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Biomarkers in Mesothelioma

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Abstract

Mesothelioma is an aggressive cancer of pleural and peritoneal cells that is difficult to diagnose and monitor. Numerous studies have attempted to identify a blood or pleural fluid based biomarker that could be used in the diagnostic pathway. More recently there has been interest in the ability of serum/plasma biomarkers to monitor mesothelioma given development of newer treatments and limitations of radiological assessment. The majority of research has focused on soluble mesothelin (SM), a soluble glycoprotein expressed by mesothelial cells. Although SM lacks the sensitivity to be used as a stand alone diagnostic marker, when measured serially rising levels indicate disease progression and poor survival. High levels of other soluble glycoproteins, such as osteopontin, fibulin-3 and VEGF are independently associated with poor prognosis at baseline, although further research is required to ascertain any role outside of clinical trials. More recent literature has focused on the development of novel biomarkers from discovery cohorts. Although many DNA and mRNA biomarkers show promise in the diagnosis or screening of mesothelioma, none have been prospectively evaluated for use in clinical practice. In this review article we highlight the potential utility of biomarkers and evaluate the existing literature.

Background

Malignant mesothelioma is an aggressive and invariably fatal cancer of pleural and peritoneal cells (ratio 4:1), and less commonly the pericardium and tunica vaginalis. [1] The incidence of mesothelioma is increasing worldwide to the extent that it is now more common than cancers of the bladder and bone. Mesothelioma is almost exclusively caused by exposure to asbestos, a link that was first published by Wagner, a pathologist in South Africa, in 1960.[2] He noticed that the incidence of pleural mesothelioma, a previously rare cancer, was increasing in areas of the Cape asbestos field which mined Cape Blue (crocidolite asbestos). The direct causal link between asbestos use in industry and mesothelioma allows for the future incidence of the disease to be predicted to some degree of accuracy. Given a mean latency of around 40 years from peak exposure [3] it is estimated that the incidence of mesothelioma in Europe will rise until between 2015 and 2020 *(add number of cases per year at peak).* [4] Given ongoing unregulated use of asbestos in countries such as China, India and Russia, mesothelioma will continue to occur despite unequivocal evidence of its harms. The other rarer causes of mesothelioma are iatrogenic chest wall irradiation (e.g. in treatment of breast cancer or lymphoma) and exposure to erionite (a mineral found in Turkey). [5-8]

There are several different mechanisms by which asbestos is purported to cause mesothelioma. The most widely accepted being that long thin asbestos fibres (over 5um in length) are inhaled into the lung, penetrating the lung epithelium and entering the pleural space. Then a continuous cycle of pleural irritation, damage and repair eventually results in the mutations giving rise to mesothelioma. The oxygen free radical hypothesis suggests that when asbestos fibres are phagocytosed there is release of oxygen free radicals that cause DNA damage and mutations.[9] The finding that asbestos fibres penetrate mesothelial cells and interfere with mitosis, as well as inducing phosphorylation and production of various pro-oncogenic protein kinases (mitogen-activated protein and extracellular signal-regulated kinases 1 and 2), is another compelling argument of pathogenesis. Finally, the same

cells release inflammatory tumour growth factor-β, platelet-derived growth factor and vascular endothelial growth factor (VEGF) which can be utilised by the malignant cells for proliferation and angiogenesis.[4] It is likely that a combination of the above as well as various host-specific factors give rise to this malignancy.

There are 4 main histological subtypes of mesothelioma (epithelioid, sarcomatoid, biphasic or mixed, and desmoplastic) which have different microscopic appearances and implications for the patient. Epithelioid is the most common variant (accounting for around 70% of cases in most series) and has the most favourable prognosis, with a median survival of 13.1 months.[10, 11] The sarcomatoid variant is associated with the poorest prognosis, with a median survival of just 4 months. The histological subtype often has implications on treatments offered by oncologists or surgeons as more aggressive subtypes are felt to be not amenable to therapy.

Clinical Presentation

The majority of patients with malignant pleural mesothelioma will present with shortness of breath, cough or chest pain. Patients less commonly present from systemic symptoms of weight loss, night sweats and fatigue, and if they do this is a poor prognostic sign as the disease is likely more advanced.[12] An abnormal chest radiograph may be the presenting complaint if some cases when performed routinely before an operation or for other medical reasons in patients without respiratory symptoms. A chest radiograph demonstrates a pleural effusion (fluid collection between the lung and chest wall) in 90% of cases, however this radiological sign is common in many malignant and non-malignant respiratory conditions.[13] In patients who present with an abnormal chest radiograph, and known past asbestos exposure, malignant pleural mesothelioma should be high on

the differential diagnosis. Even if the patient denies previous exposure to asbestos the diagnosis of mesothelioma should not be excluded given the risk of 'second-hand' or non-industrial exposures.

Although symptoms from local spread can occur (including superior vena cava obstruction, rib destruction and laryngeal nerve palsy), clinical manifestations from metastatic spread are uncommon, due to the aggressive nature of the primary disease. [1] At post mortem most common areas of spread include the thoracic lymph nodes and bone. However, tract metastases in areas where the chest wall has been operated on either for diagnostic or therapeutic purposes are more common. These metastases can be disfiguring and/or painful and research is ongoing into the role of prophylactic radiotherapy following pleural procedures. [14, 15]

Peritoneal mesothelioma presents very differently to pleural disease, commonly with diffuse abdominal pain, abdominal swelling from disease bulk or ascites (fluid accumulation in the abdominal cavity), bowel obstruction, appetite loss or nausea.

Imaging for mesothelioma

As mentioned above, at initial presentation the majority of patients will have the fairly non-specific sign of a unilateral pleural effusion on chest radiograph (Figure 1A). A minority will have pleural thickening and fewer still will have evidence of advanced pleural disease with a widespread pleural rind (Figure 1C). In most cases an unexplained unilateral pleural effusion on chest radiograph will lead to a computerised tomography (CT) scan of the chest, abdomen and pelvis with the intention of identifying or excluding malignancy. Unfortunately, diagnosing mesothelioma on CT scan alone is difficult at early stage of disease given the likely presence of pleural fluid asbestos related plaques or folded lung which can obscure the radiologist's assessment of the pleura, as well as a difficulty distinguishing benign pleural thickening from malignancy.[16] Even if the CT scan looks very suspicious for malignancy, it can still be challenging to distinguish mesothelioma from pleural metastasis from

the lung or other body systems (such as breast, ovarian or renal cancers).[17] CT scanning is currently

used as a method of monitoring disease following diagnosis e.g. response to chemotherapy,

assessing progression, etc. However, similar problems remain and given that mesothelioma does not

grow as a spherical mass but more as a pleural or peritoneal rind (Figure 1B), it can be challenging

for radiologists to quantify any change in tumour bulk. Scoring systems that have been modified for

mesothelioma's unique morphology exist but have their limitations.[18]

Figure 1; Imaging modalities in mesothelioma. A- Chest radiograph showing large right sided pleural effusion, B- Coronal CT image showing significant right sided pleural thickening and nodularity, C-Chest radiograph showing left sided pleural thickening and lung volume loss, D- Horizontal PET image showing right sided pleural enhancement posteriorly.
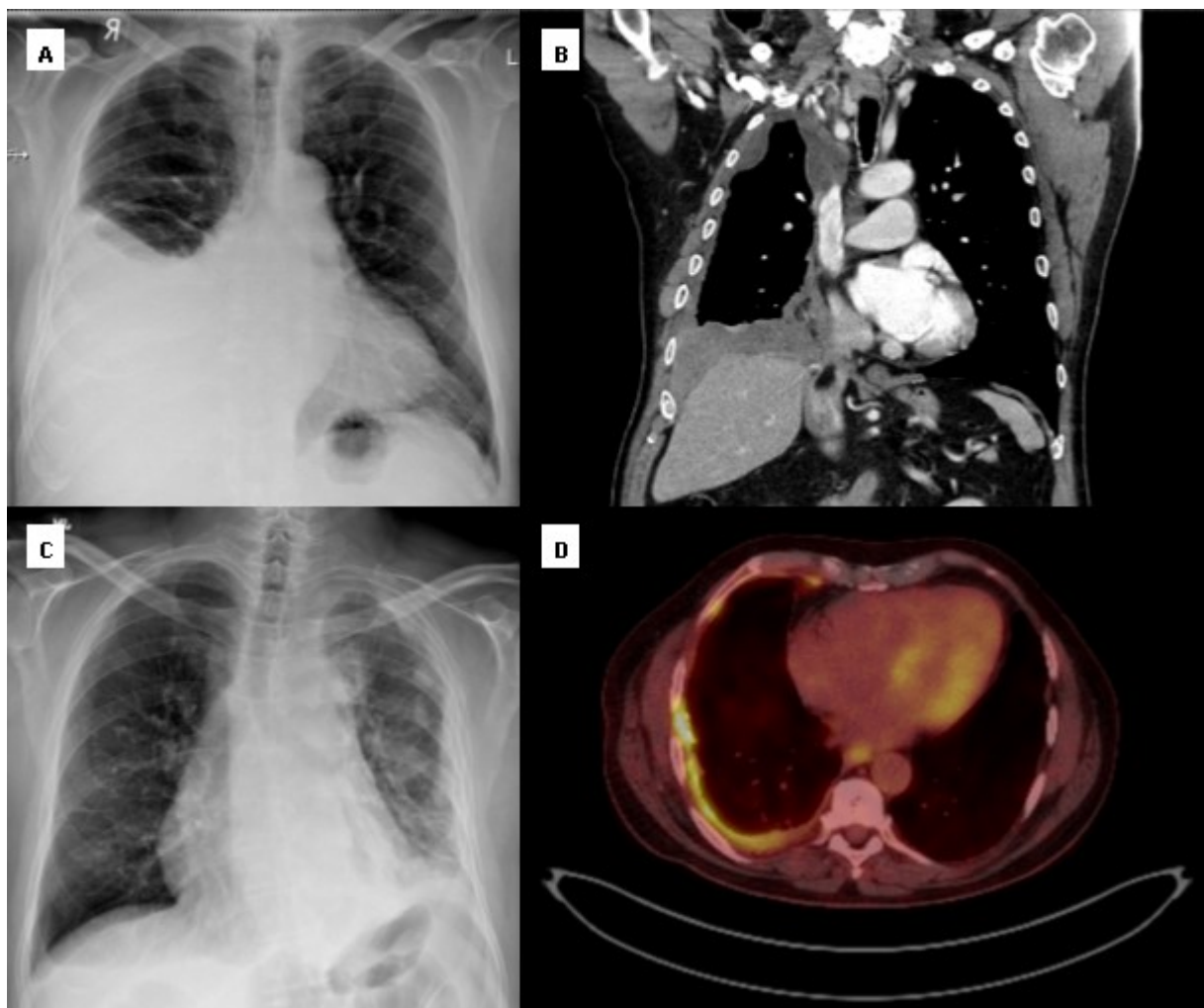
Magnetic resonance imaging (MRI) is developing as a method of diagnosing and staging mesothelioma. Using diffusion-weighted imaging, recent studies have shown the accuracy of MRI is high for differentiating benign from malignant pleural disease.[19] In addition, it may be possible to differentiate between different subtypes of mesothelioma at initial assessment.[20] PET-CT imaging is a rapidly developing area in the diagnosis and staging of mesothelioma. It relies on contemporaneously acquired CT imaging and assessment of uptake of fluourine 18-flurodeoxyglucose (FDG) into tissues. Uptake of FDG is usually higher in more metabolically active malignant tissue so will be more vivid on imaging (Figure 1D).[21] The advantage of PET-CT over standard CT or MRI is the ability to detect local and distant spread of disease so it can be more accurately staged, so is often utilised in potential surgical patients.[22] Its disadvantages include false positives in cases of pleural infection, inflammation and prior pleurodesis and limited availability outside of specialist centres.[23]

Histocytological investigations

For any patient who attends pleural/respiratory clinic with symptoms or signs suspicious for pleural mesothelioma their diagnostic pathway will depend on the presence or absence of pleural fluid on radiological imaging. Often the first line diagnostic procedure will be a diagnostic or therapeutic aspiration of pleural fluid (known as a thoracocentesis). Typically this fluid is sent to the biochemistry, microbiology and cytology laboratory and information regarding protein and glucose content, culture results and predominant cell types returned. The yield of malignant cells seen on pleural fluid cytology is notoriously low for mesothelioma (10-20%). [24] Consequently, the majority of patients will go on to have further invasive diagnostic investigations in order to obtain a tissue biopsy either via USS guided pleural biopsy, direct visualisation using thoracoscopy, or an open surgical VATS (video-assisted thoracoscopic surgery) procedure. This improves the diagnostic yield to over 90%.[25]

Immunohistochemistry

Even once a tissue sample is obtained a diagnosis of mesothelioma can be challenging because of the tumour's wide range of morphological appearances. Additionally, the pleura and peritoneum are common sites for metastases from other malignancies. Differentiating mesothelioma from adenocarcinoma (from metastases of lung or breast cancer) in a tissue biopsy poses particular difficulties. For this reason basing the diagnosis purely on microscopic appearance is not recommended and various immunohistochemical methods are employed by pathologists. Although exact methodology varies hugely between centres, guidelines generally advocate a combination of at least two positive mesothelial (Calretinin, Cytokeratin 5/6, Wilms Tumour 1, D-240) and at least two negative adenocarcinoma immunohistochemical markers (TTF1, CEA, Ber-EP4) for a positive diagnosis of mesothelioma. The pathologist should also be aware of the clinical and radiological context.

Treatment of mesothelioma

Given the invasive nature of mesothelioma, for the majority of patients, treatment has a palliative intent from diagnosis. Systemic therapies for mesothelioma have changed little in the last decade. A landmark study in 2003 found that a combination of the anti-folate Pemetrexed to platinum based therapy (cisplatin or carboplatin) improved survival in a non-placebo RCT.[26] This led to the standardisation of first line chemotherapy across the UK although there is considerable variation in the numbers of patients offered chemotherapy nationally. [27] Despite being the only NICE approved treatment, this combination only adds an average of 2 months to overall survival with a response rate of around 30% [28] and significant side effect burden.[29] The role of maintenance or second-line chemotherapy is uncertain. Maintenance pemetrexed is safe but its efficacy is yet to be

established.[30] Several second-line agents have been assessed, with positive results using vinorelbine, gemcitabine or re-challenging with pemetrexed but no national guidance exists on the topic currently. [31-34]

In the future biological therapy will play a greater role following results from the MAPS trial which demonstrated that the addition of bevacizumab to standard chemotherapy had a survival benefit of 2 months compared to chemotherapy and placebo.[35] This anti-VEGF monoclonal antibody is not yet approved by NICE but given this, and a number of other promising biological studies, the treatment for mesothelioma is likely to become more complex, with increasing cost implications and emphasis on early identification of treatment response.

Radiotherapy for mesothelioma is employed as a palliative measure to improve symptoms, such as chest wall invasion or procedure tract metastases, or as an adjunct to chemotherapy and surgery. Surgery for mesothelioma is a highly controversial topic. Several centres in the UK will offer radical surgery to patients with early stage disease and good performance status, and case series often report excellent survival. However, these series are usually based on highly selected patients with sparse randomised data. Several surgical techniques exist as do the neo-adjuvant therapies that accompany them. The MARS trial (2011) was one the first randomised trial of surgery for mesothelioma and compared extra-pleural pneumonectomy (EPP) (where all macroscopically visible tumour is removed in a large open operation) to no surgery. [36] Although there has been considerable disagreement in the interpretation of the data, the trial concluded that EPP should not be offered to patients and might actually be harmful. Another more recent RCT of a less invasive surgical technique (video-assisted thoracoscopic partial pleurectomy or VAT-PP) also demonstrated no improvement in survival or quality of life in the surgical arm.[37] There may be a role for pleurectomy decortication in some patients with mesothelioma and this the subject of a current trial in the UK (MARS2), the results of which are awaited with interest.

Potential role of biomarkers: Diagnosis

Using biomarkers to diagnose mesothelioma is an attractive concept for a number of reasons. Firstly, there is a clear 'at-risk' population in those who have been previously exposed to asbestos. Secondly, the presenting symptoms and radiological findings are difficult to distinguish from other benign and malignant pleural diseases. Thirdly, even with a high index of suspicion, current methods of histocytological diagnosis are invasive and may be inappropriate in a proportion of patients. Fourthly, once tissue is obtained the tumour can still be difficult to distinguish from other malignancies. Consequentially a large body of research has investigated the ability of serum and pleural fluid biomarkers to diagnose mesothelioma both individually and in panels.

Potential role of biomarkers: Prognosis or treatment monitoring

As discussed above, for the majority of patients, treatment for mesothelioma has a palliative intent. Any intervention therefore must careful balance improved life expectancy with quality of life. The standard of care with pemetrexed and cisplatin has a response rate of around 30% with a significant side effect profile and limited impact on symptoms. Oncologists are understandably very interested in selecting out patients who are likely to respond to chemotherapy at baseline or early in treatment. Additionally, in the few centres that offer surgery there is considerable pre-operative assessment to ensure that only patients who are most likely to benefit are put forward for surgery. However, there are very few radiological markers at baseline that can predict prognosis.

In patients who receive chemotherapy oncologists will usually perform a CT scan mid cycle to assess response. As discussed above, because mesothelioma grows as a pleural rind as opposed to spherically like many other cancers it is difficult to monitor using conventional CT scanning. A biomarker that could measure response to chemotherapy or predict recurrence would be of huge benefit to oncologists.

Mesothelin

Soluble Mesothelin (SM) is a 40kDa cell membrane bound glycoprotein overexpressed by the epithelioid component of malignant mesothelial cells. It is attached to the cell surface by phosphatidylinositol and although its role is uncertain it likely facilitates cell-adhesion and possibly in cell-to-cell recognition and signalling (see Figure 2). It was initially identified in the serum samples of patients with ovarian cancer before being found in high levels in the serum, plasma, pleural fluid and urine of patients with mesothelioma.[38] The first clinical study of SM, published in 2003, showed that serum concentrations were significantly higher in patients with mesothelioma compared to healthy controls, asbestos exposed patients or those with other inflammatory or malignant lung conditions.[39] They also demonstrated that serum SM levels are higher in epithelioid compared to non-epithelioid tumours and are positively correlated with tumour bulk. This paper reported a sensitivity of 84% (95% CI 73–93) for diagnosing mesothelioma from other pleural diseases with a specificity of 100% (91–100), although numbers were small (44 patients with mesothelioma). This finding led to a large number of subsequent and larger studies of serum SM as a diagnostic and/or screening biomarker. In 2014, Cui et al performed a meta-analysis of all studies that had examined the diagnostic ability of serum and pleural fluid SM. [40] There was considerable heterogeneity between studies in terms of patient characteristics (most notably within the control groups), ELISA kits and cut-offs used, as well as evidence of publication bias. The majority of studies used the commercial Mesomark[TM] ELISA, with 4 using other platforms. The cut-offs used to define an abnormal result ranged from 0.5nmol/L to 3.3nmol/L with most studies using a 'data specific' level as opposed to a pre-defined clinically convenient level. From the 28 studies of serum SM included in the analysis the pooled summary estimates of sensitivity and specificity were 0.61 and 0.87 respectively. For a malignancy which is otherwise difficult to diagnose and has huge implications for the individual, an inability to exclude mesothelioma with a negative result limits its clinical utility.

Given that a positive result increases the likelihood of having mesothelioma six-fold there may be a

place for serum SM in patients who are unsuitable for or decline more invasive diagnostic

procedures if the pre-test probability is high. For example, an elderly patient with a significant

history of asbestos exposure presenting with a unilateral effusion. The results presented for pleural

fluid mesothelin levels were superior to serum with pooled estimates of sensitivity and specificity of

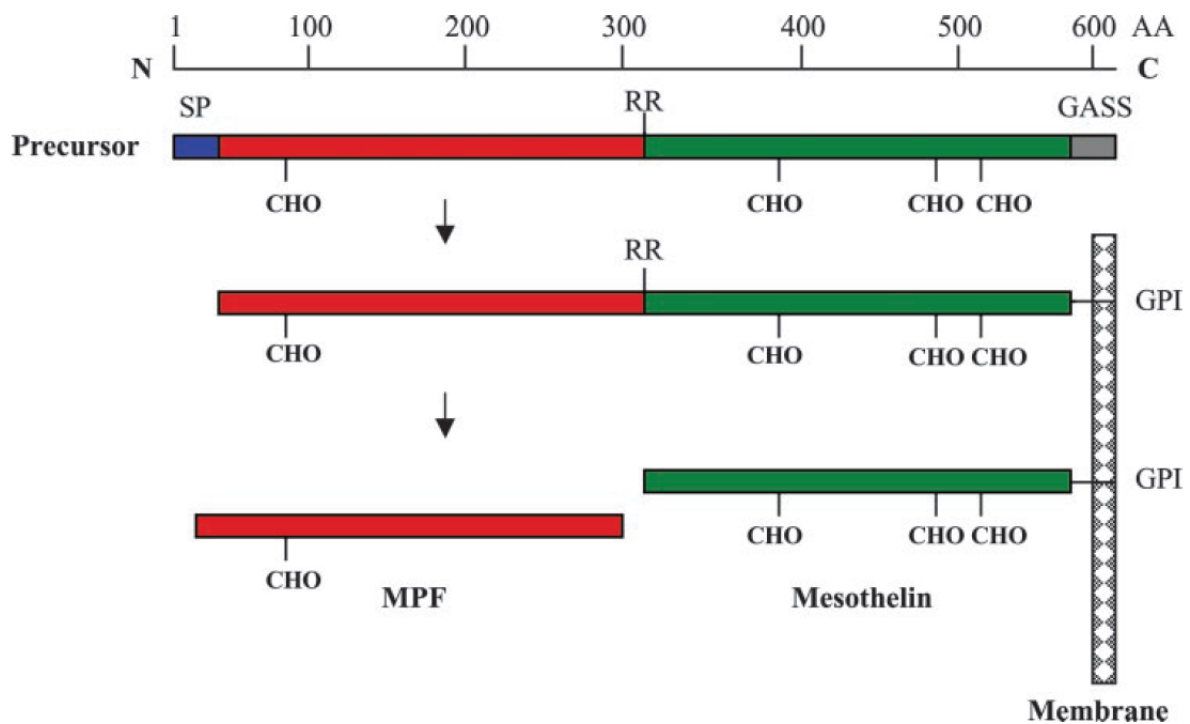0.79 and 0.85 respectively but large variation in cut-offs used (3.5 to 24.05 nmol/L).

Figure 2; Schematics showing maturation of mesothelin protein. Precursor protein for mesothelin is synthesized as a 622-amino acid polypeptide with a calculated molecular mass of 77 kDa. The potential signal peptide (*SP*) and the glycosylphosphatidyl inositol anchor signal sequence (*GASS*) are predicted at the NH2 terminus and the COOH terminus, respectively. The precursor protein has four predicted glycosylation sites (*CHO*) and a furin cleavage site (*RR*). Cleavage at the furin site generates membrane-bound mesothelin (*green*) and the secretory protein megakaryocyte-potentiating factor (*red*). Reprinted from Clinical Cancer Research , 2004, Volume 10, 3937–3942 , Hassan, Mesothelin: A New Target for Immunotherapy, with permission from AACR.

Additional work has been done on a potential role of serum SM in screening at risk populations

(asbestos exposed workers and their families). In principle mesothelioma is an attractive screening

target with a well-defined at risk population of asbestos exposed individuals. A number of studies

showed promising results when looking retrospectively [41] at SM's ability to selecting out early stage disease or those at risk of developing mesothelioma but with a false positive rate of 90% in some prospective studies (Creaney 2015 Present Status and Future Directions- book reference) it's accuracy falls below that of an acceptable screening test.

Another area where serum mesothelin has shown promise (beyond an adjunct to diagnosis) is in the monitoring of mesothelioma during treatment. Mesothelin is positively correlated with both tumour stage and radiological bulk. Several surgical case series have shown that levels fall dramatically post debunking surgery. A literature review revealed 9 studies that assessed the utility of serial mesothelin measurements (see Table 1). Although heterogeneous in terms of primary outcome and study population, all found that a falling mesothelin from baseline correlated with treatment response or improved overall survival. Despite being approved by the FDA for treatment monitoring, oncologists continue to rely on radiological markers of response. This is because many patients (especially with sarcomatoid histology) will have low or undetectable mesothelin levels despite advanced disease. Also uncertainty exists around the appropriate sampling intervals, clinically significant cut offs for monitoring and how to handle results in patients with renal dysfunction (which causes false elevation in SM levels). [42] However, given the emergence of immune therapies for mesothelioma which further invalidate current radiological markers due to infiltration of tumour by immune cells, which appears as 'pseudo-progression', and recent evidence that PET-CT adds little to treatment monitoring, [43] a large prospective study is required to evaluate the true role of mesothelin in this area.

Table 1- Nine studies assessing the role of serum mesothelin to monitor mesothelioma.

Author ,year	Treatment (no. of patients)	Threshold for SM change	Summary of results
Bonotti, 2016 [44]	Chemotherapy- 56	20%	Overall change in SM levels from baseline closely correlated with clinical response (p<0.001).
Hooper, 2015 [43]	Chemo- 58, BSC- 15	0%	Within the chemotherapy group a falling serum SM was associated with longer time to progression (p<0.001), and improved OS (p=0.031).
Hassan, 2014 [45]	Chemo & Im- 20	15%	Fall in serum SM correlated with radiological response on CT with 70% accuracy (p=0.003).
Nowak, 2013 [46]	Bio- 53	0%	Median change in serum SM correlated with sum change in tumour bulk on FDG-PET (p<0.05). % change in serum SM was associated with TTP (p<0.001) but not OS.
Franko, 2012 [47]	Chemo- 64, BSC- 4, Surgery- 10	n/a	Significantly lower mean serum SM in partial response or stable disease compared to progressive disease (p=0.001).
Hollevoet, 2011 [48]	Chemo- 57, Surgery- 5	15%	Partial response to chemotherapy correlated with a 34% fall in SM (p=0.010) compared to a 54% rise in progressive disease (p<0.001).
Creaney, 2011 [49]	Chemo- 61, BSC- 25, Surgery – 8	25%	Correlation between change in serum SM and CT (p=0.023) and FDG-PET markers (p<0.001) Also, a falling SM was associated with better OS (19 months) compared to static (13 months) or rising levels (15 months). (p=0.001).
Wheatley-Price, 2010 [50]	Chemo- 21, BSC- 13, Surgery -8	10% or 5nmol/L	Relative change in serum SM from baseline significantly associated with disease progression (p<0.010).
Grigoriu, 2009 [51]	Chemo- 20, Im- 16, BSC- 4	10%	OS higher in patients with stable SM compared to increasing (p=0.012). Rising SM levels correlated with progressive disease in 12/16 patients (had high SM levels at baseline).

Chemo- chemotherapy, Bio- biological therapy, Im- immunotherapy, BSC- best supportive care, Surg- surgery, Mod RECIST CT- Modified Response Evaluation Criteria In Solid Tumors CT, OS- overall survival, TTP- time to progression.

Megakaryocyte Potentiating Factor (MPF)

MPF, also called N-ERC/mesothelin as it is formed from the same precursor protein as SM, is a more novel biomarker. It has similar expression to SM given that it is also originates from epithelioid mesothelioma cells (see Figure x). With respect to diagnosis there is little additional benefit to SM with similar downfalls due to low levels in non-epithelioid disease. In terms of disease monitoring some studies report slightly improved accuracy but given only marginal improvement the focus of future research in this area will likely be focused on SM.[45, 48]

Osteopontin

Osteopontin (OPN) is a glycoprotein that mediates cell to cell interactions and is over expressed in many tumours including breast, lung and colon malignancies.[52] Studies have shown that it lacks the

sensitivity to be used as a solely diagnostic test. A meta-analysis of serum and plasma OPN carried out in 2014 found a pooled sensitivity and specificity of 0.57 (95%CI: 0.52-0.61) and 0.81, 95%CI: 0.79-0.84) respectively with considerable heterogeneity between the 9 studies.[53] A thrombin cleavage site impedes reproducible measurements in serum for osteopontin so more recent literature has advocated plasma sampling to improve accuracy.[54] In addition there appears to be little merit in serial monitoring of OPN. A study by Hollevoet et al showed that, unlike SM and MPF, osteopontin levels did not fall following surgery and did not correlate with treatment response from chemotherapy.[48] Despite this, a potential role for osteopontin as a baseline predictor of poor prognosis has been demonstrated. Several studies have shown that a high baseline plasma osteopontin infers poor prognosis, exclusive of histology, treatment modality or indeed other biomarkers.[48, 55] Further work is required to ascertain its utility outside of clinical trials, and given the variability in levels depending on ELISA used,[56] it is important that any future research adopts a consensus approach.

Fibulin-3

Published in the New England Journal of Medicine the first major study of fibulin-3 in serum reported a sensitivity of 100% for detecting early stage mesothelioma from an asbestos exposed population with a specificity of 94.1%.[57] These estimates would be high enough for inclusion into the routine diagnostic pathway. Unfortunately, several follow up studies using the same commercial ELISA assay were unable to replicate these results with a variety of estimates for sensitivity.[58] A recent meta-analysis of 8 studies on the topic gave pooled estimates of sensitivity and specificity of 0.87 (95% CI, 0.58 - 0.97) and 0.89 (95% CI, 0.77 - 0.95), respectively.[59] Fibulin-3 is another glycoprotein that was initially discovered in high levels in another malignancy, namely glioma. It is thought to phosphorylate epidermal growth factor and thereby promote tumour growth and invasion.[60] It is particularly overexpressed in pleural fluid from mesothelioma but estimates of

sensitivity remain too low to maintain acceptable specificity. Several studies have shown that higher pleural fluid levels at baseline infer a poor prognosis but it is unclear whether this is primarily due to higher levels being found in more aggressive tumour types or if it offers additional prognostic information.

Hyaluronic acid

Hyaluronic acid (HA) is one of the earliest studied biomarkers in mesothelioma, found in both blood and pleural fluid, although serum analysis is less useful due to rapid (2.5 to 5min half-life) clearance from the systemic circulation by stablilin-2.[61] HA is more stable in pleural fluid in the form of a large fibroblast formed polysaccharide. As a stand-alone test it is not particularly specific for mesothelioma and, given difficulties with its original testing methodology, it has had limited attention since the early 80/90s studies. More recently a combination of a more easily reproducible assay and combining results with other biomarkers has re-ignited interest in the pleural fluid analysis of HA. In 2013, Creaney et al demonstrated that when combined with pleural fluid SM the area under the curve for both biomarkers improved to 0.92 (C.I 0.86 to 0.96).[62] Interestingly, the same study followed up the 96 patients with mesothelioma and found that pleural fluid HA was biphasically distributed within the cohort. When dichotomised at 75mg/L those with high effusion HA had much better survival (18.0m) compared to patients with low levels (12.6m) (p<0.01). This confirmed the finding of a previous study [63] and although several explanations have been postulated the exact pathophysiology is uncertain. Further work is required before the clinical utility of this finding can be fully assessed.

Vascular Endothelial Growth Factor (VEGF)

Pan-VEGF and its various isoforms have been the focus of enumerate studies of malignant and non-malignant diseases of the lung. VEGF almost certainly has a role in pathophysiology of mesothelioma although it's exact role probably varies depending on the specific isoform.[64] Yasumitsu and colleagues compared levels of pan-VEGF in the serum and pleural effusion of 51 patients with mesothelioma to an asbestos exposed population.[65] They found that levels were significantly higher in mesothelioma, with highest levels in epithelioid disease but without the accuracy to be included in a diagnostic pathway (sensitivity was 70.6%, and the specificity was 88.1%). Notably, VEGF level was correlated with stage of disease and worse survival, which probably relates to its well-documented effects on tumour angiogenesis. It is of particular importance in mesothelioma given the emergence of antiangiogenic VEGF-targeted treatments (Bevacizumab) that have been shown to improve survival when given in combination with pemetrexed and cisplatin.[35] No studies have demonstrated any ability of serum VEGF to select responders from non-responders for biologic therapy, but this area demands further study given the development of promising but expensive biologicals.[46, 66]

Future biomarkers and biomarker panels

Ongoing research into biomarkers is directed at the validation of existing biomarkers or panels of existing biomarkers, and discovery of novel biomarkers. As mentioned above the combination of mesothelin and hyaluronic acid improved the overall diagnostic accuracy of both. Another study combined two molecular classes of biomarker by analysing plasma mesothelin values with the microRNA miR-103a-3p.[67] This improved the sensitivity and specificity of mesothelin alone from 74% and 89% to 95% and 81% respectively.

More novel approaches involve the protenomic discovery of previously unidentified biomarkers in serum and pleural fluid and their validation in a second cohort. Such a study was performed by Ostroff et al who demonstrated promising results using a 13 protein panel, [68] reporting area under

the curve (AUC) results of 0.98±0.04 in blinded verification cohort and 0.95±0.04 in a validation cohort (38 patients with mesothelioma). This proteomic assay is currently being validated in a multi-centre prospective trial alongside fibulin-3, it is of note that none of the 13 classifier proteins have previously been associated with mesothelioma.[69] Many studies exist using this technique of protein discovery but the importance of external validation is paramount. Another study from Morré and colleagues focused on the presence of two mesothelioma specific ENOX2 protein transcript variants in the serum of 17 patients pre and post diagnosis of mesothelioma.[70] When compared to patients who had been exposed to asbestos but without a diagnosis of mesothelioma, one or both proteins were detectable up to 10 years prior to diagnosis and with a mean of 6.2 years prior to the onset of clinical symptoms. This finding requires further prospective investigation but if useful could be used to detect mesothelioma at an early and more treatable stage.

Conclusion

The role of biomarkers in mesothelioma has been assessed at almost every stage of the disease process including screening, diagnosis, prognostication and monitoring. Despite being an attractive target for screening or diagnosis no single biomarker has sufficient reproducibility in differentiating mesothelioma from other more common benign or malignant conditions. Promising results are being seen using biomarker panels but none have been external validated in prospective trials.

 Mesothelioma is a difficult disease to prognosticate/ monitor both clinically and radiologically representing another potential role for biomarkers. Results from several studies have supported the use of serum mesothelin as a method of monitoring disease during treatment although questions remain around its utility in non-epithelioid disease. High levels of plasma osteopontin at baseline appears to be an independent poor prognostic indicator although further studies are required to assess the clinical usefulness of this finding. Given that treatments for mesothelioma are now developing following years of inertia the pressure to select responders early is growing. With the

proliferation of biomarker discovery projects and formalised tissue storage for mesothelioma

samples (i.e. MesoBank) it is essential that promising biomarkers are investigated beyond the initial

detection stage.

1.	Bibby AC, Tsim S, Kanellakis N, et al. Malignant pleural mesothelioma: an update on investigation, diagnosis and treatment. *Eur Respir Rev* 2016;25:472-486.
2.	Wagner JC, Sleggs CA, Marchand P. Diffuse pleural mesothelioma and asbestos exposure in the North Western Cape Province. *Br J Ind Med* 1960;17:260-271.
3.	Yates DH, Corrin B, Stidolph PN, Browne K. Malignant mesothelioma in south east England: clinicopathological experience of 272 cases. *Thorax* 1997;52:507-512.
4.	Robinson BW, Lake RA. Advances in malignant mesothelioma. *N Engl J Med* 2005;353:1591-1603.
5.	Carbone M, Emri S, Dogan AU, et al. A mesothelioma epidemic in Cappadocia: scientific developments and unexpected social outcomes. *Nat Rev Cancer* 2007;7:147-154.
6.	Carbone M, Pass HI, Rizzo P, et al. Simian virus 40-like DNA sequences in human pleural mesothelioma. *Oncogene* 1994;9:1781-1790.
7.	van Kaick G, Dalheimer A, Hornik S, et al. The german thorotrast study: recent results and assessment of risks. *Radiat Res* 1999;152:S64-71.
8.	Lopez-Rios F, Illei PB, Rusch V, Ladanyi M. Evidence against a role for SV40 infection in human mesotheliomas and high risk of false-positive PCR results owing to presence of SV40 sequences in common laboratory plasmids. *Lancet* 2004;364:1157-1166.
9.	Sekido Y. Molecular pathogenesis of malignant mesothelioma. *Carcinogenesis* 2013;34:1413-1419.
10.	British Thoracic Society Standards of Care C. BTS statement on malignant mesothelioma in the UK, 2007. *Thorax* 2007;62 Suppl 2:ii1-ii19.
11.	Scherpereel A, Astoul P, Baas P, et al. Guidelines of the European Respiratory Society and the European Society of Thoracic Surgeons for the management of malignant pleural mesothelioma. *Eur Respir J* 2010;35:479-495.
12.	Brims FJ, Meniawy TM, Duffus I, et al. A Novel Clinical Prediction Model for Prognosis in Malignant Pleural Mesothelioma Using Decision Tree Analysis. *J Thorac Oncol* 2016;11:573-582.
13.	Salonen O, Kivisaari L, Standertskjold-Nordenstam CG, Somer K, Mattson K, Tammilehto L. Computed tomography of pleural lesions with special reference to the mediastinal pleura. *Acta Radiol Diagn (Stockh)* 1986;27:527-531.
14.	Bayman N, Ardron D, Ashcroft L, et al. Protocol for PIT: a phase III trial of prophylactic irradiation of tracts in patients with malignant pleural mesothelioma following invasive chest wall intervention. *BMJ Open* 2016;6:e010589.
15.	Clive AO, Taylor H, Dobson L, et al. Prophylactic radiotherapy for the prevention of procedure-tract metastases after surgical and large-bore pleural procedures in malignant pleural mesothelioma (SMART): a multicentre, open-label, phase 3, randomised controlled trial. *Lancet Oncol* 2016;17:1094-1104.
16.	Rahman NM, Pepperell J, Rehal S, et al. Effect of Opioids vs NSAIDs and Larger vs Smaller Chest Tube Size on Pain Control and Pleurodesis Efficacy Among Patients With Malignant Pleural Effusion: The TIME1 Randomized Clinical Trial. *JAMA* 2015;314:2641-2653.
17.	Metintas M, Ucgun I, Elbek O, et al. Computed tomography features in malignant pleural mesothelioma and other commonly seen pleural diseases. *Eur J Radiol* 2002;41:1-9.
18.	Armato SG, 3rd, Ogarek JL, Starkey A, et al. Variability in mesothelioma tumor response classification. *AJR Am J Roentgenol* 2006;186:1000-1006.
19.	Coolen J, De Keyzer F, Nafteux P, et al. Malignant pleural mesothelioma: visual assessment by using pleural pointillism at diffusion-weighted MR imaging. *Radiology* 2015;274:576-584.
20.	Gill RR, Umeoka S, Mamata H, et al. Diffusion-weighted MRI of malignant pleural mesothelioma: preliminary assessment of apparent diffusion coefficient in histologic subtypes. *AJR Am J Roentgenol* 2010;195:W125-130.

21.   Benard F, Sterman D, Smith RJ, Kaiser LR, Albelda SM, Alavi A. Metabolic imaging of malignant pleural mesothelioma with fluorodeoxyglucose positron emission tomography. *Chest* 1998;114:713-722.

22.   Plathow C, Staab A, Schmaehl A, et al. Computed tomography, positron emission tomography, positron emission tomography/computed tomography, and magnetic resonance imaging for staging of limited pleural mesothelioma: initial results. *Invest Radiol* 2008;43:737-744.

23.   Treglia G, Sadeghi R, Annunziata S, et al. Diagnostic accuracy of 18F-FDG-PET and PET/CT in the differential diagnosis between malignant and benign pleural lesions: a systematic review and meta-analysis. *Acad Radiol* 2014;21:11-20.

24.   Renshaw AA, Dean BR, Antman KH, Sugarbaker DJ, Cibas ES. The role of cytologic evaluation of pleural fluid in the diagnosis of malignant mesothelioma. *Chest* 1997;111:106-109.

25.   Bibby AC, Maskell NA. Pleural biopsies in undiagnosed pleural effusions; Abrams vs image-guided vs thoracoscopic biopsies. *Curr Opin Pulm Med* 2016;22:392-398.

26.   Vogelzang NJ, Rusthoven JJ, Symanowski J, et al. Phase III study of pemetrexed in combination with cisplatin versus cisplatin alone in patients with malignant pleural mesothelioma. *J Clin Oncol* 2003;21:2636-2644.

27.   Beckett P, Edwards J, Fennell D, Hubbard R, Woolhouse I, Peake MD. Demographics, management and survival of patients with malignant pleural mesothelioma in the National Lung Cancer Audit in England and Wales. *Lung Cancer* 2015;88:344-348.

28.   Santoro A, O'Brien ME, Stahel RA, et al. Pemetrexed plus cisplatin or pemetrexed plus carboplatin for chemonaive patients with malignant pleural mesothelioma: results of the International Expanded Access Program. *J Thorac Oncol* 2008;3:756-763.

29.   Arnold DT, Hooper CE, Morley A, et al. The effect of chemotherapy on health-related quality of life in mesothelioma: results from the SWAMP trial. *Br J Cancer* 2015;112:1183-1189.

30.   van den Bogaert DP, Pouw EM, van Wijhe G, et al. Pemetrexed maintenance therapy in patients with malignant pleural mesothelioma. *J Thorac Oncol* 2006;1:25-30.

31.   Castagneto B, Zai S, Dongiovanni D, et al. Cisplatin and gemcitabine in malignant pleural mesothelioma: a phase II study. *Am J Clin Oncol* 2005;28:223-226.

32.   Ceresoli GL, Zucali PA, De Vincenzo F, et al. Retreatment with pemetrexed-based chemotherapy in patients with malignant pleural mesothelioma. *Lung Cancer* 2011;72:73-77.

33.   Jassem J, Ramlau R, Santoro A, et al. Phase III trial of pemetrexed plus best supportive care compared with best supportive care in previously treated patients with advanced malignant pleural mesothelioma. *J Clin Oncol* 2008;26:1698-1704.

34.   Nowak AK, Byrne MJ, Williamson R, et al. A multicentre phase II study of cisplatin and gemcitabine for malignant mesothelioma. *Br J Cancer* 2002;87:491-496.

35.   Zalcman G, Mazieres J, Margery J, et al. Bevacizumab for newly diagnosed pleural mesothelioma in the Mesothelioma Avastin Cisplatin Pemetrexed Study (MAPS): a randomised, controlled, open-label, phase 3 trial. *Lancet* 2016;387:1405-1414.

36.   Treasure T, Lang-Lazdunski L, Waller D, et al. Extra-pleural pneumonectomy versus no extra-pleural pneumonectomy for patients with malignant pleural mesothelioma: clinical outcomes of the Mesothelioma and Radical Surgery (MARS) randomised feasibility study. *Lancet Oncol* 2011;12:763-772.

37.   Rintoul RC, Ritchie AJ, Edwards JG, et al. Efficacy and cost of video-assisted thoracoscopic partial pleurectomy versus talc pleurodesis in patients with malignant pleural mesothelioma (MesoVATS): an open-label, randomised, controlled trial. *Lancet* 2014;384:1118-1127.

38.   Hassan R, Bera T, Pastan I. Mesothelin: a new target for immunotherapy. *Clin Cancer Res* 2004;10:3937-3942.

39.   Robinson BW, Creaney J, Lake R, et al. Mesothelin-family proteins and diagnosis of mesothelioma. *Lancet* 2003;362:1612-1616.

40.   Cui A, Jin XG, Zhai K, Tong ZH, Shi HZ. Diagnostic values of soluble mesothelin-related peptides for malignant pleural mesothelioma: updated meta-analysis. *BMJ Open* 2014;4:e004145.

41.   Creaney J, Olsen NJ, Brims F, et al. Serum mesothelin for early detection of asbestos-induced cancer malignant mesothelioma. *Cancer Epidemiol Biomarkers Prev* 2010;19:2238-2246.

42.   Hollevoet K, Nackaerts K, Thas O, et al. The effect of clinical covariates on the diagnostic and prognostic value of soluble mesothelin and megakaryocyte potentiating factor. *Chest* 2012;141:477-484.

43.   Hooper CE, Lyburn ID, Searle J, et al. The South West Area Mesothelioma and Pemetrexed trial: a multicentre prospective observational study evaluating novel markers of chemotherapy response and prognostication. *Br J Cancer* 2015;112:1175-1182.

44.   Bonotti A, Simonini S, Pantani E, et al. Serum mesothelin, osteopontin and vimentin: useful markers for clinical monitoring of malignant pleural mesothelioma. *Int J Biol Markers* 2016:0.

45.   Hassan R, Sharon E, Thomas A, et al. Phase 1 study of the antimesothelin immunotoxin SS1P in combination with pemetrexed and cisplatin for front-line therapy of pleural mesothelioma and correlation of tumor response with serum mesothelin, megakaryocyte potentiating factor, and cancer antigen 125. *Cancer* 2014;120:3311-3319.

46.   Nowak AK, Brown C, Millward MJ, et al. A phase II clinical trial of the vascular disrupting agent BNC105P as second line chemotherapy for advanced Malignant Pleural Mesothelioma. *Lung Cancer* 2013;81:422-427.

47.   Franko A, Dolzan V, Kovac V, Arneric N, Dodic-Fikfak M. Soluble mesothelin-related peptides levels in patients with malignant mesothelioma. *Dis Markers* 2012;32:123-131.

48.   Hollevoet K, Nackaerts K, Gosselin R, et al. Soluble mesothelin, megakaryocyte potentiating factor, and osteopontin as markers of patient response and outcome in mesothelioma. *J Thorac Oncol* 2011;6:1930-1937.

49.   Creaney J, Francis RJ, Dick IM, et al. Serum soluble mesothelin concentrations in malignant pleural mesothelioma: relationship to tumor volume, clinical stage and changes in tumor burden. *Clin Cancer Res* 2011;17:1181-1189.

50.   Wheatley-Price P, Yang B, Patsios D, et al. Soluble mesothelin-related Peptide and osteopontin as markers of response in malignant mesothelioma. *J Clin Oncol* 2010;28:3316-3322.

51.   Grigoriu BD, Chahine B, Vachani A, et al. Kinetics of soluble mesothelin in patients with malignant pleural mesothelioma during treatment. *Am J Respir Crit Care Med* 2009;179:950-954.

52.   Pass HI, Lott D, Lonardo F, et al. Asbestos exposure, pleural mesothelioma, and serum osteopontin levels. *N Engl J Med* 2005;353:1564-1573.

53.   Lin H, Shen YC, Long HY, et al. Performance of osteopontin in the diagnosis of malignant pleural mesothelioma: a meta-analysis. *Int J Clin Exp Med* 2014;7:1289-1296.

54.   Creaney J, Yeoman D, Musk AW, de Klerk N, Skates SJ, Robinson BW. Plasma versus serum levels of osteopontin and mesothelin in patients with malignant mesothelioma--which is best? *Lung Cancer* 2011;74:55-60.

55.   Grigoriu BD, Scherpereel A, Devos P, et al. Utility of osteopontin and serum mesothelin in malignant pleural mesothelioma diagnosis and prognosis assessment. *Clin Cancer Res* 2007;13:2928-2935.

56.   Anborgh PH, Wilson SM, Tuck AB, et al. New dual monoclonal ELISA for measuring plasma osteopontin as a biomarker associated with survival in prostate cancer: clinical validation and comparison of multiple ELISAs. *Clin Chem* 2009;55:895-903.

57.   Pass HI, Levin SM, Harbut MR, et al. Fibulin-3 as a blood and effusion biomarker for pleural mesothelioma. *N Engl J Med* 2012;367:1417-1427.

58.   Creaney J, Dick IM, Meniawy TM, et al. Comparison of fibulin-3 and mesothelin as markers in malignant mesothelioma. *Thorax* 2014;69:895-902.

59.   Ren R, Yin P, Zhang Y, et al. Diagnostic value of fibulin-3 for malignant pleural mesothelioma: A systematic review and meta-analysis. *Oncotarget* 2016;7:84851-84859.

60.   Hu B, Thirtamara-Rajamani KK, Sim H, Viapiano MS. Fibulin-3 is uniquely upregulated in malignant gliomas and promotes tumor cell motility and invasion. *Mol Cancer Res* 2009;7:1756-1770.

61.   Hirose Y, Saijou E, Sugano Y, et al. Inhibition of Stabilin-2 elevates circulating hyaluronic acid levels and prevents tumor metastasis. *Proc Natl Acad Sci U S A* 2012;109:4263-4268.

62.   Creaney J, Dick IM, Segal A, Musk AW, Robinson BW. Pleural effusion hyaluronic acid as a prognostic marker in pleural malignant mesothelioma. *Lung Cancer* 2013;82:491-498.

63.   Thylen A, Hjerpe A, Martensson G. Hyaluronan content in pleural fluid as a prognostic factor in patients with malignant pleural mesothelioma. *Cancer* 2001;92:1224-1230.

64.   Strizzi L, Catalano A, Vianale G, et al. Vascular endothelial growth factor is an autocrine growth factor in human malignant mesothelioma. *J Pathol* 2001;193:468-475.

65.   Yasumitsu A, Tabata C, Tabata R, et al. Clinical significance of serum vascular endothelial growth factor in malignant pleural mesothelioma. *J Thorac Oncol* 2010;5:479-483.

66.   Kindler HL, Karrison TG, Gandara DR, et al. Multicenter, double-blind, placebo-controlled, randomized phase II trial of gemcitabine/cisplatin plus bevacizumab or placebo in patients with malignant mesothelioma. *J Clin Oncol* 2012;30:2509-2515.

67.   Weber DG, Casjens S, Johnen G, et al. Combination of MiR-103a-3p and mesothelin improves the biomarker performance of malignant mesothelioma diagnosis. *PLoS One* 2014;9:e114483.

68.   Ostroff RM, Mehan MR, Stewart A, et al. Early detection of malignant pleural mesothelioma in asbestos-exposed individuals with a noninvasive proteomics-based surveillance tool. *PLoS One* 2012;7:e46091.

69.   Tsim S, Kelly C, Alexander L, et al. Diagnostic and Prognostic Biomarkers in the Rational Assessment of Mesothelioma (DIAPHRAGM) study: protocol of a prospective, multicentre, observational study. *BMJ Open* 2016;6:e013324.

70.   Morre DJ, Hostetler B, Taggart DJ, et al. ENOX2-based early detection (ONCOblot) of asbestos-induced malignant mesothelioma 4-10 years in advance of clinical symptoms. *Clin Proteomics* 2016;13:2.