Malignant Pleural Mesothelioma: From the Bench to the Bedside

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**Abstract**
Optimal management of malignant pleural mesothelioma (MPM), which is mainly based on patient characteristics and clinical stage, is not clearly defined yet, although detailed, practical guidelines for these patients have been proposed by some scientific societies. Translational research, in the field of this disease, is currently in progress and different molecular oncogenic pathways leading to the growth and progression of MPM have been characterized with recent pharmaceutical developments. However, further in-depth analysis still needs to be done for a more advanced deciphering of the step-by-step process leading from early increased mesothelial cell proliferation to invasive mesothelioma, from which we are expecting the development of definitively effective therapy. Thus, this review is an overview of the recent advances in the biology of MPM and their potential therapeutic applications in the field of MPM diagnosis and treatment.

**Introduction**
Malignant pleural mesothelioma (MPM) is a rare but highly aggressive tumor with a poor prognosis and increasing incidence. An optimal management of MPM is not clearly defined yet, although detailed, practical guidelines for these patients have been proposed by some scientific societies. These guidelines emphasize the numerous pitfalls in MPM diagnosis, and the need of innovative therapies and tools for monitoring MPM patients based on the limited and quite inconclusive results of current treatment.

Although the prospects for mesothelioma are rather pessimistic, recently research on MPM pathogenesis and biology exhibited great advances, leaving hope for significant advances in the management of these patients in the future.
Thus, this review is an overview of the recent progress in the biology of MPM and its potential therapeutic applications in cancer diagnosis and treatment. The chapter References was kept exhaustive to offer the reader the main studies in this field.

Search Strategy

A systematic analysis of the literature (2000–2012) was carried out using the databases Medline (National Library of Medicine, USA) with the following key words: mesothelioma and malignant pleural mesothelioma in combination with biology or targeted therapy. A manual search and review of reference lists in recent and relevant publications were also done in order to select the articles published in the literature. Therefore, abstracts from oncology meetings were not included in the reference list of this review.

From the Bench: The Biology of MPM

A better knowledge of the pathogenesis and molecular alterations in MPM represent a useful tool to develop predictive or prognostic biomarkers and potential therapeutic agents.

Asbestos fiber exposure is widely accepted as the main cause of MPM although exposure to other mineral fibers such as erionite, exposure to SV40 or radiation has been reported in low evidence-based reports. Although the mechanisms remain to be further elucidated, previous studies reported that the event is dependent on tumor necrosis factor (TNF)-α release by macrophages due to contact with an asbestos fiber. Subsequently, mesothelial cells express TNF-α receptor, and its interaction with ligand determines nuclear factor-κB pathway activation and resistance of cancer cells to death [1, 2]. Moreover, mesothelial cells are able to release reactive oxygen and nitrogen species (ROS and RNS) and high-mobility-group box one protein (HMGB1) leading to chronic inflammation, DNA damage and aneuploidy [3]. Asbestos-induced carcinogenesis is therefore based on a close relationship between chronic inflammatory processes and asbestos-induced mesothelial cell death offering potential targets for the prevention of mesothelioma by inhibition of the release of these molecules or the neutralization of their activity.

Other molecules, in particular cytokines [interleukin (IL)-6 and IL-8] and growth factors [vascular endothelial growth factor (VEGF), hepatocyte growth factor, transforming growth factor (TGF)-β, platelet-derived growth factor (PDGF) and insulin-like growth factor (IGF)], also implicated in MPM carcinogenesis may represent other targets for the management of MPM [see references 4–7 for details].

In patients with MPM, the following molecular alterations have been noted: chromosome alterations in tumor suppressor genes, allele loss [8, 9], gene silencing by DNA methylation in specific chromosomal regions [10], epigenetic deregulation of tumor suppressor genes by histone acetyltransferase and deacetylase (HDAC) chromatin condensation/decondensation balance [11–13] and gene mutations (leading to functional inactivation of NF2 for instance) usually found in case of loss of heterozygosity of 22q12 [14–19].

Tumor Markers

Numerous markers have been evaluated for the management of mesothelioma, but many discrepancies exist in the results obtained from different studies, as underlined in a recent review [20].

Diagnostic Value

Currently, there are a lot of markers based on the increasing understanding of the molecular and biological pathways of mesothelioma. Immunohistochemical markers have been tested for their potential to establish a diagnosis of mesothelioma on cytological grounds [21]. Genetic markers and serum markers are other promising tools, e.g. soluble mesothelin-related protein (SMRP) and megakaryocyte potentiating factor [22–25].

The most frequently evaluated immunohistochemical marker was EMA followed by BER-EP4, CEA and calretinin. Among the serum markers, the most frequently investigated were SMRP and CEA, and among effusion markers CEA, CA15-3, HA and SMRP [20]. The most valuable tissue markers for mesothelial cells are calretinin, EMA and WT-1 as positive markers and CEA, Ber-EP4 and TTF-1 (thyroid transcription factor 1) as negative markers. The most valuable tissue markers to discriminate mesothelioma cells from other malignant tumor cells appear to be CEA, Ber-EP4 and calretinin. The International Mesothelioma Panel recommends that at least 2 mesothelial and 2 carcinoma markers be used in addition to a pancytokeratin for a reliable diagnosis of MPM. None of these antibodies are 100% specific, and false positives (which often show <10% stain-
ing) can occur in either direction. One exception is the carcinoma marker TTF-1, for which no case of mesothelioma with a positive staining has been published [26]. In discriminating mesothelioma from non-malignant diseases, EMA and SMRP are considered the most valuable markers, but none of the markers had sufficient discriminatory value to differentiate mesothelioma from other diseases.

Among a large panel of markers, the MUC1 antibody (clone E29 of EMA), one of the most valuable and promising markers of which the principal isoform MUC1 or MUC1-TM is expressed at the epithelial surface in various tissues, resulted in the best sensitivity (~80%) with no false positive in MPM. The gene product MUC1 is even more important because it can be detected in the circulation and its presence (a specific glycosylated form) could result in targeted therapies [Theratope, STn, sialyl[Tn)] [27, 28].

GLUT-1 is a new promising marker in the distinction of malignant mesothelial lesions. Upregulation of glucose transport across the plasma membrane is mediated by a family of glucose transporter proteins named GLUT. Overexpression of the hypoxia-responsive GLUT-1 has been frequently observed in several carcinomas and is a very important limiting factor in the transport and metabolism of glucose in malignant cells. In contrast to malignant tumors, including mesothelioma, GLUT-1 expression has been relatively undetectable in normal tissue and benign epithelial tumors. A recent study by Kato et al. [29] showed that GLUT-1 reactivity was found in 40 of 40 mesotheliomas; whereas all 40 cases of reactive mesothelial hyperplasias were negative. Thus, GLUT-1-positive staining is a helpful marker for MPM – both epithelioid and sarcomatoid.

IMP3 is an oncofetal protein involved in embryogenesis and it promotes tumor cell proliferation, invasion and metastasis. Studies have shown that IMP3 is an important cancer-specific protein that is associated with aggressive and advanced cancers, and it is specifically expressed in malignant tumors, but it is not found in benign tissue. Shi et al. [30] recently showed that IMP3 was negative in all 64 reactive mesothelial lesions tested while it was positive in 33 of 45 (73%) malignant mesotheliomas leading to the conclusion that staining for IMP3 can be a useful immunohistochemical marker in the distinction of malignant mesothelioma from reactive mesothelial cell proliferations. In order to study promising markers, prospective accuracy studies are necessary; retrospective case-control studies could be also useful [31–33].

The added value of these markers to established diagnostic criteria such as patient characteristics and previous clinical tests should be investigated [33, 34].

Currently, the value of non-invasive markers in the diagnosis of mesothelioma remains to be determined since studies addressing this objective were of limited value and results were inconclusive due to considerable variability among the studies.

Prognostic Value

Biomarkers may help in the prognosis of MPM. A significant negative prognostic marker in MPM patients is SMRP [35], but further studies are needed. Recent studies have shown that loss of p16 is associated with poor survival [36, 37].

Angiogenesis seems to be very important in MPM progression. In fact, increased microvessel density has been associated with poor survival [38], and proteins involved in regulating angiogenesis have been implicated in the progression of MPM. The engine of angiogenesis is hypoxia. A reduced level of BAX, a tumor suppressor gene downregulated by tumor hypoxia, has been associated with a poor outcome [39]. Elevated levels of VEGF in pleural effusion are associated with diminished survival in MPM patients [6], and VEGF overexpression monitored by immunohistochemistry independently predicts poor survival in MPM patients (p = 0.0002) [40]. High levels of VEGF and fibroblast growth factor (FGF) 2, or co-expression of TGF-β, VEGF, FGF1 and FGF2 are also associated with a poor outcome [41]. PTEN expression was found as a strong predictor of survival in 126 mesothelioma patients [42].

A four-gene signature comprising KIAA097, GDIA1 (GDP-dissociation inhibitor 1), CTHBP (cytosolic thyroid hormone binding protein) and an expressed sequence tag similar to the L6 tumor antigen (which correctly classified a training sample into good and poor prognostic groups) predicted the correct outcome in a significant number of cases, encouraging future research on novel prognostic molecular markers to diagnose/monitor disease and assess treatment response [43]. The presence of an 11-gene, oncogene-driven pathway signature, correlated with a stem-cell-like expression profile, is associated with a poor prognosis in patients with MPM [44]. In the same way, a large gene expression analysis identified and validated aurora kinases as predictors of outcome.

In fact, mitosis or proliferation, diploidy and S-phase fraction were identified as significant indices, and expression of regulators of mitosis and cell cycle control was...
increased in more aggressive cancers [36]. Pass et al. [45] investigated whether specific microRNAs could help to distinguish patients with surgically treated mesothelioma into those with a good or bad prognosis.

By regulating cell functions such as growth, survival, motility/migration and invasion, the c-mesenchymal-epithelial transition (c-MET) receptor tyrosine kinase/hepatocyte growth factor axis also accounts for a critical pathway in MPM. A recent study showed that, disregarding the intracellular c-MET receptor traffic, only c-MET plasma membrane localization could be a relevant prognostic biomarker in MPM [46].

**Predictive Value**

There are no established indicators of responsiveness that can be used to optimize treatment in MPM. For targeted therapy, however, low VEGF serum levels may be useful in predicting the response to treatment with bevacizumab, and for chemotherapy, few studies have identified predictors of response to pemetrexed and/or cisplatin/carboplatin treatment in patients with MPM. It is hypothesized that low ERCC-1 expression might predict increased sensitivity to platinum-based chemotherapy, possibly due to the saturation of the enzyme complex. Conversely, high ERCC-1 levels may predict resistance to platinum-based chemotherapy.

Thymidylate synthase (TS) mRNA expression levels were inversely correlated with pemetrexed activity in different tumor cells, whereas other studies suggested a correlation between high levels of TS protein expression and reduced sensitivity to pemetrexed in colon and lung cancer cells [47, 48]. Furthermore, TS mRNA and protein expression were predictive of treatment response in patients with advanced breast cancer treated with pemetrexed alone and in patients with non-small cell lung cancer treated with pemetrexed/gemcitabine neoadjuvant therapy, respectively [49]. In a recent retrospective analysis, Righi et al. [50] observed that low TS protein levels are predictive of improved time to progression and overall survival (OS). Another retrospective analysis of TS and ERCC-1 protein expression by immunohistochemistry in 99 MPM patients treated with the carboplatin/pemetrexed regimen found that TS expression was a predictor of clinical outcome [51].

Retrospective studies on candidate predictive biomarkers in MPM specimens may provide a strong rationale for future trials. However, to be able to identify the best biomarkers for personalized MPM chemotherapy in prospective trials, optimization and standardization of methodologies are required, as well as the use of large and uniformly treated cohorts and the incorporation of both emerging candidate biomarkers and genotype studies. Probably, considering the rarity of this disease, the creation of a collaborative network which would allow analyzing the role of several biomarkers in an appropriate number of uniformly treated patients with MPM may be a good strategy. Surely, the detection of novel biomarkers will be one of the major targets of MPM research in the future.

**To the Bedside: New Treatment Approaches**

The role of surgery and radiotherapy remains controversial in MPM and should be further explored. Platinum-based combination chemotherapy with antimitabolites (pemetrexed/raltitrexed) became the cornerstone of first-line treatment [52, 53] during the last decade, but results were of limited value with respect to patient survival. Issued from an increasing knowledge on MPM pathogenesis, targeted agents and novel therapeutic strategies under investigation in preclinical models and clinical trials are presented below.

**Epithelial Growth Factor Receptor**

The epithelial growth factor receptor (EGFR) plays a role in cell proliferation, differentiation, migration, adhesion and survival [54], and it is overexpressed at both protein and transcriptional levels in >50% of MPM patients [55].

The Cancer and Leukemia Group B (CALGB) 30101 phase II trial enrolled 43 chemotherapy-naïve patients with MPM, and all patients received 500 mg gefitinib once a day until disease progression or unacceptable toxicity. The results showed a 3-month progression-free survival (PFS) of 40%, and this was compared with a similar historic control group of 337 chemotherapy-naïve MPM patients from the CALGB database [56]. This was higher than that seen in the gefitinib trial (40% PFS at 3 months), and the authors therefore concluded that single-agent gefitinib was not active in malignant mesothelioma [57]. In the second phase II trial, 63 chemotherapy-naïve patients with advanced or recurrent MPM and a performance status of 0–1 were treated with erlotinib. There were no responses, although 14 of 33 (42%) patients had stable disease (SD) [58], and the authors therefore concluded that single-agent erlotinib was not effective in MPM.

One explanation for the low efficacy of EGFR inhibitors despite EGFR overexpression might be the rarity of EGFR mutations in mesothelioma [59]. Some research
groups reported that there was no relationship between EGFR overexpression and outcome in patients with MPM [55, 60, 61], and others have reported that EGFR overexpression is associated with improved outcome [62–64]. However, EGFR overexpression in MPM is seen more commonly in the epithelial histological subtype, which is associated with improved patient survival, but it is not an independent prognostic marker [63, 64].

There is significant cross-talk between the EGFR pathway and other receptor signaling pathways. Downstream proteins within the EGFR signaling pathway, such as PI3K and AKT, are utilized by other tyrosine kinase receptor growth factor pathways, including the c-MET and IGF-1 receptor pathways [65–67]. Immunohistochemically, c-MET protein was found to be overexpressed in MPM and in samples of normal pleura. In mesothelioma cell lines, treatment with the small molecule c-MET inhibitor SU11274 resulted in dose-dependent growth inhibition [68]. The IGF-1 receptor has been shown to be important in the malignant phenotype of MPM101 and treatment of mesothelioma cell lines with the IGF-1 receptor inhibitors NVP-AEW541 and AG1024 resulted in dose-dependent growth inhibition [69]. AG1024 was also shown to enhance the cytotoxic effect of cisplatin in mesothelioma cells [70].

Significant cross-talk exists between EGFR and cyclooxygenase 2 (COX2) [71]. COX2, which is overexpressed in many solid tumors, is also a potential therapeutic target [72–74]. In MPM, immunohistochemical analysis of the COX2 protein revealed overexpression in 59–100% of tumor samples [75–77]. Treatment of mesothelioma cell lines with COX2 inhibitors induced cytotoxicity and also enhanced the effects of pemetrexed [78, 79].

**K-ras, BRAF and PI3KCA Mutations**

Screening for K-ras gene mutations at codons 12, 13 and 61 in several mesothelioma cell lines did not reveal any mutation [80, 81], which was in agreement with a study by Ni et al. [82] in 17 mesothelioma samples.

In MPM, BRAF gene mutations were absent in 53 tumors and in 6 tumor cell lines [83]. Finally, Suzuki et al. [84] studied 21 mesothelioma cell lines and did not find any PI3KCA gene mutation.

**PTEN**

PTEN gene deletion in 1 of 9 mesothelioma cells lines and loss of PTEN protein expression in 2 of 26 mesothelioma samples, both resulting in elevated AKT activity, were reported [85]. In an immunohistochemical study of 19 MPM tissue samples for AKT pathway-related proteins, PTEN protein expression was absent in 16% and mild-to-moderate PTEN expression was found in 86%. Suzuki et al. [84] reported loss of PTEN protein expression in 2 and low expression in 11 of 21 mesothelioma cell lines, resulting in constitutive activation of AKT. In a larger series of 206 MPM tissue samples, PTEN protein expression was lost in 62% of the cases (score 0) whereas 14% had weak (score 1), 10% moderate (score 2) and 14% strong PTEN expression. Loss of PTEN protein expression can be considered an independent poor prognostic biomarker in 126 of 206 MPM patients. Median survival was significantly longer in patients with PTEN-positive tumors than in those with PTEN-negative tumors [42]. Loss of the PTEN protein, resulting in constitutive activation of AKT, may induce resistance to both EGFR tyrosine kinase inhibitors and anti-EGFR monoclonal antibodies as they act upstream of PTEN.

**VEGF/VEGF Receptors**

Preclinical studies have shown that VEGF and VEGF receptors (VEGFR) are expressed in MPM, and circulating VEGF levels are higher in malignant mesothelioma (MM) patients than in healthy individuals or in patients with other malignancies [86]. Increased VEGF levels are positively correlated with microvascular density and associated with a poor prognosis [87]. VEGF levels increased with MPM progression [88]. VEGF stimulates MPM cells in a dose-dependent manner, and MPM cell growth was found to be inhibited by anti-VEGF antibodies [6].

A phase II trial combining erlotinib and bevacizumab reported that 50% of patients had SD; median PFS was 2.2 months and median OS was 5.8 months [89].

Development of hypertension is reported as a possible surrogate marker for bevacizumab activity and a potential significant predictor of outcome.

One phase II trial evaluating vatalanib in previously untreated patients showed no correlation between baseline VEGF or PDGF levels and response, PFS or survival [90].

Cediranib also seems to be a potent pan-VEGFR inhibitor that has antitumor activity in several solid tumors [91–93]. One phase II trial by Garland et al. [94] included 54 patients with MPM who had received prior treatment with platinum-based chemotherapy; a partial response (PR) was noted in 9% of patients, SD in 33%, with a median PFS of 2 months and a median OS of 10 months.

Semaxanib is an inhibitor of the VEGF-1 receptor and, less potently, the PDGF receptor (PDGFR) and c-kit; due

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to the low oral bioavailability of this molecule, intravenous administration is required [95].

A phase I trial tested thalidomide in MPM patients, including 33% of chemotherapy-naïve patients. There was no PR or complete response; 27.5% of patients were free of progression after 6 months, and OS was 7.6 months [96]. Its use as maintenance therapy following cytotoxic chemotherapy showed no evidence of improvement in either PFS or OS.

In patients with unresectable mesothelioma, sorafenib had limited activity, which supported the results with other VEGFR tyrosine kinase inhibitors [97]. In a phase I study, sorafenib (400 mg b.i.d.) was combined with doxorubicin (60 mg/m²), and this combination was well tolerated. The increased doxorubicin exposure with sorafenib did not result in significantly increased toxicity. These results justify further clinical investigation [98].

Sunitinib was tested in a phase II trial in MPM as second-line treatment after chemotherapy with platinum and antimetabolites but did not reach the main objective of the study with the following results: PR 12%; SD 65%; median time to progression of 3.5 months and OS of 7 months (Nowak et al., IMIG 2011, unpubl. data).

High levels of VEGF and FGF2 or co-expression of TGF, VEGF, FGF1 and FGF2 have been found to be associated with a poor outcome [99]. MPM exhibits high expression levels of the surrogate marker of hypoxia, hypoxia-inducible factor 1 [100].

Imatinib is a highly selective inhibitor of the bcr/abl mutated tyrosine kinase, as well as of both c-kit and PDGFRs. Several phase II studies have been conducted with imatinib mesylate in MPM refractory to chemotherapy or chemo-naïve patients, but negative results were reported [101–103]. In vitro and in vivo experiments demonstrated that STI-571 can cause MPM cell apoptosis and death through inhibition of the AKT/PI3K pathway and that it can also enhance MPM sensitivity to gemcitabine or pemetrexed [104]. Patients with MPM are currently being enrolled in a phase I study of imatinib combined with cisplatin and pemetrexed [105].

**PDGF/PDGFR**

PDGFαR is overexpressed in mesothelioma cells, and increased secretion of PDGF is thought to cause thrombocytopenia, which is considered a prognostic factor of adverse events and occurs in many patients with MPM [106]. Indeed, high serum PDGF levels in MPM patients seem to be an independent predictor of poor survival [107].

Overexpression of PDGFα has been shown in MPM cell lines, and blocking PDGFR has led to growth inhibition in vitro [108]. Combined with the fact that c-kit expression is seen in 26% of MPM patients, this spurred clinical trials investigating imatinib in MM [109].

Inhibition of PDGFR with imatinib and paclitaxel has been shown to lower interstitial fluid pressure with a possible subsequent improvement in drug delivery and increased efficacy in vitro [110]. In a phase I trial with imatinib in combination with gemcitabine including 5 MM patients, 1 patient had PR [111]. In preclinical trials, dasatinib had cytotoxic effects and resulted in decreased migration and invasion in mesothelioma cell lines [112]. In another phase II trial conducted in 46 inoperable patients with no responders, PFS was 2 months and median OS 4.8 months [113].

**PI3K/AKT/mTOR Pathway**

Sirolimus is approved as an immunosuppressant used especially in kidney transplants, and it has an antiproliferative effect on the PI3K/AKT/mTOR (mammalian target of rapamycin) pathway through the tyrosine kinase mTOR. The PI3K/AKT/mTOR pathway is often aberrant in MPM, and in vitro studies have shown that inhibition of the pathway may induce apoptosis in MPM cell lines [85, 114]. Temsirolimus, a derivative of rapamycin, has been evaluated in a phase I trial including 2 MM patients. None responded to the treatment [115]. In an in vitro study, combination therapy with cisplatin and sirolimus had synergistic antitumor effects in MPM cell lines [116].

**Mesothelin**

Mesothelin is highly expressed in several cancers, including ovarian cancer, pancreatic cancer, some squamous cell carcinomas and mesotheliomas (epithelioid subtype cells only) [117, 118]. The high membrane expression of mesothelin in MM and the limited distribution of mesothelin in normal tissue raised interest for mesothelin as an antitumor target [119]. A phase I trial has been conducted, but none of the MM patients showed a response [120].

Several agents presenting activity in preclinical models are being developed to target mesothelin: a recombinant immunotoxin (SSIP), a humanized monoclonal antibody (MORAb-009) and an attenuated *Listeria* vector that encodes human mesothelin (CRS-207). SSIP and MORAb-009 have completed phase I evaluation. For SSIP, 4 of the 33 evaluable patients treated had minor responses, 19 had SD, while 10 had progressive disease [121]. The result of this study and previously reported synergistic effects of SSPI in combination with chemotherapy [122] triggered an ongoing phase II study.
**Ribonuclease**

Ranpirnase is a ribonuclease that degrades RNA, and the irreparable RNA damage may constitute a death signal for apoptosis and also contributes to the inhibition of cell growth and proliferation. For this reason, ranpirnase has been tested in a phase II trial and 4.9% of patients responded to therapy; OS was 6 months. Several patients were excluded from the study due to adverse events, e.g., renal insufficiency, allergic reactions, arthralgia and peripheral edema [123]. Whether this small advantage is of enough clinical value to continue further research with this drug remains presently unclear.

**Asparagine-Glycine-Arginine-Human TNF-α**

TNF-α has antitumor activity through activation of apoptosis. However, TNF treatment is associated with severe toxicities, which only allow TNF to be administered in doses at least tenfold lower than the dose effective in preclinical models [124, 125]. NGR-human TNF consists of human TNF-α fused to the tumor-homing peptide asparagine-glycine-arginine (NGR) able to selectively bind an aminopeptidase N-isoform overexpressed in tumor blood vessels. A phase II trial by Gregorc et al. [126] evaluating NGR-human TNF included 57 patients: PR was seen in 1 (2%) patient. Eighteen (31%) patients with SD had a median PFS of 4.4 months. Overall, PFS and OS were 2.8 and 12.1 months, respectively. Treatment was well tolerated.

**HDACi**

Histone proteins exist in either acetylated or deacetylated configurations, and the equilibrium between the two forms is regulated by histone acetyltransferase and HDAC. Inhibition of histone acetylation results in acetylation of histone proteins and expression of genes associated with cell cycle arrest, apoptosis and tumor suppression. Moreover, HDACIs induce acetylation of nonhistone proteins leading to other anticancer effects such as inhibition of angiogenesis, motility and invasion of tumor cells. Many specific or pan-HDACis have been tested in MPM, including suberoylanilide hydroxamic acid (SAHA/vorinostat), panobinostat or valproic acid (VPA), either alone or in combination with chemotherapy. Vorinostat has already gained FDA approval for the treatment of cutaneous T-cell lymphoma [13].

Preliminary evidence from a phase I trial suggested that vorinostat might exert clinically meaningful activity in patients with mesothelioma [127]. However, a phase III trial (Vantage 014) comparing vorinostat with placebo in 660 pretreated mesothelioma patients who had progressed on one or two prior therapies failed to demonstrate a significant improvement in OS [128]. Vorinostat or placebo was given 3 days per week during a 21-day cycle. Although the median PFS for vorinostat was 6.3 versus 6.1 weeks, this did not translate into a statistically significant OS benefit for vorinostat compared with placebo. In a phase I trial by Ramalingam et al. [129], combination treatment with vorinostat, carboplatin and paclitaxel led to SD in the 1 MPM patient included. The same team evaluated another HDACi, belinostat, in a phase II trial in 13 patients. There were no responders, and PFS was only 1 month and OS was 5 months. Only 2 patients (15%) had SD [130]. In vitro studies suggest increased efficacy of HDACi in combination with other agents [131].

In vitro data suggest that VPA has proapoptotic effects in MPM, and synergistic effects of VPA with chemotherapeutic agents such as doxorubicin were noted. In a recent phase II study by Scherpereel et al. [132], the effect and safety of VPA combined with doxorubicin were evaluated in a total of 45 MPM patients with performance status 0–2 after at least 1 chemotherapy (platinum/pemetrexed). Response rate was 16% (95% CI 3–25%), and disease control rate was 36% (95% CI 22–51%). OS was 6.7 months (95% CI 4.9–8.5 months). New clinical trials testing HDAC (vorinostat or VPA) combined with cisplatin and pemetrexed are now proposed for chemonaïve MPM patients.

**CBP501 EIMC-A12**

Since most cancer cells are dependent on the G2 checkpoint to survive, this has led to the development of CBP501, a G2 checkpoint abrogator. One phase I trial by Shapiro et al. [133] included 3 patients treated with CGP501 combined with cisplatin. One patient had PR and PFS of 9.7 months. Two patients had SD that lasted 11 and 3 months, respectively. A combined phase I/II trial is currently ongoing and enrolling patients with solid tumors (phase I) and MPM patients (phase II). MPM patients will be randomized to pemetrexed and cisplatin with or without CBP501.

IMC-A12 is an antibody targeting IGF-1. Inhibition of the IGF-1 receptor has led to decreased cell proliferation and enhanced the cytotoxic effect of cisplatin in vitro [70].

**Immunotherapy and Gene Therapy**

Immunotherapy with systemically administered IL-2 has limited efficacy but substantial adverse effects [134, 135] whereas intrapleural administration of IL-2, which is well tolerated and results in objective responses, needs...
further study to assess its superiority to conventional treatment [136]. Nevertheless, further studies of systemic IL-2, as well as artificial upregulation of endogenous IL-2 by gene transfer, are underway, based upon data from murine models of mesothelioma [137].

Rapamycin, a natural macrolide approved as immunosuppressor, was found to exert antiproliferative effects by inhibition of serine/threonine kinase, which is called mTOR in mammals. Synthetic derivatives or ‘rapalogs’ have been developed to improve the pharmacological properties of rapamycin: everolimus, temsirolimus and deforolimus.

Bortezomib is a potent inhibitor of the 20S proteasome, which has shown cytotoxic effects in vitro and in MM xenografts in vivo [138, 139]. On the basis of promising preclinical data, two phase II trials of bortezomib have been initiated in Europe. One trial is exploring single-agent activity in the second-line setting and in patients with a performance status of 2 in the first-line setting [140].

Interferon (IFN)- α 2a and IFN-γ 1b have been combined with various standard chemotherapies for mesothelioma. The response rates in these trials have been variable (for IFN-α 2b plus cisplatin, 27–36% [141]; for IFN-α 2b with doxorubicin alone, 11% [142] and for IFN-α 2b with cisplatin plus doxorubicin, 29% [143]).

High-dose methotrexate was combined with IFN-α and IFN-γ in a series of 24 evaluable patients with advanced mesothelioma. Seven (29%) had a PR, and the 1- and 2-year survival rates were 62 and 31%, respectively [144]. A small study of epirubicin plus IL-2 showed only 1 PR among 25 patients with advanced disease [145].

Vaccine approaches are also under investigation for the treatment of MPM. WT-1 peptide epitopes that stimulate T-cell immunity are currently under evaluation for the treatment of mesothelioma. Preliminary results from ongoing studies have documented the safety of this vaccine.

Interesting preliminary results were observed after administration of Mycobacterium vaccae in a limited number of patients. This needs to be confirmed before exploring this treatment further.

Exploiting the immunostimulatory capacities of dendritic cells (DCs) holds great promise for cancer immunotherapy. Currently, DC-based immunotherapy is evaluated clinically in a number of malignancies, including melanoma and urogenital and lung cancer, showing variable but promising results. In a murine model, Hegmans et al. [146] demonstrated that immunotherapy using pulsed DCs may emerge as a powerful tool to control mesothelioma outgrowth. This team showed that mesothelioma is infiltrated by immune effector cells but also contains cytokines and regulatory T cells that suppress an efficient immune response.

Immunotherapy of mesothelioma might be more effective when combined with drugs that eliminate or control regulatory T cells [147]. The results of a phase I clinical trial addressing the safety and feasibility of tumor lysate- or exosome-pulsed DCs to induce tumor-specific cytotoxic T-lymphocyte responses in patients with MPM were recently published [148]. The goal of this trial in 10 MPM patients was to assess the safety and immunological response induced by the intradermal and intravenous administration of tumor lysate-pulsed DCs at 2-week intervals after chemotherapy. The treatment was safe with no grade 3 or 4 toxicities associated with the vaccines or any evidence of autoimmunity; moderate fever was the only side effect. Interestingly, local accumulation of infiltrating T cells was found at the site of vaccination. Immunological response to tumor cells was detected in a subgroup of mesothelioma patients.

Intracavitary Therapy

The pleural space provides an easy access for molecules to the body and novel intrapleural therapies for mesothelioma are under clinical development [149]. Intraoperative intracavitary chemotherapy has been used in an effort to improve local disease control. In a series of 92 patients, hyperthermic intracavitary perfusion with cisplatin (225 mg/m²) was performed following extrapleural pneumonectomy [150]. Recurrence of pleural mesothelioma was seen in 51% of the patients, but the value of this approach remains uncertain due to selection bias and the lack of randomized trials.

Intrapleural instillation of a replication-deficient, recombinant adenovirus has been used to insert the gene for the herpes simplex virus thymidine kinase gene, thus making tumor cells sensitive to the normally nontoxic antiviral drug ganciclovir or to deliver the gene for human type I IFNs (β/α) to induce antitumor immune responses [151–153]. A number of long-term clinical responses suggest that this approach has some promise, and also has the capacity to induce significant humoral and cellular antitumor immune responses [153, 154].

One of the most attractive targets for therapy is mesothelin, a tumor differentiation antigen that is overexpressed by most epithelial mesotheliomas but not by normal cells [155]. Three mesothelin-targeted agents are in various stages of clinical development. These include SSIP (anti-mesothelin dsFv-PE38), a recombinant immunotoxin composed of an anti-mesothelin Fv fragment
linked to a truncated Pseudomonas exotoxin [156–158], MORAb-009, a chimeric anti-mesothelin monoclonal antibody [159], and CRS-207, a live-attenuated Listeria monocytogenes vector encoding human mesothelin [160]. The rationale for mesothelin as a tumor vaccine is that mesothelin elicits a strong T-cell response in patients [161]. Phase I trials have been completed with SSIP and MORAb-009, and some evidence of minor antitumor activity has been observed with SSIP [162].

Conclusion

It is obvious that a continuous collaboration between clinicians, pathologists [163] and basic researchers will be a crucial step to improve the treatment of mesothelioma patients. Thus, during these last 5 years, many studies on VEGF/VEGFR, PI3K/AKT/mTOR pathway, or on mesothelin, HDACi and immunotherapy/gene therapy from the bench to the bedside that are very encouraging. However, further in-depth analysis is required for a more advanced deciphering of the step-by-step process leading from early increased mesothelial cell proliferation to invasive mesothelioma, from which we are expecting the development of definitively effective therapy. Moreover, many questions remain to be answered such as: how long should we give first-line treatment? Which second-line treatment should we use? What is the role of targeted therapies, either alone or combined with chemotherapy, surgery and/or radiotherapy? Therefore, as already emphasized by all mesothelioma experts, it is essential that all MPM patients should be recruited in clinical trials and research studies to fasten translational research and to improve the management of this rare and aggressive cancer [164].

References


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