Personalizing treatment for malignant pleural mesothelioma

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CHAPTER 3

3

A Catalogue of Treatments and Technologies for Malignant Pleural Mesothelioma

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Abstract

Malignant pleural mesothelioma is an aggressive fatal malignancy with a prognosis that has not significantly improved in the last decades. This review summarizes the current state of treatment and the various attempts that are made to improve overall survival for patients with malignant pleural mesothelioma. It also discusses technologies and protocols to test new and hopefully more effective compounds in a more individualized manner. These developments are expected to improve the prognosis for this group of patients.

Introduction

Malignant pleural mesothelioma (MPM) is an aggressive tumor that arises by neoplastic transformation of the mesothelial cells lining the pleural cavity [1–4]. In the United States, the incidence is approximately 1.05 cases per 100,000 persons [5]. In Europe, the incidence in males is higher, around 3 cases per 100,000 persons [6]. The occurrence of MPM is associated with asbestos exposure. There is a latency period of around 30-50 years between asbestos exposure and development of MPM. Even though all handling of asbestos is strictly regulated in Europe since 2005, the incidence is not expected to decrease before 2020[4–9]. In addition, outside Europe, some other developed countries have only controlled the import or still produce asbestos and less-developed countries still use or even expand the use of asbestos [5–7]. This results in an estimated 125 million asbestos-exposed people and 43,000 annual deaths due to asbestos-related diseases worldwide [4,9].

The prognosis for patients with MPM is poor. If untreated, most patients die in the first year after diagnosis [4,8]. First-line chemotherapy treatment consists of a platinum-based combination with pemetrexed [3,6,10]. This combination provides a 3-month survival benefit over cisplatin alone and a 6-month survival benefit over nontreated patients [11,12]. Around 40% of the patients with MPM respond to the combination [8,11,13,14]. For patients that do not respond to first-line chemotherapy or become progressive after treatment, there is no standard second-line regimen [6,14]. European Society for Medical Oncology Clinical Guidelines recommend enrolling eligible patients in clinical trials [6,7].

First-line treatment in mesothelioma

Almost every chemotherapy regimen has been tested in mesothelioma [15–17]. The most effective anticancer drugs are cisplatin, antimetabolites (methotrexate and pemetrexed), and anthracyclines (doxorubicin and daunorubicin). Anticancer drugs with no or minor activity in MPM are the taxanes, topoisomerase inhibitors, alkylating agents, and the vinca-alkaloids with the exception of vinorelbine. The most studied anthracycline is doxorubicin. This drug showed some activity in a number of clinical trials with varying response rates [15–17].

Until 2000, nearly all studies tested single agents. In 2002, a meta-analysis suggested that combination therapy gave better response rates than single agent therapy [18]. The first clinical trial that compared single agent therapy to a combination was performed by Vogelzang et al. [11]. This resulted in the standard first-line treatment combination of cisplatin and pemetrexed. This combination therapy combines two drugs with different activities. Cisplatin is a platinum ion with two chloride atoms and two amine groups. One chloride is first removed for a hydroxyl group yielding $PtCl(H_2O)(NH_3)_2^+$. This form binds strongly to the G basis in deoxyribonucleic acid (DNA). Here, the second chloride atom can be removed yielding a cross-linking molecule between two G bases on different DNA strands. While the

majority of interactions are between two G–G bases, other interaction, such as G-A, can also be detected. DNA strand crosslinking obviously induces substantial problems with DNA strand separation during mitosis and is supposed to be the major mechanism of cell death [19]. Pemetrexed is an antifolate that inhibits the biosynthesis of purine and pyrimide nucleotides by inhibiting the enzymes dihydrofolate reductase, thymidylate synthase (TS), and glycinamide ribonucleotide formyltransferase (RNF). Pemetrexed enters the cell by the reduced folate carrier. Folylpolyglutamate synthetase polyglutamates pemetrexed to a form that has a 100-fold greater affinity for the enzymes TS and RNF. As a result, cell growth is attenuated due to a reduced amount of DNA bases available for DNA replication. Both drugs have serious side effects cisplatin can cause nephrotoxicity that is controlled by expanding the kidney fluid volume before treatment. Antifolates induce elevated levels of h omocysteine. Homocysteine accumulation causes severe toxicities such as neutropenia, thrombocytopenia, and diarrhea. With supplementation of vitamin B12 and folic acid, homocysteine can be recycled into methionine or converted into cysteine [11,20,21].

The search for new treatment options for MPM

A phase III trial by Vogelzang et al. showed patients receiving cisplatin with pemetrexed had an overall survival (OS) of 12.1 versus 9.3 months for patients receiving cisplatin. Also time to progression (TTP) was higher in the cisplatin with pemetrexed group (5.7 months) compared to the cisplatin group (3.9 months). Approximately 40% of the patients had a partial response (PR). A retrospective analysis of the follow-up data showed that patients receiving two or more lines of treatment had a significant longer survival. Sixty-two percent of the patients received single-agent therapy and 38% combination therapy. For patients with two or more lines of chemotherapy, the median survival time (MST) from start of first-line treatment was 15.3 months for those receiving first-line pemetrexed and cisplatin versus 12.2 months for patients that previously received first-line cisplatin. For patients that did not receive second-line chemotherapy, MST was 9.8 months in the cisplatin/pemetrexed group and 6.8 months in the cisplatin group. This analysis suggests that a selected group of eligible patients could benefit from a second-line treatment, but the most effective second-line treatment for this patient population has not yet been identified [22]. Since then, various other second-line phase II trials have been conducted as will be discussed below.

Inhibitors of growth factors

Growth factors and their receptors play an important role in the development of mesothelioma. The epidermal growth factor receptor (EGFR) plays a role in cell proliferation, differentiation, migration, adhesion, and survival. EGFR is highly overexpressed in mesothelioma. However, EGFR tyrosine kinase inhibitors erlotinib and gefitinib as well as the EGFR antibody cetuximab did not show any response. EGFR is not a tumor driver as suggested from the absence of sensitizing mutations in the EGFR tyrosine kinase domain, which may explain the lack of response to EGFR inhibitors [4,20,23].

Another transmembrane tyrosine kinase is activated by the platelet-derived growth factor (PDGF) and plays a role in cell proliferation. Imatinib and dasatinib are anticancer drugs that inhibit the kinase activity of the PDGF receptor, but phase II studies with these drugs in patients with MPM were disappointing [4,8,20,23].

Inhibitors of angiogenesis

A third growth factor activating kinase receptor is the vascular endothelial growth factor (VEGF) which plays a role in angiogenesis. VEGF expression levels are high in a large portion of MPM tumors and they may activate the VEGF receptor to induce angiogenesis in tumors. Therefore, different VEGF-receptor inhibitors were consequently tested in phase II studies. These include small kinase inhibitors sorafenib, sunitinib, vatalanib, and cediranib, which did not improve response rates or OS for patients with MPM [4,8,10,20,23]. Thalidomide was the most promising agent; however, no benefit in TTP or OS was observed in a large randomized phase III study [24]. Bevacizumab, an antibody binding VEGF, has recently been tested in a phase III trial in combination with cisplatin and pemetrexed. In patients who were able to receive bevacizumab, the OS was significantly extended in the pemetrexed/cisplatin/bevacizumab (PCB) group (18.8 months) versus the pemetrexed/cisplatin (PC) group (16.1 months). Second-line treatment with pemetrexed or with a platinum containing treatment was allowed in this study protocol and may have affected the OS. An improvement in progression-free survival (PFS) for the PCB group (9.2 months) versus the PC group (7.3 months) was also observed. Even though more patients stopped treatment in the PCB group due to toxicity, the quality of life in this group was considerably better than in the control group. However, absence of masking could have influenced the quality-of-life results, so these results should be interpreted with caution [25].

Other targeted agents

Other targeted agents investigated as second-line treatment are bortezomib, vorinostat, everolimus, defactinib, asparagine-glycine-arginine human tumor necrosis factor alpha (NGR-hTNFα), and amatuximab.

Bortezomib, an inhibitor of the 20S proteasome, was tested in two phase II studies. As a single agent in second-line treatment, it was not active. Also, in combination with cisplatin, bortezomib failed to meet the primary objectives [26,27].

Vorinostat is a histone deacetylase (HDAC) inhibitor. HDACs are regulatory enzymes that manipulate histone modifications resulting in changes in the cell epigenetics. Inhibiting HDACs results in expression of genes associated with cell cycle arrest, apoptosis, and tumor suppression [20,23]. Preclinical and phase I data showed promising results, which could not be confirmed in a randomized double-blind phase III study with single agent vorinostat [28].

A percentage of 35-40 of the patients with MPM have mutations in the neurofibromatosis type 2 (NF2) gene that encodes the protein merlin. Merlin downregulates the activity of the kinase mammalian target of rapamycin (mTOR) and blocks focal adhesion kinase (FAK) activation. Mutations in NF2 then results in activated mTOR and FAK [4,10]. Everolimus is an inhibitor of mTOR that was tested in patients with MPM, yet the phase II study did not meet its primary endpoint [29]. Another compound targeting the NF2-pathway is defactinib, a FAK-inhibitor. While preclinical data again were promising, the placebo-controlled phase II study was early terminated due to reasons of futility [30]. Possibly the inhibition of the NF2/mTOR/ FAK pathway was not sufficient to control MPM. Tumor necrosis factor alpha (TNF-α) is a secreted protein that induces apoptosis in endothelial-tumor cells via caspase activation. To target the protein to the tumor tissue and at the same time limit general side effects of TNF-α, TNF-α was fused to the tumor homing peptide sequence NGR [8,10,23]. A single agent phase II trial in 57 patients with MPM showed promising results [31]. In the following randomized phase III trial, patients who progressed on first-line treatment received weekly NGR hTNFα or placebo in combination with gemcitabine, vinorelbine, doxorubicin, or best supportive care. In the intention to treat analysis the OS was not significant different between the NGR-hTNFα group and placebo group [32]. Currently, a maintenance phase II trial with NGR-hTNFα is ongoing, the primary objective is TTP (NCT01358084) (Table 1).

Amatuximab (MORab-009) is a chimeric monoclonal antibody that binds with high affinity to mesothelin [8,10,20,33]. Mesothelin is a tumor-differentiating antigen, present at mesothelial cells lining the pleura, peritoneum, and pericardium. Its biological function is unknown [4,20,33]. Mesothelin is highly expressed in epithelial MPM, but not in sarcomatoid MPM. The limited expression in normal mesothelial cells and high expression in tumor cells makes i an attractive target [23,33–35]. Preclinical studies showed that amatuximab has activity against mesothelin expressing tumor cells [20,36]. In a single-arm phase II study, cisplatin and pemetrexed were combined with amatuximab for six cycles, which was followed by amatuximab-maintenance therapy in case of response or stable disease (SD). The primary endpoint, 3-month improvement in PFS compared to historical controls, was not met. However, with a PR in 39% of the patients and SD in 51% of the patients, the study concluded that amatuximab has activity in MPM [33]. Finding biomarkers to select patients for whom this drug would be effective is important. A randomized placebo-controlled study to investigate survival benefit is planned.

Oncolytic viral therapy

A different approach in cancer therapy employs oncolytic viruses that are emerged to selectively eliminate cells with particular driver mutations. Different viruses including adenovirus, measles virus, vesicular stomatitis virus, replication competent retrovirus, and the genetic engineered Newcastle disease virus have been tested in preclinical studies with good results [37–44]. To date, one phase I/IIa study is testing the safety, tolerability, and biological effect of the selectively replication-competent herpes simplex virus HSV1716 (NCT01721018) (Table 1).

Immunotherapy in MPM

There are reported cases of spontaneous regression of MPM, which were associated with lymphocyte infiltration in the tumor. Lymphocyte infiltration in MPM is also associated with improved survival [45–47]. These data suggest that MPM could be an immunogenic tumor, which makes immunotherapy an interesting therapeutic option [45,48,49].

There have been several different immunotherapy approaches tested. One of those is an antibody-drug conjugate. SS1P is a recombinant pseudomonas toxin coupled to the variable fragment of an anti-mesothelin antibody [35,50]. In phase I clinical trials, the vast majority of patients developed antibody responses to SS1P after one cycle of treatment, preventing further treatment unless this response is eliminated. Pentostatin and cyclophosphamide are drugs that deplete lymphocytes, preventing the formation of antitoxin antibodies. A phase II trial showed that pretreatment with these agents allowed patients to receive more cycles of treatment with SS1P, resulting in improved clinical responses [50].

While we discussed reagents directly targeting MPM, specific activation of immune responses in patients would be an alternative way of immunotherapy. A new wave of antibodies controlling checkpoints in immune cell control has shown strong responses in other tumors including non-small-cell lung cancer and melanoma [51–57]. These antibodies block the activities of programmed cell death protein 1 (PD-1), programmed death ligand 1 (PD-L1), and cytotoxic T-lymphocyte antigen 4 (CTLA-4).

PD-L1 is expressed in many tumor cells, including MPM [48,49,58–61]. Binding of PD-L1 to its receptor PD-1 on T cells inhibits proliferation and activation of T-cells and quenches immune responses against the tumor. As a result, tumors that express PD-L1 evade cytotoxic T-cell control. Consequently, blocking PD-1 with antibodies allows activation of cytotoxic T-cells. Mansfield et al. showed positive PD-L1 expression in 40% of MPM tissues by immunohistochemistry (IHC) staining. Cedres et al. reported that 20.8% of the cases are positive for PD-L1 expression. Both articles report a higher incidence of PD-L1 expression in sarcomatoid MPM than in epitheloid MPM and describe that PD-L1 expression is associated with a poor prognosis [48,49].

In a phase I study, pembrolizumab, a PD-1 receptor antibody, was not only safe and tolerable for patients, also a disease control rate (DCR) of 76% was observed. Twenty-five patients with MPM received pembrolizumab after first-line treatment. Seven patients had a PR and 12 experienced SD [62]. Recently, a phase II study with second-line pembrolizumab treatment in MPM has opened for patient accrual (NCT 02399371). The first primary objective is determining the overall response rate in an unselected patient population and in a patient population with PD-L1 positive MPM. The second primary objective is to determine the threshold for PD-L1 expression using 22C3 antibody-based IHC in correlation to tumor response (Table 1).

Table 1: Ongoing phase II and III trials in mesothelioma.

	Drug	Clinical trial number	Primary outcome	Description
Growth factor inhibitor	IMC-A12	NCT01160458	CRR	Evaluate the safety and effectiveness of IMC-A12, an antibody blocking type I insulin like growth factor in patients that previously received chemotherapy
	cetuximab	NCT00996567	PFS	Multicenter open phase II study testing cetuximab in combination with pemetrexed and cisplatin or carboplatin as first line treatment
Targeted agents	Alisertib	NCT02293005	DCR	Evaluate the safety and effectiveness of alisertib an inhibitor of aurora kinase A protein
	Defactinib	NCT02004028	Biomark respons	Assess biomarker response from tumor tissue of patients that received defactinib prior to surgery
	NGR-hTNFα	NCT01358084	PFS	Randomized double blind phase II study to determine efficacy of NGR-hTNFα as maintenance treatment
	amatuximab	NCT02357147	OS	Multicenter, double blind randomized phase II study evaluating the safety and efficacy of amatuximab in combination with pemetrexed and cisplatin as first line treatment.
Oncolytic viruses	HSV1716	NCT01721018	Safety, tolerability	Phase I/IIa of the safety, tolerability and biological effect of single and repeat administration of the herpes simplex virus
Immunotherapy	Pembrolizumab	NCT02399371	Ability PD-L1 to predict response, OS	Phase II study to evaluate the effect of pembrolizumab on OS.
	Nivolumab	NCT02497508	DCR	Single arm phase II study to determine if nivolumab will improve DCR from 20% to 40% at 12 weeks.
	Tremelimumab	NCT01843374	OS	Phase IIb, randomized double blind study to determine the effect of tremelimumab on OS.
	Tremelimumab + MEDI4736	NCT02588131	ORR	NIBIT-MESO1 is a phase II, open label, single arm study evaluating the efficacy of tremelimumab in combination with the αPD-L1 MEDI4736
Vaccine	DC vaccination	NCT02649829	Number patients *	MESODEC is a phase I/II trial to show the feasibility and safety of WT-1 targeted DC vaccination in combination with chemotherapy prior to surgery.
	WT-1 vaccination	NCT01890980	One year PFS	Phase II study determining if PFS is extended for patients receiving WT1 vaccine and montanide + GM-CSF after multimodality treatment compared to patients receiving montanide + GM-CSF after multimodality treatment
	WT-1 vaccination	NCT01265433	One year PFS	Phase II study determining if PFS is extended for patients receiving WT1 vaccine and montanide + GM-CSF after multimodality treatment compared to patients receiving montanide + GM-CSF after multimodality treatment

NGR-hTNFα: peptide asparagine-glycine-arginine – human tumor necrosis factor alpha, DC: dendritic cell, CRR: clinical response rate, PFS: progression free survival, DCR: disease control rate, OS: overall survival, ORR: objective response rate.* number of resectable patients with feasible and safe DC vaccine product and the number of patients receiving DC vaccination in combination with chemotherapy within the proposed time frame of surgery.

Nivolumab, another PD-1 receptor antibody, is currently evaluated in a single-arm phase II study in patients with recurrent MPM (NCT02497508). The primary objective of this study is the DCR at 12 weeks, which is expected to increase from 20% to 40% (Table 1).

Tremelimumab is a monoclonal antibody against CTLA-4. Blocking CTLA-4 will activate cytotoxic T-cells directly. Two single-arm phase II studies have been conducted, both showing encouraging clinical activity [63,64]. Therefore, a randomized double-blind placebo-controlled phase II study is now evaluating the efficacy of tremelimumab. The primary objective is demonstrating a 50% improvement in OS from 7 to 10.5 months (NCT01843374). Tremelimumab is also tested in combination with the anti-PD-L1 checkpoint inhibitor durvalumab. The primary outcome of this phase II study is immune-related objective response rate (NCT02588131) (Table 1).

While these checkpoint inhibitors allow an OS improvement of 20% in melanoma patients, the current studies should show whether these could be reproduced for mesothelioma patients or whether it predominantly induces PRs with only limited survival benefit.

Vaccines

Vaccines against mesothelioma cells may increase immune responses against the tumor. In 2005, Hegmans et al. reported that vaccination with antigen-pulsed dendritic cells (DCs) prevented tumor outgrowth in mice [65]. In the following phase I study, 10 patients received mature DCs, pulsed with the patient's own tumor lysate after chemotherapy. The treatment was feasible and safe and in some patients antitumor immune responses were detected. Whether this has any effects on survival of patients with mesothelioma should be further tested [66]. The DCs in this study were pulsed with tumor extracts in which only a minor portion of the antigens are tumor specific and relevant for the immune system. Pulsing DCs with only one tumor-associated antigen should provide more specific responses. The MESODEC study is a phase I/II trial in which patients are treated with DCs that are loaded with Wilms tumor 1 (WT-1) antigens. WT-1 is a transcription factor, which is highly overexpressed in mesothelioma cells. The general objective of the MESODEC study is to show the feasibility and safety of WT-1-targeted DC vaccination in combination with chemotherapy. Whether this treatment enables the induction of a systemic or immune response is also evaluated (NCT02649829) (Table 1). Another strategy focusing on WT-1 is vaccination of patients with ynthetic peptides derived from the WT-1 protein sequence. WT-1 could be targeted with a T-cell-based immunotherapeutic approach because it is processed and presented at the cell surface in the context of major histocompatibility complex class I molecules. A pilot study showed that the vaccine gave minimal toxicity and induced immune responses against WT-1 in a high proportion of patients [67]. Currently, two phase II studies with WT-1 vaccination are ongoing. In both studies, WT-1 vaccination in combination with granulocyte-macrophage-colony-stimulating-factor with or without

the vaccine adjuvant (montanide), is given after combined modality therapy. Primary outcome is 1-year PFS (NCT01890980 and NCT01265433) (Table 1).

Immunotherapy against cancer is a fast-developing treatment strategy with antibody-drug conjugates, new reagents to overcome immune checkpoints in order to boost immune responses, and vaccination strategies that are all tested in phase II studies on patients with mesothelioma. The prospects are bright for a subgroup of patients but these have to be selected.

Preclinical models in translational research for MPM

If clinical trials reveal one thing, it is that many drugs fail in phase II studies. Most of the drugs described in this review were active in preclinical studies, but lacked antitumor activity in the clinical setting. It is apparently difficult to predict clinical outcome with preclinical models. Selection of compounds for further clinical development is challenging. This is even more urgent in MPM since the disease is heterogeneous, the patient population is small and many new drugs are generated. Preclinical models are essential for a better selection process. Several factors are important in a good preclinical model. First of all, the preclinical model should resemble the patients' tumor, ideally with a representation of the stroma surrounding the tumor cells, the surrounding immune cells and vasculature. With many new drugs generated, it is important to be able to test multiple drugs at the same time; therefore, the preclinical model should be easy to handle and reproducible in its readout. Another factor is time; it is important to get results within a short period of time, so a preclinical model should not be time-consuming. There are many preclinical models available, each with their own advantages and disadvantages.

Cell lines

Most preclinical models are based on cell-line experiments. Cell lines are typically passaged for many years, making them highly selected clonal subpopulations of the original tumor, with many additional genetic aberrations. They then become a relatively poor representation of the original tumor [68–71]. Cell lines can be cultured in monolayer or in spheroids. Spheroids are tumor cells organized in a three-dimensional (3D) arrangement [70]. Monolayer cultures are easy to handle and suitable for large scale drug testing. Spheroids are more laborious but may better reflect the natural conditions of the tumor. They are not suitable for large-scale drug testing since read out of cell survival and quantification is challenging. MPM is a tumor extremely resistant to chemotherapy, mostly due to resistance to apoptosis [70,72]. Spheroids acquire multicellular resistance to a variety of treatments, which mimics the chemoresistance in patients [73,74]. Some drugs exhibit sensitivity in monolayer culture but resistance in spheroids. The proteasome inhibitor bortezomib, for example was found to be very effective in monolayer MPM cell-line cultures [75–77]. However, the phase II studies with this drug were disappointing.

Lack of activity was also observed in spheroid cultures [26,27]. Barbone et al. showed that spheroids treated with bortezomib were resistant due to upregulation of Noxa, a BH3-protein that displaces Bim and thereby mediates apoptosis [73].

Perfused microfluidic systems in combination with spheroids, may better reflect the in vivo situation, because regulation of drug exposure and mass transport is possible. Ruppen et al. compared static 3D-cultures with perfused 3D-cultures. For perfused 3D-cultures, a microfluidic chip was used. This chip contained two identical channels, each with eight trap ping sections and in each section a spheroid. Spontaneous formed spheroids were trapped in the sections, after which nutrients, oxygen, and drugs were delivered by diffusion from the main channel. Interestingly, perfused spheroids were twice as resistant to cisplatin compared to static spheroids [74].

Primary tumor cultures

Primary tumor cultures are cultures of single cells isolated from patients, which are propagated for a short period of time in order to prevent formation of clonal subpopulations. Multiple groups generated primary tumor cultures from cells isolated from pleural effusions of patients with MPM. These cultures resemble the original tumor closely regarding histological and molecular features [14,71,78,79]. Szulkin et al. used primary tumor cultures for chemosensitivity assays and observed a large patient-to-patient variability in sensitivity to drugs. Many cultures were resistant to drugs as was also observed in the clinical setting [14].

Xiang et al. generated spheroids from primary tumor cells. The spheroid of one primary cell line resembled cell line spheroids, while the spheroid of another primary cell line formed mostly loose aggregates [79]. It was not reported how long these primary cells were cultured and how often they were passaged, which makes it difficult to conclude that single cell spheroid formation from primary tumor cultures is a reproducible system. Tumor fragment spheroids are small biopsies of the tumor cultured on a collagen layer in order to grow out as spheroids. These tumor fragment spheroids exhibit the same complexity of cell types and extracellular matrix as the tumor. They retain many characteristics of the original tumor. Chemosensitivity assays on these tumor fragment spheroids are possible, but only for a very limited number of conditions [72,73,80,81].Techniques allowing a simple, individual tumor-based drug screen remain challenging.

Mouse models

Animal models are also very important in preclinical drug development. One advantage of animal models is that they can mimic the 3D-structure of a tumor and the vasculature around it. Furthermore, it also considers the pharmacokinetics, pharmacodynamics, and toxicity of a compound and in some models even the contribution of the immune system. There are different types of models reported, most of them mouse- based. In older

models, mesothelioma tumors were induced by intrapleural or intrabronchial exposure to carcinogens-like asbestos fibers, other natural and synthetic fibers and metals. Mouse models with mesothelial specific expression of oncogenes like SV40, NF2, or p53 were used to accelerate the induction of MPM in asbestos-exposed mice [82–84]. While these models resembled human mesothelioma in terms of latency, superficial growth, shedding of tumor cells, and growth as spheroids, these models had no loss of function of genes known to be inactivated in human MPM. This made it difficult to understand the molecular mechanism underlying the tumor [82]. Jongsma et al. developed the first genetic mouse model of MPM. Knockout-mice, deficient in the NF2 gene, were crossed with INK4A/ARF or p53-deficient mice. The offspring mice rapidly developed mesothelioma, with a high incidence and without further exposure to carcinogens [82,84]. The tumors that arise in these mice are not representative of the human tumor, but can be constructed with genetic mutations common to most of the patients with MPM. With increasing knowledge about genetic mutations in human mesothelioma, it is important to introduce the most prevalent mutations in these genetic mouse models. This will better resemble the human tumor. In other animal models, cell lines were injected in the pleural cavity of the mice. Most available cell lines however, do not form tumors in mice [71]. Those that do, may be selected for survival under mouse conditions and may not reflect human MPM. Patient-derived xenografts (PDX) are tumor biopsies or tumor cells from pleural effusions transplanted in nude mice. Kalra et al. showed that a PDX-mouse-model for MPM resembles the primary tumor culture and primary tumor regarding both histological and molecular features [71]. A disadvantage of this type of model is that it can only be generated in immune-deficient mice. The immune system may have a role in tumor clearance and sometimes chemotherapy responses, which complicates evaluation of the PDX-mouse-models. Although there are drawbacks, PDX-mouse-models could be very useful in evaluating efficacy of therapeutic agents.

We summarized various cell-based models and mousemodels that are available to improve translational research (Table 2). Each model has its own advantages and disadvantages and no model is perfect. Which model should be used depends on the aim of the research. Most important, none of the models have been validated by a strong corresponding chemotherapy response between the model and the corresponding patient.

Expert commentary and five year view

The prognosis for patients with MPM has not improved over the last decade. The current standard of care, cisplatin in combination with pemetrexed, has not been replaced by another treatment regimen in 12-year time. Although many therapies have been tested on patients with MPM, none were effective in phase II trials. There are various reasons for the limited progress in the treatment of mesothelioma. The first reason is the relatively small size of the patient population. This limits the interest of the pharmaceutical industry but

also complicates the execution of large randomized studies. This may be further complicated when mesothelioma is a more diverse tumor than anticipated. It is very difficult to define personalized treatment options unless obvious biomarkers related to treatment success are defined. These are currently lacking.

Table 2. Overview of the available preclinical models and the features based on resembling the tumor, drug testing, and time

Preclinical model		Resemble patient cells of tumor	Resemble natural conditions of tumor	Drug testing	Time
Cell line models	Monolayer	No	No	Multiple	Fast
	3D spheroids	No	Only to chemo resistance	View	Slow
Primary tumor models	Monolayer	Yes	No	Multiple	Fast
	3D spheroids	Yes	Only to chemoresistance	View	Slow
	Tumor fragments	Yes	Stroma composition	View	Slow
			chemoresistance		
Mouse models	Asbestos induced	No	Yes	One	Slow
	Genetic	No	Yes	One	Fast
	Xenograft cell lines	No	Yes, however, no immune system	One	Slow
	Patient-derived xenograft	Yes	Yes, however, no immune system	One	Slow

Yet there are a number of developments that can be expected to improve the prospects for, at least a subgroup of, patients with MPM. First, the genome of many mesothelioma tumors is being sequenced and defines genes that are often mutated, including the gene encoding the breast cancer-associated protein 1 (BAP1) [85–87]. BAP1 loss may affect the activity of the histone-methyltransferase EZH2 resulting in unusually high H3K27me3 modifications [88]. This epigenetic marker is also observed in other tumors and suggests that drugs affecting this epigenetic marker may be more selective and effective against MPM. This is indeed suggested in preclinical models. Second, drug screens can be performed on primary tumor cultures of MPM cells or, possibly, spheroids of these cells [14]. The detected drugs responses could be coupled to the patient that donated these tumor cells. This will allow personalized treatment for patients with MPM and ex vivo testing of larger series of anticancer drugs to select the best combination for the individual patient. Prediction should be accurate to prevent false-negative predictions and inadequate treatment of patients with MPM. This is critical before personalized screening on basis of patients tumor cells will be introduced in the clinic. Third, the latest addition to the cancer-drug repertoire, is immunotherapy with check-point inhibitors. Proteins like

PD-1, PD-L1, and CTLA-4 can dampen the adaptive immune response against tumors. Antibodies blocking these proteins establish the local immune responses against cancer, in fact starting a controlled auto-immune response. This new therapy can be effective for tumors with a high mutational load, which does not include MPM. Yet, the unique and high expression of proteins in tissues or tumors may also unleash an immune response and this will be tested for MPM in the near future.

Although the prospects for MPM treatment have not improved over the last decade, there are various developments that may finally lead to a step forward in the treatment of this tumor. The next decade will show serious progress in the fundamental understanding of MPM which in turn will improve the prospects of these patients.

Key issues

- MPM is an aggressive tumor with a poor prognosis. For patients that do not respond to first-line treatment or become progressive after treatment there is no standard second-line treatment available.
- Many inhibitors of growth factors are tested in MPM, most with negative results. Bevacizumab is the most promising agent.
- For other targeted agents, large phase II and phase III trials have been conducted.
- Immunotherapy is a new development in MPM, studies testing antibodies against PD-1 and CTLA-4 are ongoing.
- Other ongoing trials are focusing on primed DC-vaccination and WT-1 vaccination.
- Many drugs that were active in preclinical models, fail in phase II studies, indicating it is difficult to predict clinical outcome with preclinical models.
- A good preclinical model resembles the patients' tumor, is able to test multiple drugs at the same time and generate results within a short period of time.
- Each model, cell-based or mouse, has its own advantages and disadvantages; no model is perfect. Which model should be used depends on the aim of the research.
- Genomesequencing, drug screens performed on primary MPM cells, and immunotherapy with checkpoint inhibitors, are developments that can be expected to improve MPM.

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