Immunotherapy advances for mesothelioma treatment

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Abstract

Introduction: Mesothelioma is a rare type of cancer that is strongly tied to asbestos exposure. Despite application of different modalities such as chemotherapy, radiotherapy and surgery, patient prognosis remains very poor and therapies are ineffective. Much research currently focuses on the application of novel approaches such as immunotherapy towards this disease.

Areas Covered: The types, stages and aetiology of mesothelioma are detailed, followed by current treatment options such as radiotherapy, surgery and chemotherapy which are then discussed, followed by a description of innate and adaptive immunity and the principles and justification of immunotherapy. Clinical trials for different immunotherapeutic modalities are described, and lastly the article closes with an Expert Commentary and Five Year View, the former of which is summarised below.

Expert Commentary: Current efforts for novel mesothelioma therapies have been limited by attempting to apply treatments from other cancers, an approach which is not based on a solid understanding of mesothelioma biology. In our view, the influence of the hostile, hypoxic microenvironment and the gene expression and metabolic changes that resultantly occur should be characterised to improve therapies. Lastly, clinical trials should focus on overall survival rather than surrogate endpoints to avoid bias and inaccurate reflections of treatment effects.

Keywords

Mesothelioma; Immunotherapy; Immune Checkpoint Inhibition; Novel Therapies; Clinical Trials; Clinical Trial Endpoints

1. Introduction

Mesothelioma is a general term referring to numerous different cancers that are typically related to asbestos exposure and develop from the normal mesothelial cells that line various organs. Mesothelioma is typically divided into four classifications: pleural mesothelioma (mesothelioma of the lungs); peritoneal mesothelioma (mesothelioma of the abdomen); pericardial mesothelioma (mesothelioma of the heart) and mesothelioma of the tunica vaginalis (testicular mesothelioma) [1,2]. Histologically, there are primarily three broad categories of mesothelioma: epithelioid (approximately 80%-90% of cases); biphasic (approximately 10-20% of cases) and sarcomatoid (also approximately 10-20% of cases) [1,3]. Epithelioid mesothelioma is characterised through rounded to cuboidal cells, whilst sarcomatoid mesothelioma is characterised by spindle-shaped cells and cause bulky and aggressive tumours [3]. Lastly, biphasic mesothelioma contains a mixture of sarcomatoid and epithelioid cells. The histological subtype of mesothelioma may be an important determinant of its treatability; although sarcomatoid mesothelioma is relatively rare, it is commonly known as an aggressive cancer and patients have a very poor prognosis [4].

Current treatments for mesothelioma remain ineffective, with no standard second-line therapy and no treatments that dramatically improve survival. This therefore represents a significant unmet need, as it is anticipated that the disease will peak between 2015-2030 due to the latency period of the disease [5]. Incidence rates for pleural mesothelioma vary among different countries, with approximately 2000-3000 cases per year in the USA, 1000-1500 cases per year in the UK, and 1000 cases per year in Germany [4]. The latency period between asbestos exposure and disease onset leads to the delayed expected incidence peak, in addition to the fact that a quarter of a million deaths are expected from the disease in Europe within the next few years, due to occupational exposure [4].

This review will detail the pathogenesis of mesothelioma, in addition to its stages and the current approaches to treatment, both surgical (tumour resection and palliative care) and pharmacological (drugs such as gemcitabine, pemetrexed and cisplatin). After this, the basics of immunotherapy and its types and potential advantages will be discussed, followed by examples of application of immunotherapy to mesothelioma and current clinical trials. Lastly, the review closes with an Expert Commentary and Five Year View detailing our opinion on mesothelioma treatment and research directions in the present and coming years.

2. Body

2.1 Pathogenesis of Mesothelioma

Development of mesothelioma is thought to be influenced by a variety of factors such as simian virus 40 (SV40), which has been shown to be present in some human mesothelial tumours. Further evidence for the role of SV40 in mesothelioma development can be seen through the fact that its intracardial injection into mice leads to the development of malignant mesothelioma in 60% of cases [6]. Other factors indicated as contributing to the development of mesothelioma include genetic susceptibility, in addition to ionising radiation; previous comprehensive reviews have indicated that although a definitive causal link could not be established, there was sufficient evidence for radiation exposure to at least be considered [7,8]. An additional risk factor is erionite, a naturally occurring mineral that has properties similar to asbestos [5].

It is widely believed that asbestos exposure is perhaps the most common driver for mesothelioma development, as it has been estimated that approximately 70-80% of mesothelioma cases are related to occupational or environmental asbestos exposure [4]. It is significantly more probable that men develop the disease rather than women (at a ratio of approximately 4:1 – 8:1), again likely due to workplace exposure [9]. It is also recognised that there is a long latency period between asbestos exposure and mesothelioma development (consisting of multiple decades), which has led to the expectation that the disease will peak between 2015-2030 [5].

Asbestos is thought to contribute to mesothelioma development through the inhalation of asbestos fibres, which remain trapped in the lower third of the lung [2]. The unsuccessful clearance of these fibres leads to a chronic inflammatory state which may contribute to mesothelioma development [10]. It has been thought that the release of high-mobility group box 1 protein following induction of necrosis by asbestos exposure may cause a chronic inflammatory state, accumulation of macrophages and TNFα secretion. TNFα then promotes survival of mesothelial cells that have been genetically damaged through asbestos exposure via the activation of NF-κB [7,11,12]. In addition to asbestos alone, concomitant smoking may enhance the risk of developing a malignancy (non-small cell lung cancer) by up to sixty-fold [2].

Despite the above, it is also recognised that not all individuals exposed to asbestos will develop mesothelioma, which implicates a role for the additional factors such as genetic susceptibility. Further evidence for this is that mesothelioma clustering can be seen within some families [13]. In recent years BRCA1-associated protein 1 (BAP1) germline mutations have been identified as predisposing factors to mesothelioma pathogenesis, with some important differences in clinical outcomes observed between mesothelioma patients with germline BAP1 mutations, and those without [9,13]. In particular, it has been identified that mesothelioma patients with germline BAP1 mutations have a significantly higher survival (up to seven-fold, irrespective of gender and age) than patients with sporadic mesothelioma [9]. Thus, identification of genetic factors driving mesothelioma development and patient stratification based on these findings may improve patient prognosis and clinical outcomes.

2.2 Symptoms and Diagnosis of Mesothelioma

Diagnosis of mesothelioma is complicated by the fact that its symptoms are often nonspecific, as most patients present with a cough, shortness of breath, and difficult, laboured breathing [2,14]. Chest pain is also commonly presented, as are pleural effusions and dysphagia (difficulty in swallowing) may also occur, though typically later in the course of the disease. Peritoneal mesothelioma may present symptoms such as nausea, vomiting, loss of appetite and diarrhoea or constipation [14]. Thus, the lack of symptoms specific to the disease makes early diagnosis a difficult task.

In order to effectively diagnose mesothelioma, a combination of pathology insight, examination, radiology and knowledge of the history of the patient’s asbestos exposure is required. For patients presenting with pleural effusion, cytological analysis of this may allow confirmation of diagnosis; in one-third of cases pleural fluid is positive for malignant cells [14]. However, the presence of malignant cells within pleural fluid alone is usually not enough to confirm diagnosis, and corroboration with clinical, radiological and cytological data is preferred [14]. A pleural biopsy may confirm diagnosis, and a computed tomography

(CT) scan can identify the extent of the disease, whilst radiological approaches in general are essential for the diagnosis, staging and management of the disease [14]. Radiological approaches used for the diagnosis of mesothelioma include x-rays, CT scans, magnetic resonance imaging (MRI) scans and positron emission tomography (PET) imaging. The challenge with mesothelioma diagnosis, in addition to the lack of specific symptoms, is the fact that distinguishing malignant tumours from benign pleural diseases can be challenging. However PET imaging has been shown in one study to have 96.8% sensitivity and 88.5% specificity in distinguishing malignant from benign pleural disease [14,15].

2.3 Stages of Mesothelioma

Various staging protocols have been established for mesothelioma development, usually for malignant pleural mesothelioma (MPM) as there is a lack of staging for other mesothelioma types [2]. Examples of staging protocols include the Butchart system (based on the spread of the disease irrespective of histology) and the Brigham system (based on surgical resectability and involvement of lymph nodes), however the most widespread staging system in terms of usage is that developed by the International Mesothelioma Interest Group (IMIG), which is more detailed and incorporates information on the tumour, node involvement, and metastasis (hence TNM system) [2,14,16].

Stage I MPM under the staging system developed by the IMIG includes lymph node-negative patients with minimal visceral pleural involvement and minimal tumour growth restricted to the parietal pleura, whilst stage II MPM is lymph node-negative and confluent superficial tumour growth on all pleural surfaces or involvement of the lung parenchyma or diaphragm [16]. Stage III, which represents the most common stage of disease presentation, consists of tumours which have metastasised to areas such as lymph nodes, or whose tumour has extended into the soft tissues of areas such as the chest well or pericardium. Lastly, stage IV MPM contains features such as locally advanced tumours invading the spine or ribs and patients may present with distant metastases [16]. A CT-PET staged series demonstrated that 3% of patients presented with stage I, 9% stage II, 48% stage III and 40% at stage IV [17].

The staging system described above was based on analysis of a small retrospective surgical series and as such expansion of the patient cohort would be desirable [18]. To address this IMIG and the International Association for the Study of Lung Cancer Staging and Prognostic Factors Committee together initiated a multinational database based on twenty-nine centres on four continents and included 2,460 patients who passed the initial eligibility screen. This database indicated that current T descriptors should be maintained with the caveat that T1a and T1b be placed in a single T1 category. Similarly, pleural thickness had prognostic significance and was indicated to be further examined on future revisions of the staging system. It was proposed that N1 and N2 merge into N1, whilst N3 is relabelled to N2 [18].

Based on additional analyses the optimal stage groupings for the eighth edition of the staging system was: “stage IA (T1N0), stage IB (T2-3N0), stage II (T1-2N1), stage IIIA (T3N1), stage IIIB (T1-3N2 or any T4) and stage IV (any M1)” [18]. Developing a precise staging system is crucial, as the progress of mesothelioma and the stage at which it is diagnosed can have important implications for its treatability, as detailed in the next section.

2.4 Current Treatments for Mesothelioma

Mesothelioma may be treated through combination or individual application of radiotherapy, chemotherapy, and surgery. The stage of the disease is an important determinant of which approach to use, with earlier stages being generally more treatable and potentially resectable by surgery.

2.4.1 Palliative and Curative Treatments for Mesothelioma

Regrettably, current treatments for mesothelioma do not dramatically improve survival, and there is no standard second-line therapy. However, there are established treatments and therapeutic options which are broadly split into palliative care (to provide relief from symptoms and alleviate patient suffering) and curative treatments (to treat and eliminate the actual disease). Suggested criteria to stratify patients to curative treatments or palliative care have been described, such as the decision to employ palliative care if the patient has a poor nutritional state and general condition, stage III or IV mesothelioma, or biphasic or sarcomatoid mesothelioma regardless of its stage [19]. As previously described, sarcomatoid mesothelioma is especially difficult to treat and its patients have a very poor prognosis [4]. Converse to the justification for palliative therapy, treatment with a curative intent may be undertaken if the patient is less than seventy years old, has no significant cardiopulmonary compromise, has epithelioid mesothelioma and has no relevant accompanying disease [19].

2.4.2 Surgical Treatment of Mesothelioma

Surgery may be employed as either palliative care or used with curative intent. Generally, curative surgery is attempted only for resectable tumours, which are generally stage I or stage II. Although the ideal result from surgery would be a complete removal of the tumour, this is applicable only for a minority of patients as over 80% of mesothelioma diagnoses occur in stage III or IV [16,20,21]. Thus, surgery is generally employed as palliative care, with the aim being reduction of the tumour mass to alleviate symptoms. Surgery may be employed individually for patients with resectable tumours, however it is generally combined with other therapeutic modalities such as chemotherapy or radiotherapy, as it has been shown that patients undergoing multimodality therapy had a median survival of twenty months against ten for surgery alone [22].

2.4.3 Radiotherapy for Mesothelioma

Radiotherapy is a strategy which employs the use of high energy waves to damage cellular genetic material, thereby preventing tumour cell proliferation, and may be used with either a curative or palliative intent [23]. Although radiotherapy is employed to treat mesothelioma, for MPM it is difficult to achieve tumouricidal concentrations due to the growth pattern of the tumour around the lung [16] and thus for many years it was largely used as an adjuvant after surgery [24]. However, intensity-modulated radiotherapy (IMRT) which aims to deliver a homogenous dose to the entire tumour at a concentration low enough that underlying organs are not damaged [16], has also been employed. IMRT initially demonstrated troubling levels of toxicity, though later reports indicated that it can be delivered safely in the setting of surgery. However, questions regarding its use still remain [24].

2.4.4 Chemotherapy for Mesothelioma

Despite the relatively poor efficacy and clinical outcome, chemotherapy is employed for the treatment of mesothelioma, as some improvements in survival have been observed [4]. The cornerstone of chemotherapy for the treatment of mesothelioma is combination chemotherapy, due to the fact that combination chemotherapy generally leads to better therapeutic outcomes than single agent chemotherapy. Anti-folates (such as pemetrexed) are often used in conjunction with platinum-based therapy (such as cisplatin) as a first-line therapy for unresectable advanced-stage MPM, however there is no standard second-line therapy for patients who do not respond to this [25,26]. Other drugs, including gemcitabine, etoposide and doxorubicin have also been used for the treatment of mesothelioma, however outcomes remain poor as median survival for patients treated chemotherapy after diagnosis is only four to twelve months [3,27]. Thus, surgery, radiotherapy and chemotherapy, which are the cornerstones for treatment in other cancer types, are all of limited use in mesothelioma and therefore new approaches are urgently needed.

2.5 Immunity and Immunotherapy

2.5.1 Innate and Adaptive Immunity

Protective mechanisms within the human body may generally be divided into innate or adaptive. Innate immunity is the first-line response to a pathogen but cannot provide long-term protection, and may generally be through anatomic or chemical barriers, in addition to cellular responses [28]. Examples of anatomical barriers include the epithelial skin surfaces and glandular and mucosal surface, whilst examples of chemical barriers include antimicrobial substances such as lysozyme within acidic environments [28]. Cellular-based innate immunity requires either recognition of conserved molecular components on the surface of the pathogen, or recognition of intracellular receptors within the pathogen. These conserved molecular components are generally referred to as pathogen-associated molecular patterns (PAMPS) and cells such as dendritic cells (DCs), macrophages, neutrophils and monocytes recognise the PAMPS and through phagocytosis remove pathogens [28].

In addition to the cells listed previously, natural killer (NK) cells are also involved in innate immunity, providing protection through surveillance and detecting cells infected with viruses. Additionally, NK cells may target tumour cells or other normal cells through their lack of major histocompatibility complex (MHC) molecules, and can be activated through interleukin stimulation such as interleukin-2 (IL-2) treatment [28,29]. DCs also serve as a link between innate and adaptive immunity through two broad mechanisms. First, DCs are "sentinels" and capture, process and present antigens and migrate to lymphoid tissue to select T cells that react to the antigen. Secondly, DCs are "sensors" and thus respond to numerous environmental stimuli via differentiation or maturation [30]. NK cells have also been shown to serve as a bridge of sorts through the promotion of cross-presentation of tumour-derived antigens by DCs through the release of interferon-gamma (IFNγ) and tumour necrosis factor alpha (TNFα) which ultimately promotes antigen-specific CD8+ T cell (also known as cytotoxic T lymphocytes) activation [28,31].

Unlike innate immunity, adaptive immunity confers long-lasting protection against infectious agents through recognition and memory of specific antigens. Whilst innate immunity relies on the recognition of highly repetitive molecules, adaptive immunity instead requires recognition of specific antigens [28]. Adaptive immunity is dependent on receptors that are custom made based on recombination of gene segments and involves T lymphocytes, which mature in the thymus and are responsible for effecting cellular immune responses, and B lymphocytes, which are responsible for producing antibodies [32]. Mature T cells are activated following interaction between their T cell receptor (TCR) with antigenic peptides in complex with MHC molecules on antigen-presenting cells (APCs), with CD8+ T cells recognising MHC Class I molecules whilst CD4+ T cells (also called T helper cells) recognise MHC Class II molecules [32]. CD8+ T cells comprise the majority of circulating T cells and thus serve to remove cells harbouring pathogens such as viruses and transformed cells, whilst CD4+ cells produce cytokines that assist in the activity of other T cells [32]. Thus, innate and adaptive immunity together provide a robust defence for the body against pathogens and harmful cells.

2.5.2 Immunotherapy

Of all the new therapeutic modalities under investigation for cancer treatment, immunotherapy is one that has received significant attention [5,33]. Immunotherapy is an innovative approach that, rather than targeting cancer cells themselves via drug treatment, aims to stimulate the immune system to promote an anti-tumour immune effect [34]. Specific to MPM, the potential application of immunotherapy may be sound given that it has been shown that lymphocyte infiltration within the tumour mass correlated with improved patient prognosis [35,36].

Immunotherapy as a whole may be largely split into active immunotherapy and passive immunotherapy [37]. The difference lies in whether the immune system of the patient is stimulated *in situ*; passive immunotherapy generally isolates effectors *in vitro* before applying them to the patient, whilst active immunotherapy aims to stimulate the patient's own immune system, primarily through vaccination [37]. It has been stated previously that a common shortcoming of passive immunotherapy is that it is likely to yield only a temporary benefit, whilst active immunotherapeutic strategies may offer long-term disease control through education of the host's immune system [38]. Passive immunotherapy may typically be used when the patient's immune system is weak or poorly responsive, whilst active immunotherapy requires that the patient's immune system is responsive to challenge, will be competently stimulated and promote effector actions [39].

There are many types of immunotherapy (Figure 1) [39]. Examples of active immunotherapeutic approaches include vaccination (such as peptide-based and DC-based) and immune checkpoint inhibition whereas cytokine administration and adoptive cell transfer are examples of passive immunotherapy [39]. Numerous different immunotherapeutic approaches have been applied to mesothelioma, detailed below in the relevant subsections.

2.6 Application of Immunotherapy to Mesothelioma

2.6.1 Immunotherapeutic Vaccination

Immunotherapeutic vaccination aims to induce tumour-specific immune responses *in vivo* and there are multiple types such as peptide vaccination (the most common) or DC-based vaccines [39]. Peptide vaccines contain immunogenic epitopes from tumour-specific or tumour-associated antigens (TSAs or TAAs respectively), which can arise from numerous sources including but not limited to mutated oncogenes or tumour suppressor genes, oncofoetal proteins, oncogenic viruses, cancer-testis antigens or overexpressed self-proteins [39]. It is thought that immunogenic recognition of these TSAs/TAAs can lead to specific immune responses against the tumour, hence why it is such an attractive prospect. This is particularly relevant for personal peptide-based vaccination (PPV) as it is based on the concept of strengthening the host's existing immune response.

DCs represent an ideal “vehicle” for cancer vaccines due to their ability to affect both the innate and adaptive immune responses, and DC-based vaccination primarily works through two approaches: *in vivo* direct targeting of antigens to DC receptors, and *ex vivo* generation of antigen-loaded DCs [39]. DC-based vaccination is an approach that remains under a significant degree of investigation, due to the long-held interest and potential of the approach. One method that has garnered particular interest is the use of autologous tumour cells as a source for TAAs, as theoretically they should provide the most comprehensive coverage of tumour-specific components available. Allogeneic tumour cells are also useful in providing TAAs through their continuing culture *in vitro* and thus may theoretically provide limitless TAAs and allow for large-scale production of vaccines that can be consistent between vaccine batches thus allowing for improved comparison of clinical outcomes [39]. Autologous tumour cell vaccination requires transfection of tumour cells to make them produce cytokines and co-stimulatory molecules followed by irradiation of the tumour cells. Irradiation of the tumour cells renders them inactive and thus their injection into patients is safe as the cells do not proliferate and present TAAs to T cells, thus promoting a tumour-specific immune response [39].

Specific to mesothelioma, one frequently investigated protein is Wilms tumor 1 (WT1) due to the fact that it is overexpressed in MPM and immunohistochemical staining of WT1 is routinely used to aid in MPM diagnosis [40]. Numerous clinical trials of immunotherapeutic vaccination relating to mesothelioma have been performed, with examples listed in Table 1 below:

Table 1: Examples of clinical trials relating to vaccine immunotherapy in mesothelioma. An asterisk (*) indicates that the number of patients is the projected accrual, not the number actually enrolled.

Clinical Trial Identifier	**Study Title**	**Phase**	**Number of Patients**	**Status/Outcome of Study**
NCT01265433	Randomized Study of Adjuvant WT-1 Analogue Peptide Vaccine in Patients With Malignant Pleural Mesothelioma (MPM) After Completion of Combined Modality Therapy	II	31	Ongoing
NCT01890980	Phase II Study of Adjuvant WT-1	II	60*	Ongoing

	Analogue Peptide Vaccine in MPM Patients After MSK10-134			
NCT02649829	Autologous Dendritic Cell Vaccination in Mesothelioma (MESODEC)	I/II	20*	Recruiting
NCT02408016	Genetically Modified T Cells in Treating Patients With Stage III-IV Non-small Cell Lung Cancer or Mesothelioma	I/II	20*	Recruiting
NCT01258868	Tumor Cell Vaccines With ISCOMATRIX	I	44	Terminated

	Adjuvant and Celecoxib in Patients Undergoing Resection of Lung and Esophageal Cancers and Malignant Pleural Mesotheliomas			
NCT00006216	Vaccine Therapy and Ganciclovir in Treating Patients With Mesothelioma	I	3-16*	Unknown
NCT00398138	Vaccine Therapy and GM-CSF in Treating Patients With Acute Myeloid Leukemia, Myelodysplastic	I	22	Completed – results indicate T cell stimulation in leukaemia patients but median disease-free

	Syndromes, Non-Small Cell Lung Cancer, or Mesothelioma			survival was not reached [41].
NCT00003974	Vaccine Therapy in Treating Patients With Stage I, Stage II, or Stage IIIA Non-small Cell Lung Cancer or With Stage I or Stage II Mesothelioma	I	20*	Completed, but no results posted on ClinicalTrials.gov
NCT00280982	Dendritic Cell-based Immunotherapy in Mesothelioma	I	10	Completed – results indicate that the use of autologous tumour lysate-pulsed DCs

				was feasible, well tolerated and induced an immune response in patients. Though preliminary, results indicate that a subset of patients may benefit from this approach without significant side effects [42]
NCT01569919	A Phase II Trial to Assess TroVax® Plus Chemotherapy in Patients With Malignant Pleural	II	26*	Unknown

	Mesothelioma (SKOPOS)			
NCT02054104	Adjuvant Tumor Lysate Vaccine and Iscomatrix With or Without Metronomic Oral Cyclophosphamide and Celecoxib in Patients With Malignancies Involving Lungs, Esophagus, Pleura, or Mediastinum	I/II	21	Participant recruitment suspended
NCT01675765	Safety and Efficacy of Listeria in	I	60	Ongoing

	Combination With Chemotherapy as Front-line Treatment for Malignant Pleural Mesothelioma			
NCT01291420	Dendritic Cell Vaccination for Patients With Solid Tumors	I/II	10*	Unknown – data presented at ASCO 2014 suggest that the overall survival data is indicative that adjuvant DC-based therapy provides a clinical benefit for MPM patients [43]
NCT02151448	αDC1 Vaccine + Chemokine	I/II	168*	Recruiting

	Modulatory Regimen (CKM) as Adjuvant Treatment of Peritoneal Surface Malignancies			
NCT00003263	Cisplatin, Interferon Alfa, Surgery, and Radiation Therapy in Treating Patients With Malignant Pleural Mesothelioma	I	6	Completed, but no results posted on ClinicalTrials.gov
NCT01503177	Intrapleural Measles Virus Therapy in Patients With Malignant Pleural Mesothelioma	I	36*	Recruiting

NCT02395679	Dendritic Cells Loaded With Allogeneous Cell Lysate in Mesothelioma Patients (MesoCancerVa)	I	9*	Unknown
NCT00002475	Cyclophosphamide Plus Vaccine Therapy in Treating Patients With Advanced Cancer	II	40*	Completed, but no results posted on ClinicalTrials.gov
NCT01241682	Dendritic Cell-based Immunotherapy Combined With Low-dose	I	10	Completed – results indicate that the treatment was safe, with the only side

	Cyclophosphamide in Patients With Malignant Mesothelioma (PMR-MM-002)			effect being moderate fever. Overall survival data was promising, with 70% of patients surviving for at least two years and two patients alive after 50 and 66 months [44]
NCT02661100	A Trial of CDX-1401 in Combination With Poly-ICLC and Pembrolizumab, in Previously Treated Advanced Solid Tumor Patients	I/II	N/A	Withdrawn prior to enrolment

NCT01997190	Intrapleural AdV-tk Therapy in Patients With Malignant Pleural Effusion (MpeTK01)	I	19*	Ongoing – results presented at ASCO 2016 indicate that Phase II studies may be warranted and that the intrapleural treatment can be safely administered, though side effects such as cytokine release syndrome, fever, nausea and chills were seen in some patients [45]
NCT00423254	Safety and Immune Response to a Multi-	I	24	Completed – there was no partial or

	component Immune Based Therapy (MKC1106-PP) for Patients With Advanced Cancer.			complete response according to RECIST criteria. Further investigation for specific clinical indications may be justified given that 15 of 24 evaluable patients showed an immune response, whilst several patients showed stable disease for six months or longer [46]
NCT02714374	Safety and Effect of GL-ONC1	I	36*	Recruiting

	Administered IV With or Without Eculizumab Prior to Surgery to Patients With Solid Organ Cancers Undergoing Surgery			
NCT02661659	A Phase Ib Trial of a Maintenance Multipeptide Vaccine (S-588210) in Patients With Unresectable Malignant Pleural Mesothelioma Without Progression	I	N/A	Withdrawn prior to enrolment

	After First-Line Chemotherapy			

2.6.2 Immune Checkpoint Inhibition

Within the healthy body, the immune system is tightly regulated both positively and negatively to ensure an appropriate immune response. Among these regulatory elements, immune checkpoints comprise a number of inhibitory pathways that serve as a way to maintain self-tolerance and minimise immune-mediated damage through modulating the length and strength of the response [47]. However, it is now apparent that this regulation is hijacked during the process of carcinogenesis and aberrantly regulated to allow cancer cells to evade immune detection. Cytotoxic T-lymphocyte-associated antigen 4 (CTLA4) and programmed cell death protein 1 (PD1) are two key negative regulators of the immune system and thus their blockage through antibodies or other treatments is currently under a significant degree of investigation as it is thought that if their expression is reduced, immune-mediated tumour death could be enhanced (Figure 2) [47].

Immune checkpoint blockade has shown success in some other cancer types such as melanoma, where blockage of CTLA4 through ipilimumab (a mAb against CTLA4) has been approved for its treatment [21]. Similar to ipilimumab is tremelimumab, another mAb against CTLA4, which remains under investigation in many clinical trials though it is not yet approved for use in the clinic [5]. After the success of CTLA4 inhibition, the effects of PD1 inhibition (through either targeting PD1 itself or its ligands) have also been assessed in clinical trials and currently pembrolizumab and nivolumab are approved for the treatment of metastatic melanoma [35,48].

Despite the application of immune checkpoint blockade in melanoma, these outcomes have not transferred to mesothelioma. One of the biggest trials for immune checkpoint blockade in mesothelioma was DETERMINE, which as of data presented at ASCO 2016 consisted of 571 patients randomised to either placebo (n=189) or treated with tremelimumab (n=382) as a second- or third-line therapy [49]. The results for this trial presented at ASCO 2016 showed that 81% of patients died, whilst no statistically significant difference in overall survival between treated (median=7.7 months) and placebo (median 7.3 months) was observed [49]. The most frequent adverse events included diarrhoea, decreased appetite, dyspnoea, fatigue and nausea [49]. Results for PD1 inhibition initially appeared more promising; preliminary results for KEYNOTE-028, a trial assessing pembrolizumab in PD1-positive mesothelioma patients demonstrated a disease control rate of 76% and the drug was generally well tolerated [50]. Updated results (published May 2017) confirmed that the drug appeared to be well tolerated, indicated that pembrolizumab "might confer anti-tumour activity in patients with PD-L1-positive malignant pleural mesothelioma" and suggested that further investigations were warranted [51].

Although CTLA4 and PD1 are two of the most investigated markers, there are numerous other immune regulators which offer therapeutic potential. Among these are LAG3, TIM3, BTLA, 2B4, TIGIT and CD160 [52-54]. These are all involved in immune regulation in multiple ways; for instance LAG3 exerts a negative effect on T cell activation and effector functions via inhibition of CD4-dependent downstream signalling. Additionally, LAG3 blockade has been demonstrated to lead to a reduction in the activity of immunosuppressive T regulatory cells [54], which distinguishes it from other targets such as CTLA4. Detailed coverage of the molecular signalling and potential therapeutic benefit of these and other markers have been comprehensively reviewed by Catakovic and colleagues [54]. To date, according to ClinicalTrials.gov, it appears that there are no trials for these targets in mesothelioma. However, at ASCO 2017 data on screening of PD-L1, PD-L2 and TIM3 from

329 patients was presented which indicated that although co-expression can occur, these expressions were mutually exclusive in a large proportion of patients [52]. Therefore it has been argued that although CTLA4 and PD1 are among the most investigated targets, the role of these less well-understood markers and their clinical potential should be fully elucidated [52,53]

Numerous trials have been completed or are ongoing using different immune checkpoint inhibitors in mesothelioma, examples of which are shown in Table 2:

Table 2: Examples of clinical trials relating to immune checkpoint blockade in mesothelioma. An asterisk (*) indicates that the number of patients is the projected accrual, not the number actually enrolled.

Clinical Trial Identifier	**Study Title**	**Phase**	**Number of Patients**	**Status/Outcome of Study**
NCT01843374	Randomized, Double-blind Study Comparing Tremelimumab to Placebo in Subjects With Unresectable Malignant Mesothelioma (Tremelimumab)	II	658	Ongoing – preliminary results presented at ASCO were detailed previously, but in brief 81% of patients died and there was no statistically significant difference in overall survival between placebo and tremelimumab [49]

NCT03075527	A Phase 2 Study of Durvalumab in Combination With Tremelimumab in Malignant Pleural Mesothelioma	II	40*	Recruiting
NCT02588131	A Study of Tremelimumab Combined With the Anti-PD-L1 MEDI4736 Antibody in Malignant Mesothelioma (NIBIT-MESO-1)	II	40*	Recruiting
NCT02592551	MEDI4736 Or MEDI4736 +	II	20*	Recruiting

	Tremelimumab In Surgically Resectable Malignant Pleural Mesothelioma			
NCT02141347	Ph1 to Assess Safety, Tolerability of Tremelimumab/ Tremelimumab+MEDI4736 in Japanese Solid Malignancies/ Mesothelioma	I	73	Completed, but no results posted on ClinicalTrials.gov
NCT01655888	The Anti-CTLA-4 Monoclonal Antibody Tremelimumab in Malignant Mesothelioma	II	29	Unknown – results published in 2015 indicate that one patient (3%) achieved a partial response and

				11 (38%) achieved disease control rate with a good safety profile. The most frequent treatment-related adverse effects were gastrointestinal, fever and dermatological [55]
NCT01649024	A Clinical Study With Tremelimumab as Monotherapy in Malignant Mesothelioma	II	29	Unknown – results published in 2013 indicate that no patients achieved a complete response, whilst only two (7%) had a durable partial

				response and the primary endpoint was not reached. Treatment-emergent adverse effects were observed in 93% of patients [56]
NCT03126110	Phase 1/2 Study Exploring the Safety, Tolerability, and Efficacy of INCAGN01876 Combined With Immune Therapies in Advanced or	I/II	450*	Recruiting

	Metastatic Malignancies			
NCT02899299	Study of Nivolumab Combined With Ipilimumab Versus Pemetrexed and Cisplatin or Carboplatin as First Line Therapy in Unresectable Pleural Mesothelioma Patients (CheckMate743)	III	600*	Recruiting
NCT03048474	Ipilimumab and Nivolumab in the Treatment of	II	33*	Recruiting

	Malignant Pleural Mesothelioma (INITIATE)			
NCT02716272	Nivolumab Monotherapy or Nivolumab Plus Ipilimumab, for Unresectable Malignant Pleural Mesothelioma (MPM) Patients (MAPS2)	II	125	Ongoing
NCT02054806	Study of Pembrolizumab (MK-3475) in Participants With Advanced Solid	I	477	Recruiting/Ongoing – results published in May 2017 indicate that the drug was well tolerated, might

	Tumors (MK-3475-028/KEYNOTE-28)			confer an anti-tumour effect and that further investigations were warranted [51]
NCT02707666	A Pilot Window-Of-Opportunity Study of the Anti-PD-1 Antibody Pembrolizumab in Patients With Resectable Malignant Pleural Mesothelioma	I	15*	Recruiting
NCT02991482	PembROlizuMab Immunotherapy Versus Standard Chemotherapy for	III	142*	Not yet recruiting

	Advanced prE-treated Malignant Pleural Mesothelioma (PROMISE-meso)			
NCT02784171	Pembrolizumab in Patients With Advanced Malignant Pleural Mesothelioma	II	126*	Recruiting
NCT02959463	Adjuvant Pembrolizumab After Radiation Therapy for Lung-Intact Malignant Pleural Mesothelioma	I	24*	Recruiting
NCT03126630	Pembrolizumab With or Without Anetumab	I/II	134*	Recruiting

	Ravtansine in Treating Patients With Mesothelin-Positive Pleural Mesothelioma			
NCT02399371	Pembrolizumab in Treating Patients With Malignant Mesothelioma	II	65*	Recruiting
NCT02758587	Study of FAK (Defactinib) and PD-1 (Pembrolizumab) Inhibition in Advanced Solid Malignancies (FAK-PD1)	I/II	59*	Recruiting

NCT02661100	A Trial of CDX-1401 in Combination With Poly-ICLC and Pembrolizumab, in Previously Treated Advanced Solid Tumor Patients	I/II	N/A	Withdrawn prior to enrolment
NCT02628067	Study of Pembrolizumab (MK-3475) in Participants With Advanced Solid Tumors (MK-3475-158/KEYNOTE-158)	II	1350*	Recruiting
NCT02856425	Trial Of Pembrolizumab And	I	18*	Recruiting

	Nintedanib (PEMBIB)			
NCT02419495	Phase IB of Selinexor in Combination With Standard Chemotherapy in Patients With Advanced Malignancies	I	588*	Recruiting
NCT03063450	CheckpOiNt Blockade For Inhibition of Relapsed Mesothelioma (CONFIRM)	III	336*	Recruiting
NCT02341625	A Study of BMS-986148 in Patients	I/II	407*	Recruiting

	With Select Advanced Solid Tumors			
NCT03071757	A Study of the Safety, Tolerability and Pharmacokinetics of ABBV-368 as a Single Agent and Combination in Subjects With Locally Advanced or Metastatic Solid Tumors	I	100*	Recruiting
NCT02497508	Nivolumab in Patients With Recurrent Malignant	II	33	Ongoing

	Mesothelioma (NivoMes)			
NCT02458638	A Study of Atezolizumab in Advanced Solid Tumors	II	725*	Recruiting
NCT03074513	Atezolizumab and Bevacizumab in Rare Solid Tumors	II	160*	Recruiting
NCT01772004	Avelumab in Metastatic or Locally Advanced Solid Tumors (JAVELIN Solid Tumor)	I	1706*	Recruiting – preliminary safety data indicates an acceptable safety profile in pre-treated populations [57]

2.6.3 *Oncolytic Virotherapy*

Oncolytic viruses are either genetically engineered or naturally occurring viruses that preferentially target tumour cells over healthy cells. In addition to this, they are capable of impairing abnormal vasculature and promote immune functions. Due to its potential, this approach has garnered increased interest in recent years, though questions over issues such as administration routes and the injection frequency required to achieve a therapeutic effect and theorising how the oncolytic viruses may evade immune detection remain [58]. Despite these issues, the challenging clinical management of diseases such as mesothelioma makes such diseases suitable candidates for innovative therapies. Particularly for MPM, treatment of pleural effusions requires access to the pleural cavity, and thus local injections of oncolytic viruses is a possibility [58].

The application of oncolytic virotherapy to the treatment of mesothelioma has been comprehensively reviewed by Boisgerault and colleagues in 2015 [58]. There are numerous types of oncolytic viruses such as herpesvirus, adenovirus and RNA viruses. An ongoing Phase I/II study sponsored by Virttu Biologics Limited (ClinicalTrials.gov identifier NCT01721018) is assessing the effect of intrapleural administration of HSV1716, a mutated herpes simplex virus. 12 MPM patients have been enrolled but the study's results have not been posted yet (estimated completion is June 2017). Multiple trials have been initiated or are completed relating to virotherapy in mesothelioma (summarised in Table 1 in the manuscript by Boisgerault and colleagues [58]). A new Phase I/II trial (registered July 2016, ClinicalTrials.gov identifier NCT02879669) aims to assess the effects of ONCOS-12, an oncolytic adenovirus that primes the immune system in the treatment of unresectable MPM. The study aims to recruit 30 patients and its expected primary completion date is December

2018. Another new Phase I study (registered March 2016, ClinicalTrials.gov identifier NCT02714374) aims to investigate GL-ONC1, a genetically modified vaccinia virus that may have an anti-tumour effect. The study aims to recruit 36 patients with solid tumours and has an estimated primary completion date of March 2018, with the overall study being completed in March 2020. Thus, in addition to the numerous trials described by Boisgerault and colleagues [58], trials remain ongoing and therefore oncolytic virotherapy is still under a degree of investigation.

2.6.4 Cytokine Administration

Cytokine administration was among the first therapeutic interventions in the treatment of cancer, though its use as a monotherapy is less common and it is now generally used in combination therapy [39]. Cytokine administration aims to provide a short-term "boost" to anti-tumour effects through temporary stimulation of the host's immune system [35]. Numerous cytokines are utilised for this [39] but a common one is interleukin-2 (IL2). An early Phase II study with intrapleural IL2 administration with 22 mesothelioma patients indicated that there may be some beneficial effects of cytokine administration, with median survival of responders (28 months) being significantly longer than the median survival for non-responders (8 months) [53,59]. However, this initial positive result was hampered by further research and trials which demonstrated high treatment-related toxicity and side effects [53].

Despite the apparent failure of IL2 administration as a monotherapy, it is currently under investigation as part of multimodality therapy. A study sponsored by the University Health

Network, Toronto is currently recruiting participants for a Phase I/II study that aims to examine the effect of tumour-infiltrating lymphocytes and low-dose IL2 therapy in pleural mesothelioma patients following treatment with cyclophosphamide and fludarabine. The study (ClinicalTrials.gov identifier NCT02414945) aims to recruit ten patients and has an estimated completion of November 2025.

2.6.5 Monoclonal Antibody Therapy

The overarching principle behind the use of mAbs in cancer therapy is to target cancer-specific or cancer-associated antigens which the antibody binds to, ultimately resulting in an anti-tumour effect through a variety of mechanisms [39]. For mesothelioma, common antibody targets include mesothelin (highly expressed in mesothelioma) and TGF-β [35]. Monoclonal antibodies may be combined with other therapeutics to serve, for example, as vehicles for drugs.

Mesothelin represents an interesting target as its high level of expression in mesothelioma results in it commonly being used as a serum biomarker for mesothelioma diagnosis [60] and thus there are several trials investigating its potential as a biomarker. Antibodies targeting mesothelin function through the antibody dependent cellular cytotoxicity (ADCC) mechanism, which confers specificity against mesothioloma tumors. In particular anti-mesothelin antibodies recognize specifically the antigen (mesothelin) via their Fab domain whereas via their Fc domain they bind receptors on NK cells and recruit these cytotoxic cells in the neoplastic tissue thereby killing tumor cells overexpressing mesothelin. Comparatively, TGF- β is an interesting protein as in normal epithelial cells it is a potent growth inhibitor and promotes cellular differentiation. However, in the case of tumour progression, cancer cells lose their ability to respond to TGF-β and thus TGF-β becomes a key stimulator of angiogenesis, affects the microenvironment and causes immunosuppression [61]. Mesothelin and TGF-β are both thus interesting targets and there are numerous trials targeting them, with some examples of initiated, ongoing or completed trials listed in Table 3 below:

Table 3: Examples of clinical trials relating to the use of monoclonal antibodies in mesothelioma. An asterisk (*) indicates that the number of patients is the projected accrual, not the number actually enrolled.

Clinical Trial Identifier	Study Title	Phase	Number of Patients	Status/Outcome of Study
NCT00325494	A Study of MORAb-009 in Subjects With Pancreatic Cancer, Mesothelioma, or Certain Types of Ovarian or Lung Cancer	I	24	Completed – results indicate that MORAb-009 (also known as amatuximab) was well tolerated and that of the 24 patients, 11 had stable disease [62]
NCT00738582	An Efficacy Study of MORAb-009 (Amatuximab) in Subjects With Pleural	II	89	Completed, but no results posted on ClinicalTrials.gov

	Mesothelioma (Amatuximab)			
NCT01413451	Amatuximab for High Mesothelin Cancers	I	7	Terminated
NCT01521325	A Single-Dose Pilot Study of Radiolabeled Amatuximab (MORAb-009) in Mesothelin Over Expressing Cancers	I	6	Completed, but no results posted on ClinicalTrials.gov
NCT02357147	Study of the Safety and Efficacy of Amatuximab in Combination With Pemetrexed and Cisplatin in Subjects	II	108	Ongoing

	With Unresectable Malignant Pleural Mesothelioma (MPM). (ARTEMIS)			
NCT01112293	Anti-TGF Monoclonal Antibody (GC1008) in Relapsed Malignant Pleural Mesothelioma	II	13	Completed – results indicate all patients tolerated the therapy, in addition to the fact that three patients showed stable disease at three months, although no partial or complete radiographic responses were observed [61]

NCT01105390	AMG 102, Pemetrexed Disodium, and Cisplatin in Treating Patients With Malignant Pleural Mesothelioma	II	N/A	Withdrawn prior to enrolment
NCT01160458	Phase II Study of IMC-A12 in Patients With Mesothelioma Who Have Been Previously Treated With Chemotherapy	II	20	Ongoing
NCT01445392	SS1(dsFV)PE38 Plus Pemetrexed and Cisplatin to Treat	I	24	Completed – results indicate that SS1P when given with

	Malignant Pleural Mesothelioma			pemetrexed and cisplatin was safe, well tolerated, and demonstrated an anti-tumour effect in unresectable advanced MPM [63]
NCT01898156	Two-Part, Open-Label, Multi-Center, Phase 1/2 Study of BIW-8962 as Monotherapy in Subjects With Lung Cancer	I/II	37	Terminated

NCT00996567	A Study of Cetuximab Combined With Cisplatin or Carboplatin/Pemetrexed as First Line Treatment in Patients With Malignant Pleural Mesothelioma. (MesoMab)	II	22	Completed, but no results posted on ClinicalTrials.gov
NCT03126630	Pembrolizumab With or Without Anetumab Ravtansine in Treating Patients With Mesothelin-	I/II	134*	Not yet recruiting

	Positive Pleural Mesothelioma			
NCT01362790	SS1P and Pentostatin Plus Cyclophosphamide for Mesothelioma	I/II	55	Ongoing
NCT01355965	Autologous Redirected RNA Meso-CIR T Cells	I	18	Ongoing – initial results indicate that the treatment was safe, feasible, and without clear evidence of off-target cytotoxicity, in addition to an anti-tumour effect [64]

NCT02341625	A Study of BMS-986148 in Patients With Select Advanced Solid Tumors	I/II	407*	Recruiting
NCT02369198	MesomiR 1: A Phase I Study of TargomiRs as 2nd or 3rd Line Treatment for Patients With Recurrent MPM and NSCLC	I	27	Completed
NCT01134250	Combination Therapy of F16IL2 and Paclitaxel in Solid Tumour Patients	I/II	96*	Unknown

NCT03007030	Trial of Adcetris in CD30+ Malignant Mesothelioma	II	50*	Recruiting
NCT01486368	A Phase II Study of PF-03446962 in Patients With Advanced Malignant Pleural Mesothelioma	II	17	Completed
NCT01439152	Phase I Study to Determine the Maximum Tolerable Dose of BAY94-9343 in Patients With Advanced Solid Tumors.	I	147	Ongoing – results presented at ASCO 2016 indicated adverse events to include reversible keratopathy and gastrointestinal disorders though

				serious drug-related adverse events were low and there were no drug-related deaths. Preliminary data showed durable partial responses in patients with advanced mesothelioma [65]
NCT02610140	Phase II Anetumab Ravtansine as 2nd Line Treatment for Malignant Pleural Mesothelioma (MPM)	II	248	Ongoing

NCT02639091	Phase Ib Study of Anetumab Ravtansine in Combination With Pemetrexed and Cisplatin in Mesothelin-expressing Solid Tumors	I	30*	Recruiting
NCT00024674	Study of SS1(dsFv)-PE38 (SS1P) Anti-Mesothelin Immunotoxin in Advanced Malignancies: Continuous Infusion for 10 Days	I	N/A	Withdrawn prior to enrolment

NCT00024687	Study of SS1(dsFv)-PE38 (SS1P) Anti-Mesothelin Immunotoxin in Advanced Malignancies: IV Infusion QOD x Six Doses	I	N/A	Withdrawn prior to enrolment
NCT02661100	A Trial of CDX-1401 in Combination With Poly-ICLC and Pembrolizumab, in Previously Treated Advanced Solid Tumor Patients	I/II	N/A	Withdrawn prior to enrolment

NCT03000257	A Study of ABBV-181 in Participants With Advanced Solid Tumors	I	158*	Recruiting
NCT02714374	Safety and Effect of GL-ONC1 Administered IV With or Without Eculizumab Prior to Surgery to Patients With Solid Organ Cancers Undergoing Surgery	I	36*	Recruiting
NCT02628535	Safety Study of MGD009 in B7-H3-expressing Tumors	I	114*	Recruiting

NCT02485119	Phase I Dose Escalation Study of BAY94-9343 Given by Intravenous Infusion Every 3 Weeks in Japanese Subjects With Advanced Malignancies	I	15*	Ongoing
NCT03126110	Phase 1/2 Study Exploring the Safety, Tolerability, and Efficacy of INCAGN01876 Combined With Immune Therapies in	I/II	450*	Recruiting

	Advanced or Metastatic Malignancies			

2.6.6 *Adoptive Cell Transfer*

Adoptive cell transfer is a strategy that primarily relies on immune cells that have been "educated" *ex vivo* before the application of these autologous cells to the patient. The application of adoptive cell transfer through cells such as tumour-infiltrating lymphocytes represents a promising therapeutic strategy as it is backed by preclinical support and the autologous nature of the approach may improve its efficacy [66,67]. Education of these T cells may be via the transfection or transduction of chimeric antigen receptors (CARs) to the T cells, which allows the T cell to recognise tumour cells expressing TSAs or TAAs [53]. Frequent targets for adoptive cell transfer include fibroblast activation protein (FAP) and the aforementioned mesothelin [53]. Numerous trials relating to adoptive cell transfer in mesothelioma have been initiated, are ongoing or are completed, with examples listed below in Table 4:

Table 4: Examples of clinical trials relating to the use of adoptive cell transfer in mesothelioma. An asterisk (*) indicates that the number of patients is the projected accrual, not the number actually enrolled.

Clinical Trial Identifier	**Study Title**	**Phase**	**Number of Patients**	**Status/Outcome of Study**
NCT01722149	Re-directed T Cells for the Treatment (FAP)-Positive Malignant Pleural Mesothelioma	I	6*	Recruiting
NCT02408016	Genetically Modified T Cells in Treating Patients With Stage III-IV Non-small Cell Lung Cancer or Mesothelioma	I/II	20*	Recruiting

NCT01355965	Autologous Redirected RNA Meso-CIR T Cells	I	18	Ongoing – initial results indicate that the treatment was safe, feasible, and without clear evidence of off-target cytotoxicity, in addition to an anti-tumour effect [64]
NCT02159716	CART-meso in Mesothelin Expressing Cancers	I	19	Ongoing
NCT02414269	Malignant Pleural Disease Treated With Autologous T Cells Genetically	I	24*	Recruiting

	Engineered to Target the Cancer-Cell Surface Antigen Mesothelin			
NCT03054298	CAR T Cells in Mesothelin Expressing Cancers	I	30*	Recruiting
NCT02580747	Treatment of Relapsed and/or Chemotherapy Refractory Advanced Malignancies by CART-meso	I	20*	Recruiting
NCT01583686	CAR T Cell Receptor Immunotherapy Targeting Mesothelin	I/II	136*	Recruiting

	for Patients With Metastatic Cancer			

3. Conclusions

The “orphan” status of mesothelioma in terms of the lack of beneficial treatments creates a pressing need to uncover novel therapeutics to improve the outcomes for this disease whose incidence peak is anticipated to occur in the coming years. The fact that therapies proven effective for multiple other cancer types such as tyrosine kinase inhibition have demonstrated little therapeutic benefit other than bevacizumab for newly-diagnosed mesothelioma is surprising and indicates mesothelioma as a particularly aggressive or unique tumour.

Innovative approaches such as immunotherapy have been successfully applied and approved in other cancer types, leading to the investigation of such approaches in mesothelioma, as is apparent from the large number of clinical trials that have been initiated, are ongoing, or are completed. Although there has been a significant degree of hope for the role of immunotherapy as a treatment for mesothelioma, clinical trial results remain largely disappointing. Immunotherapy for mesothelioma is likely complicated by the chronic inflammatory state that drives mesothelioma development. Different approaches to immunotherapy, includingcytokine administration or immune checkpoint blockade, have demonstrated relatively high levels of treatment-related adverse events and the therapeutic benefit remains unclear.

Despite the negative outcomes of numerous trials, it is clear that there are many approaches under investigation at different clinical trial phases. It is hoped that these investigations could yield a therapeutic benefit to alleviate the suffering of mesothelioma patients, though in addition to monitoring effects on survival (particularly overall instead of progression-free) the degree and frequency of treatment-related adverse events should be carefully monitored.

4. Expert Commentary

In recent years there has been an increased interest in developing improved therapeutics for the treatment of mesothelioma. Although undoubtedly welcome, efforts have been focussed largely on applying existing therapies from other cancers such as immune checkpoint blockade or tyrosine kinase inhibition to mesothelioma. Though such approaches have promoted improved clinical outcomes in numerous cancer types, such success has generally not been seen in mesothelioma, with most clinical trials reporting negative results.

It is therefore our opinion that the development of novel therapeutics should focus not on applying therapies from other cancers, but should instead be based on a detailed understanding of the specific factors driving the carcinogenesis and resistance of mesothelioma. In particular, it is widely established that the hypoxic microenvironment of mesothelioma can have significant influence on the gene expression profile of mesothelioma cells, with microenvironment constituents such as stromal cells playing key roles in this.

The chronic inflammation that drives mesothelioma leads to an altered immune response, which may partially explain the general lack of efficacy of immunotherapy. Similarly, the low mutational load of mesothelioma relative to other cancer types presents an additional explanation for potential reasons for therapy failure. If one also considers the difficulty to apply the immune-related response criteria to MPM and the rate and degree of side effects it is obvious that particular caution is needed before drawing conclusions. Although many trials for immunotherapeutic modalities have shown failure or limited benefit, some results from pembrolizumab still need to be validated in randomized clinical trials aiming to assess its real impact on the overall survival of patients.

Examination of the mechanisms that mesothelioma cells use to survive within their hostile microenvironment and tolerate the gene and metabolic changes that occur should be examined in detail, as elucidation of these mechanisms may provide “druggable” targets in addition to insight into altered pathways driving mesothelioma development. In our view, 3D tissue culture models such as organoids, accurate *in vitro* simulation of the microenvironment and immune system, and lastly the use of primary cells wherever possible will all facilitate our improved understanding of factors contributing towards the carcinogenesis of mesothelioma.

It has recently been demonstrated that surrogate end points for clinical trials such as progression-free survival do not fully reflect overall survival for immunotherapy trials and may often be significantly different [68]. Caution has therefore been advised when analysing results containing only progression-free survival and not overall survival data. Future trials should aim to include information on overall survival wherever possible so as to provide the most comprehensive information available.

Ultimately, despite the fact that mesothelioma is still very much an orphan disease, there has been an increased interest in recent years and we hope that this increased interest will eventually lead to improved therapies and clinical outcomes for patients. By achieving a precise understanding of mesothelioma carcinogenesis we may be able to isolate essential factors and thus identify potential routes for the development of novel therapeutics.

5. Five-Year View

Immunotherapy remains highly investigated across numerous different diseases, including mesothelioma, likely due to the success of the approach in diseases such as melanoma. Although approaches such as interleukin-2 administration have demonstrated a therapeutic effect, these benefits are limited through treatment-related toxicity and side effects. Similarly, although immune checkpoint blockade remains highly investigated, results for mesothelioma have been disappointing.

We anticipate that in the coming years immunotherapy will continue to be investigated due the high number of clinical trials (detailed in the tables of this article) that are in the recruitment or pre-recruitment stages. As suggested by Thapa and colleagues, it may be worth uncovering the biology behind immune checkpoints other than PD1 and CTLA4 and developing clinical trials for inhibitors against them as these under-investigated targets may represent novel therapeutic avenues [53].

Researching immunotherapy should take into consideration the role of the microenvironment as it is readily apparent that hypoxia and the chronic inflammation that defines mesothelioma leads to an altered immune response. Both of these factors are under increased investigation and we anticipate that elucidation of these factors may alter and improve research focus.

Lastly, the recent argument that surrogate endpoints (such as progression-free survival) do not fully reflect the endpoint of overall survival in terms of treatment effect size [68] may promote an altered approach to trials, by aiming to report overall survival and base conclusions on this rather than potentially misleading surrogate endpoints.

Key Issues

- Mesothelioma is a rare, aggressive cancer whose incidence is expected to peak in the coming years
- Current treatments do not significantly prolong survival, and clinical outcomes remain poor
- Immunotherapy is a widely-investigated approach that aims to utilise the patient's own immune system against tumours
- Numerous immunotherapeutic approaches such as peptide, DC-based and autologous or allogeneic vaccination, immune checkpoint blockade and cytokine administration exist
- Although immune checkpoint blockade has shown effect in melanoma, this approach has demonstrated a very limited benefit in mesothelioma
- In addition to monitoring of overall survival instead of progression-free survival or other surrogate endpoints, side effects of immunotherapeutic approaches should also be considered

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**Paper of considerable interest

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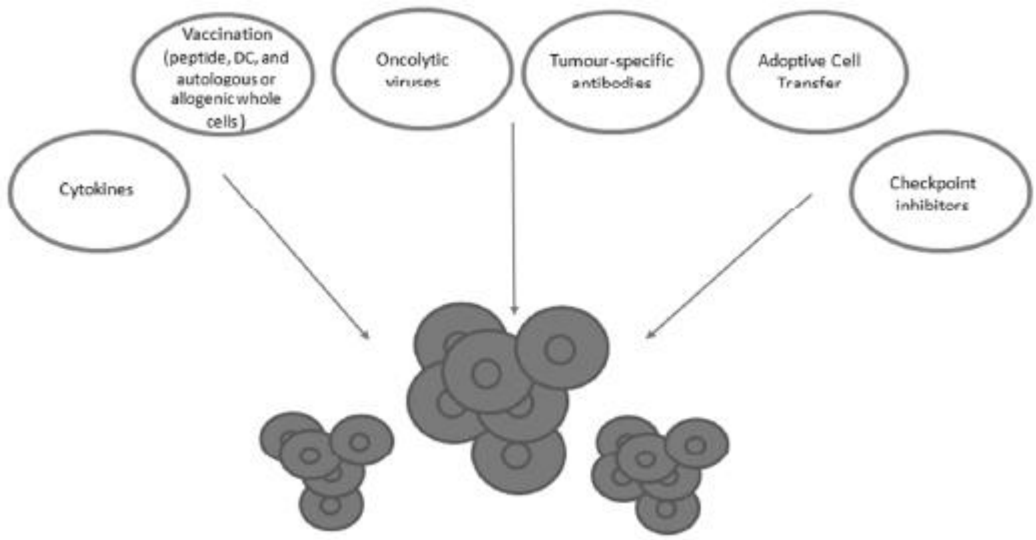

Figure 1: Overview of different immunotherapeutic strategies.

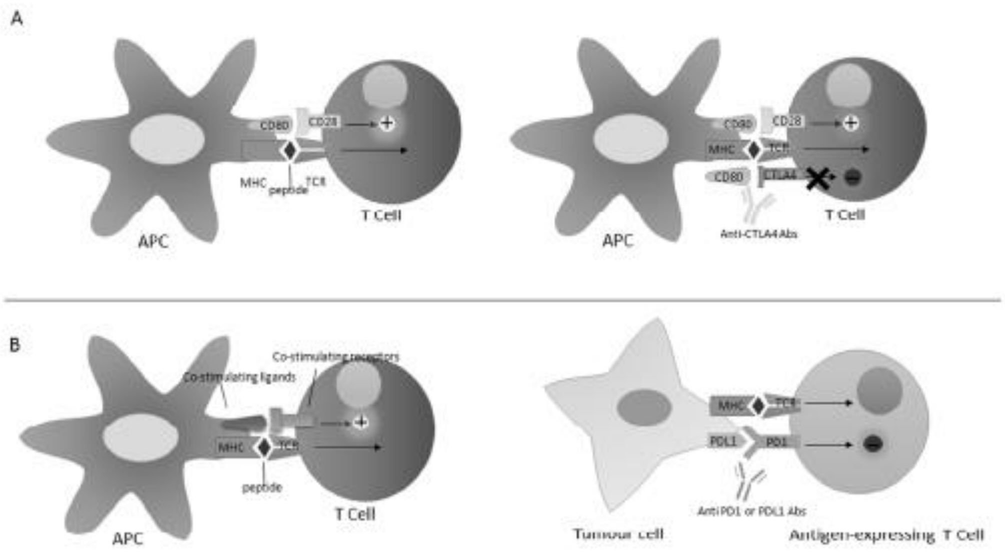

Figure 2: A) The activation of naïve T cell requires signalling from TCR (signal 1) and CD28 (signal 2) which are expressed on their surface while CTLA4 is expressed intracellularly until TCR interacts with antigen from APC cells which promotes the migration of CTLA4 on the surface to attenuate the T cell response from TCR and CD28. Anti-CTLA4 antibodies block the interaction of CTLA4 with the ligand (CD80), allowing for enhanced anti-tumour response. B) PD1 is upregulated on T cell following the activation of T cell by TCR signalling and positive signals. PD1 receptor interaction with the ligand PDL1 leads to attenuation of immune response. Anti-PD1/PDL1 antibodies block this signalling to enhance a antitumor immune response.
Abbreviations : CTLA4, cytotoxic T-lymphocyte antigen 4; PD1, programmed death 1; PDL1, programmed death ligand 1; APC, antigen presenting cell; MHC, major histocompatibility complex; TCR, T cell receptor.