Serum soluble mesothelin concentrations in malignant pleural mesothelioma: relationship to tumor volume, clinical stage and changes in tumor burden

Jenette Creaney¹,², Roslyn J. Francis¹,⁴, Ian M. Dick¹, Arthur W. Musk¹, Bruce W. S. Robinson¹,²,⁵ Michael J. Byrne¹,³ and Anna K. Nowak¹,³

¹ National Research Centre for Asbestos Related Diseases, School of Medicine and Pharmacology, University of Western Australia, Sir Charles Gairdner Hospital, 4th Floor, G Block, Verdun St, Nedlands, WA 6009, Australia.
² The Australian Mesothelioma Tissue Bank, Sir Charles Gairdner Hospital, Verdun St, Nedlands, WA 6009, Australia.
³ Department of Medical Oncology, Sir Charles Gairdner Hospital, Verdun St, Nedlands, WA 6009, Australia.
⁴ Department of Nuclear Medicine, Sir Charles Gairdner Hospital, Verdun St, Nedlands, WA 6009, Australia.
⁵ Department of Respiratory Medicine, Sir Charles Gairdner Hospital, Verdun St, Nedlands, WA 6009, Australia.
⁶ School of Population Health, University of Western Australia, Nedlands, WA 6009, Australia.

Research Support: This study was funded in part by research grants from the National Health and Medical Research Council of Australia and the Insurance Commission of Western Australia.

Corresponding Author:

Prof. Jenette Creaney, National Research Centre for Asbestos Related Disease, UWA
Tel: +(618) 9346 2005, FAX: +(618) 9346 2816, creaneyj@cyllene.uwa.edu.au
Running Title: Serum mesothelin in mesothelioma

Key words: mesothelioma, biomarker, SMRP, mesothelin, response
Statement of Translational Relevance

Radiological measurement of response to therapy presents particular challenges in malignant pleural mesothelioma, with tumors growing as a rind around the pleural cavity rather than as discrete measurable masses. Existing radiological response criteria may not fully reflect changes in tumor bulk. Serum biomarkers have a role in the monitoring of tumor response in other cancers. These prospectively collected data suggest that in the clinical context of the management of an individual patient an elevated level of serum mesothelin may provide useful guidance for the clinician. Higher baseline levels suggest larger tumor volume, more advanced tumor stage and bode a poorer prognosis. A decrease in serum mesothelin following chemotherapy increases the likelihood that the tumor is responding to therapy and predicts for improved survival. Larger, multicentre confirmatory studies are needed before measurement of serum mesothelin is implemented in routine clinical practice for assessment of prognosis or tumor response.
Abstract

**Purpose:** To examine the clinical utility of soluble mesothelin in patients with malignant pleural mesothelioma.

**Experimental Design:** Ninety-seven patients (11F:86M) were prospectively enrolled, longitudinal serum samples collected, and mesothelin concentrations determined. Baseline mesothelin levels were analysed relative to tumor stage, presence of metastatic disease, the Positron Emission Tomography (PET) parameters maximum standardized uptake value (SUVmax), tumor volume, total glycolytic volume (TGV) and survival. Changes in mesothelin level were correlated to objective response to chemotherapy as assessed radiologically and by PET imaging, and with patient survival.

**Results:** Baseline mesothelin levels greater than 5nM were a significant negative prognostic indicator (HR=2.25; (95%CI) 1.20-4.21) and correlated with tumor stage and volume. In 55 patients who received chemotherapy, change in mesothelin correlated with radiological response (Chi-square 11.32; p=0.023) and change in metabolically active tumor volume (r=0.58; p<0.01). Median survival for patients with a reduction in mesothelin following chemotherapy (19 months) was significantly longer than for patients with increased mesothelin (5 months; p<0.001).

**Conclusion:** These findings demonstrate the potential value of changes in mesothelin levels for prognostication and monitoring of treatment response in mesothelioma.
Introduction

Malignant mesothelioma is an aggressive, asbestos induced tumor of serosal surfaces (1, 2). Palliative use of first-line chemotherapy for advanced pleural mesothelioma is widely accepted (3) and, although controversial, some patients also receive surgical interventions (4-6). Early phase clinical trials commonly use radiological endpoints such as objective response rate, time to progression, and 6-month progression free survival to assess the efficacy of novel therapies. The utility of early phase clinical trials in predicting the outcomes of subsequent randomised phase III investigations depends upon these radiological endpoints being robust, reproducible, and clearly related to overall survival and patient benefit. Unfortunately, these conditions are not well met with assessment of response in malignant pleural mesothelioma (7, 8). The unique morphology of mesothelioma presents some difficulties for quantitative radiological measures. The tumor tends to grow as a “rind” around the pleural surface, rather than as a spherical mass. While a modification of the RECIST criteria (9) has in part addressed the specific application of RECIST to the anatomical distribution of mesothelioma (10), these measurements are still difficult to reproduce, vary between observers, and the relationship of the Modified RECIST measurements to tumor volume is unclear (11, 12).

Previously, we and others have shown that serial FDG-PET scanning can predict response to chemotherapy and patient survival (13-15). Nevertheless, change in FDG uptake can be difficult to interpret in the setting of surgery or prior pleurodesis, due to false positive uptake in inflammatory tissue (16). Pleurodesis is a common procedure in patients with mesothelioma, and limits the proportion of patients who can be monitored this way.

Tumor markers offer an attractive means of monitoring tumor response in patients, being less expensive and less invasive for repeated use. Indeed it is in such a setting that markers are most clinically useful in other forms of cancer (17-22). For mesothelioma...
patients, tumor markers could offer a quantitative measure of tumor response and a potential alternative endpoint for clinical trials of novel therapies.

Mesothelin, a differentiation glycoprotein present on the surface of mesothelial cells and over-expressed in several neoplasms including mesothelioma (23), is a strong candidate tumor marker for monitoring tumor response in this disease. Levels of soluble mesothelin significantly correlate with tumor size and increase with disease progression (24). Pass et al showed that mesothelin levels following surgery were reduced (25) and increasing serum mesothelin levels have been associated with poor patient outcome (26, 27). While the diagnostic utility of serum mesothelin has been chiefly reported in epithelial mesothelioma tumors, the use of mesothelin for monitoring purposes may also be applicable to mesothelioma tumors with a sarcomatoid histology, as these variants have also been shown to express the protein (unpublished data and (27), albeit at low levels.

In this paper we report the relationship of serum mesothelin levels to clinical tumor stage and to tumor volume and tumor glycolytic volume (TGV) as assessed by FDG-PET scans. We also describe the changes with disease response or progression following therapy and relate these changes to changes in measures of disease bulk and with patient survival.
Materials and Methods

Patients

Patients were prospectively recruited to a longitudinal cohort study of imaging and biomarkers from October 2003 until 2007. All eligible consecutive patients referred to a tertiary outpatient oncology service were approached to participate. Eligibility criteria included confirmed diagnosis of malignant pleural mesothelioma, age over 18 years, no prior chemotherapy and Eastern Cooperative Oncology Group performance status 0-2. At enrolment each patient was radiologically staged using the UICC TNM system (28). This was not an intervention study and patient management was at the discretion of the treating physician and the patient. During the study period, the initial standard of care for systemic treatment was chemotherapy with a platinum and gemcitabine, and since 2004, treatment with a platinum and pemetrexed. Some patients received best supportive care only. A small proportion of patients were treated on a combined modality protocol comprising extrapleural pneumonectomy, adjuvant chemotherapy, and hemithoracic radiotherapy.

Blood samples were taken at baseline. For patients receiving best supportive care only, further blood samples were taken at approximately 3 monthly intervals. For patients undergoing chemotherapy, blood samples were taken before the start of each chemotherapy cycle and three-monthly on completion of chemotherapy. Patients undergoing trimodality therapy had blood samples taken pre-operatively, post-operatively, with each cycle during chemotherapy, and 3-monthly thereafter. Blood sampling was timed to coincide with clinical and imaging assessments.

The study was approved by the institutional Human Research Ethics Committee, and all patients provided informed written consent.
CT imaging

Each patient had a contrast-enhanced thoracic and upper abdominal helical computed tomography (CT) scan with 5-mm slices. For patients receiving chemotherapy a CT scan was performed up to 4 weeks before the first cycle, and following the first and third or fourth cycle, with an additional CT scan to confirm response when appropriate. Radiological response to therapy was assessed using the modified RECIST criteria for mesothelioma (10). CT scans were assessed by a thoracic radiologist, experienced in mesothelioma measurement and unaware of patient outcome. Radiological complete response (CR) was defined as the disappearance of all target lesions with no evidence of tumor elsewhere, and radiological partial response (PR) was defined as at least a 30% reduction in the summated unidimensional tumor measurement. A confirmed response required a repeat observation on 2 occasions at least 4 weeks apart. Radiological progressive disease (PD) was defined as an increase of at least 20% in the total tumor measurement over the nadir measurement, or the appearance of one or more new lesions. Patients with stable disease (SD) were those who fulfilled the criteria for neither PR nor PD.

18F-FDG PET

Whole body FDG PET imaging was performed on a germanium oxyorthosilicate Phillips Allegro PET scanner. Patients fasted for 6 hours and were imaged 90 minutes following 215MBq/m2 FDG administration. Scans were performed at baseline, repeated before commencing chemotherapy, if the patient had undergone a period of observation before starting treatment, and then repeated after the first and fourth cycle of chemotherapy. In view of the potential confounding effect of talc pleurodesis, only patients without talc
pleurodesis in the previous 6 months were included in the PET scan related analyses. Each FDG-PET scan was staged by two experienced PET physicians, using UICC TNM staging criteria (28), with consensus reporting used to attain agreement. The PET physicians were blinded to CT stage and patient outcome, but were permitted to utilise the CT scan for anatomical correlation.

Semi-quantitative analysis of PET scans was performed using a previously described 3D iterative region growing algorithm to define tumor volumes of interest and derive a total glycolytic volume (TGV) and maximum standardized uptake value (SUVmax) (13, 29). All SUV measurements were calculated using body weight. The region growing software was implemented in Interactive Data Language version 6.1 on a Windows platform. The growing function of the algorithm uses an adaptive threshold to delineate 3–dimensional tumor boundaries. The threshold, which is re-applied at each iteration, is determined by the current mean, the activity in neighbouring pixels and the maximum normal background activity in liver (29). Contiguous 3-dimensional volumes of interest (VOI) are generated, and the operator is required to re-seed on non-contiguous tumor elements. TGV and SUVmax were then determined from the tumor VOI, with TGV being a composite measure of metabolic activity and volume of the whole tumor mass while SUVmax represents the maximum SUV value in the tumor VOI.

Mesothelin

Mesothelin concentrations were determined following the manufacturer’s instructions using the MESOMARK™ assay (Fujirebio Diagnostics, Malvern, PA). All assays were performed on coded samples by investigators who were unaware of the patient’s clinical characteristics.
Statistical Analysis

The work reported in this paper was performed as a sub-study of a study powered to answer imaging questions; hence the sample size was determined independently of this pilot sub-study.

Measurements between groups were compared using the non-parametric Mann-Whitney U test. Correlations between measurements were analysed using the non-parametric Spearman’s test. The association of mesothelin concentration with survival was analysed by entering it into a Cox proportional hazards regression model as either a continuous or as a grouped variable, both with and without covariates. A p value of less than 0.05 was considered significant. The statistical analysis was performed using the PASW version 18 statistics package (SPSS Inc, Chicago IL).
Results

Patients

97 patients were prospectively recruited over 5 years. Two patients were ineligible because of concomitant non-mesothelioma tumors detected on FDG-PET scan. Almost one quarter of the patients received best supportive care, 61 received first-line chemotherapy, seven underwent trimodality therapy and two received radiotherapy (Figure 1). The demographic characteristics of the patients and the histological characterization of their tumors are shown in Table 1. As the study addressed multiple independent questions, some patients were assessable for different aspects of the study. For example, those patients who were not eligible for FDG-PET scanning due to prior talc pleurodesis or surgery remained eligible for aspects assessing the relationship between radiological tumor response and mesothelin levels. At the time of censoring ten patients were alive; median survival for the overall group of 95 patients was 15 months.

Prognostic significance of baseline mesothelin

Serum for baseline mesothelin measurement was collected as close as possible to the time of diagnosis of mesothelioma, with a median (range) time difference of 2 (-3 – 33) weeks from the date of diagnosis. For this study, soluble mesothelin levels at baseline ranged from undetectable to 103nM, with a median concentration of 2.16nM. When patients were grouped into tertiles by baseline serum mesothelin level, the median survival from the time of diagnosis for patients with the lowest tertile of mesothelin level (below 1nM) was 20 (0 – 57) months, significantly longer than those in the highest tertile of mesothelin level (above 5nM; 12.5 (1 - 44) months; \( p=0.012 \)) (Figure 2A). Univariate analysis of mesothelin levels entered as a continuous variable indicated that baseline mesothelin was not a significant predictor of survival; however a mesothelin level greater
than 5nM was associated with decreased survival compared to a mesothelin level of less than 1nM (Table 2). Univariate analysis indicated that sarcomatoid histology, CT Stage IV disease, and high baseline PET volume and TGV were associated with decreased survival (Table 2). After adjustment for age, gender, serum creatinine levels, histology and treatment in a Cox regression model (Table 2; Model A), baseline mesothelin entered into the model as a continuous variable was a significant predictor of survival (p=0.038). However when the Cox Regression model was adjusted for PET measures (Table 2; Model B) mesothelin measurements were not a significant prognostic predictor of patient survival. Adjustment of the model for CT staging resulted in neither mesothelin measures nor CT staging being significant prognostic predictors of survival (data not shown).

**Correlation of baseline serum mesothelin levels with tumor stage**

Baseline serum mesothelin concentrations were obtained within a mean (±SD) of 0.1 ± 3.5 weeks of CT imaging, and correlated with tumor stage. There was a modest significant increase in serum mesothelin with more advanced radiological stage (Figure 2B). The median (range) mesothelin level was 0.88 (0.34 – 2.71) nM for the Stage I group and 2.5 (0.38 – 103.17) nM for Stage IV (p=0.008). Nevertheless, some individuals with Stage IV disease had low serum mesothelin. There was no significant difference in mesothelin levels between patients with (n=16) or without (n=70) CT evidence of metastatic disease (p=0.118) (data not shown).

In the 55 eligible patients there was no significant increase in mesothelin concentrations with increasing stage as assessed by FDG-PET scan (Figure 2C). FDG-PET identified unrecognised metastatic disease in 4 patients; mesothelin levels did not differ between those patients with and without PET-identified metastases (data not shown).
Correlation of mesothelin concentration with tumor burden

Staging represents the anatomical extent of tumor involvement, and may not always correspond to tumor burden in this disease. Correlation of metabolically active tumor volume with serum mesothelin levels was examined in 52 patients with serum mesothelin concentrations available within four weeks of the FDG-PET scan. Excluded patients were those who did not have a FDG-PET scan for any reason, those with pleurodesis within 6 months of the scan and three patients who had minimal $^{18}$F-FDG avid disease that was unable to be quantified. Mesothelin levels correlated significantly with metabolically active tumor volume as measured by FDG-PET Total Glycolytic Volume (TGV) (Spearman’s $r=0.419$; $p=0.002$). Mesothelin levels also correlated with tumor volume ($r=0.409$; $p<0.001$) and with the intensity measure, SUVmax ($r=0.291$; $p<0.05$) (Figure 2).

Change in mesothelin concentration following therapy

Six patients underwent extrapleural pneumonectomy with macroscopic clearance of tumor but positive histological margins. Five patients had samples taken between 2 months and 24 hours pre-operatively and within 24 to 72 hours post-operatively; in these patients, mesothelin levels decreased to a mean of 54% of the baseline level (range 27 to 83% of baseline). It is unclear whether subsequent more profound declines would have occurred, as further post-operative mesothelin levels were not available on any of these patients, three of whom died before additional testing. In the sixth patient, a post-operative mesothelin level was not taken until 5 months following surgery and approximately 9 months before recurrence and at that time was 50% of the initial baseline measurement. The half life of the protein and the trajectory of decline following macroscopic resection is
unknown and should be explored by serial measurements over the immediate post-operative period to examine the nadir reached after macroscopic complete resection and the relationship of this nadir to subsequent clinical outcomes.

Of the patients receiving chemotherapy in this study 55 were analysable for changes in mesothelin levels; 39 received gemcitabine and a platinum agent, 16 received pemetrexed combined with cisplatin. The number of treatment cycles ranged from one to six.

Changes were assessed by comparing an individual patient’s pre-chemotherapy mesothelin level with the lowest level achieved during treatment. Following chemotherapy, serum mesothelin decreased by 25% or greater of baseline levels in 24 of 55 patients (decreased group); remained within 25% above, or below, of baseline in 23 patients (stable group), and increased by more than 25% of baseline in 8 patients (increased group). There was a significant correlation (Chi-square 11.32, p=0.023) between radiological response as determined by the modified RECIST criteria and changes in serum mesothelin (Table 3). Of the 17 patients with a partial response to chemotherapy, a reduction in mesothelin levels was seen in 12; none had increased mesothelin levels.

Twenty eight of the 55 patients had concurrent assessment of tumor changes by FDG-PET. There was a significant correlation between the percentage change from baseline mesothelin levels and the percentage change in PET assessed TGV (r=0.58, p<0.001) and volume (r=0.61, p<0.001) (Figure 3). Mesothelin concentrations over time as well as PET and CT scans pre and post chemotherapy are shown for a representative patient in Figure 3.

Change in mesothelin concentration following chemotherapy and survival
When examined as a continuous variable, Cox regression analysis demonstrated that percentage change in mesothelin level in the 55 patients available for analysis following chemotherapy was predictive of patient survival (HR=1.05, 95% confidence interval, 1.02–1.07, p<0.001, per 10% change in mesothelin level). When categorized on the percentage change in mesothelin levels into three groups. Both the stable mesothelin group (HR 2.02, 95%CI 1.10-3.13, p<0.001) and the increased mesothelin group (HR 23.03, 95%CI 7.47–70.92, p<0.001) had an increased risk compared to the decreased mesothelin group. Kaplan-Meier analysis (Figure 4) illustrates the significant relationship between percent change in mesothelin levels following chemotherapy and survival.

**Discussion**

In this study we demonstrated that change from baseline mesothelin level in response to chemotherapy reflects patient prognosis, suggesting a possible role for mesothelin in monitoring response to treatment. Confirming and extending recent findings that showed a correlation between change in mesothelin levels and radiological response (26). Tumor markers are widely used in ovarian cancer, breast cancer, colorectal cancer, and prostate cancer both in clinical trials and in routine clinical practice. In some cases measurement of CA 15-3 is used as an adjunct to monitoring response clinically and by imaging, and to decrease the requirement for additional investigations or increase the duration between tests, despite baseline levels not being prognostic or predictive (30).

Our data demonstrate a modest correlation between baseline mesothelin level and tumor stage, and no value of baseline mesothelin in differentiating between patients with or without metastatic disease. However, mesothelin levels were more strongly correlated with tumor burden and metabolically active tumor volume as measured by FDG-PET. This disconnect between stage and tumor burden is not unexpected, as tumor stage is...
determined by anatomical site and extent of tumor involvement and not necessarily related
to tumor bulk. We have previously shown that tumor volume on FDG-PET is strongly
associated with survival on univariate analysis (p=0.008) (15). Here, the correlation
between tumor volume and mesothelin level is also reflected in the observation that
patients with high baseline mesothelin levels have a significantly reduced survival. This
finding that high baseline mesothelin correlates with poor survival has been previously
shown by others (25, 31, 32). In our study this was independent of age, sex, histology and
treatment. The addition of histological type to the analysis increased the significance of
mesothelin examined as a trend variable, because it adjusted the analysis for the
decreased survival time of the sarcomatoid histology type, which had lower mesothelin
levels than the epithelioid and biphasic types. The addition of either tumor volume or TGV
removed the significance of mesothelin, examined as either a trend or grouped variable,
as a predictor of survival, which suggests that mesothelin does not have any significant
predictive value for survival beyond its relation with tumor size, as measured by PET
analysis. Indeed from the data presented here the prognostic information provided by
baseline mesothelin is mainly associated with its relationship to tumor volume, therefore
the blood-based assay could be used as a surrogate for tumor volume as determined by
FDG-PET.

Changes in serum mesothelin levels reflected changes in tumor burden. Despite the
small patient numbers examined, mesothelin levels invariably decreased in response to
debulking surgery, suggesting a clear relationship between decrease in volume of viable
tumor and levels of the tumor marker. This relationship is strengthened by our observation
that mesothelin levels changed in concert with observations of objective radiological
response, and with FDG-PET quantified tumor volume and metabolically active tumor
volume. Similar findings have recently been reported in a smaller patient group but without assessment of metabolic tumor volume (26).

Radiological response as assessed by the Modified RECIST Criteria uses unidimensional measurements and does not provide a quantitative evaluation of change in tumor burden. It has been shown to correlate with important endpoints such as survival, and pulmonary function (10). Similarly, we have demonstrated the relationship between change in mesothelin levels on therapy, and overall survival.

Limitations to this study include the small patient numbers and heterogeneous clinical treatment received. Some patients had mesothelin levels below the limits of detection reducing the number with quantifiable results. Furthermore, the study was primarily designed and powered to answer another primary study question. Nevertheless, a comprehensive imaging study including both CT and FDG-PET techniques is an ideal setting in which to explore the role of a serum biomarker.

While none of the currently available tools for monitoring response in patients with mesothelioma represents a “gold standard”, additional information is needed clinically. Serial monitoring of radiological response is difficult to assess in this disease. Formal measurement of response is time consuming and readily confounded by the presence of pleural effusion and atelectasis. The measurement of soluble serum mesothelin may represent a cheaper, less invasive technique that can provide support for clinical judgement and potentially decrease the frequency of radiological monitoring in routine clinical practice, particularly with regards to monitoring the efficacy of therapy. Mesothelin may also prove an informative surrogate endpoint in clinical trials. Further prospective, multicentre validation studies are necessary before these findings can enter routine clinical practice, and ongoing Australian clinical trials in MPM are currently collecting serial mesothelin measurements to contribute to this validation process.
These data suggest that in the clinical context of the management of an individual patient an elevated level of serum mesothelin may provide useful guidance for the clinician. Higher baseline levels suggest larger tumor volume, more advanced tumor stage and bode a poorer prognosis. A fall in the level following chemotherapy increases the likelihood that the tumor is responding to therapy, may encourage continuation of treatment and increases the likelihood of a longer patient survival.

Acknowledgements

We thank the invaluable assistance of Judy Innes-Rowe and Hema Rajandran in data management, Dr Jan Boucek and Dr. Karen Tucker in total glycolytic volume analysis, Deborah Yeoman and Hanne Dare in performing mesothelin assays, Dr Arman Hasani in response analysis, Dr Amanda Segal for pathology review, and the Western Australia PET Service and Cyclotron staff.
Table 1: Patient Characteristics

<table>
<thead>
<tr>
<th></th>
<th>ALL</th>
<th>No Chemotherapy</th>
<th>Chemotherapy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>95</td>
<td>28</td>
<td>67</td>
</tr>
<tr>
<td>Age median (range)</td>
<td>66 (41-94) years</td>
<td>72 (47-94) years</td>
<td>66 (41-80) years</td>
</tr>
<tr>
<td>Gender</td>
<td>11F:84M</td>
<td>2F:26M</td>
<td>9F:58M</td>
</tr>
<tr>
<td>Histology</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Epithelioid</td>
<td>68</td>
<td>18</td>
<td>50</td>
</tr>
<tr>
<td>Biphasic</td>
<td>18</td>
<td>7</td>
<td>11</td>
</tr>
<tr>
<td>Sarcomatoid</td>
<td>9</td>
<td>3</td>
<td>6</td>
</tr>
<tr>
<td>Median Survival(a) (range)</td>
<td>15 months (1-66)</td>
<td>7 months (1-44)</td>
<td>15 months (1-66)</td>
</tr>
</tbody>
</table>

\(a\) – from diagnosis
### Table 2: Cox regression model of mortality from the time of measurement of baseline mesothelin.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Univariate</th>
<th>P value</th>
<th>Model A</th>
<th>P value</th>
<th>Model B</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mesothelin (per 5 nM increase)</td>
<td>1.04 (0.96-1.11)</td>
<td>NS</td>
<td>1.09 (1.00-1.19)</td>
<td>0.038</td>
<td>1.01 (0.92-1.11)</td>
<td>NS</td>
</tr>
<tr>
<td>Mesothelin grouped (cf &lt;1 nM)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(1-5 nM)</td>
<td>1.35 (0.80-3.31)</td>
<td>NS</td>
<td>1.48 (0.79-2.78)</td>
<td>NS</td>
<td>1.45 (0.76-2.75)</td>
<td>NS</td>
</tr>
<tr>
<td>(&gt; 5 nM)</td>
<td>2.25 (1.20-4.21)</td>
<td>0.011</td>
<td>2.31 (1.11-4.81)</td>
<td>0.025</td>
<td>1.42 (0.61-3.32)</td>
<td>NS</td>
</tr>
<tr>
<td>S creatinine (per unit increase)</td>
<td>0.99 (0.97-1.00)</td>
<td>0.066</td>
<td>0.98 (0.96-0.99)</td>
<td>0.006</td>
<td>0.99 (0.98-1.01)</td>
<td>NS</td>
</tr>
<tr>
<td>Age (per year increase)</td>
<td>1.02 (1.00-1.04)</td>
<td>NS</td>
<td>1.04 (1.01-1.19)</td>
<td>0.011</td>
<td>1.04 (1.01-1.07)</td>
<td>0.017</td>
</tr>
<tr>
<td>Gender (Females cf Males)</td>
<td>0.47 (0.38-1.47)</td>
<td>NS</td>
<td>0.58 (0.27-1.24)</td>
<td>NS</td>
<td>0.48 (0.21-1.10)</td>
<td>NS</td>
</tr>
<tr>
<td>Treatment (cf none)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pemetrexed</td>
<td>0.87 (0.41-1.77)</td>
<td>NS</td>
<td>1.25 (0.58-2.68)</td>
<td>NS</td>
<td>0.73 (0.28-0.98)</td>
<td>NS</td>
</tr>
<tr>
<td>Gemcitabine</td>
<td>0.96 (0.54-1.72)</td>
<td>NS</td>
<td>0.74 (0.41-1.32)</td>
<td>NS</td>
<td>0.52 (0.28-0.98)</td>
<td>0.045</td>
</tr>
<tr>
<td>Histology (cf epitheloid)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Biphasic</td>
<td>1.11 (0.60-2.04)</td>
<td>NS</td>
<td>1.19 (0.73-2.54)</td>
<td>NS</td>
<td>0.90 (0.46-1.75)</td>
<td>NS</td>
</tr>
<tr>
<td>Sarcomatoid</td>
<td>3.65 (1.61-8.29)</td>
<td>0.002</td>
<td>4.41 (1.89-10.29)</td>
<td>0.001</td>
<td>3.40 (1.28-9.00)</td>
<td>0.014</td>
</tr>
<tr>
<td>Pleurodesis (cf not done)</td>
<td>0.87 (0.52-1.46)</td>
<td>NS</td>
<td>1.15 (0.62-2.11)</td>
<td>NS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CT stage (cf Stage I)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stage II</td>
<td>1.21 (0.40-3.65)</td>
<td>NS</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stage III</td>
<td>1.61 (0.75-3.48)</td>
<td>NS</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stage IV</td>
<td>2.21 (1.04-4.68)</td>
<td>0.04</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PET measurements</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Volume (per 1000 unit increase)</td>
<td>1.50 (1.15-1.95)</td>
<td>0.003</td>
<td>1.77 (1.23-2.54)</td>
<td>0.002</td>
<td>1.11 (1.03-1.19)</td>
<td>0.003</td>
</tr>
<tr>
<td>TGV (per 1000 unit increase)</td>
<td>1.08 (1.02-1.13)</td>
<td>0.003</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Results are Hazard Ratios and 95% Confidence Intervals.

Model A: Mesothelin, measured at the time of diagnosis, was entered into the model as a categorical or as a trend variable. Survival was measured from the time of diagnosis.
Model B: Mesothelin, measured at the time of the PET scan, was entered into the model as a categorical or as a trend variable. Either TGV or Volume was entered into the model. Adjusted mesothelin result shown is after adjustment for PET volume. A similar result for mesothelin after adjustment for the TGV was observed.
Table 3: Correlation of change in mesothelin concentration with radiological response

<table>
<thead>
<tr>
<th>Mesothelin (% change of baseline)</th>
<th>Response (CT modified RECIST)</th>
<th>PR</th>
<th>SD</th>
<th>PD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Decrease</td>
<td>12 (7)</td>
<td>10 (11)</td>
<td>2 (5)</td>
<td></td>
</tr>
<tr>
<td>Stable</td>
<td>5 (7)</td>
<td>12 (11)</td>
<td>6 (5)</td>
<td></td>
</tr>
<tr>
<td>Increase</td>
<td>0 (2)</td>
<td>4 (4)</td>
<td>4 (2)</td>
<td></td>
</tr>
</tbody>
</table>

Results are number of patients observed (expected number of patients), $\chi^2=11.32$, $p=0.023$

Response: PR – Partial Response, SD – Stable Disease, PD – Progressive Disease

Mesothelin: Decrease - levels reduced by 25% or greater of baseline values, Stable – levels within 25% of the baseline value, Increase- levels increased by 25% or greater of baseline values
**Figure 1:** Flow chart of treatment patients enrolled on the study received. (EPP – extrapleural pneumonectomy).

**Figure 2:** Relationship of baseline serum mesothelin with clinical parameters. (A) Kaplan-Meier survival curve of overall survival (months) from diagnosis for patients with high (>5nM; thick line) and low (<1nM; thin line) baseline mesothelin concentrations. $P$ value represents value of log rank test. (B & C) Correlation of mesothelin levels with tumor stage (B) as determined by CT and (C) FDG-PET. Significant difference at a level of * $p<0.05$ and **$p<0.01$. ns – not significant. (D - F) Correlation of serum mesothelin with FDG-PET measures (D) total glycolytic volume (TGV), (E) volume (Vol), (F) and maximum standardised uptake value (SUVmax).

**Figure 3:** Relationship of change in serum mesothelin levels with clinical parameters (A) Change in mesothelin concentration from baseline to the nadir value that occurred during chemotherapy for individual patients compared with change in baseline FDG-PET total glycolytic volume (TGV) and (B) volume (Vol). (C-E) Representative data from a 69 year old male patient with epithelial mesothelioma: (C) Mesothelin levels (◇) plotted against months following diagnosis, the patient received two courses of gemcitabine and cisplatin (▲) and four course of gemcitabine and carboplatinum (◆). Time of death is indicated by vertical dashed line. (D) Coronal slices of a FDG PET scan taken at baseline and after one cycle of chemotherapy. (E) Transaxial CT slices before and after one cycle of chemotherapy.
Figure 4: Kaplan-Meier survival curve of overall survival (months) from diagnosis for patients with decreased (thick line), stable (thin line) and increased (dashed line) mesothelin concentrations following chemotherapy. $P$ value represents value of log rank test compared to the group with a decrease in mesothelin levels following chemotherapy (* $p<0.05$; *** $p<0.0001$).
References

Enrolled (n = 97)

Ineligible (n = 2)

- EPP (n = 7)
  - Adjunct Chemotherapy (n = 5)
  - No Adjunct Chemotherapy (n = 2)
- Chemo-therapy (n = 81)
  - Gemcitabine & Platinum (n = 46)
  - Pemetrexed & Platinum (n = 15)
- Radio-therapy (n = 2)
- Best Supportive Care (n = 25)
Figure 2
Serum soluble mesothelin concentrations in malignant pleural mesothelioma: relationship to tumor volume, clinical stage and changes in tumor burden


Clin Cancer Res  Published OnlineFirst December 21, 2010.

Updated version
Access the most recent version of this article at:
doi:10.1158/1078-0432.CCR-10-1929

Author Manuscript
Author manuscripts have been peer reviewed and accepted for publication but have not yet been edited.

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.