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Current and prospective pharmacotherapies for the treatment of pleural mesothelioma

Abstract

Introduction

Mesothelioma is a rare asbestos-linked cancer with an expected incidence peak between 2015-2030. Therapies remain ineffective, thus developing and testing novel treatments is important for both oncologists and researchers.

Areas Covered

After describing mesothelioma and the shortcomings of current therapies, the article discusses numerous therapies in turn such as immunotherapy (passive and active), gene therapy (such as suicide gene therapy) and targeted therapy such as tyrosine kinase inhibitors. The bases for different therapies and clinical trials at different phases are also described. The article concludes by detailing possible reasons for therapy failure.

Expert Opinion

Despite the many attempts to uncover new therapeutic options, mesothelioma is still an orphan disease, complicated by factors such as the inflammatory microenvironment and low mutational load. Our opinion is that uncovering the biological mechanisms behind mesothelioma development will assist therapy development. The lack of efficacy of tyrosine kinase inhibitors and modest anti-angiogenic activity indicates a less relevant role for tumour cell proliferation and neoangiogenesis, thus the shortcut of treating mesothelioma with therapies from other cancers may be unsound. Conversely, many lines of evidence indicate that focussing on the survival mechanisms that tumour cells exploit may yield better therapeutics, particularly nutrition and cellular machinery.

1. Introduction

Mesothelioma is an uncommon form of cancer arising from mesothelial cells which line the membranes of organs including the heart (pericardial mesothelioma), testes (testicular mesothelioma), abdomen (peritoneal mesothelioma) and the lungs (pleural mesothelioma) [1]. Of these four subtypes, peritoneal and pleural mesothelioma account for the vast majority of cases in mesothelioma (upwards of 90%), whilst pleural mesothelioma is overall the most prevalent, accounting for 68-85% of all mesothelioma cases [2].

Malignant pleural mesothelioma (MPM) affects significantly more men than women, at a ratio of 4:1, usually at advanced age (>65 years old) [3]. More men than women are affected by the disease due to workplace exposure. Approximately 2500 cases of MPM occur per year in the United States per year, whilst 5000 patients in Western Europe die from the disease each year [3]. Although a rare disease, there is an urgent need to develop new therapeutics as the fatalities from the disease are expected to increase over the next few years, in part due to the long latency period (approximately 50 years) from asbestos exposure to disease onset [4, 5]. It is anticipated that the disease incidence will plateau between 2015-2030, and given the poor clinical outcome of current treatments there is a clear, urgent need to develop new therapeutics to improve patient care and address the oncoming surge of MPM cases [5].

This review will cover current treatment options for malignant pleural mesothelioma – both pharmacotherapies and other options such as surgery – in addition to discussing prospective novel therapies for the disease. A variety of topics will be discussed, including drugs used in the clinic at present such as cisplatin, pemetrexed and gemcitabine, as well as immunotherapeutic options such as tremelimumab and other immune checkpoint inhibitors such as nivolumab and pembrolizumab that are currently under investigation in early phase clinical trials. Lastly, the review closes with an Expert Opinion summarising the contents of the article and arguing the strengths and weaknesses of the different approaches discussed throughout the review.

2. Body

2.1 Current treatments for mesothelioma

As stated in the Introduction, malignant pleural mesothelioma presents an unmet challenge due to the anticipated surge in cases in the coming years and the current poor clinical outcome. There is also a high interest in this cancer due to it being largely a man-made epidemic through the use of asbestos [6]. Despite it being established that asbestos is linked to MPM development, many countries have been slow to implement its removal, thus elevating the chances of further diagnoses in the future. Further complicating the issue is the fact that the fire-retardant and insulating properties of asbestos mean that it has been used as part of the structural support of numerous buildings, and should these buildings be damaged millions of people could be exposed to it and potentially develop mesothelioma [3].

MPM is treated in different ways (including surgery, radiotherapy and chemotherapy) which may vary depending on the stage of the cancer. Stage I MPM is characterised by minimal tumour growth, isolated to the parietal pleura with possible involvement of the visceral pleura. Stage II MPM is characterised by superficial tumour growth on all pleural membranes, or involvement of the diaphragmatic muscle or lung parenchyma. Notably, Stage I and II MPM patients have a tumour that may be resectable and thus treatable by surgery [3]. However, patients are commonly diagnosed at later stages, reducing the treatability of the disease by surgery.

Stage III MPM represents the most common stage at clinical diagnosis, and represents patients whose tumour has metastasised to areas such as lymph nodes, whilst Stage IV is characterised by the tumour invading the spine or ribs, with potential distant metastases, as well as other key clinical features [3].

Unfortunately, no treatment regimen for MPM has demonstrated real capability to improve these patients' survival even though standard therapies for MPM do exist. They are of two kinds: one with more "curative" intent, and the other as palliative care to provide relief from symptoms. Opinions on suggested criteria for which approach is taken have been detailed previously [5] such as deciding that curative intent should be taken if the patient is below seventy years old, has a cancer not in the advanced stages, has no significant cardiopulmonary complications, and has no relevant accompanying disease, whilst palliative care may be employed when the patient has a poor general condition and nutritional state, has advanced stage cancer or has sarcomatoid or biphasic mesothelioma at any stage [5]. Sarcomatoid mesothelioma, although a very rare form of mesothelioma, is a notoriously difficult cancer to treat and has a very poor clinical outcome [7].

Early stages of the cancer may be treated by surgery, with the desirable outcome being complete resection of the tumour but this is applicable only for a minority of patients due to the fact that most diagnoses occur at advanced tumour stages [3, 8, 9]. Surgery may also be used as a palliative therapy, serving to reduce symptoms and eliminate the bulk of the tumour mass (this is known as cytoreduction). Multiple types of surgery are employed, such as extrapleural pneumonectomy and pleurectomy/decortication [10]. Surgery alone, for those with resectable tumours, improves clinical outcomes. However, more effective outcomes are obtained when surgery is combined with adjuvant therapy such as chemotherapy or radiotherapy, with survival increasing slightly from ten months to twenty months [8, 11, 12]. Despite this, a systematic review carried out by Papaspyros and Papaspyros indicates that results from surgery are conflicting, with some studies indicating poor survival or no difference between patients treated surgically and those not, whilst the overall thought is that surgery as part of trimodality therapy offers long-term survival [13]. However, the authors also indicated that specialised centres demonstrated better results, which may present a complication in translating observed therapeutic benefits to the wider population [13].

In addition to surgery, adjuvant chemotherapy and radiotherapy may be used in the treatment of MPM. Preclinical studies have demonstrated that radiotherapy may enhance the efficacy of an immune checkpoint inhibitor, however data remains largely preliminary [8, 14]. Ultimately, single-modality therapy is generally less effective than multimodal therapy. Adjuvant chemotherapy is also supposed to target distant metastases [9].

The cornerstone of chemotherapy for MPM is platinum-based drugs such as cis-platinum, which are often in combination with anti-folate agents as first-line therapy for advanced stage MPM when the tumour cannot be resected [15]. However, although some patients respond to this therapy there is no standard second-line therapy [16]. Other chemotherapy drugs utilised in the treatment of MPM include etoposide, doxorubicin, pemetrexed and gemcitabine. Generally, combination treatments of different chemotherapeutic drugs have shown more effective outcomes [17]. One combination that has been shown to achieve a slight improvement of survival is combination of pemetrexed with cisplatin [18, 19]. However, although this study was an improvement in survival (of 2.8 months), successful therapy for MPM is obviously still lacking.

The lack of effective therapy for MPM highlights a very clear need to develop novel compounds and treatments to address the poor survival rate. There are several emerging therapies for mesothelioma which are at different stages of clinical development and usage, one of which is immunotherapy.

2.2 Immunotherapy

Immunotherapy in general refers to the idea of killing the cancer cells not by drugs targeting the cancer cells, but by using drugs or other therapeutic agents to facilitate immune-mediated anti-tumour effects. For MPM, it has been shown that lymphocyte infiltration to the tumour correlated with better prognosis in patients, highlighting the justification and potential of harnessing the power of the immune system [20, 21]. A detailed review on different approaches to immunotherapy for MPM can be found by Grégoire (2010) [21].

Before developing immunotherapeutic treatments for mesothelioma, it is important to first understand the immunophenotype of mesothelioma patients. Studies have shown that although leukocyte counts in patients were not altered overall, there was a shift in the subtypes that

promoted tumour growth – for example there was a marked reduction in the levels of cytotoxic t-lymphocytes [21, 22]. It has been shown in several cases that lymphocytes infiltrate the solid mesothelioma tumour and that immune cell-tumour associations are also present in pleural effusions; however, despite this, immune systems of patients are often tolerant towards the cancer growth [21, 23]. Thus enhanced understanding of the reasons behind this is crucially important prior to designing immunotherapeutic agents, so as to target the facets of the immune system that are over- or underactive.

The immune response can be harnessed for therapeutic targets through passive immunotherapy and active immunotherapy. Passive immunotherapy has been described as an approach whereby effectors are isolated, "trained" *in vitro* and then re-injected into the patient to promote an anti-tumour effect. By contrast, active or adaptive immunotherapy refers to the approach of stimulating the immune system (i.e. through antigen presentation), thus triggering an immune response against the cancer. [21]. Generally, one problem with passive immunotherapy is that it is probable that the therapeutic benefit will be short-term, whereas active immunotherapy may have a more long-term approach to disease control [24].

2.2.1 Passive Immunotherapy

There are numerous approaches to passive immunotherapy such as the use of cytokines, monoclonal antibodies, and activated T-lymphocytes [18, 21]. It has been shown that cytokines such as interleukins stimulate the immune system against viruses and tumours, and it is hypothesised that this stimulation could be harvested to reduce tumour growth. One phase II study utilised interleukin-2 treatment for mesothelioma patients and found that those who responded to therapy had a statistically significant increase in median survival compared to non-responders [25]. However, conflicting findings on interleukin-2 treatment have been reported [18], potentially due to different administration methods, and toxicity and side-effects of interleukin-2 treatment have been reported [26] This and the conflicting reports provide a clear example of the challenge of harnessing the power of the immune system.

Although immune checkpoints are crucial within a healthy body for regulating self-tolerance and protecting healthy tissues during the immune response, it is increasingly clear that immune checkpoints are hijacked during the process of cancer development – for comprehensive coverage of blocking immune checkpoints in cancer as therapy, see Pardoll (2012) [27]. In brief, tumour resistance to the immune system typically arises through overexpression of inhibitory ligands that blunt T-cell effector functions, with this overexpression occurring either on the tumour cells or on other cells within the tumour microenvironment. In contrast, however, tumour-mediated immune evasion is not generally due to overexpression of factors that stimulate T-cell activation [27].

Therefore one approach that is currently receiving a significant amount of attention is the use of immune checkpoint inhibitors. .Although there was promising data (and FDA approval) for other cancer types [28], this success has not yet transferred to mesothelioma. There is much focus on blockage of PD-1 (programmed cell death protein 1) and CTLA-4 (cytotoxic T-lymphocyte associated protein 4) which are two key negative regulators of the immune system [28]. CTLA-4 is under particular scrutiny due to the fact that ipilimumab, another antibody against it, has been approved for the treatment of melanoma [29]. Immune checkpoint blockade in mesothelioma has been comprehensively reviewed by Marcq and colleagues [30]

Tremelimumab is a monoclonal antibody against CTLA-4 (similar to ipilimumab), which is currently under investigation in clinical trials. Unfortunately, an announcement by AstraZeneca stated that tremelimumab as a monotherapy does not significantly improve survival and that the end point of the study was not reached [31]. At the American Society of Clinical Oncology 2016 meeting, data on the DETERMINE trial (ClinicalTrials.gov identifier NCT01843374) was presented, consisting of 571 patients either untreated or treated with tremelimumab as a second or third-line therapy [32]. Unfortunately again, 81% of the patients died and there was no statistically significant difference between placebo and treated patients in terms of survival. Ultimately despite the sound scientific justification the use of monoclonal antibodies to immune check point inhibitors is still in its early stages for MPM and thus requires significantly more research (especially randomized clinical trials exploring the impact of these drugs on patients' overall survival) so as to not to misinform those who suffer from this disease, both directly and indirectly.

PD-1, the other primary immune checkpoint marker of interest, has also been under investigation. Pembrolizumab is a monoclonal antibody that blocks the interaction of PD-1 with its ligand, which should lead to a removal of the inhibition of T-cell activity against the cancer. The findings of a clinical trial (KEYNOTE-028) demonstrated that the drug was well-tolerated by patients and demonstrated a robust anti-tumour effect in patients with PD-1 ligand-positive MPM [33]. Though promising, and although this highlights potential therapeutic use of immunotherapy, antibodies against other targets have shown less positive results.

The potential of combination immunotherapy against multiple markers has also been assessed. Another PD-1 inhibitor is nivolumab, which again has been approved for treatment of melanoma [34], though its use in mesothelioma is less well-established. One phase II clinical trial (NCT02716272) is investigating the combination of nivolumab (to inhibit PD-1) with ipilimumab (to inhibit CTLA-4) in mesothelioma, though results are yet to be released. Combination of nivolumab with ipilimumab has already been carried out for other cancer types, providing the basis for this approach [35]. Other clinical trials investigating nivolumab in mesothelioma are NCT02341625, NCT02497508 and NCT02899299, though these are all in the recruitment stage or are ongoing. Combination immunotherapy for melanoma, using nivolumab and ipilimumab, demonstrated high rates of side-effects (with them occurring in 55% of patients) at grade three or four [36]. This thus also represents an additional factor when investigating the potential of combination immunotherapy in mesothelioma.

Although monoclonal antibody therapy is currently a "hot-topic" as an approach to immunotherapy, there are many other possibilities such as active immunotherapy. Active immunotherapy is the therapeutic approach whereby the aim is the education and activation of the immune system to attack cancer cells. There are different strategies that have been introduced in the clinical setting for MPM and they have raised the attention of medical professionals, being now in the centre of many discussions.

2.2.2 Active Immunotherapy

Active immunotherapy may involve therapeutic vaccines, with peptide and cell vaccines being approaches investigated under clinical examination. It has been demonstrated in an early clinical trial that Wilms tumor 1 (WT1), highly expressed in mesothelioma, when used as peptide vaccination induced quantifiable specific immune responses, although no clinical improvement was seen [37]. Two clinical trials are currently ongoing using a derived product of WT1 alone or in combination with chemotherapy (NCT01890980 and NCT01265433). Other approaches include the use of modified bacterial organisms such as *L. monocytogenes*

expressing mesothelin, which is overexpressed in mesothelioma and which may be involved in tumour invasion [38]. This cancer vaccine (CRS-207) has been developed to promote an immune response against the tumour-associated antigen mesothelin and is currently part of an ongoing trial (NCT01675765).

Cell vaccines use dendritic cells loaded with tumour-associated antigens; the technique consists of *ex vivo* manipulation of these cells obtained by peripheral blood precursors and modification with a tumour-specific antigen, and then vaccinating the patient with these cells [39]. There are several clinical trials and a few are completed (NCT00280982 and NCT01241682), whilst others are recruiting or their status is unknown (NCT02395679, NCT02649829 NCT01291420). The phase 1 trial NCT00280982 showed that the vaccine was feasible, well-tolerated, and cytotoxic activity against autologous tumour cells was detected in a subgroup of patients [40].

2.3 Gene Therapy

One other mode of therapy which is attracting many researchers is gene therapy, which is a new strategy consisting of genetic manipulation for a therapeutic purpose. It has been shown that several genes are modified in mesothelioma and numerous preclinical studies using different genes and vector systems demonstrated a therapeutic effect with promising results for the clinical setting. Numerous vector systems are available for gene therapy, including (but not limited to) adenoviruses, vaccinia viruses and non-viral options such as antisense oligonucleotide therapy [41, 42].

Adenoviruses represent the most common vector within gene therapy, and have previously been applied in both preclinical and clinical studies in mesothelioma. One study on immunocompetent Fischer rats demonstrated that the herpes simplex virus-thymidine kinase gene when carried by an adenovirus vector resulted in a reduction in tumour burden, though the authors also stated that only small increments in survival were seen [43]. However, a similar approach was later utilised at a Phase I trial, detailed below [44]. The use of vaccinia vectors has also been shown, with vaccinia-vector-cytokine constructs that were intratumourally administered demonstrating tumour regression [45]. Non-viral approaches to gene therapy have been demonstrated to be effective in mesothelioma cell lines, with Smythe and colleagues demonstrating that antisense oligonucleotides against Bcl-xL, an anti-apoptotic member of the Bcl-2 family, promoted apoptosis with or without liposomal delivery [42].

One form of gene therapy is suicide gene therapy, which uses a virus to deliver a transgene which encodes for a specific enzyme that metabolises prodrugs into toxic metabolites, leading to tumour cell death or "suicide" [46]. A Phase I trial investigated the potential of suicide gene therapy in mesothelioma [44]. The intrapleural administration of this treatment was safe and well tolerated in mesothelioma patients. 34 patients were enrolled but only 2 resulted in long-term durable responses, though as a Phase I study it is too preliminary to draw any conclusions.

As previously mentioned, cytokines represent a potential route through which therapy can be improved. Cytokine gene therapy is a genetic manipulation strategy which uses a viral vector encoding a specific cytokine (such as interleukin-2 [IL-2], IL-12, tumour necrosis factor [TNF] or interferons [INF -α, β, or γ]) to increase their expression, triggering a direct cytotoxic effect on tumour cells [47]. This approach has the advantage of reducing toxicity and increases the local concentration of these molecules. A phase I study was conducted to evaluate the safety and

feasibility of single-dose intrapleural IFN-β gene transfer using an adenoviral vector (Ad.IFN-β) in mesothelioma patients, which showed that it was generally well-tolerated and that transient lymphopenia was the most common side-effect [48]. Antitumour immune responses were seen in 70% of the patients, whilst 40% of patients showed a meaningful clinical response (as assessed by disease stability and/or regression). Two clinical trials (Phase I) as gene therapy alone for MPM patients have been under assessment and their status is either ongoing or unknown (NCT00299962, NCT00066404).

Another gene therapy strategy is using genetically modified T cells engineered with lentiviral or retroviral vectors to produce T-cell receptors that will specifically attack cancer cells [49]. An *in vivo* study showed that antimesothelin-engineered T-cells recognised mesothelin-expressing cells and induced significant tumour regression [50]. A current phase I study "Autologous Redirected RNA Meso-CIR T Cells" is ongoing (NCT01355965). Another target that can be attractive for gene therapy is fibroblast activation protein (FAP) since it appears to be mainly expressed on the surface of reactive tumour-associated fibroblasts as well cancer cells. Schuberth and colleagues demonstrated that re-directed T cells specific for FAP were cytotoxic towards FAP positive targets *in vitro* and *in vivo* [51], supporting a phase I trial that is currently recruiting to evaluate the safety of a single administration of adoptively transferred FAP-specific re-directed T cells (NCT01722149).

2.4 Targeted Therapy

Although the molecular mechanisms involved in MPM are still unclear, in recent years promising pathways have attracted the attention of translational medicine researchers, and as a result several drugs are in the development pipeline and at various stages of preclinical and clinical studies. The main molecules under investigation are responsible for molecular signalling and the aforementioned immune response. Examples of approaches include targeted therapy (direct action against cancer-specific molecules and signalling pathways, resulting in limited nonspecific toxicity), tyrosine-kinase inhibitors, epidermal growth factor receptor (EGFR) targeting, vascular endothelial growth factor (VEGF) targeting and RNA targeting.

Recently small molecule inhibitors, which are able to pass through the membrane and interact with the intracellular domain of receptors and downstream intracellular signalling, have been studied, such as tyrosine kinase inhibitors (TKIs) [52].

2.4.1 Tyrosine Kinase Inhibitors Against EGFR

TKIs are primarily effective in the targeted treatment of various malignancies and most of them act as competitor with the ATP binding site of the catalytic domain of numerous tyrosine kinases [53]. TKIs are oral drugs with an established safety profile and can be efficiently combined with other forms of treatment including chemotherapy or radiation therapy [54]. Numerous TKIs have showed an effective antitumor activity in various cancer types and have been approved for the clinic. TKIs are currently under investigation in mesothelioma.

EGFR is one such target of TKIs, which is involved in downstream signalling pathways related to oncogenes and activates the RAS/RAF/MAPK cascade which plays a role in cell proliferation, metastasis, and invasion [55] and the PI3KCA/AKT/mTOR pathway, which determines the inhibition of apoptosis [56]. Therefore, abnormal EGFR signalling contributes to the development of a malignant phenotype in several varieties of epithelial cancers as well as MPM [57, 58]. It has been reported that EGFR is overexpressed in 60–70% of MPM tissue

specimens [59] and inhibition of EGFR-dependent signalling pathways in mesothelioma cell lines also causes reduced cell survival [57]. Gefinitib (ZD1839, Iressa), a potent TKI has been studied in a phase II study in 43 patients with unresectable disease but it was not active despite the fact that in 97% of patients EGFR was overexpressed [60]. Another EGFR TKI, erlotinib (OSI-774, Tarceva) was used in a phase II trial in previously untreated patients with MPM but single-agent erlotinib was not effective [61]. Another clinical trial of erlotinib (Tarceva) plus bevacizumab (Avastin) in previously treated patients with MPM did not show an effective response [62]. One of the major obstacles for the use of TKIs against EGFR in mesothelioma is that although EGFR is overexpressed in the majority of MPM patients, this overexpression does not correlate with clinical outcomes following use of TKIs [63]. Elucidation for clinical failure may be that mutations analysed in patients with other malignancies may not be different from MPM patients [59], or alternatively the frequency of mutation may be too low in mesothelioma patients [64], resulting in the lack of clinical response in non-selected patients.

Another class of EGFR inhibitors is anti-EGFR monoclonal antibodies (mABs), which interact with the extracellular domain of EGFR, competing with EGF ligand. Cetumixab (Ertibux) showed a therapeutic efficacy on blocking cell growth in MPM cell lines and mouse models [65] and a phase II study with cetuximab in combination with cisplatin or carboplatin/pemetrexed as first line treatment has been completed, though no study results have yet been posted (NCT00996567).

2.4.2 Tyrosine Kinase Inhibitors Against VEGF

Neoangiogenesis is a hallmark of cancer which contributes to tumour growth and metastatic dissemination [66]. It is well-established that vascular VEGF is the most powerful angiogenic promoter, released by several malignancies including MPM [67, 68]. The role of VEGF has been detected in MPM in studies, showing high levels (detected by immunohistochemistry) in the tissue specimens of patients with MPM [69] and as free circulating molecules [70]. All this evidence has highlighted that an anti-VEFG therapy could have a therapeutic effect in MPM patients.

VEGF or VEGF receptor (VEGFR) inhibitors have been used in several clinical trials as a single therapy or in combination with chemotherapy for MPM patients as reported in Table 1. Bevacizumab (Avastin), a humanized monoclonal antibody against VEGF, the most potent growth factor involved in tumour angiogenesis, was approved in the EU in 2005 for the treatment of carcinoma, non-small-cell lung cancer, colorectal cancer, carcinoma, renal cell cancer, and ovarian cancers [71-74] . Bevacizumab has been evaluated as first-line treatment in a phase II clinical trial with cisplatin and pemetrexed in patients with advanced mesothelioma. The trial failed to achieve the primary endpoint [75]. A phase III multicentre, randomized, controlled study of bevacizumab in addition to cisplatin and pemetrexed in untreated mesothelioma patients has also been carried out [76]. The overall survival was statistically significantly longer in patients treated with the triple therapy regimen than cisplatin and pemetrexed. Though statistically significant the difference was only 2.7 months, highlighting the need for further research. The majority of VEGFR tyrosine kinase inhibitors such as vatalanib (PTK787/ZK 222584), sorafenib, sunitinib, imatinib, and cediranib (AZD2171) have been studied in MPM and have shown limited or absent levels of activity, resulting in a lack of clinical benefits [77-82]. Nintedanib (BIBF 1120), an oral potent triple angiokinase inhibitor, is under investigation in two Phase II clinical trials as single treatment and in combination with pemetrexed and cisplatin followed by maintenance nintedanib compared with chemotherapy alone in patients with unresectable MPM (NCT02568449, NCT01907100, respectively).

2.4.2 Histone Deacetylase (HDAC) Inhibition

Another class of drugs that are under investigation as targeted therapy include vorinostat and belinostat, drugs with inhibitory activity against histone deacetylase (HDAC) enzymes, which control gene expression through histone modifications [83] and it has been demonstrated that HDAC inhibitors promote susceptibility of mesothelioma cell lines to tumour necrosis factor-related apoptosis-inducing ligand (TRAIL) [84]. Vorinostat, one of the common HDAC inhibitors, has shown activity against mesothelioma in phase I trials [85]. A phase III, multicentre trial (VINTAGE-014) of vorinostat versus placebo as a second-line or third-line therapy failed to show an improved overall survival [86]. A phase II trial with Belinostat (PXD 101) as second-line therapy in advanced mesothelioma did not show clinical activity [87].

2.4.3 Focal Adhesion Kinase (FAK) Inhibition

One the most frequently mutated tumour suppressor genes detected in mesothelioma cells is NF2, which inactivates merlin (the protein product of the NF2 gene) and results in an increase of the activity of focal adhesion kinase (FAK). FAK is a cytoplasmic protein, a tyrosine kinase that is involved in cell proliferation, survival, migration, invasion and cancer stem cell (CSC) renewal [88]. A study reported that FAK was overexpressed in mesothelioma cell lines and implicated in promoting invasiveness [89]. Defactinib (VS-6063) is a highly potent, selective FAK inhibitor. A phase II randomized multicenter trial (COMMAND) of defactinib in previously treated MPM was performed but the study has been terminated to lack of efficacy (NCT01870609). Recently another FAK inhibitor (GSK2256098), has been tested in a phase I study in patients with advanced cancer, including mesothelioma patients [90]. The initial pharmacologic and pharmacokinetic studies showed an acceptable and safe profile in patients with mesothelioma, in particular those harbouring merlin loss [90]. In addition, another phase I trial has been completed, though no study results have been posted yet (NCT01938443). This study investigated the effect of dose escalation of GSK2256098 in combination with trametinib, a mitogen-activated protein kinase (MEK) inhibitor in patients with advanced solid tumours, including mesothelioma.

2.4.4 Other Targeted Therapies

Bortezomib is a selective inhibitor that acts via downregulation of nuclear factor-κB and thus promotes apoptosis. A phase II study investigating the activity of Bortezomib as first-line and second-line therapy in MPM patients demonstrated a lack of clinical activity and thus did not warrant further investigations [91].

In addition to the above is BNC105P, an inhibitor that selectively blocks tubulin, which through polymerisation promotes cancer cell proliferation. It showed effective activity in preclinical and phase I studies [92]. A phase II study with BNC105P investigated its efficacy and safety as second line therapy in MPM but the response was insufficient to justify further studies [92].

Cixutumumab (Agent IMC-A12) is a humanized antibody against insulin growth factor-I receptor (IGF-IR), which in cooperation with its ligands (IGF-I) play a role in cell proliferation, invasion, and metastasis though IGF signalling pathway [93]. A study demonstrated that cixutumumab decreased the growth of mesothelioma compared to the control group in *in vivo* models [93]. A phase II clinical trial of cixutumumab in previously treated patients with MPM is currently ongoing (NCT01160458).

Another example of targeted therapy which involves RNA as a therapeutic target is ranpirnase which targets tRNA and is able to promote impaired protein synthesis and cell cycle arrest, showed that it has an antitumor activity [94]. In mesothelioma cell lines, ranpirnase inhibited cell growth, both *in vitro* and *in vivo* [95]. A phase II study with ranpirnase as single drug in untreated patients has showed that ranpirnase may have an activity and a tolerable toxicity profile and phase III trial of ranpirnase was conducted in comparison with single-agent doxorubicin showing no significant difference (only 2 months; 11.3 vs 9.1 months) [94]. Another phase III randomized study was conducted with ONCONASE (ranpirnase) plus doxorubicin versus doxorubicin alone [94]. The clinical outcomes, however, are not yet available.

Human tumour necrosis factor-alpha (hTNF-α), has an antitumor activity in many solid tumours, including malignant mesothelioma [96]. However, several studies have shown that administration of hTNF-α lead to a toxic effect, and therefore NGR-hTNF-α has been developed as a fusion protein with a cyclic tumour-homing peptide (CNGRCG), asparagine-glycine-arginine and tested in a phase II as maintenance treatment in patients with advanced MPM. At the moment this study is recruiting participants (NCT01358084). NGR015 is a randomized double-blind phase III study of NGR-hTNF-α plus Best Investigator's Choice (BIC, a choice of different chemotherapy drugs) versus placebo plus BIC as second line therapy in patients with advanced MPM is also ongoing but not recruiting participants (NCT01098266).

GC1008 is an anti–TGFβ antibody. TGFβ is involved in tumour progression due to its abilities to stimulate vessel formation, modify the stromal environment, and, mainly, promote local and systemic immunosuppression [97]. Additional investigational strategies evaluated the role of heat shock protein 90 (HSP90) inhibitor (ganetespib), enhancer of zeste homolog 2 (EZH2) inhibitor (Tazemetostat), inhibitor of the MET receptor tyrosine kinase (Tivantinib), Cancer Stem Cell (CSC) inhibitor (Napabucasin/BBI608) and TargomiRs (a mimic microRNA treatment). These trials are all active trials and some of them are recruiting (Table 1).

2.5 Conclusions

The lack of effective treatment for mesothelioma highlights an urgent need to develop novel therapeutics. It is surprising that treatments which are proven effective for other cancer types such as tyrosine kinase inhibitors do not show clinical benefit for mesothelioma. Although immunotherapy has been successfully applied to melanoma, immune checkpoint blockade requires more research before its application to treat mesothelioma due to recent clinical trials setbacks. Similarly, the failure of many clinical trials for other promising therapies remains problematic. Isolating the driving issues behind the failure of these treatments to show significant effects may assist in resolving this long-standing issue.

Possible causes for failure of these therapies at the clinical level are numerous. Immunotherapy, despite high hopes for approaches such as immune checkpoint blockade, has generally not resulted in a clinical benefit for mesothelioma. Primarily, it has been shown useful for melanoma and side-effects have been observed [28]. Resistance to immune checkpoint inhibition has been indicated to arise through stromal cells mediated by chemokine (C-X-C motif) ligand 12 (CXCL12) which promoted immune evasion, based on model of pancreatic ductal adenocarcinoma [98]. FAP has been shown to be expressed on tumour-associated fibroblasts and cancer cells, and use of human CD8+ T cells transduced with anti-FAP demonstrated growth inhibition of FAP-positive tumour cells [51], thus providing further evidence for the role of the stroma. It has been argued that once the toxicity of the immune checkpoint inhibitors reaches

acceptable levels approaches such as combination checkpoint therapy could be employed, or their role as adjuvant therapy considered [29]. However, further research is required prior to the clinic for immune checkpoint blockade in mesothelioma.

Mesothelioma is a cancer characterised by a low mutational load, which may represent an explanation for the failure of TKIs. For instance, it has been demonstrated that in peritoneal mesothelioma, patients lack the EGFR mutations that would render them sensitive to TKIs [99]. Similarly, in a patient cohort (n=38) of pleural and peritoneal mesothelioma, EGFR mutations were detected only in six (16%) patients [100]. An additional factor that may contribute towards therapy failure is the hypoxic environment that defines mesothelioma. It has been shown that hypoxia induces NOTCH1 whose inhibition promoted apoptosis in mesothelioma cells [101]. Furthermore, hypoxia has also been shown to promote motility and invasiveness of MPM cells [102]. Thus, characterisation of the effects of hypoxia on the tumour cell's gene expression profile could represent a route through which therapies could be improved.

Ultimately further research is required to improve therapeutic outcomes. A significant effort has been employed to discover new therapies for mesothelioma yet this cancer remains difficult to treat. It is possible that a focus on different pathways may yield improved outcomes, as expanded upon in the Expert Opinion.

3. Expert Opinion

Despite the attempts to find new therapeutic routes, MPM is still an "orphan" disease and the lack of treatment really improving the prognosis of these tumours is urging us to take action.

There is no doubt that any achievement for this tumour should be based on a better understanding of the genetic and biological mechanisms underlying its carcinogenesis and progression. The inflammatory microenvironment (including the stromal cells), the low mutational load, along with the marked immune suppression are just some of the features that pose as difficult hurdles to the identification of new treatments.

The asbestos fibre-dependent inflammation affects the immune response, whereas the low mutational load of MPM cells does not allow the expression on a sufficient "battery" of neo-antigens necessary to trigger a robust immune reaction. On the other hand, the role of tumour cell proliferation and neoangiogenesis looks to be significantly less relevant than in other tumours if one considers the lack of efficacy or the modest activity of the treatment of this tumour with TKIs or anti-angiogenic agents respectively. The absence of driving genes and the onset of selective pressure exerted by TKIs provide a possible explanation of the failure of these therapies for MPM and prompt us to rethink their use i.e. combined treatments and alternative pathways of growth.

Therefore it seems clear that the shortcut of treating MPM with drugs because they have shown a certain degree of activity in other tumour is likely not to be the best option.

As opposite it is imperative to explore new avenues offered by the mechanism that MPM cells exploit to survive within the hostile microenvironment and with particular attention to the type of nutrients and their cell machinery. With regard to this other authors have already shown how MPM is an extremely hypoxic tumour [101, 103, 104] and we have already demonstrated how MPM cell metabolisms is highly dependent on glycolysis, providing the evidence for targeting this metabolic reprogramming [105].

Eventually, if one considers the current front line therapy for MPM is the combination treatment of chemotherapy plus antiangiogenic agents [76] improves the survival only by three months compared to chemotherapy alone, it is clear that more research is the first mandatory step in the direction of more effective treatments for MPM.

Highlights Box

1) Current treatments for mesothelioma do not significantly enhance survival

2) Despite the application of immunotherapy in melanoma treatment, challenges remain for this therapy to be effective for mesothelioma

3) The use of targeted therapy may lead to improved clinical outcomes, however the presence of protein overexpression and use of inhibitors does not always follow through at the clinical level

4) Although neoangiogenesis and VEGF expression are undoubtedly a feature of mesothelioma, anti-VEGF/R treatments do not appear to have a clinical benefit, excepting triple combination therapy with cisplatin and pemetrexed which extends survival by 2.7 months

5) Improved understanding of the basic mechanisms mesothelioma cells use to survive in their hostile environment (with particular attention on nutrition and cellular machinery) could lead to new development pipelines and therapeutic possibilities

Table 1: Summary of completed and ongoing clinical trials in mesothelioma

Intervention	Clinical trial	N patients	Phase	Status
Immunotherapy strategies				
Immune checkpoint blockade				
Pembrolizumab	NCT02054806	477	I	ongoing
Nivolumab	NCT02716272	125	II	ongoing
	NCT02341625	407*	I/II	recruiting
	NCT02497508	33	II	ongoing
	NCT02899299	600*	III	recruiting
Tremelimumab	NCT01843374	658	II	ongoing
Wilms Tumor-1 (WT1) vaccine				
WT-1-vaccine	NCT01890980	60*	II	ongoing
Montanide	NCT01265433	31	II	ongoing
Anti mesothelin vaccine				
CRS-207	NCT01675765	60	I	ongoing
Dendritic Cell-based vaccine				
Tumour lysate-loaded autologous dendritic cells	NCT00280982	10	I	completed
	NCT01241682	10	I	completed
	NCT02395679	9*	I	unknown
	NCT02649829	20*	I/II	recruiting
	NCT01291420	10*	I/II	unknown
Gene therapy				

Adenoviral-mediated IFN-β				
BG00001	NCT00299962	18*	I	ongoing
	NCT00066404	-	I	unknown
Adoptive T Cell Immunotherapy				
Autologous T cells	NCT01355965	18	I	ongoing
Adoptive Transfer of re-directed T cells	NCT01722149	6*	I	recruiting
Tyrosine kinase inhibitors				
EGF inhibitors				
Gefinitib (ZD1839, Iressa)	NCT00025207	40	II	completed
Erlotinib (OSI-774,Tarceva)	NCT00039182	55	II	completed
	NCT00137826	37	II	completed
Cetuximab (Ertibux)	NCT00996567	22	II	completed
Anti-angiogensis inhbitors				
Vatalanib	NCT00053885	47	II	completed
Sorafenib	NCT00794859	54*	II	unknown
Sunitinib	NCT00392444	39	II	completed
Imatinib (Glicev)	NCT00402766	19	I	completed
	NCT02303899	22	II	ongoing

Cediranib (AZD2171)	NCT00243074 NCT00243074 NCT01064648	54 54 116*	II II I/II	completed completed ongoing
Nintedanib	NCT02568449 NCT02863055 NCT01907100	55 * 116* 537*	II II III	recruiting not yet recruiting recruiting
Bevacizumab	NCT00295503 NCT00651456	53 448	II III	completed completed
Histone deacetylase inhibitors				
Vorinostat (MK-0683)	NCT00128102	662	III	completed
Belinostat	NCT00365053	13	II	completed
FAK inhibitors				
Defactinib (VS-6063)	NCT01870609	344	II	terminated
GSK2256098	NCT01938443	34	I	completed
NF-κB pathway inhibitor				
Bortezomib	NCT00513877	33	II	completed
IGF-IR inhibitor				
Cixutumumab	NCT01160458	20	II	ongoing
Cytotoxic RNase				
Ranpirnase (ONCONASE)	NCT00003034	300	III	unknown

A recombinant protein hTNF-α fused with a peptide				
NGR-hTNF	NCT01358084 NCT01098266	100* 390*	II III	recruiting ongoing
Anti-TGF Monoclonal Antibody				
GC1008	NCT01112293	14	II	completed
HSP90 inhibitor				
Ganetespib	NCT01590160	27	I/II	ongoing
EZH2 inhibitor				
Tazemetostat	NCT02860286 NCT02875548	67* 300*	II II	recruiting recruiting
MET receptor tyrosine kinase inhibitor				
Tivantinib	NCT01861301 NCT02049060	18 31	II I/II	terminated ongoing
CSC inhibitor				
BBI608	NCT02347917	24	I/II	ongoing
Mimic microRNA treatment				
TargomiRs	NCT02369198	27	I	completed

* Estimated number of participants

Conflict of Interest Statement

All authors have nothing to disclose.

Table 1 Legend:

A detailed overview of numerous clinical trials across numerous therapeutic approaches in mesothelioma. Targets under investigation and the specific drug or inhibitor used are detailed, as is a hyperlink of the ClinicalTrials.gov identifier, in addition to the number of patients enrolled, the phase of the trial, and its current status.

Figure 1 Legend:

Overview of different therapeutic strategies. Closed arrows represent inhibition whilst directed arrows represent activation or stimulation.

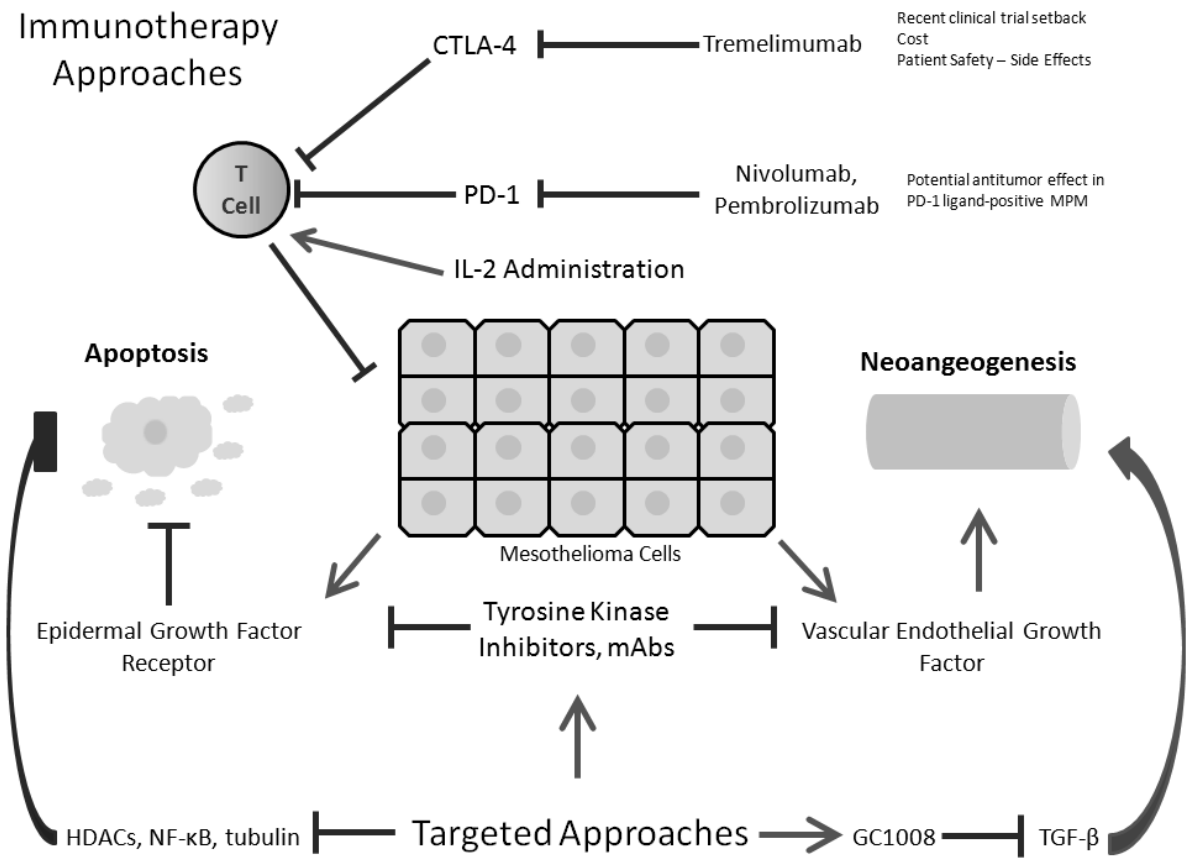

Figure 1: Overview of different therapeutic strategies. Closed arrows represent inhibition whilst directed arrows represent activation or stimulation.

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