Title

PROGRESS AND CHALLENGES IN MESOTHELIOMA: FROM BENCH TO BEDSIDE

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ABSTRACT

Malignant Pleural Mesothelioma (MPM) is currently an incurable cancer with a typical survival of 1 year from the time of diagnosis. The recent genomic and transcriptomic characterisation of MPM presents new opportunities and challenges for MPM researchers. Recent advances in clinical and laboratory diagnostics, and proposals for an updated, data-driven, staging system, also present new challenges for clinicians and hospital services involved in MPM care. The aim of this review is first to introduce the reader to the topic of MPM, a disease that is causally linked to prior, typically occupational, exposure to asbestos fibres. Secondly, we will discuss MPM from the clinical and laboratory perspectives, including reviews of current and evolving therapies and our present understanding of the molecular basis of the disease. Finally, we will attempt to identify critical knowledge gaps that currently prevent more effective treatment, including the challenges involved in early detection and chemoprophylaxis.

INTRODUCTION

Malignant Pleural Mesothelioma (MPM) is a locally invasive and currently incurable thoracic malignancy. A causative link to prior exposure to fibrous silicates (collectively termed ‘asbestos’) was first reported by Wagner and subsequently validated in workers previously exposed to mainly crocidolite and amosite [1], commonly in the ship-building, construction and mining industries . As a result, asbestos is prohibited in most countries, but a legacy of asbestos-insulated buildings remains. More worryingly, asbestos continues to be used without controls in many developing nations, and MPM will therefore remain a global threat for decades to come. Recent years have seen major advances in the study of MPM, including the first detailed characterisation of the MPM tumour genome, the development of an updated, data-driven, staging system and a range of novel treatments and diagnostic tools. The aim of this article is review these advances from the clinical and laboratory perspectives, and to highlight remaining critical knowledge gaps.

THE CLINICAL PERSPECTIVE

Presentation

Patients frequently present as an acute admission to hospital, often with breathlessness and/or chest pain associated with a unilateral pleural effusion [2,3]. MPM is more common in males and a long latency between asbestos exposure and disease is typical (40-50 years). In females, a direct exposure history may be absent but indirect (non-occupational) contact may be traceable via a spouse or parent. Right-sided disease tends to predominate [4] but routinely available tests, such as Computed Tomography (CT) imaging [5,6], pleural fluid cytology [7] and closed pleural biopsies [8] offer poor sensitivity. Patients should therefore have early access to specialist diagnostics, including Local Anaesthetic Thoracoscopy (LAT), expert pathology review and the support a Mesothelioma Clinical

Nurse Specialist and MPM-focused multi-disciplinary team.

Imaging

The key investigations: Chest radiography, Ultrasound and Computed Tomography

Chest radiography (CXR) will typically reveal a pleural effusion +/- loss of hemi-thoracic volume or a pleural mass. Pleural plaques are neither sensitive nor specific for MPM [4] but are useful to corroborate a history of prior asbestos exposure. Thoracic ultrasound (TUS) should be performed at the first opportunity, allowing estimation of effusion volume, identification of pleural nodules and planning of diagnostic/therapeutic fluid aspiration +/- subsequent LAT. Computed Tomography (CT) scanning, with venous-phase contrast enhancement, may add valuable diagnostic information, but non-specific 'benign' features (e.g. a bland pleural effusion) should not dissuade invasive sampling. 35-46% [5,6] of patients with pleural malignancy will have a 'benign' CT report in routine practice, and the negative predictive value (NPV) of CT is particularly low when arterial-phase contrast (e.g. CT pulmonary angiography) or non-thoracic radiologist reporting is employed [6] (see Figure 1). Therefore, a new effusion +/- pleural thickening should prompt invasive sampling.

The evolving role of PET-CT and MRI

Positron Emission Tomography (PET)-CT is not commonly used for primary diagnostic purposes, but FDG uptake is typically higher in MPM than in benign pleural disease (reported mean SUVmax 6.5 (3.4)) vs. 0.8 (0.60), respectively [9]). PET-CT sensitivity is likely to be reduced in low volume tumours, therefore false negatives may occur in early stage MPM. Additionally, false positives may result from inflammatory or infectious pleurites, such as rheumatoid pleuritis, tuberculosis, and in patients who have had prior

talc pleurodesis [10,11]. These data are reflected in recent meta-analyses, which report variable performance in differentiating benign from malignant pleural disease (sensitivity 81%-95%, specificity 74%-82%) [10,11]. A negative PET-CT should not, therefore, in isolation dissuade further investigation, e.g. by LAT, in patients in whom MPM is suspected. PET-CT can also be used to target the best site for histological sampling in suspected MPM. The randomised, multi-centre TARGET trial (ISRCTN14024829) is currently recruiting in the UK to determine whether this approach improves diagnostic yield in patients in whom a first biopsy has proven non-diagnostic.

The primary role of PET-CT in MPM is as a staging tool, since is delivers the highest diagnostic accuracy (relative to CT and Magnetic Resonance Imaging (MRI)) for stage III disease [12], which has traditionally been the threshold for surgical intervention in previous studies. PET-CT is particularly usefully in identifying extra-thoracic metastases [13] but has limited sensitivity for both mediastinal node involvement (60%) and extra-pleural invasion (50%) [14]. MRI offers superior sensitivity to extra-pleural invasion [15] and should be considered if T4 disease is suspected prior to surgery (e.g. multi-focal chest wall invasion). Moreover, direct, histological assessment of the mediastinum should be considered, particularly in light of emerging data regarding the prognostic importance of mediastinal nodal involvement in MPM (see 'Staging'). Importantly, MRI also allows more precise estimation of pleural tumour volume [16] and acquisition of functional data [17] that may be useful in future T-staging and early detection strategies.

Invasive Pleural Sampling

Histological confirmation is currently recommended in all patients [18]. In those with effusion, LAT is preferable to closed pleural biopsy (e.g. Abram's) since the latter offers

insufficient sensitivity (47% in a recent prospective study [8] and 57% in a series of 2893 cases) [7], frequently resulting in repeated chest wall punctures which increase the risk of subsequent needle tract metastases. LAT is well-tolerated, safe (mortality 0.34% in 4736 cases) and accurate (sensitivity 92.6%, specificity 100% in 1369 cases [19]), and allows simultaneous fluid evacuation and either talc pleurodesis or placement of an indwelling pleural catheter. General anaesthetic (GA) thoracoscopy offers similar performance but given the requirement for GA should be reserved for complex pleural spaces or used when LAT is unavailable or preferred by the patient. In patients with pleural thickening, CT- or TUS-guided cutting pleural biopsies offer high sensitivity (87%-94%) at minimal risk [7]. The latter can be performed on the table if LAT fails due to a fused pleural space [20].

Pathology

Laboratory Assessment of Diagnostic Samples

International guidelines have not previously recommended fluid cytology for primary diagnostics. This reflects low sensitivity and specificity in earlier series. However, recent years have seen the development of a range of techniques [21], including fluorescent in situ hybridization (FISH) testing for homozygous deletion (HD) of the 9p21 locus (harbouring the p16/CDNK2A gene, which is frequently lost in MPM tumours [22]). HD of p16 offers high specificity (near 100% in the relatively small studies recently reported) in fluid cytology and pleural biopsies [23] (see Figure 1). However, this high specificity requires prior demonstration using immunocytochemistry that the cell population being tested is mesothelial in origin, since p16 loss is associated with other cancers. Overall, the sensitivity of fluid cytology, even with p16 FISH, remains low and pleural biopsies are required in negative cases.

Histological assessment of pleural biopsies may reveal an invasive front of MPM tumour cells. This should be confirmed using an appropriate panel of two positive mesothelial immune-histochemical (IHC) markers and two negative adenocarcinoma markers. However, sub-pleural invasion may not be present in early stage disease even if large, deep biopsies are taken, probably because of the spatially heterogeneous distribution of this component at this stage. However, Hida recently reported that the presence of either p16 HD by FISH or loss of BRCA1-associated protein 1 (BAP1, encoded by the commonly inactivated BAP1 gene, [22]) IHC results in improved sensitivity (92.5%) [24] at high (100%) specificity, relative to assessment for sub-pleural invasion alone. While the data regarding these new molecularly-targeted laboratory tests should be interpreted with caution given the small samples sizes involved, their development may greatly improve the accuracy of early sampling for MPM.

The importance of Histological Sub-type

Histological sub-type is the strongest and most consistent predictor of survival in MPM [18] (see Figure 2). Epithelioid MPMs (50-60%) look morphologically similar to carcinomas and commonly present with pleural effusion, while sarcomatoid MPMs (10-20%) look like sarcomas and frequently present with pleural mass. These differing phenotypes and outcomes suggest epithelioid and sarcomatoid MPM are different diseases, making biphasic MPM (defined histologically by a mixture of epithelioid and sarcomatoid elements) difficult to explain. Comertpay recently reported that MPM is polyclonal based on the methylation status of the human androgen receptor gene [25]. Therefore, biphasic tumours may arise through synchronous evolution, or the intermingling of separate pools of epithelioid and sarcomatoid foci.

Biomarkers

MPM diagnostics are difficult, outcomes are heterogeneous and radiological responses to therapy are difficult to define. A biomarker-directed approach is therefore attractive. The most promising circulating diagnostic markers are mesothelin (or serum mesothelin-related protein (SMRP) [26], fibulin-3 [27], high mobility group box-1 (HMGB-1) [28] and an aptamer-based proteomic classifier [29]. Of these, mesothelin is the most widely studied and the only marker with FDA approval. However, a large meta-analysis reported poor sensitivity (32%) at 95% specificity [26], so negative results are of little value, particularly in non-epithelioid disease. The other markers require prospective validation, which is currently ongoing [30]. The most important future role for circulating markers may be as early indicators of treatment response, given the cost of emerging therapies.

Staging

The first globally adopted MPM staging system was defined in 1994, based on expert consensus and relatively small surgical series [31]. In assigning a T-stage, much emphasis was placed on the visible extent of pleural surface involvement at surgery and the system proved difficult to use in routine practice, where most patients are managed non-surgically. Subsequent validation studies confirmed poor separation of early stage (T1-T2) cases and significant discordance between surgical and clinical staging results [32]. This prompted a large international prospective staging project, which included more non-surgically treated patients and culminated recently in recommendations for an updated staging system (see Figure 3). The proposed TNM 8 system delivers improved prognostic performance and should be easier to deliver since surgical observations have become less important for allocation of T-stage, which is now based on ‘clinical’ (predominantly imaging) results [33]. In addition, under the new system, nodal staging [34] is better aligned with documented

patterns of pleural drainage from animal and human cadaveric studies, having been previously based on patterns of lung cancer nodal spread. Animal studies demonstrate that the parietal pleura drains ventrally towards nodes along the internal thoracic artery and dorsally towards the internal intercostal artery nodes, near the heads of the ribs [35]. This probably facilitates direct drainage of malignant MPM lymph into mediastinal (previously N2) glands, not necessarily involving intrapulmonary (previously N1) glands. This data is concordant with human cadaveric studies reported by Okiemy [36] and is reflected in the observations of Rahman, who reported frequent involvement of mediastinal (previously N2) nodes in patients with pleural invasion in the absence of intrapulmonary (previously N1) nodes [37]. Furthermore, Rusch *et al* recently reported no difference in survival between patients with intrapulmonary (previously N1) vs. mediastinal (previously N2) disease (median survival 17 months versus 13 months, HR 1.11 (p=0.2771)), but a significant difference in those with no node involvement (N0) relative to those with any node involvement' (previous N1 HR 1.26 for, p=0.0071 vs. N0; previous N2 HR 1.40, p <0.0001 vs. N0) [38]. As a consequence, the updated staging system proposes merging the previous N1 and N2 categories into a single new N1 grouping [34], emphasising the importance of any node-positive disease (see Figure 3). While it is hoped this system will perform more efficiently it continues to be based on predominantly surgical data, and has yet to benefit from large-scale staging studies testing the relative value of pathways combining modern tools such as PET-CT, MRI and invasive mediastinal staging.

Current and Emerging Therapies

In this section, the evidence for a range of MPM treatments is reviewed. The focus is on systemic, radiation and surgical techniques. However, effective management of associated pleural effusion is extremely important in symptomatic patients and may be the only

treatment delivered in many cases. In a recent randomised study involving all forms of malignant pleural effusion, talc slurry (TS) pleurodesis and indwelling pleural catheter (IPC) placement delivered equivalent rates of pleurodesis success and symptom control [39], although non-comparative data suggest that pleurodesis may be less successful in patients with MPM [40]. Ideally, patients should be offered a choice between an attempt at TS or IPC insertion, based on the relative risks and benefits of these techniques, unless non-expansile (trapped) lung is clinically obvious, in which case an IPC is preferable [41].

Palliative Chemotherapy

In 2003, Vogelzang reported an objective radiological response (ORR) in 41% of patients treated with a platinium/anti-folate doublet, comprised of Cisplatin and Pemetrexed (Cis-Pem). This was associated with an overall survival (OS) benefit of 2.8 months, relative to treatment with Cisplatin alone [42]. Comparable results were subsequently reported using an alternative anti-folate, Raltitrexed, in a similarly designed Phase III trial, although this drug is no longer available. Carboplatin(Carbo)-Pem has been shown to offer similar efficacy to Cis-Pem [43] in a post-licensing comparison and this combination has been the only licensed MPM therapy since, and the comparator for all Phase III trials of novel agents. This evidence base is clearly weakened by the lack of randomised evidence of a survival benefit over no chemotherapy (i.e. all supportive care), although indirect evidence such as improved survival in MPM registries might support this [44]. The placebo-controlled MSO1 failed to demonstrate any survival benefit for chemotherapy relative to ASC, but importantly involved an older regimen not including an anti-folate (Mitomycin, Vinblastine & Cisplatin) and failed to reach its adjusted recruitment target [45].

As with many therapies, the ORR to Cis/Carbo-Pem has proven lower in post-licensing studies (13%-26.3%[43,46]). When combined with the lack of a reliable predictive biomarker [47], an elderly patient population (64% of UK patients are > 70 years old) [3] and evidence of short survival in chemo-resistant patients [48], cautious use of Cis/Carbo-Pem in some centres is understandable. In a recent audit in England & Wales, treatment rates varied from 46% to 71% (see later website link for UK National Lung Cancer Audit Mesothelioma Report (2016)). This variation leads to associated challenges in recruitment of large numbers of patients to 1st line trials involving Cis-Pem, and 2nd line studies that require previous treatment with Cis-Pem. A reliable predictive marker for Cis-Pem response would therefore be the first and most important step in delivering stratified MPM management in the future.

In the recently reported MAPS study [49], the addition of Avastin (Bevacizumab), a vascular endothelial growth factor (VEGF) inhibitor demonstrated superior OS, relative to Cis-Pem alone. However, the OS gain was modest (2.7 months) with significant associated side effects (71% of patients experienced Grade 3-4 adverse events) and unfortunately, no predictive biomarker to assist in patient selection. At present, the MAPS triplet regime has yet to be licensed in Europe, the US or the UK. In our opinion, while chemotherapy is a valuable option for some patients, new approaches are urgently needed. Chemotherapy may, for example, prove to be an important part of future multi-modality immunotherapy schedules, given its ability to deplete tumour promoting immunosuppressive cells. Maintenance [50] and second-line chemotherapy studies have previously been negative [51] and no standard of care exists in this context.

Surgery

Several previous non-randomised series reported long-term survivors following extra-pleural pneumonectomy (EPP), which involves removal of the diseased pleura, in addition to the lung, pericardium and hemi-diaphragm, usually as part of a multi-modality regime including neo-adjuvant chemotherapy and adjuvant hemi-thoracic radiotherapy [18]. However, these data were systematically biased in favour of EPP, since they incorporated stringent surgical selection criteria. The Mesothelioma and Radical Surgery (MARS) trial was a randomised feasibility study of EPP, as part of multi-modal approach. MARS took over 3 years to randomise 50 patients and failed its feasibility end-points, but importantly demonstrated excess mortality in the EPP arm (adjusted HR 2·75 (1·21–6·26; p=0·016)) [52]. A recent meta-analysis also reported higher 30-day and 2-year mortality following EPP, relative to lung-sparing (pleurectomy/decortication (P/D)) surgery [53]. Despite much initial debate, the potential for harm associated with EPP is now generally accepted, prompting a move towards a lung-sparing approach. Lung-sparing surgery for MPM has been variably defined but should be categorised according to the consensus statement jointly made by the International Mesothelioma Interest Group and the International Association for the Study of Lung Cancer in 2011 [54]. This document defines P/D as a parietal and visceral pleurectomy performed with the intention of removing all visible tumour. Extended P/D additionally includes resection of the diaphragm and/or pericardium if these surfaces are affected. In contrast, Partial Pleurectomy (also referred to as Partial P/D) may involve removal of parietal and/or visceral tumour, potentially facilitating re-inflation of a non-expansile (trapped) lung, but always leaves visible tumour behind, and is generally performed as a combined diagnostic and palliative procedure. In 2014, the MesoVATS trial reported no survival advantage in patients allocated to Partial Pleurectomy (HR for death at 1-year 1·04 [95% CI 0·76–1·42]; p=0·81) relative to a those treated by a

simple TS pleurodesis. Partial P/D was also associated with a longer hospital stay, more complications and increased cost [55]. A large non-randomised Italian series has also recently reported inferior OS in patients treated by Partial P/D relative to Extended P/D, although this may reflect the selection criteria of the surgeons involved [56]. Meso-TRAP is currently recruiting in the UK to specifically determine the effect of Partial P/D on symptoms in patients with symptomatic non-expansile (trapped) lung, since these were largely excluded from MesoVATS [55]. At present, Meso-TRAP is a randomised feasibility study, which if positive will lead to a future Phase III trial randomising patients between Partial P/D and IPC placement. Also in the UK, the MARS-2 trial (NCT02040272) is currently recruiting patients with potentially resectable disease, having completed an initial feasibility phase. The primary objective of this important Phase III study is to determine the effect of Extended P/D, in combination with Cis-Pem, on OS and quality of life, relative to Cis-Pem alone. While the outcomes of these studies are eagerly awaited, surgery has at present, no proven therapeutic role in MPM.

Radiotherapy

Intensity-modulated radiotherapy (IMRT) now allows effective moulding of large RT doses, even to the complex morphology of the pleura. However, some of the apparent radio-resistance associated with MPM may reflect intrinsic radio-resistant tumour biology, and development of effective radio-sensitizers may be necessary for maximum effect. Combinations of RT with relevant agents, e.g. DNA-damage repair inhibitors (e.g. PARP inhibitors) and/or immunotherapies may deliver new treatment options. For example, fractionated RT has been shown to augment the response to CTLA-4 checkpoint inhibitors in pre-clinical cancer models [57].

For decades, palliative RT has been routinely offered for MPM pain, despite widely varying response rates (0-69%) and an inconclusive systematic review in 2014 [58]. In 2016, the SYSTEMS study, a multicentre, single-arm, phase II study reported an improvement in pain in only 35% of patients following 20Gy/5♯ [59], which is the standard dose in most centres. SYSTEMS-2, a randomised Phase III study is currently recruiting, comparing dose escalation (to 36Gy/6♯) to 20Gy/5♯, for which there is now robust efficacy data based on the results of the original SYSTEMS study.

Radical RT has most commonly been used as part of multi-modality strategy based around EPP. Most previous studies were therefore non-randomised case series, however these demonstrated that up to 54Gy/30♯ could be delivered post-EPP and that Radiation Pneumonitis (RP) rates could be minimised using IMRT [60]. In 2015, the randomised SAKK 17/04 trial failed to detect any difference in loco-regional relapse-free survival in patients allocated to IMRT after EPP, but the study was under-powered as only 73% patients had been allocated to IMRT at early closure [61]. Furthermore, in the post-EPP era, it is more important that radical RT can be delivered safely with two lungs in-situ. Therefore, Rimner's 2016 report of tolerable IMRT toxicity (Grade 3 RP in 2/27 (7%)) following P/D is important data [62] and a phase III study is planned.

Previous studies regarding the effect of prophylactic RT on the incidence of procedure-tract metastases were contradictory and relatively small. In 2016, the SMART trial reported no reduction in procedure-tract metastases incidence in 203 patients randomised equally between immediate (prophylactic) RT and deferred RT if a tract metastasis developed [63]. Results from the broadly similar and recently completed PIT (Prophylactic Irradiation

of Tracts) trial are expected imminently, but at present there is little to support this approach.

Mesothelin-targeted agents

Mesothelin is an attractive, relatively selective target for MPM therapy since it is over-expressed in most MPM tumours and expression is limited to normal pleural, peritoneal and pericardial surfaces. Immunotoxins target this expression specifically, including SS1P and the more recently reported RG7787 [64]. SS1P is a recombinant immunotoxin comprised of an anti-mesothelin variable fragment and the PE38 portion of Pseudomonas exotoxin A. The agent is cytotoxic to mesothelin-expressing cell lines but ineffective as monotherapy, due to development of anti-SS1P antibodies in over 90% patients. In 2013, Hassan and colleagues reported major regressions in 3/10 patients treated with a combination of SS1P and cyclophosphamide and pentostatin [65], which selectively deplete T- and B-cells respectively, leaving myeloid cells relatively preserved. Several other strategies targeting mesothelin are currently the subject of open trials. These include a chimeric monoclonal antibody to mesothelin (amatuximab) [66] and a mesothelin tumour vaccine (CRS-207) [67]. A randomised Phase II, 2nd line trial of the drug-antibody conjugate, anetumab ravtansine (comprised a tubulin inhibitor bound to SS1P) recently reported failure to meet its primary end-point (Progression Free Survival) [68]. While the ongoing studies in this area may yet validate highly targeted approaches to MPM therapy this result is a significant disappointment. If ultimately fruitful, the potential utility of serum mesothelin as a predictive and response marker [64] could be considerable advantage for mesothelin-targeted agents.

Immunotherapy

Immunotherapy offers great hope for MPM patients, after exciting positive clinical trials in other cancers. However, recent land-mark data demonstrate that mutational burden is low in MPM [22] and success may require multiple agents or combination with other modalities. Lung-sparing surgery may, for example, have an important role to play by reducing tumour bulk and creating a host environment more amenable to immunotherapy (releasing tumour neo-antigens, reducing the ratio of tumour cells to T-effector cells). Comprehensive reviews of this rapidly evolving field have recently been published [69-71]. Our impressions of the progress so far and the best hopes for future success are laid out below.

Cytokines, including interleukins (ILs) and interferons (IFNs), activate host immune response to viruses and cancer cells. Almost 20 years ago, Astoul reported tumour regression in selected patients (12/20 (54%), all epithelioid) in response to intra-pleural IL-2. However subsequent clinical data have been contradictory [72]. Intra-pleural IFN-γ has previously produced a 20% response rate [73], but excessive (flu-like) toxicity has greatly limited further study. More recent studies of adenovirus-encoding IFN-β (Ad–IFN-β) [74,75] and IFN-α2b (Ad–IFN-α2b) [76] suggest these cytokines may have useful activity in MPM. However, their effects are severely limited by development of adenovirus-neutralizing antibodies and the immunosuppressive tumour microenvironment. Importantly, a recent Phase I study has reported acceptable side-effects of a multi-modal approach to address this, combining chemotherapy (promoting tumour neo-antigen exposure), COX-2 inhibition (suppressing the immunosuppressive tumour micro-environment) and IFN-α2b immunogene therapy [77]. Future randomised efficacy studies are therefore expected.

The considerable therapeutic potential of Chimeric Antigen Receptor (CAR)-T Lymphocytes in MPM has been explored over recent years. CARs specific to MPM tumour antigens can be introduced into circulating T-lymphocytes *ex-vivo*, thereby directing their cytotoxic potential towards antigen-bearing MPM cells on re-infusion. These products are Major Histocompatibility Complex (MHC)-independent, meaning they can be utilised in diverse populations. Anti-mesothelin and anti-fibroblast activation protein (FAP; which is expressed ubiquitously in the MPM tumour microenvironment) CAR T-lymphocytes have both been shown to induce regression in MPM xenograft tumour models and can be delivered locally, to the pleura, using an indwelling pleural catheter [78,79]. Several Phase I studies testing the safety of these approaches are currently underway.

Immune checkpoints exist to dampen immune responses to native tissues and are generally mediated by receptor-ligand interaction. Allison was the first to discover that established tumours expressed cytotoxic T-lymphocyte antigen-4 (CTLA-4) and this mediated an immune-tolerant, pro-tumour effect. This spawned the development of CTLA-4 inhibitors as cancer therapies. However, a recent Phase I study of the CTLA-4 blocker, tremelimumab, showed ORR in only 2/29 patients, and no extension in OS in a subsequent Phase IIb (DETERMINE) trial [80]. Programmed Death receptor-1 (PD-1) is a T-lymphocyte associated checkpoint that has emerged as an alternative potential target for MPM therapy [71]. PD-1 is expressed on effector lymphocytes, while its natural ligands PD-L1 and PD-L2 are expressed on tumour cells or in the surrounding microenvironment. PD-1/PD-L1 blockade unleashes down-regulated T-cells, mediating tumour rejection. PD-1 expression is highly variable in MPM cohorts (20-70% in previous studies), with stronger

expression in sarcomatoid sub-types and an inverse correlation with survival [71]. Alley et al recently reported interim results of the KEYNOTE-028 Phase Ib trial of the PD-1 blocker, pembrolizumab, summarising responses in 50 patients with unresectable, PD-L1 positive MPM (defined by >1% PD-L1 expression in the tumour or surrounding stroma). This analysis suggests significant potential activity, including PR and stable disease (SD) rates of 20% and 52%, respectively and a median duration of response of 5·6 months (95% CI 3·6–12·0)[81]. An open-label Phase II study of pembrolizumab is currently recruiting, and importantly includes subjects without PD-L1 expression, in whom some responses have been reported. One of the aims of this trial is to determine a threshold of PD-L1 expression for anti-PD-L1 activity [82]. Given the cost and potential duration of these treatments this data will be of enormous interest to funders and regulatory bodies. Early phase studies of nivolumab and avelumab have also been reported, with disease control rates (PR + SD) of 50-57%, in cohorts without entry criteria based on PD-L1 expression, as recently summarised by Mansfield [71]. In addition, pre-clinical studies on other, so far neglected, elements of the checkpoint pathway (e.g. PD-L2, LAG-3, TIM-3)[69,70] may yield further targets for MPM treatment, and a means of long-term control may evolve from this exciting field of research.

Molecularly targeted agents

For reasons described below, there are few obvious targets for direct pharmacological inhibition in MPM. Phase II studies involving ertolinib [83] and gefitinib [84] (small-molecule epidermal growth factor receptor (EGFR) tyrosine kinase inhibitors (TKIs)) and defactinib (a focal adhesion kinase (FAK) inhibitor) have previously been negative [85]. However, interest in this field is currently increasing, following positive recent results in the Phase III MAPS study, involving the VEGF inhibitor, bevacizumab [49](see ‘Palliative

Chemotherapy') and the Phase II LUME-Meso, study, involving the multi-targeted angiokinase inhibitor, nintedanib. In the latter, addition of nintedanib to 1st line Cis-Pem resulted in improved median PFS (from 5.7 (5.5 - 7.0) to 9.4 (6.7 - 11.2) months, (HR for 0.54 (0.33 to 0.87), p = 0.01) and a Phase III study is currently ongoing [86].

THE LABORATORY PERSPECTIVE

General molecular features, new sub-types and driver mutations

A broad molecular characterization of 216 cases of MPM was recently published [22]. Based on exome sequencing, few protein-altering mutations were reported (average 24±11 per tumour). This is low relative to most cancers, particularly lung cancer, where coding mutations typically number in their hundreds [22]. Indeed, the mutation signature of smoke-related DNA-damage was entirely absent, suggesting that smoking does not contribute to MPM. Instead, a reactive oxygen species (ROS)-induced DNA damage signature predominated, consistent with known effects of asbestos fibres and macrophage-driven inflammation [87].

Unsupervised clustering of transcriptomic data revealed four distinct tumour subtypes, rather than the histologically defined previous three. Epithelioid and sarcomatoid classifications were retained but biphasic tumours were split into biphasic-E and biphasic-S [22]. 68% of tumours histologically classified as epithelioid were molecularly classified as biphasic-E, which had a significantly worse prognosis than other molecular sub-types. Sarcomatoid tumours also expressed higher levels of PD-L1 and had higher estimated numbers of immune-inhibitory M2 macrophages and infiltrating lymphocytes. This suggests this subgroup may be more amenable than other sub-types to immunotherapy [22] and hints that this new data may allow better stratification of patients in future studies.

One of the most striking molecular features identified was the paucity of recognizable driver mutations in well-characterized oncogenes, in particular those for which small molecule inhibitors are presently available. It is thus highly unlikely that large numbers of MPM patients will benefit from off-the-shelf targeted therapeutics, as borne out in previous molecularly-targeted studies [85]. Instead, MPM is typified by loss-of-function mutations in tumour-suppressor genes and mutations of unknown consequence. This lack of obviously 'druggable' targets presents the greatest single challenge to the immediate development of novel treatments.

The main genetic suspects in MPM

BAP1

Germline mutations of BAP1 give rise to multiple tumours including MPM, as recently reviewed elsewhere[88], while sporadic BAP1 mutations occur in approximately 50% of cases [89]. BAP1 is a de-ubiquitylating enzyme that removes ubiquitin tags from substrate protein targets, including BRCA1-associated protein, BARD1, and a transcription co-factor HCF1 [90],[91]. Ubiquityaltion has multiple roles in regulating protein function and signal transduction and the effect of BAP1 loss on each of its substrates will need to be empirically determined [92]. Loss of BAP1 also alters polycomb-regulated gene expression with potentially far-reaching effects [93] and tailored therapeutic strategies will be required to target these pathways effectively.

CDKN2A/B

Deletion of CDKN2A occurs in up to 50% of MPM cases [94]. CDKN2B is syntenic with CDKN2A on chromosome 9, so the vast majority of cases exhibit loss of both loci [95]. Both genes encode inhibitors of cyclin-dependent kinases (p16 by A; p15 by B), which

serve to block or delay progression through the cell cycle. The frequency of these deletions suggests that cellular senescence may play an important role in delaying the emergence of MPM, and suppression of senescence could represent a “gate-keeper” event in pathogenesis. Notably, cellular senescence is also induced by oxidative damage and both the inflammatory microenvironment and asbestos fibers are known sources of ROS [96].

NF2/LATS1 – The Hippo Pathway

Loss of NF2 (Neurofibromatosis type 2, or Merlin) in MPM was first reported in 1995 and occurs in 19-50% of cases [22]. NF2 is part of the Hippo signalling pathway, which is dysregulated in a various cancers [97]. In MPM, mutations are found in multiple Hippo genes including NF2 (more frequent in sarcomatoid and biphasic-S), LATS1 and MST1 (more frequent in epithelioid and biphasic-E). Cross-talk between specific pathway components and other signalling pathways, such as WNT, TGF-β and AMPK [98,99] may determine the eventual phenotypic outcome and better understanding is required for development of precision therapies.

SETD2

SET domain histone methyltransferases are epigenetic regulators of gene expression. SETD2 tri-methylates histone H3 on lysine 36, a modification that is associated with active transcription [100]. This gene was one of several novel MPM-associated mutation targets recently reported,along with other members of the SETD gene family [22].. A distinct role for SETD2 in methylation of microtubules during mitosis and cytokinesis was recently described: Deletion of SETD2 ablated lysine 40 methylation of γ-tubulin, resulting in mitotic spindle defects and aneuploidy [101]. Whole and partial chromosome number

alterations are widely reported in MPM and mutations in SETD2 provide a plausible mechanistic explanation for at least some of this observed aneuploidy.

TP53

Tp53 is the single most-frequently mutated gene across all cancers, yet its mutation rate in MPM is relatively low, present in only 8% of MPMs and, strikingly, in none of the epithelioid tumours analyzed by Bueno & colleagues [22]. It is possible that loss of p14ARF as part of the CDKN2A locus relieves the selective pressure to inactivate p53 although DNA damage can activate p53 independently of p14ARF status [102]. Alternatively, p53 may play a non-canonical role in this disease. In particular, recent work has shown that functional p53 plays an important role in protecting cells from oxidative damage [103]. Oxidative stress in MPM is thought to derive from asbestos-driven chronic inflammation and reactive oxygen species (ROS) can alter protein function and promote ectopic cell proliferation [104]. However, high levels of ROS can be cytotoxic. Given the potential role of ROS in driving disease development or progression it is possible that MPM tends to select for wild-type p53 in order to suppress ROS-driven cytotoxicity. It may be possible to exploit this feature of MPM through strategies that drive or enhance p53-dependent apoptosis, for instance via emerging BH3-mimetic therapies [105]. However, such an approach would not be without its risks as widespread activation of p53 can be harmful to normal tissues [106].

CRITICAL KNOWLEDGE GAPS AND FUTURE DIRECTIONS

The Development of Effective Therapies

The recent genetic characterization of MPM constitutes a watershed moment for MPM research, but has also exposed key knowledge gaps. Few of the genetic players in MPM

are well understood and little is known about how they contribute to the disease. For example, what targetable vulnerabilities arise from mutation of the tumour suppressors commonly identified? What role does the tumour microenvironment play in supporting disease? Does asbestos contribute to MPM beyond the acquisition of key mutations? Can immunotherapy be exploited despite the low mutation burden? These and other questions require the development and widespread adoption of better *in vitro* (patient-derived, 3D and co-culture) and *in vivo* (combined use of conditional alleles with asbestos exposure) models that a) permit investigation of early disease evolution and b) better reflect response to therapies than current 2D monoculture. Fortunately, the low number of common mutations will focus research efforts on a small number of pathways and, with the appropriate tools, progress is poised to accelerate further.

Early Detection

Additional but equally important questions surround the role of early detection of MPM, which was identified as a key priority for patients during the recent James Lind Alliance Priority Setting Partnership (see associated website information). However, based on the literature to date, we can infer that in incident cases of MPM only 1/3 of patients will have visible pleural plaques [4] and only 2/3 will have a positive Mesothelin blood test (the current best of the circulating markers) [26] or a CT scan with obvious morphological evidence of pleural malignancy [5,6]. This suggests that we do not currently have the necessary detection tools to run an accurate screening programme, particularly since the prevalence of MPM will be considerably than lower in this setting than in the symptomatic, incident populations included in the studies referenced above. This is reflected in 2 previous negative screening studies, in which few cases were detected [107,108]. However, these used low-dose non-contrast CT, which is insensitive to early stage MPM

and recruited a relatively unselected cohort of ex-asbestos workers. Therefore, if early detection/screening is to be successful more accurate diagnostic tests, and better selection criteria are urgently needed. Patients, grant funders and ethics committees also need to be confident that the benefits of screening would be worth the risk and costs, including the burdens placed on those with false positive results. At present this is probably not the case but may change over coming years. Therapeutic options appear likely to increase in the near future. MRI may also prove to be a better screening tool than CT, since it does not involve exposure to ionising radiation and offers superior precision [16] and complementary (but as yet unproven) diagnostic functional end-points [17]. In addition, recent data suggests that reconstruction of cumulative life-time asbestos exposure and accurate modelling of lifetime MPM risk is possible [109], and may better select individuals most likely to benefit. This selection may be further improved by use of novel biomarkers such as ENOX2 protein transcript variants, which can be detected in the serum of patients destined to develop MPM up to 10 years prior to diagnosis [110]. Earlier detection is likely to improve outcomes, not least by allowing the maximum possible number of patients to enter clinical trials of new treatments. Given the considerable expansion of MPM research over recent decades we believe this should be pursued aggressively, in parallel with, not following the development of effective therapies.

Chemoprophylaxis

Chemoprophylaxis involves use of preventative therapies with minimal or no side effects to modify the biology associated with carcinogenesis, thereby reducing cancer incidence. Unlike screening, chemoprophylaxis does not require development of expensive diagnostic tools but could utilise similar lifetime MPM risk models [109] to identify those most likely to benefit. MPM is typically preceded by decades of chronic asbestos-related

inflammation and the production of ROS [87], which in turn has recently been associated with the common genetic lesions found in MPM tumours [22]. However, previous studies using plausible chemoprophylactic agents have been disappointing. In a transgenic mouse model of peritoneal mesothelioma, driven by SV40 Tag and induced by asbestos injection, anti-inflammatories (aspirin and cyclo-oxygenase-2 (COX-2) inhibitors) and anti-oxidants (Selenium, Vitamin A or Vitamin E) did not demonstrate useful chemoprophylactic effects [111]. In a xenograft (immunodeficient) peritoneal MPM mouse model, aspirin has recently been reported to suppress HMGB-1 levels and tumour growth [112], which may be relevant since HMGB1 is implicated in MPM pathogenesis and detectable blood at presentation in many patients [28]. We agree with others who recently encouraged a re-evaluation of chemoprophylaxis in MPM [113], but acknowledge the time and large sample sizes required to perform meaningful studies in this area. More precise pre-clinical data are required to design future human studies. This is likely to require high-throughput drug screening combined with animal models that more accurately recapitulate early disease biology, ideally in an immunocompetent system. MPM is preceded by up to 2 years by ‘Benign Asbestos Pleural Effusion (BAPE)’ (or Benign Fibrinous Pleurisy) in a significant minority of patients [114]. Since the nutritive and potentially pro-tumourigenic nature of pleural effusion has only recently been described [115], this component of early MPM biology may not have been accounted for in previous pre-clinical studies. Studies involving sequential biopsies in patients with BAPE may therefore better define the key events involved in the transition from chronic pleural inflammation to MPM, and should be considered.

CONCLUSION

There has been enormous progress in MPM over the last decade, including the development of an updated, data-driven staging system and the rigorous assessment of new (and some older) therapies in global clinical trials. Better diagnostic tools and methods for palliation of associated pleural effusion are now available. The MPM tumour genome has recently been characterised in detail for the first time, opening up new opportunities for MPM researchers and new hope for patients and their families. Further significant progress is therefore expected in the near future.

SEARCH STRATEGY AND SELECTION CRITERIA

References for this review were identified by searching PubMed using the search term 'Mesothelioma', combined with one or more additional terms depending on the area of interest, e.g. 'Chemotherapy', 'Surgery', 'Immunotherapy', 'Biomarker'. The authors' publication libraries were also reviewed and relevant abstracts selected from recent scientific meetings. Only references since 2000 were included.

USEFUL WEBSITES

- Outcomes of the recent James Lind Alliance Priority Setting Partnership, with regard to current research priorities in MPM: http://www.psp.nihr.ac.uk/mesothelioma
- Recently published (December 2016) national audit data regarding outcomes in MPM patients diagnosed in England and Wales during 2014: https://www.rcplondon.ac.uk/projects/outputs/national-lung-cancer-audit-pleural-mesothelioma-report-2016-audit-period-2014
- The Mesothelioma Applied Research Foundation (formerly known as MARF): http://www.curemeso.org/

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CONFLICTS OF INTEREST

None declared.

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FIGURE LEGENDS

Figure 1

Panel A & B: Axial Computed Tomography images of a patient with diagnosed with early stage Malignant Pleural Mesothelioma (MPM) in our unit. These demonstrate a large pleural effusion (PE), but no obvious areas of pleural thickening. Panel C & D: Local Anaesthetic Thoracoscopy (LAT) images recorded in the same patient demonstrating widespread parietal pleural tumour (some highlighted by white arrows) after complete evacuation of the large pleural effusion. Note the descending thoracic aorta (Ao) also covered by tumour, the deflated left lower lobe (LLL) and the left hemi-diaphragm (LHD). Panel E: H&E staining (x 20 magnification) of parietal pleural biopsies taken during LAT, demonstrating an invasive front of proliferating mesothelioma cells, extending from the pleural surface (not present in the image because of the depth of the biopsy) into the sub-pleural fat (red arrows). The pleural surface lies just beyond the black arrow in the top right corner. Islands of invasive tumour cells are also highlighted (*). Panel F: The lower image demonstrates homozygous deletion of the P16 locus by fluorescent in-situ hybridisation (FISH) in exfoliated MPM cells in pleural fluid. Homozygous deletion requires loss of 2 red signals in at least 20% of nuclei. There is loss of one red signal in heterozygous deletion. The lower image shows a normal p16 test for comparison, demonstrating benign mesothelial cells, which exhibit two green signals (chromosome 9 centromeric probe) and two red signals (locus-specific probe to P16/INK4A (CDKN2A) gene).

Figure 2

Baseline clinical data and overall survival (OS) outcomes were recorded retrospectively in 404 patients diagnosed with Malignant Pleural Mesothelioma (MPM) in the West of Scotland, between January 2008 and April 2017. In 370/404 OS data were available. Kaplan-Meier methodology was used to determine the impact of histological subtype on OS in 342/404 (28/404 patients in whom a histological sub-type was not recorded were excluded). OS was significantly influenced by histological sub-type (log rank $p < 0.0001$, chi square 27.5); OS was longest in patients with epithelioid MPM, intermediate in biphasic cases and shortest in sarcomatoid MPM (log rank for trend $p < 0.0001$, chi square 26.8). Relative to epithelioid MPM, the log-rank hazard ratio (HR) for death in patients with sarcomatoid (HR 1.93 vs epithelioid, $p < 0.0001$) and biphasic MPM (HR 1.75, vs epithelioid, $p = 0069$) were significantly increased. HR for death was not different between sarcomatoid and biphasic cases (HR 1.14, $p > 0.05$).

Figure 3

Summary of the current staging system of Malignant Pleural Mesothelioma, as presented in the current 7th edition of the staging manuals of the Union for International Cancer Control (UICC) and the American Joint Commission on Cancer (AJCC). The proposed revisions for the imminent 8th edition are presented alongside (with updated descriptors highlighted in pink), in addition to updated stage groupings and associated overall survival curves. The survival curves are reproduced from Rusch *et al*. J Thorac Oncol 2016;11(12): 2112-2119. IM; internal mammary, PC; peri-cardiac, PD: peri-diaphragmatic, SC; supra-clavicular.