily beyond 40 years.

Disclosure: No significant relationships.

P5.27: JAPANESE GENERAL SCREENING STUDY FOR ASBESTOS-RELATED DISEASES (JSGARD): RESULTS OF 2 YEAR FOLLOW UP

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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. The aim of this study was to prospectively evaluate the actual situation of asbestos exposure and the prevalence of asbestos-related diseases in a group of Japanese general population.

Methods: From 2006 to 2008, 9,810 subjects (mean age, 57 years; 54% male and 50% smokers) underwent chest radiography and low-dose CT (LDCT) in 26 institutions in Japan. Among them, 6,286 (64.1%) subjects underwent subsequent screening after 2 years of interval. Clinical information such as histories of residential and occupational asbestos exposure for all life was reviewed. Images were interpreted independently by 15 experienced radiologists or pulmonologists.

Results: Self-reported history of occupational exposure was definitely and possibly present in 1,103 (11.2%) and 1,702 (17.3%) subjects, respectively, whereas self-reported history of residential exposure was definitely and possibly present in 262 (2.7%) and 931 (9.5%) subjects, respectively, although asbestos factory in their residential areas actually existed in 2870 (29.3%) subjects. Pleural plaque, pleural thickening, and non-calcified pulmonary nodule/mass were identified in 264 (2.7%), 245 (2.5%), and 1003 (10.2%) subjects, respectively, on LDCT, resulting in approximately 4 times higher detectability compared with chest radiography. As malignant tumor, lung cancer was pathologically confirmed in 29 (0.3%) subjects, including 3 subjects with pleural plaque on LDCT. However, self-reported history of asbestos exposure was not identified in them. Similarly, it was not confirmed in 77 (29.2%) of 264 subjects with pleural plaque on LDCT. Based on logistic regression analysis, presence of pleural plaque on LDCT was significantly correlated with male (odds ratio OR, 2.32), age 60 years and more (OR, 1.75), smoking (OR, 1.60), self-reported history of asbestos exposure (OR, 3.92), residential period in asbestos factory area (OR every 10 years, 1.13), and asbestos-related work period (7 works identified, OR every 10 years, 1.3-3.2). Similarly, presence of lung cancer was significantly correlated with age 60 years and more (OR, 2.67) and presence of pleural plaque (OR, 4.17). After repeated LDCT screening, among 6,286 subjects consisting of 139 and 6,147 subjects with and without pleural plaque on LDCT at baseline screening, respectively, 5 (3.6%) of 139 and 7 (0.1%) of 6,147 subjects showed marked progression and new onset of pleural plaque, respectively. Furthermore, lung cancer was pathologically confirmed in 8 (0.1%) subjects.

Conclusion: Our results indicate the potential risk of asbestos exposure among Japanese general population and also the importance of public relations and enlightenment for asbestos exposure among them.

Disclosure: No significant relationships.

P5.29: THE PREDICTIVE VALUE OF X-RAYS IN IDENTIFYING ERIONITE-RELATED PLEURO-PULMONARY CHANGES OVER A 6-YEAR PERIOD AMONG ADULTS LIVING IN TUZKOY, TURKEY.

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Background: Due to environmental exposure to erionite, malignant pleural mesothelioma (MPM) is a common tumor in three villages in Central Turkey. Of these, Tuzkoy is the most well known and the largest village. Between 1980 and 1988, MPM accounted for more than one-fifth of the total deaths and approximately half of the cancer-related deaths in Tuzkoy.

Methods: Chest X-rays of 231 adults 18 years of age older living in Tuzkoy were taken in 2006. Mortality outcomes were investigated retrospectively in 2012 using verbal autopsy methods. The X-rays were examined and compared to mortality outcomes to determine their predictive value in identifying malignant mesothelioma (MM) over the 6-year period.

Results: One hundred and forty-nine (64%) X-rays were of female patients. The mean and median ages of patients were 47 ± 15 years and 47 years, respectively (Range: 18-85 years). Of the 231 X-rays, 56 (24%) were abnormal: 14 (6%) suggested pleural calcification; 11 (5%) had diffuse pleural thickening; 13 (5.7%) had loss of the costophrenic angles; 5 (2%) had apparent thickening in the minor fissure; and 1 (<1%) had pleural effusion. Four chest X-rays were unreadable. Over the 6-year period, 15 deaths were recorded. 9 (4 males and 5 females) of which were due to M: five died from pleural and 4 from peritoneal mesothelioma. Mean ages of
Results: There were 286 patients exposed to asbestos in an occupational setting; 259 (90.6%) developed pleural mesothelioma, 27 (9.4%) developed peritoneal mesothelioma. Mean age at first exposure was 18.3 years (SD 0.4). Mean age at diagnosis was 67.3 years (SD 11.2). Mean latency was 49.1 years (SD 10.9). Median survival was 19.7 months. There were 98 patients exposed to asbestos in a non-occupational setting: 46 (46.9%) developed pleural mesothelioma, 52 (53.1%) developed peritoneal mesothelioma. Mean age at first exposure was 9.1 years (SD 10.2). Mean age at diagnosis was 51.3 years (SD 16.3). Mean latency was 42.7 years (SD 15.5). Median survival was 56.7 months. There was no significant difference in survival between bystanders and primary exposure after adjusting for age at diagnosis and gender (HR=0.36 p <0.001) compared to thoracic mesothelioma patients, after adjusting for age at diagnosis and gender (p=0.86). Irrespective of exposure status, peritoneal mesothelioma patients had significantly better survival (HR=0.36 p <0.001) compared to thoracic mesothelioma patients, after adjusting for age at diagnosis and gender.

Conclusion: The observed findings suggest that individuals with occupational asbestos exposure are more likely to develop pleural than peritoneal mesothelioma whereas bystanders develop either pleural or peritoneal mesothelioma. Although there was no survival difference between the exposure groups, peritoneal patients had an overall better survival than pleural patients. This study emphasizes the importance of assessing the patient’s environmental exposure history. Early recognition of this risk factor can hopefully alter the prognosis of this dreadful disease.

Disclosure: No significant relationships.
**P5.34: THE ROLE OF PERIOPERATIVE SYSTEMIC CHEMOTHERAPY IN DIFFUSE MALIGNANT PERITONEAL MESOTHELIOMA PATIENTS TREATED WITH CYTOREDUCTIVE SURGERY AND HYPERTHERMIC INTRAPERITONEAL CHEMOTHERAPY**

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**Background:** Provided that studies on CRS and HIPEC to treat DMPM usually have not explored the prognostic significance of perioperative systemic treatments, we attempted to evaluate the effects of preoperative systemic chemotherapy (CT) on overall (OS) and progression free survivals (PFS). As secondary endpoint we evaluated the effects of preoperative systemic chemotherapy on short-term surgical outcomes regarding morbidity and completeness of cytoreduction.

**Methods:** Data from 116 DMPM patients treated with CRS and HIPEC from August 1995 to October 2011 were retrospectively analyzed from a prospectively collected database in the NCI of Milan. Sixty cases underwent preoperative CT, 30 underwent postoperative CT, and 26 did not perform any CT. Fifty-five cases used the perioperative combination of platinum and pemetrexed. We tested whether covariates related to clinical, histologic, perioperative CT, and surgical treatment were correlated with completeness of cytoreduction (CC), postoperative G3-5 morbidity, and progression free (PFS) and overall survivals (OS). Univariate and multivariate analysis were performed.

**Results:** factors independently associated with CC were ECOG performance status (PF) =0, and PCI <20. Factors independently associated with postoperative G3-5 morbidity were ECOG PS>1, bowel anastomosis and number of peritonectomy procedures. Preoperative platelet count>400x10^3/mm3, histological subtype (biphasic and sarcomatoid vs epithelial), CC, and G3-5 morbidity were independent prognostic factors. Preoperative CT was not associated with CC or G3-5 morbidity. There was no significant difference in terms of survival between the preoperative CT, postoperative CT and no-CT groups.

**Conclusions:** the CC, G3-5 and OS were not influenced by aspects related to perioperative CT. Such a comparative analysis should be repeated in a multi-institutional context with a judicious selection of control groups well-balanced regarding the distribution of risk/prognostic factors.

**Disclosure:** No significant relationships.


**P5.35: AN ORAL PAN-CLASS I PI3-KINASE INHIBITOR, NVP-BKM120, INHIBITS TUMOR GROWTH IN HUMAN MALIGNANT PERITONEAL MESOTHELIOMA MURINE XENOGRAFTS**

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**Background:** Malignant peritoneal mesothelioma (MPeM) is a cancer of the abdomen that frequently recurs after surgical resection and is relatively resistant to chemotherapy. We have recently reported that over expression of PI3K and mTOR pathways in tumor samples from patients with MPeM are associated with shortened patient survival. In this study our aim was to test the effectiveness of a novel class I PI3-kinase specific inhibitor for the treatment of MPeM.

**Methods:** MPeM cells (Meso-1, Meso-2 and Meso-3) were treated with BKM120 (50 nM/L) and cell proliferation rates were assessed in vitro. Downstream PI3K/mTOR signaling was studied by analyzing RNA by qPCR and protein expression by Western blot. Anti-tumor effects of BKM120 were tested in an MPeM murine xenograft model.

**Results:** BKM120 treatment for 48 h significantly (p<0.05) inhibited MPeM cell proliferation. There was no clear difference in anti-proliferative activity of BKM120 in phosphatase and tensin homolog (PTEN) positive versus PTEN null cell lines. Treatment of cells with BKM120 significantly reduced PI3K downstream signaling; PI3K-p110α, phospho p85, AKT and phospho AKT protein expression were markedly less in treated cells compared with the control group. Although BKM120 is a specific inhibitor of class I PI3-kinase, mTOR and phospho mTOR expression were also reduced in treated cells. S6 kinase activity showed no significant difference in the control versus treated groups whereas phospho S6 kinase activity decreased in BKM120 treated group. Oral treatment of BKM120 (20mg/every alternate day) for one month significantly reduced xenograft growth compared to placebo controls.

**Conclusion:** BKM120 is a potential inhibitor of PI3K and mTOR signaling in MPeM. Tumor growth can be suppressed in MPeM xenograft models with oral administration of BKM120. Further studies are warranted to evaluate the action this compound in tumor relapse and PI3K associated pathways. This work was supported by a grant from the Mesothelioma Applied Research Foundation (MARF).

**Disclosure:** No significant relationships.
Methods: Consecutive patients registered at the pathology department at the Yuyao Peoples Hospital with suspected mesothelioma diagnosis by clinical findings and histology by Hematoxylin-Eosin (HE) staining from 2005-2010. The histological diagnosis was verified through immunohistochemistry with the positive markers calretinin, WT1, D2-40, CK5-6, AE1-AE3, CAM5.2, EMA and the negative markers CEA, BerEP4, MOC31, TTF-1 and the hormone receptors ER and PgR. Asbestos exposure data were obtained from the patient files and/or by contacting the patients relatives.

Results: Among 38 suspected mesothelioma cases based on HE and clinical picture, 27 were verified with immunohistochemistry, 16 peritoneal and 11 pleural. Peritoneal mesothelioma (n=16) were all females with a mean age at diagnosis of 51 years (range 43-71) and of those were occupational information could be obtained (n=13) there was a 92% occupational asbestos exposure by hand spinning of asbestos ranging from 1-12 years. The females with pleural mesothelioma (n=10) had a mean age at diagnosis of 57 years (range 38-82) and an occupational asbestos exposure by hand spinning of asbestos of 100% ranging from 7-10 years. Only one male aged 67 was registered with pleural mesothelioma, with unknown asbestos history. Median survival was only 5 months on the cases where survival was registered (n=7). Revised diagnosis after immunohistochemistry in 11 cases included various carcinomas, among them a mucinous adenocarcinoma, uterine cervix carcinoma and pulmonary adenocarcinoma. 4/9 females (44%) had a history of heavy asbestos exposure, ranging from 5-20 years of hand spinning of asbestos. Three males also had a revised diagnosis, two with carcinoma where one had worked 5 years in an asbestos factory, and one benign asbestosis.

Conclusion: In this retrospective study of a mesothelioma cases from a single hospital in Yuyao, there were gross overrepresentation of women, 38:1, and of peritoneal mesothelioma, 68%. Importantly, 90-100% of these cases, where an occupational history was obtained, had a history of several years of asbestos by hand spinning. In a study published in 1985 from Cixi, a city close to Yuyao, described small asbestos factories and household spinning with high registered asbestos dust concentration were women used to work from 10 to 12 hours daily [1]. The median latency time of mesothelioma from asbestos exposure is 40 years, so the women described here were likely exposed very young as the mean age at diagnosis was only 51. Moreover, other cancers were seen in these highly exposed individuals. As the Yuyao area still harbor small asbestos factories, such clusters of mesothelioma will be expected in the future.

Disclosure: No significant relationships.
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