Immune checkpoint inhibition for the treatment of mesothelioma

Expert Opinion On Biological Therapy

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Abstract (147 words)

Introduction: Combination chemotherapy is currently standard care for advanced mesothelioma. Checkpoint blockade is a promising new treatment.

Areas covered: This review covers clinical use and biomarkers of checkpoint blockade. Medline search used keywords ‘mesothelioma’ combined with ‘checkpoint blockade’ OR ‘PD-L1’ OR ‘PD1’ OR ‘anti-CTLA4’; the search terms AND ‘clinical trial’ or AND ‘biomarker*’ were added. Handsearching covered abstracts from relevant meetings from 2016-2018 and reference lists. Data informed a narrative review.

Expert Opinion: Single agent anti-CTLA4 blockade is inactive in mesothelioma. Single agent PD-1 blockade as second or subsequent treatment gives 20-29% partial responses; no randomised comparisons against placebo or chemotherapy are available. Biomarkers of response have been difficult to identify. There is no consensus as to whether tumor PD-L1 expression predicts outcomes. Combination checkpoint inhibitors (CTLA4 and PD1 blockade) provide a small incremental increase in response rates and progression free survival. Chemoimmunotherapy is the next frontier.

Article highlights box:

- In malignant pleural mesothelioma, treatment with the single agent anti-CTLA4 antibody tremelimumab did not improve overall survival or other outcomes over placebo in the second line setting
- Objective tumor response rates to single agent anti-PD1 checkpoint blockade range from 20 to 29% in the second line and subsequent setting. Results from ongoing randomised clinical trials comparing these agents to second line chemotherapy or placebo are not yet available
- Objective tumor response rates to combination checkpoint blockade with anti-PD1/PD-L1 and anti-CTLA4 agents range from 25 to 29% in mostly pretreated patients. A single non-comparative randomised phase II study demonstrated better outcomes in all parameters for combination treatment over anti-PD1 therapy alone but small patient numbers limit definitive conclusions
- Malignant pleural mesothelioma is often a bulky and heterogenous tumor; tumor biomarker discovery may be challenged by spatial and temporal heterogeneity of PD-L1 expression and other putative biomarkers. Further work is needed to define optimum biomarkers and there is currently no role for patient selection in clinical trials or routine practice.
- Combining chemotherapy with checkpoint blockade is under active investigation.

Introduction

Malignant mesothelioma is a cancer of the mesothelial cells which line body cavities including the thorax, abdomen, and pericardium. Whilst considered a 'rare' cancer, the incidence of mesothelioma varies widely worldwide, paralleling use of, and environmental exposure to, the known carcinogen asbestos and similar fibres[1-3]. Great Britain, Australia, Italy and France have amongst the highest reported crude mortality rates, however there is likely to be widespread under-reporting of mesothelioma in many under-developed countries, despite their widespread use of asbestos[1, 4]. The current global burden of disease is estimated at almost 40,000 deaths worldwide[1]. Mesothelioma usually presents with pleural effusion, chest pain, dyspnoea, or systemic symptoms such as weight loss and night sweats[5]. Imaging usually demonstrates unilateral pleural thickening or pleural masses, often accompanied by a pleural effusion, with contraction of the affected hemithorax in more advanced cases[6]. A diagnosis is preferably made on histology from tumor biopsy, taken thoracoscopically or percutaneously, but can also be made on pleural effusion cytology in epithelioid subtype tumors[7]. The three main histological subtypes are epithelioid, biphasic, and sarcomatoid histology, although less common poor prognosis subtypes such as transitional and pleomorphic are becoming more widely recognised[8].

Current management of mesothelioma

The role of surgical management of mesothelioma is controversial, and its availability differs by geographic location. Surgery is usually used as part of multimodality therapy, either preceded by, or followed by, chemotherapy and/or radiotherapy, with no randomised evidence to support a specific best practice[9-11]. However, most patients worldwide do not have access to, or are not suitable for, radical surgery. Hence, systemic therapy has been the mainstay of anti-cancer treatment for people

with mesothelioma for more than a decade[12]. The combination of cisplatin and pemetrexed for up to six cycles of first line therapy improves survival by a small duration, with similar results from the less-used cisplatin with raltitrexed[13, 14]. Carboplatin can be substituted for cisplatin with some confidence that the efficacy is relatively similar[15-17]. More recently, the combination of cisplatin, pemetrexed and bevacizumab has shown an improvement in overall survival over cisplatin/pemetrexed alone, with a hazard ratio of 0.77[18] and an increase in median survival of 2.7 months; however this combination is not widely available worldwide due to lack of FDA approval for mesothelioma, lack of reimbursement in many jurisdictions, and the high cost of bevacizumab. The median survival for the intervention arm in this study was 18.8 months; although this is one of the highest median survivals reported to date in this disease, mesothelioma is still an invariably fatal malignancy with a short survival.

Following first line therapy, there is no systemic treatment for mesothelioma which has shown a survival benefit in a randomised clinical trial. Whilst a number of systemic therapy options have been trialled, there has been little progress with cytotoxic chemotherapy or with targeted agents[19, 20]. Reintroduction of platinum/pemetrexed, and the use of vinorelbine or gemcitabine, are commonly used therapies, but have modest efficacy. Hence, the second and subsequent line setting has provided an ideal setting in which to test immunotherapies, specifically immune checkpoint blockade, in the last 5 years.

Immunotherapy in mesothelioma

The historical context of mesothelioma immunotherapy

Immunotherapy has been trialled in mesothelioma over more than 25 years, with early clinical trials studying interferon alpha[21], intratumoral GM-CSF[22], autologous tumor cell vaccines with adjuvant[23], and TGFbeta blockade[24] amongst other strategies. Nevertheless, most of these treatments were ineffective, logistically difficult, not scalable, or all of these. Sporadic radiological responses, occasional reports of spontaneous regression and serological evidence of immune responses raised the

possibility that mesothelioma could be an immunogenic tumor[25, 26]. However, it was not until checkpoint blockade became available as an 'off the shelf' cancer therapy that more consistent responses and reports of patient benefit were seen, with PD-1 blockade widely available and no requirement for tumor samples, treatment personalisation, or intratumoral injection.

Immunotherapy enters standard treatment in other cancers

Immunotherapy is now a standard therapeutic modality in some other solid cancers, following the success of checkpoint blockade, initially alone, then as checkpoint blockade combinations, and now together with other modalities including chemotherapy and radiotherapy. Whilst the initial approvals in advanced melanoma were for the anti-CTLA4 antibody ipilimumab, the efficacy of anti-PD1 therapy and the combination of both ipilimumab and nivolumab has led to long term disease control in many patients, and even potential cures[27]. Following positive clinical trials in the adjuvant setting, checkpoint blockade is now also standard therapy in resected stage III melanoma[28]. Patients with metastatic non-small cell lung cancer (NSCLC) and high PD-L1 expression also derive a survival benefit from single agent nivolumab or pembrolizumab [29, 30]. More recently, the PACIFIC trial demonstrated a dramatic hazard ratio of 0.52 favouring sequential chemoradiotherapy followed by durvalumab over chemoradiotherapy alone in stage IIIA NSCLC[31]. Furthermore, the combination of PD-L1 blockade with chemotherapy has also now demonstrated strong initial proof of concept in NSCLC, with a hazard ratio for overall survival of 0.70[32]. Hence, checkpoint blockade immunotherapy is now in routine use in the clinic.

Reported clinical trials of checkpoint blockade in mesothelioma

The first clinical trials studying checkpoint blockade in mesothelioma were developed following the initial success of anti-CTLA4 in melanoma. The anti-CTLA4 antibody tremelimumab was administered in an open label phase II trial to 29 patients with previously treated mesothelioma at a dose of 15mg/kg every 90 days, with imaging also performed on a 90 day cycle. This initial study showed 7%

partial responses (PR), 24% stable disease (SD), and 69% progressive disease (PD) as best response. The median progression-free survival (PFS) was 6.2 months and median overall survival (OS) 10.7 months [33]. These findings spurred the development of a second study by the same group, using a more intensive treatment schedule of tremelimumab 10mg/kg 4 weekly for 6 doses, then 12 weekly, again in 29 patients. Results were similar, with 3% PR, 34% SD, 62% PD, and a similar PFS (6.2 months) and OS (11.3 months) [34]. On the basis of the progression free survival data and the proportion of patients with stable disease, a randomised double blinded phase III clinical trial, the DETERMINE study, was initiated, comparing tremelimumab 10mg/kg 4 weekly for 7 doses (followed by 12 weekly dosing) with placebo infusion on an identical schedule. Eligible patients had good performance status (ECOG 0-1) and all were pre-treated; randomisation was 2:1 to the active agent. DETERMINE recruited 571 patients, and unfortunately was a negative study, with a hazard ratio of 0.92 (95% CI 0.76-1.12, p=0.41) for OS, and a median OS of 7.7 months (tremelimumab) vs. 7.3 months (placebo)[35]. Nevertheless, the rates of PR and SD were similar to the two previous studies, although more frequent imaging allowed investigators to determine progression at an earlier timepoint. There is no role for single agent anti-CTLA4 antibody as second or subsequent line treatment in mesothelioma.

The next study to be reported was KEYNOTE-028, a multicohort study in which mesothelioma comprised one cohort. Pembrolizumab was given at a dose of 10mg/kg 2-weekly, a higher dose than that which would usually be given in current contemporary practice. Participants were selected for PD-L1 positivity using the 22C3 clone on archival biopsy samples, with positive PD-L1 expression ~~being~~ defined as membranous PD-L1 expression in ≥ 1% of tumor and associated inflammatory cells, or positive staining in stroma. Importantly, 80 evaluable samples were tested to find just under half which were PD-L1 positive. Patients were mostly pretreated, had good performance status, and ~~had~~ measurable disease. Those with stable disease or better continued to receive treatment for up to 24 months, and those with progression or unacceptable toxicity discontinued treatment. Response was assessed 8-weekly. There were no complete responses, however there were objective PRs in 20% of

patients, SD in 52%, and PD in 16%. There was no clear association between the extent of PD-L1 expression and outcome, however patient numbers were small. Most patients with partial response and some with stable disease experienced prolonged disease control. The median time to response was 1.9 months, and the median duration of response 12 months; the median duration of stable disease was 5.6 months. The median progression free survival was 5.4 months (95% CI 3.4-7.5 months) and the median OS was 18 months (95% CI 9.4 months – not reached)[36].

The first study of single agent nivolumab in mesothelioma has recently been reported. In this study, nivolumab was given at 3mg/kg 2 weekly, and patients had pre-treatment and on-treatment biopsies taken. There was no eligibility requirement for expression of PD-L1. From 33 evaluable patients, the objective PR rate was 24%, with 23% having SD. Treatment stopped at 12 months, with some patients experiencing ongoing partial response after the end of treatment. PD-L1 expression was seen in 27% of samples and there was no apparent correlation between PD-L1 expression and outcomes. With a median PFS of 2.6 months, and a median OS of 11.8 months, it is clear that phase III randomised controlled trials will be critical to help us interpret the outcomes of these studies[37]. Additional studies of single agent checkpoint blockade have been reported in abstract form, with results, as available at the time of writing, shown in Table 1.

Moving forward from single agent checkpoint blockade, a number of clinical trials are combining anti-CTLA4 antibodies with PD pathway blockade, a logical next step to enhance the efficacy of immunotherapy in mesothelioma. Results have been reported for the MAPS-2 trial (nivolumab and ipilimumab versus nivolumab alone), the INITIATE trial (nivolumab plus ipilimumab), and the NIBIT-Meso trial (tremelimumab plus durvalumab) [38-40]. The IFCT-1501 MAPS2 trial was a prospective, multicentre two arm noncomparative phase II study in patients with unresectable pleural mesothelioma, undertaken at 21 centres in France[40]. Patients had received 1-2 prior treatment regimens including platinum containing chemotherapy, and there was no selection on PD-L1 status.

The primary endpoint was disease control rate (DCR) at 12 weeks. Most patients had epithelioid disease and most had one prior treatment regimen. 125 patients in total were randomised in a 1:1 ratio to either nivolumab 3mg/kg 2 weekly, or nivolumab 3mg/kg 3 weekly followed by ipilimumab 1mg/kg 6 weekly, with open label treatment continued for up to two years. Objective tumor responses were centrally assessed, with 19% PR in the nivolumab arm and 28% PR in the combination therapy arm in the evaluable population. The 12 week DCR was 40% in the nivolumab group and 52% in the combination group. With a median follow up on 20.1 months, the median PFS and OS were 4.0 months and 11.9 months respectively in the nivolumab group and 5.6 months and 15.9 months respectively in the combination group. Adverse events were as expected from the global literature of these agents. NIBIT-Meso-1 was an open label single arm phase II study in which patients with pleural or peritoneal mesothelioma could be enrolled; 70% had experienced prior treatment. Patients received tremelimumab intravenously, 1mg/kg 4 weekly for a maximum of 4 treatments, in combination with durvalumab 20mg/kg 4 weekly ongoing[38]. The primary endpoint was objective radiological response, with 25% of patients achieving a partial response by mRECIST; 28% by irmRECIST. With a median follow up of over 19 months, the median PFS was 5.7 months and median OS 16.6 months. Results from all three studies as reported are shown in Table 2. Finally, the INITIATE study was a prospective single centre single arm phase II study, also in people with unresectable pleural mesothelioma who had received prior treatment[39]. As per the MAPS-2 study, the primary endpoint was DCR at 12 weeks. Most patients had epithelioid disease, and 54% were PD-L1 expression negative on tumor cells. Patients received nivolumab 240 mg 2 weekly and ipilimumab 1mg/kg 6 weekly, for up to four doses of ipilimumab but with nivolumab continuing for up to two years. Of 34 patients, 29% had PR, and 68% had disease control at 12 weeks. The median PFS was 6.2 months and median overall survival not reached at publication. Hence, it appears that the addition of either ipilimumab or tremelimumab to PD pathway blockade adds a modest increment to the objective tumor response rate, with the only randomised study demonstrating a signal for a longer progression free survival and overall survival with the combination. Nevertheless, these results suggest that combination immunotherapy will not

be efficacious for a majority of patients, and there is still no clear indication as to how to best select patients for combination therapy. Although those with highest tumor PD-L1 expression may be more likely to respond to treatment, the sensitivity and specificity of PD-L1 expression as a predictive biomarker is low.

Chemoimmunotherapy in mesothelioma

The design of the first clinical trial of chemoimmunotherapy in mesothelioma pre-dated evidence of benefit in NSCLC, and was informed by a substantial body of murine experimental data. The investigator team had shown that chemotherapy with gemcitabine preserved and indeed enhanced the T cell response in a mouse model of mesothelioma[41]. Furthermore, gemcitabine increased antigen cross-presentation in the context of tumor cell apoptosis[42]. Finally, dramatic synergy was observed between gemcitabine chemotherapy and immunotherapy with CD40 activation[43]. Concurrently, other researchers were also demonstrating the potential for combining chemotherapy with immunotherapy in animal models, reinforcing the potential of this approach, against the prevailing concept of the era that chemotherapy and immunotherapy would be antagonistic [44-46].

The first human clinical trial of chemoimmunotherapy in mesothelioma combined the CD40 activating antibody CP-870,893 with cisplatin and pemetrexed in a phase I clinical trial in patients who had not previously received any treatment for their disease. Patient had measurable disease and a good performance status. Chemotherapy was received in standard doses on day 1 of a 21 day cycle, and CP-870,896 was received on day 8 of the cycle. Patients received up to 6 cycles of combined therapy and up to a further 6 cycles of CP-870,893 if the disease was responding or stable. Whilst this phase I study aimed to identify the maximum tolerated dose of the combination, efficacy outcomes and changes in immune cell subsets were secondary endpoints. In 15 patients, six partial response (40%)

and nine patients with stable disease (53%) were seen, with a median overall survival of 16.5 months and three patients surviving beyond 30 months. Monitoring of immunopharmacodynamic markers of CD40 activation was challenging, as chemotherapy caused a dramatic cyclical change in numbers of all lymphocyte subsets, generally with a decrease from baseline at day 8, a partial restoration by day 15, and a peak at day 21 as the cycle restarted[47]. CD40 activation, whilst still under some investigation, has not yet found a niche in oncology treatment in any cancer.

Subsequently, a small number of chemo-immunotherapy clinical trials were initiated almost concurrently around the world. The Australian single arm phase II DREAM clinical trial (DuRvalumab and chEmotherApy in Mesothelioma) started in late 2016 and rapidly recruited 54 patients to a combination of cisplatin and pemetrexed (at standard doses) with the anti PD-L1 antibody durvalumab given on the same day, three weekly, for a maximum of six cycles of combined treatment. Patients were subsequently able to receive single agent durvalumab for up to a year total durvalumab treatment. At the time of writing, promising results have been presented in abstract form with the study meeting its primary endpoint in terms of 6 month progression-free survival[48]. A clinical trial with a similar design has been completed by the PrECOG group in the USA, but is unreported as yet. A Canadian clinical trial (NCT02784171) comparing nivolumab with chemotherapy to chemotherapy is continuing to recruit patients.

Biomarkers of checkpoint blockade in mesothelioma

Despite the promising results of immunotherapy for mesothelioma, there is a need to identify suitable biomarkers for patient stratification and monitoring of response on treatment. Biomarkers of response can help guide patient selection for therapy, targeting treatment to those who have the

capacity to respond. PD-L1 expression, tumor mutation burden, and characteristics of the microenvironment are key candidate biomarkers which will be discussed in detail.

PD-L1 Expression

PD-L1 expression on tumor cells is a logical biomarker for the prediction of treatment response to immune checkpoint therapies targeting the PD pathway. PD-L1 expression occurs in 15-40% of mesothelioma tumors, in particular the non-epithelial subtypes and has been associated with poor patient outcome [49-56]. In studies of PD-L1 expression in mesothelioma, a variety of antibodies, scoring metrics and cut-offs have been used. Although SP-263 has been the most commonly used antibody[38-40, 53, 54, 56], other studies have used clone E1L3N[49, 51] and 28-8[37]. In the context of immune checkpoint blockade, single agent studies investigating second line anti-PD1 therapy in patients with mesothelioma have shown that expression of PD-L1 in pre-treatment biopsies did not correlate with patient outcome. In the initial phase Ib KEYNOTE-028 trial, clinical benefit of pembrolizumab therapy was observed in 20% of patients with MM, despite pre-selection for PD-L1 positive tumors [36]. The percentage or intensity of baseline PD-L1 expression did not correlate with response to nivolumab treatment[37, 57] or combination therapy with tremelimumab and durvalumab [38] in the NIBIT-Meso study. Nevertheless, in the single arm INITIATE study of nivolumab and ipilimumab, there was some indication that patients with a greater proportion of tumor cells staining positive for PD-L1 derived more benefit at a range of cutpoints, however with only 34 patients in this study, testing in larger numbers of patients and well powered randomised studies are required to confirm this. This observed lack of clear correlation between PD-L1 expression within the tumor and response to treatment can potentially be explained by the use of different staining procedures, different PD-L1 antibody clones and differing cut-offs for positivity. For example, in the KEYNOTE-028 clinical trial, the 22C3 antibody clone was used and a PD-L1 positive tumor was defined as PD-L1 expression in ≥ 1% of tumor and associated inflammatory cells, or positive staining in stroma [36] . In contrast, the single agent nivolumab clinical trial used the 28-8 antibody clone with a cut-off of PD-L1 expression in ≥ 1% of tumor cells

alone [37]. In addition, PD-L1 is a dynamic biomarker and its expression may differ across tumor tissue, between tumor lesions and during treatment. Furthermore, in many of these studies, PD-L1 expression has been examined on archival tissue in patients who have subsequently received first line treatment. As yet there has been no head to head comparison of different antibodies, and nor is there consensus on appropriate cut-offs, on whether only tumor cells or both tumor and immune infiltrate should be scored, and on whether nuclear staining, cytoplasmic staining, or both should be examined. It would be appropriate to give a numerical score, to examine both tumour and immune infiltrate but be able to report separately, and to report on a range of cut-points including >1%, >5%, and >50%. While these initial studies suggest that pre-treatment PD-L1 expression in mesothelioma ~~tumors~~ may not be a useful indicator of those patients most likely to succeed on single agent anti-PD-1 treatment regimens, harmonisation of methods for PD-L1 testing in mesothelioma is required before it can be ruled out as a predictive biomarker.

Tumor Mutation Burden

Immune checkpoint blockade has been most successful in tumors with a naturally high mutation rate caused by exposure to exogenous carcinogens such as UV light and smoking (melanoma and lung cancer)[58]. Somatic mutations in tumors have the potential to generate mutation-derived antigens or neo-antigens that may generate a tumor-specific immune response. While only a minority of mutations will go on to generate immunogenic neo-antigens, the more somatic mutations a tumor has, the more neo-antigens it is likely to form and as such the tumor mutational burden (TMB) is used as a surrogate of the tumor neo-antigen load. In line with this, a high TMB has been associated with response to anti-CTLA-4 therapy in melanoma [59] and anti-PD-1 in NSCLC [60]. NSCLC is the first indication~~s~~ for TMB application as a biomarker. Mesothelioma, despite being caused by the carcinogen asbestos, has been shown to have a low TMB[61, 62]. As such TMB may not be a useful biomarker of response to immune checkpoint blockade in this disease. However, a recent report by Mansfield et al. showed that neo-antigen expression may be driven by chromosomal rearrangements

in mesothelioma in addition to the more commonly described single nucleotide variants [63]. Importantly the predicted neo-antigens correlated with clonal expansion of tumor infiltrating lymphocytes (TILs) and were proven to bind patient specific HLA molecules. Finally, T cells responsive to predicted neo-antigens were detected within peripheral blood of patients with mesothelioma [63]. This may explain the observed benefit of immune checkpoint blockade in a proportion of patients with mesothelioma, however further work is required to determine the relationship between TMB, chromosomal rearrangements and response to treatment.

The first FDA approval based on the concept of mutation burden was for pembrolizumab for patients with microsatellite instability high (MSI-H) and/or DNA mismatch repair deficient (dMMR) unresectable or metastatic solid tumors. This was the first FDA approval of a treatment based on a patient's biomarker status, rather than histology. Defective mismatch repair in tumors leads to higher somatic mutational load, producing a larger pool of neo-antigens for immune recognition, and has been frequently observed within several types of cancers, most commonly colorectal, endometrial and gastric adenocarcinomas. The MSI status of mesothelioma is not fully known. Two conflicting reports regarding MSI in mesothelioma have recently been reported. Bonneville et al. demonstrated that MSI was detected in 2.4% of cases (n=83) by analysing tumor-normal pairs in the TCGA cohort with their MSI-calling software MANTIS [64]. In contrast, Aralananda et al analysed 335 mesothelioma patient biopsies for deficiency of mismatch repair proteins and assessed MSI via PCR in deficient biopsies [65]. Only 6 samples were identified as deficient in mismatch repair proteins and of these all were confirmed as MSI negative by polymerase chain reaction (PCR). This discordance of results might be explained by the different methods used to identify MSI-H/dMMR. While immunohistochemistry and PCR are in routine use for clinical MSI testing, inferring MSI via next-generation sequencing (MSI-NGS) of tumors is an alternative method for MSI determination, with two FDA-authorized NGS platforms now incorporating MSI-calling algorithms. Given the low TMB and MSI observed in

mesothelioma together with the response rates to checkpoint blockade that have already been demonstrated it is unlikely that these will be useful biomarkers of response in this disease.

Tumour immune microenvironment

As the main effector cell of the anti-cancer immune response, the presence and number of tumor infiltrating T cells is associated with improved prognosis in several cancer types[66], regardless of therapy. Several studies have identified a link between T cell infiltration and outcome in patients with mesothelioma [67-70], however whether T cell infiltration is a predictor of response to immunotherapy has not been established ~~for MM~~. However, with the advent of single cell technologies such as mass cytometry and next generation sequencing, the immunological milieu of the tumor microenvironment is being analysed in greater detail than simply T cell infiltration. Gene expression profiling signatures that identify tumors with a T cell inflamed phenotype have recently shown promising results predicting response to immune checkpoint blockade[71] . In line with this, a recent study by Lee et al, showed that the tumor immune microenvironment (TiME) of mesothelioma could be separated into two tumor types [57], with the good-TiME signature significantly associated with a favourable prognosis. When this signature was applied to pre-treatment biopsies from 10 patients with mesothelioma who underwent second line therapy with anti-PD-1, the good-TiME molecular signature was associated with improved response to treatment[57]. Of the 5 patients who demonstrated a good-TiME signature, 3 had a complete response (modified-RECIST), 1 had a partial response and 1 stable disease. While these signatures need to be validated in a larger cohort of patients, gene expression profiling in mesothelioma may be useful to identify which patients should be considered for anti-PD-1 therapy.

Potential Biomarkers

Despite rapid advances in biomarker research, to date only PD-L1 expression and MSI/dMMR have shown clinical relevance for predicting response to immune checkpoint blockade in any cancer.

However neither of these biomarkers have been validated for patients with mesothelioma. New and emerging predictive biomarkers for immunotherapy are under investigation, however their relevance to patients with mesothelioma has not been established. Another important consideration is, where should we look for biomarkers? While tumor biopsy is the main method for obtaining a biomarker signature such as PD-L1 expression or MSI/dMMR, liquid biopsy is emerging as a novel method to reveal tumor specific information by analysing peripheral blood or other fluid samples. This is of particular relevance to patients with mesothelioma, in whom cytoreductive surgery is rarely performed and collection of tissue by biopsy is not only invasive, but can lead to sampling error that may affect downstream tumor analysis. Liquid biopsies, including circulating tumor cells (CTC) and circulating cell free tumor DNA (ctDNA) are minimally invasive, allow repeated access to the tumor and are a surrogate of tumor burden. CTCs have been used to assess tumor PD-L1 expression both prior to and on treatment in breast, bladder and lung cancer [72] and plasma levels of ctDNA are known to correlate with tumor burden [73-76], and response to therapy [77-79]. While the isolation and clinical utility of CTCs and ctDNA for mesothelioma has proved difficult in the past, two recent proof of concept studies have moved this field forward. A novel microfluidic system has been developed by Yoneda et al which captures mesothelioma specific CTCs with improved efficiency [80, 81]. Likewise, Hylebos et al detected the presence of ctDNA in treatment naïve patients by performing whole exome sequencing of tumor tissue then searching for patient specific variants in ctDNA [82]. These methods will open up new avenues for biomarker development in patients with mesothelioma, whereby CTCs and ctDNA could be used to monitor PD-L1 expression, tumor burden and tumor mutation status both prior to and during treatment. Tumor derived exosomes are also gaining attention in biomarker research for their ease of isolation and expression of tumor derived proteins. A recent study in patients with metastatic melanoma showed that circulating levels of exosomal PD-L1 positively correlated with tumor burden and poor prognosis [72]. In addition, pre-treatment exosomal PD-L1 was significantly higher in patients who failed to response with pembrolizumab [72]. While this is a relatively new field of research, exosomes have been isolated from pleural effusions in patients with mesothelioma [83].

Pleural effusion (PE) associated with MM contains a mixture of malignant cells, immune cells and cytokines and thus provides a unique opportunity to sample events at the tumor site which might prove useful for biomarker monitoring.

Special considerations for clinical trials of immunotherapy in mesothelioma

There are a number of special considerations for clinical trials of immunotherapy, most of which are not unique to mesothelioma. Whilst the original clinical trial response assessment tools were developed and studied in the context of chemotherapies, treatment responses in immunotherapy may not always follow the same predicable pathway of uniform reduction in tumor burden as seen with chemotherapy. The revised modified RECIST 1.1 for mesothelioma addresses this issue by recommending that clinical trials incorporate immunotherapy-specific assessment guidelines in the protocol which incorporate the principles of iRECIST[84, 85]. In practice, this allows for the potential of pseudoprogression by enabling patients to stay on treatment through progression, and introducing the concepts of unconfirmed and confirmed progressive disease. Pseudoprogression may manifest through initial increase in size of target lesions prior to subsequent response, or the development of new lesions. At the moment, it is unclear whether and how often this occurs in mesothelioma treated with immunotherapy, however this potential response pattern should be considered when designing clinical trials. The frequency of imaging is also important in immunotherapy clinical trials. Whilst chemotherapy studies have typically imaged every 6 to 9 weeks to avoid continuing an inactive but potentially toxic therapy, some immunotherapy clinical trials have taken the first imaging timepoint up to 12 week after baseline[33]. This may risk artificially increasing the median time to progression in an ineffective treatment in a single arm study, and may have contributed to the optimism around single agent tremelimumab before the failure of the DETERMINE randomised clinical trial[35].

Other considerations for immunotherapy clinical trials include the importance of collecting translational research samples, where possible, to inform future development of these therapies. For second-line therapy, contemporaneous pre-treatment tumor biopsies may have value, as archival biopsies represent the tumor state before first line treatment. It is still unknown how first line treatment, whether that be chemotherapy or combination therapies, may change the tumor microenvironment or potential biomarkers such as PD-L1 expression. Unfortunately, a single biopsy also has inherent limitations, as it is unlikely to fully represent the biomarker landscape of a large tumor involving multiple areas of the hemithorax. One way in which more nuanced post-treatment information can be obtained is through 'window of opportunity' trials. In this scenario, patients who are clinically appropriate for aggressive surgical resection would receive a biopsy prior to treatment, and then undergo immunotherapy or combination treatment on the clinical trial protocol. Following a defined period of treatment, usually two to three treatment cycles, surgical resection will be performed, and the entire specimen can then be examined, enabling a better understanding of the spatial heterogeneity of the tumor microenvironment and biomarkers. At least one such study, using pre-surgical pembrolizumab, is currently underway in the USA (NCT02707666).

In immunotherapy clinical trial design, other important considerations include stratification and the appropriate selection of endpoints. PD-L1 expression is consistently greater in sarcomatoid and biphasic mesothelioma. Whilst stratification for any non-epithelioid histology is standard practice in mesothelioma trials, the differing expression of checkpoints between subtypes adds further importance to this practice. It is yet unclear whether stratification by PD-L1 expression is appropriate, and until further information is available on the role of PD-L1 expression in response to immunotherapy, this stratification is not required. Stage is usually not incorporated into clinical trial eligibility or stratification as staging currently has a limited role in determining management strategies, and decisions on surgical resectability are made by a multidisciplinary team and incorporate histological subtype, patient preference and comorbidities. Endpoint selection is also important but

problematic, particularly for single arm studies. Whilst it has been tempting to consider stable disease as a beneficial effect of immunotherapy, many patient with indolent mesothelioma can demonstrate stable disease even without treatment. The negative results of the DETERMINE clinical trial should warn against interpreting stable disease as evidence of efficacy of immunotherapy[33-35]. Progression free survival and 6 month progression free survival are also open to patient selection bias in clinical trials and can lead to more positive interpretations of the data than are justified if patients have indolent disease. Ideally, randomised phase II trials should be encouraged in signal-seeking studies, in which case PFS or PFS6 are robust endpoints. Independent radiological review of imaging should be considered where response rates or PFS are endpoints, and overall survival is the only robust endpoint in this setting.

Future directions for research in mesothelioma immunotherapy

As mesothelioma is an uncommon disease, and patient numbers for clinical trials are limited, refining checkpoint blockade and immunotherapy needs to be informed by work in animal models. With an expanding number of checkpoint blockade and antigen specific therapies available, preclinical testing of combinations, in particular, should be actively pursued. Using this approach, our team was able to demonstrate that the combination of anti-CTLA4 and anti-OX40 was synergistic against murine mesothelioma, whilst other immunotherapy combinations gave additive results [86].

Another opportunity for research and clinical trials in mesothelioma is the combination of immunotherapy with radiotherapy. Whilst the abscopal effect – reduction in tumor bulk distal to the radiotherapy treated area – appears to occur with higher frequency in patients with other cancers treated with checkpoint blockade and radiotherapy, it has not been reported in human mesothelioma. Nevertheless, it has been reported in the laboratory setting [87], although there is much to learn about

the interaction between radiotherapy and the immune response, and how this may be augmented by immunotherapies.

Finally, increasingly sophisticated bioinformatics provides many opportunities to enhance our understanding of immunotherapy in mesothelioma, and to consider rational combinations. For example, it is possible to interrogate RNA sequencing or RNA expression data for differences between responders and non-responders, both in animal models and in patient tumor datasets. Network analysis can identify gene hubs which could be further activated or suppressed to enhance the desired therapeutic response, and drug databases then interrogated for compounds which may phenocopy the optimal genetic profile of responders[88, 89]. Most importantly, therapeutic combinations must not be solely driven by licensing or ownership of drug or antibody, but by a solid platform of preclinical evidence to support any clinical trial strategy. Only in this way will we successfully move new treatments to the clinic and study our limited pool of patients wisely.

Expert opinion (499 words)

Malignant pleural mesothelioma is an uncommon cancer with a known aetiological agent, asbestos. Standard care in patients with advanced mesothelioma has been combination chemotherapy for over 15 years, with a recent demonstration that adding bevacizumab to chemotherapy may improve overall survival. Highly selected patients may benefit from aggressive surgical management. Despite historical interest in immunotherapy in mesothelioma, the availability of checkpoint blockade has precipitated the first widespread study and use of this modality in mesothelioma. Single agent anti-CTLA4 blockade has no survival benefit over placebo in pre-treated mesothelioma and should not be used. However, clinical trials using single agent PD-1 blockade in the second and subsequent line setting have demonstrated partial response rates of between 20 and 29% using either nivolumab or pembrolizumab, with some of these responses being durable, but no randomised comparisons against placebo or second line chemotherapy being available as yet. There are no signals of additional toxicities in this population. As there is no standard second line chemotherapy for mesothelioma, the use of single agent anti-PD-1 antibody is a reasonable second line strategy for patients with mesothelioma who have no contraindications to immunotherapy, or those who may be unfit for or refuse first line chemotherapy. There is no evidence for any specific duration of therapy, and treatment may be continued until progression, toxicity, or patient wish to cease therapy. Patient monitoring for immunotherapy toxicities and for treatment outcomes should follow standard practice as for other indications. Current clinical trials are studying combinations of checkpoint inhibitors, with two single arm and one randomised phase II study to date suggesting a small incremental increase in response rates and possibly progression free survival from adding CTLA4 blockade to PD-1 inhibition. At the moment, such combinations are best used within an appropriate clinical trial. First line chemotherapy with or without bevacizumab continues to provide a higher response rate and known survival benefits. Biomarkers of response have been difficult to identify, with no clear consensus as yet as to whether tumor expression of PD-L1 is predictive of outcomes. At the present time, PD-L1 expression, tumor mutation burden, or other putative biomarkers are not sufficiently validated as a

basis to select patients for treatment. There is no evidence for the use of checkpoint blockade as neo-adjuvant or adjuvant therapy in patients who have aggressive surgical management, although this should be studied in clinical trials and window of opportunity studies. Combinations of checkpoint blockade with chemotherapy are the next frontier in these clinical trials in mesothelioma, with promising initial reports and ongoing studies. Again, the use of such combinations remains experimental and should be done within the structure of a clinical trial. Monitoring response in clinical trials of checkpoint blockade requires consideration of immune response criteria, and the phenomenon of pseudoprogression has been observed in this disease. Incorporating well-designed biomarker testing into current and future clinical trials is also critical for our future interpretation and understanding of appropriate patient selection for checkpoint inhibition, and the design and use of rational combination therapies.

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