Malignant Pleural Mesothelioma: 
from the bench to the bedside

Introduction
Malignant pleural mesothelioma (MPM) is a highly aggressive, rare form of cancer with a growing rate of incidence and an often unfavorable prognosis. Optimal management of this disease has not yet been defined due to the lack of an indisputedly effective therapy, although various scientific societies have proposed practical guidelines that are being applied in standard clinical practice. These guidelines emphasize the difficulty of diagnosing MPM and note the poor results of current treatments, thus emphasizing the need for innovative therapies and methods to monitor patients suffering from this disease. Although the prognosis of MPM is often unfavorable and the prospects pessimistic, recent studies investigating the pathogenesis and biology of this disease have revealed some interesting findings, pointing to promising significant progress for the future treatment of these patients. Translational research in this disease is making great progress and different molecular oncogenic pathways leading to the growth and progression of MPM have been characterized and better defined, leading to exciting pharmaceutical developments. However, more in-depth analysis still needs to be done to further define the processes, including increased early mesothelial cell proliferation to the progression of invasive mesothelioma. All this information will help define an effective, more personalized treatment for these cancer patients.

The purpose of this bibliographic review of the scientific literature is to provide an overview of the recent advances in our knowledge of the biology of MPM and their potential therapeutic-diagnostic applications. This article is written in a non-scientific style to make it more available and accessible to a varied audience. We therefore refer interested readers and experts to the chapter containing the references, which could prove useful to anyone who would like to learn more about the studies analyzed in this review.

New therapeutic approaches
The role of surgery and radiotherapy for the treatment of MPM is still controversial and further studies may eventually provide more information in this regard. However, medical therapy is considered the standard treatment in clinical practice, and the combination of platinum-based chemotherapy with antimetabolites (pemetrexed/raltitrexed) in particular has been considered the best first-line therapy for patients with MPM (1,2). Results obtained thus far have been limited, however, especially in terms of survival. The main goal is to increase knowledge about the pathogenesis of MPM to define and improve target therapies and new therapeutic agents currently under investigation. The purpose of this

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review of the scientific literature is to describe the main therapeutic approaches currently being investigated in preclinical and clinical studies.

**Epithelial Growth Factor Receptor**

The epithelial growth factor receptor (EGFR) performs a role in the proliferation, differentiation, migration, adhesion and survival of cells (3) and is overexpressed in over 50% of MPM patients (4). The expression of the receptor in mesothelioma cells has led to the hypothesis that a therapy targeted against EGFR will inhibit it and prevent its uncontrolled and often harmful activity. This is why a number of studies have investigated the efficacy of EGFR inhibitors such as gefitinib and erlotinib in chemotherapy-naive patients. These studies have demonstrated that these drugs are not very effective as first-line therapy in MPM when they are administered as monotherapy, so not in conjunction with any standard chemotherapy (5, 6, 7). However, although the EGF receptor is overexpressed in mesothelioma, the reason why EGFR inhibitors are not very effective may be because mutations of this receptor are rare (8).

Some studies disagree about the correlation between the overexpression of EGFR in mesothelioma cells and the response to treatment with inhibitors of this receptor. Some research groups have proven that there is no relationship between the overexpression of EGFR and the clinical outcome of MPM patients (9,10), while others have shown that patients who overexpress the receptor may have a better outcome (11-14). These discrepancies confirm the need for further research to better define the results. In any case, it has been shown that overexpression of EGFR in MPM is more common in the epithelial histological subtype, which is associated with better patient survival but is not an independent prognostic marker (13,14).

Recent results indicate the presence of an important communication network between the EGFR pathways and other cellular signaling pathways. For example, some proteins such as PI3K and AKT which play a role in the EGFR signaling pathway, also act in other cellular growth pathways and interact with other factors such as c-MET and IGF-1 (15,17). The histological overexpression of the c-MET protein has been documented in MPM and also in some normal pleura samples. According to this rationale, c-MET inhibitors have been investigated in mesothelioma cell lines; preliminary results have shown a dose-dependent inhibition of tumor growth (18).

This type of dose-dependent inhibition was also observed in MPM cell lines subjected to IGF receptor inhibitors (19). Moreover, the cytotoxic effect of cisplatin also increased when administered together with these inhibitors (20).

An important biological communication also exists between EGFR and cyclooxygenase 2 (COX-2) (21). COX2 is overexpressed in many solid tumors and so it is considered a potential therapeutic target (22-24). Immunohistochemical studies of this protein in MPM demonstrated that it was overexpressed in 59-100% of the tumor samples analyzed (25-27). They also showed that treating
mesothelioma cell lines with COX2 inhibitors induces cytotoxicity and increases the effect of pemetrexed (28-29).

K-ras, BRAF and PI3KCA mutations
In the search for therapeutic targets, researchers have investigated the presence of genetic mutations associated with neoplastic pathogenesis, leading to studies of K-ras, BRAF and PI3KA gene mutations.
Unfortunately, genetic mutations of K-ras were not seen in the initial studies (30-32), thus changing expectations about this protein as a potential therapeutic target.
Studies of BRAF gene mutations have shown that they are absent in various tissues and tumor cell lines (33); other authors (34) have studied different MPM cell lines to analyze the PI3ka gene, but no mutation has been seen.

PTEN
PTEN is a protein that has been investigated in MPM to evaluate a potential therapy that would interact with this pathogenetic pathway.
Recent studies of various mesothelioma samples have shown that there is a loss of expression of the protein and that the mutation of this protein expression can be considered a negative prognostic value. In fact, patients with a decreased or lack of PTEN expression had a worse prognosis, whereas those with no genetic mutation had a greater survival rate (35).
It was also observed that the loss of PTEN expression might result in increased AKT activity, another important factor associated with the pathogenesis of cancer (36, 34). The loss of PTEN expression, which activates AKT, may induce resistance to various biological treatments such as EGFR inhibitors or anti-EGFR monoclonal antibodies. Therefore, these mutations also have consequences on other pathogenetic pathways, demonstrating the complexity of the pathogenesis and the intersecting network between these biological factors.

VEGF / VEGF Receptors
VEGF receptors have also been studied in mesothelioma cells and preclinical studies have shown they are expressed in both tumor tissue and peripheral blood in MPM patients (37).
The rationale for using drugs that inhibit this biological pathway is because they are expressed in greater levels in MPM patients than in healthy subjects. Also, the increased levels of VEGF are associated with increased microvascular density and appear to be associated with an unfavorable prognosis (38) as well as the likelihood of disease progression (39-41).
Anti-VEGF antibodies that actively inhibit this factor have been investigated. Studies have also tested the efficacy of combined VEGF and EGF inhibitors, in which these combinations achieved
disease stabilization in 50% of patients, progression-free survival of 2.2 months and a median survival of 5.8 months (42, 43).

Drugs currently being investigated include vatalanib and cediranib, which are VEGF receptor inhibitors with antitumor activity in a variety of solid tumors (44-47).

Semaxanib is another VEGF-1 receptor inhibitor, but it also acts on the PDGF receptor (PDGFR) and c-kit (48). Another drug is thalidomide, which has been investigated in MPM patients with the following results: no partial or complete responses, 27.5% of patients were progression free after 6 months, and median overall survival of 7.6 months (49).

Sorafenib has shown limited activity in non-resectable MPM patients (50). However, it has also been studied in combination with doxorubicin, which confirmed that this combination is well tolerated thus justifying further clinical studies (51).

Sunitinib has been investigated in a Phase II study in MPM as second-line treatment after chemotherapy with platinum and antimetabolites, with the following results: partial response in 12% of patients; disease stabilization in 65% of patients; mean time to progression of 3.5 months and overall survival of 7 months (Nowak et al., IMIG 2011, unpubl. data).

Various Phase II studies have been conducted to determine the efficacy of imatinib mesylate in MPM patients refractory to chemotherapy or chemotherapy-naive patients (52-54). Combination studies between imatinib, cisplatin and pemetrexed are currently underway (55). New research is investigating the utility of these drugs, which appear to be active in MPM due to their ability to induce apoptosis of the tumor cells and by inhibiting various metabolic pathways such as AKT/PI3K, for example; the efficacy of these drugs has also been demonstrated due their ability to increase the sensitivity of the tumor to chemotherapy with gemcitabine or pemetrexed (56).

**PDGF / PDGFR**

The discovery that the PDGF receptor is highly expressed in mesothelioma cells has further defined the rationale for targeting treatment against this molecule (57).

The increased secretion of PDGF appears to be associated with thrombocythemia, which is considered a prognostic factor of adverse events that is seen in many MPM patients (58-59). In fact, high serum levels of PDGF in MPM patients appear to be a predictive factor of an unfavorable prognosis.

The expression of c-kit in MPM cells has also been shown in 26% of patients, prompting a number of clinical studies investigating imatinib in this disease (60).

Inhibition of PDGFR using imatinib combined with paclitaxel reduced interstitial fluid pressure, thereby enhancing the effect of the drugs and increasing in vitro efficacy (61). A partial response was seen in a Phase I study of imatinib in combination with gemcitabine (62). Dasatinib has shown cytotoxic effects in preclinical studies, leading to decreased migration and invasion in mesothelioma cells (63-64).
**PI3K/AKT/mTOR Pathway**
The PI3K/AKT/mTOR biological pathway is often aberrant in MPM, and various in vitro studies have shown that inhibiting this intracellular pathway may induce apoptosis in MPM cell lines (36, 65).

Sirolimus is a drug that has been approved as an immunosuppressant and which is currently used mainly in kidney transplantation and has an anti-proliferative effect on the PI3K/AKT/mTOR pathway.

Temsirolimus, a derivative of rapamycin, was investigated in a Phase I study but did not produce meaningful results (66). Studies investigating the combination of cisplatin and sirolimus are also underway, which have shown synergistic anti-tumor effects in MPM cell lines (67).

**Mesothelin**
Mesothelin is highly expressed in a number of cancer types, including ovarian, pancreatic, some squamous carcinomas and the epithelial subtype MPM (68, 69).

Overexpression of the membrane protein mesothelin in MPM and its limited distribution in normal tissue has raised interest in this protein as a potential anti-tumor target (70).

Preliminary studies have not yet produced meaningful results (71); however, a number of drugs with anti-mesothelin activity are currently being investigated (72). Synergistic effects from the combination of these new agents with chemotherapy have been observed (73) offering promising results.

**Ribonucleases**
Ribonucleases are proteins that act on cellular RNA. Ranpirnase belongs to this group of proteins and has been investigated for its potential to induce apoptosis of tumor cells and inhibit cellular growth and proliferation.

However, various treatment-related adverse events have been observed, such as renal insufficiency, allergic reactions, arthralgia and peripheral edema (73).

**Asparagine-Glycine-Arginine-human**
TNF has known anti-tumor activity which is activated by inducing apoptosis of tumor cells. Studies of systemic treatment with this drug have shown that it is highly toxic and so it must be administered in such low doses to avoid disabling side effects that it is rendered ineffective (75-76). Researchers have investigated a molecule consisting of a TNF fused to a peptide (tumor-homing peptide asparagine-glycine-arginine (NGR)) which is capable of selectively binding to mesothelial cells and has shown good tolerability as well as promising responses (77).
**HDACi**

Histone deacetylase inhibitors (HDACi) have been shown to alter the growth of numerous cancerogenic cell types. These molecules, many of which are derived from natural sources, have shown that they can inhibit proliferation, induce differentiation, and induce apoptosis of tumor cells. Preliminary data from a Phase I study suggest that vorinostat has clinically significant activity in mesothelioma patients (78).

However, other studies have shown that this drug does not increase survival (79). Vorinostat has also been investigated in combination with carboplatin and paclitaxel (80) and shown disease stabilization in some cases.

Belinostat is another drug that belongs to this group but has not proved to be superior to the other drugs (81). However, in vitro studies have demonstrated increased efficacy of these inhibitors when they are administered in combination with other agents (82, 83).

**CBP501 EIMC-A12**

Cells constantly undergo control processes to verify whether they are free of mutations and can continue in their cell cycle and multiply, or whether there are anomalies, in which case their life cycle must stop, lead to apoptosis and destruction of the cells to prevent more extensive damage. There are actually check-points that the cells must pass to obtain “permission” to proceed in their cellular cycle. Although they are anomalous, tumor cells can overcome these controls and can escape from being destroyed.

Drugs that act on these checkpoints have been defined to block the cell cycle of tumor cells which otherwise would continue proliferating and multiplying (84). Studies have also documented partial responses to treatment with these drugs when administered in combination with cisplatin (20), by enhancing the chemotherapy induced by these drugs.

**Immunotherapy and Gene Therapy**

Immunotherapy is another treatment that has contributed to significant advances and is currently being studied. An example of this treatment is the systemic administration of IL-2, which unfortunately had only limited efficacy and some side effects (85-86). Intrapleural administration of IL-2 has also been investigated and found to be well tolerated with objective responses, although further studies are needed to evaluate whether it offers more benefits than conventional treatment (87). Studies are also investigating systemic therapy with IL-2 by gene transfer of endogenous IL-2 as well as artificial regulation (88). Rapamycin is a natural macrolid that has been approved as an immunosuppressant and appears to have anti-proliferative effects by inhibiting some kinases such as mTOR. Synthetic derivatives of rapamycin known as “rapalogs” have been developed to
improve the pharmacological properties of this macrolide; several examples are everolimus, temsirolimus and deforolimus. Bortezomib is a potent proteasome inhibitor that has shown interesting cytotoxic effects in vitro and in vivo (89-90). Several studies are currently underway based on promising preclinical data (91). Studies are evaluating the combination of interferon and various standard chemotherapy regimens, which have shown variable response rates to the treatment (92-95). Vaccine therapies have also been studied, aiming to stimulate immune activity against tumor cells in MPM patients. Some very interesting studies aimed at activating the immunostimulant ability of dendritic cells have also demonstrated variable but promising results (97-99).

**Intrapleural therapy**

The pleural cavity provides easy access for therapeutic molecules and intrapleural administration of drugs active in this disease could certainly offer excellent therapeutic prospects (100). Various studies have evaluated the intracavitary administration of chemotherapy even after surgical resection, with the goal of enhancing local control of the disease (101-103). Results have shown a 50% relapse of the disease after resection together with intrapleural chemotherapy, but further studies are needed to confirm these results that could probably show better responses. Studies are also evaluating the intrapleural administration of recombinant viruses to try to sensitize tumor cells to drugs administered later (104-106). Anti-mesothelin agents have also been injected into the pleural cavity (107-112), with the aim of inducing an immune response that could also act against tumor cells (113).

**Conclusion**

It is clear that clinicians, pathologists (114) and basic researchers must collaborate constantly to improve the treatment of rare but very aggressive diseases which often have an unfavorable prognosis such as MPM. Numerous studies have been conducted over the last few years to investigate targeted molecular therapy and the biological pathways involved in the pathogenesis of this disease. We need to gain a better understanding of the basic mechanisms of the development of this cancer in order to sufficiently understand the biomolecular pathways that play a role in cancerogenesis and so more effectively inhibit them. All these new studies, including those currently underway, have contributed to new advances or encouraging findings. However, further studies and more in-depth analyses carefully conducted and controlled will be able to confirm the results obtained thus far, as well as provide new information in order to achieve an effective therapy.
As MPM experts have already emphasized, all MPM patients must be afforded the opportunity to participate in ongoing clinical studies, not just to contribute to translational research but mainly to gain access to treatments which, even though they are still being defined and investigated, can contribute to as yet undefined treatment lines regimens (115).

References


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