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Latest developments in our understanding of the pathogenesis of mesothelioma and the design of targeted therapies

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Abstract

Malignant mesothelioma is an aggressive cancer whose pathogenesis is causally linked to occupational exposure to asbestos. Familial clusters of mesotheliomas have been observed in settings of genetic predisposition. Mesothelioma incidence is anticipated to increase worldwide in the next two decades. Novel treatments are needed, as current treatment modalities may improve the quality of life, but have shown modest effects in improving overall survival. Increasing knowledge on the molecular characteristics of mesothelioma has led to the development of novel potential therapeutic strategies, including: (i) molecular targeted approaches, i.e. the inhibition of vascular endothelial growth factor with Bevacizumab; (ii) immunotherapy with chimeric monoclonal antibody, immunotoxin, antibody drug conjugate, vaccine and viruses; (iii) inhibition of asbestos-induced inflammation, i.e. aspirin inhibition of HMGB1 activity may decrease or delay mesothelioma onset and/or growth. We elaborate on the rationale behind new therapeutic strategies, and summarize available preclinical and clinical results, as well as efforts still ongoing.

Keywords

Mesothelioma; Carcinogenesis; Polyclonal tumors; Asbestos; BAP1; HMGB1; Aspirin; Molecular therapy; Immunotherapy

Introduction

Malignant mesothelioma (MM) is a rare tumor arising from the mesothelial cells lining the pleural and peritoneal cavities, or less commonly from the pericardium and the tunica vaginalis of the testis [1].

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In the US and Europe up to 80% of MMs occur in the pleura and are defined as malignant pleural mesothelioma (MPM), since asbestos, after inhalation in the lungs, reaches the pleura via the lymphatic system. The association of MPM with asbestos exposure is well established [2]; further dissemination of asbestos to the peritoneum may occur when exposure is high [3]. The mean age at diagnosis is 74 years old, and since the main source of asbestos exposure is occupational in origin, the male to female sex ratio is 4:1–8:1 (highest in areas where asbestos exposure is high). MPM prognosis remains very poor, with a median survival of 6–12 months, and a 5-year survival <5% [1]. MPM is resistant to chemotherapy, although the combination of pemetrexed and cisplatin led to an overall survival benefit of about 11 weeks [4]; presently this is the most commonly used chemotherapy.

Peritoneal mesotheliomas, instead, are less common, representing about 20% of all MMs in the US, and are rarely associated with asbestos exposure, partially explaining why they are less frequent than MPM [1]. Peritoneal mesotheliomas often occur in a younger age group and the male to female sex ratio is close to 1. All of these findings are expected in MMs that are not related to occupational exposure. In peritoneal mesotheliomas, cytoreductive therapy together with hyperthermic intraperitoneal chemotherapy can significantly improve prognosis [5–7]. Thus, peritoneal mesotheliomas often present different pathogenesis and clinical challenges than MPM.

In this review, we choose to focus on MPM. The increase in MPM incidence has been associated with widespread use of asbestos in the past century. The latency period of asbestos-associated MPM is on average 30–60 years [8], which accounts, at least in part, for the fact that MPM incidence is still raising worldwide despite working bans on the use of asbestos in the early 1990s [9]. Moreover, many economical emerging countries have not (yet?) prohibited asbestos usage; therefore, in these countries an even higher number of MPM patients is expected in the future [10]. Asbestos refers to a family of six mineral fibers that were used commercially in the '70s, that are classified into two subgroups: (i) the amphiboles, a group of rod-like fibers including amosite (brown asbestos), crocidolite (blue asbestos), anthophyllite, actinolite and tremolite; and (ii) the serpentine group, consisting of chrysotile (white asbestos) [11]. The mechanisms responsible for the genesis of MPM as a result of asbestos exposure are being elucidated. What has emerged so far, is that the inhalation of long and thin asbestos fibers induces a chronic inflammatory response at sites of fibers deposition that over time may lead to malignant cell transformation. Three main contributing mechanisms have been proposed. (i) Mesothelial cells and macrophages exposed to asbestos fibers generate reactive oxygen species (ROS) and reactive nitrogen species, which lead to DNA damage [12,13]. (ii) Asbestos fibers absorb a variety of proteins and chemicals, which may result in the accumulation of hazardous molecules including carcinogens [14]. (iii) Asbestos-exposed mesothelial cells and macrophages release a variety of cytokines and growth factors, including high-mobility group box 1 (HMGB1) and tumor-necrosis factor- α (TNF- α) [15–17], which induce inflammation and facilitate malignant transformation of mesothelial cells that have accumulated DNA damage [18].

In the US and Europe, exposure to asbestos is the main, but not the exclusive, risk factor connected with MM. The fact that fewer than 5% of asbestos miners who mined asbestos for more than 10 years developed MM [1], indicates that –fortunately– most people exposed to

high amounts of asbestos do not develop MM. Additional well-established risk factors for MM include exposure to the naturally occurring asbestos-like mineral fibers, such as erionite, antigorite, etc., chest irradiation, and germline *BRCA1-associated protein 1 (BAP1)* mutations [1,19–24].

In this review we briefly summarize established therapies, and then we focus on the current knowledge of the molecular pathogenesis of MM, and the rationale behind investigating novel targeted approaches. We discuss three main areas: (i) molecular therapies, (ii) immunotherapy, (iii) targeting asbestos-induced inflammation. For each topic we review available results on agents under clinical investigations, and ongoing efforts to improve MPM treatment.

Established therapies

Treating MPM patients remains a challenge. Current approaches are: chemotherapy, and multimodal treatment including surgical resection combined with chemotherapy and/or radiotherapy, photodynamic therapy (PDT), and hyperthermic perfusion of the pleura followed by resection [25]. Residual microscopic disease persists even after the most complete surgical resection, and therefore resection is often associated with a local adjuvant treatment in order to kill residual tumor cells [25]. Pleurectomy is currently the surgery of choice as extrapleural pneumectomy has higher morbidity without showing significant survival advantages [26,27]. Although MM in general is sensitive to radiation, the overall efficacy of radiation therapy is limited because irradiation to a widespread tumor area with a high radiation dose causes severe adverse effects, including pneumonitis, myocarditis, and myelopathy due to spinal cord toxicity. Radiation therapy is therefore mostly used for palliative purposes or in combination with surgery. Among local adjuvant treatments to resection, PDT is a light-based cancer treatment. The effect of PDT requires the interaction of three components: a photosensitizer, oxygen, and light with the specific wavelength activating the photosensitizer. None of these are individually toxic, but when combined they induce a tumoricidal photochemical reaction [28,29]. The first, and so far only, phase randomized III clinical trial conducted by Dr. Pass and colleagues at the NCI, found no differences in survival by randomizing patients to surgery with intraoperative PDT versus surgery without PDT (14.4 versus 14.1 months survival, respectively) [30]. It has been suggested that the technique of intrapleural PDT has improved through the years of practice, leading to a better tolerance of this treatment [28,29]. Two recent studies suggested that PDT offers an improved survival even in patients with locally advanced stages of MPM [31–33]. The hypothesis that PDT improves survival in MM patients needs to be confirmed by a new randomized clinical trial.

Surgery in combination with radiation and chemotherapy can be used for healthy patients with early stage disease, but most patients have unresectable disease at the time of diagnosis and are often treated only with palliative chemotherapy [34]. Cisplatin and pemetrexed (an antifolate) combination chemotherapy is the current standard first-line therapy approved by the US Food and Drug Administration (FDA) for patients with advanced and unresectable MPM. Two Phase III trials have demonstrated improved survival of about 11 weeks with an antifolate plus cisplatin over treatment with cisplatin alone [4,35]. However, most MPM

patients do not respond to this or other therapies, and even those MPM that responds to therapy, rapidly become resistant to it. MPM patients have therefore a limited life expectancy usually associated to poor quality of life [36]. Second-line therapy is currently not well defined [37].

Our recent finding that MM are polyclonal tumors, i.e., formed by the coalescence of different independent subclones, may account for a high degree of intratumoral heterogeneity and contribute to the emergence of drug-resistant subpopulations [38]. The polyclonal origin of MM [38] highlights the need to attack simultaneously several different molecular targets in order to eliminate the different clones, as each clone may carry its own distinct set of molecular alterations.

Molecular genetics and molecular therapies

Germline and somatic BAP1 mutations: opening of new frontiers

In 2001 we discovered that, in some Turkish families, an extremely high susceptibility to develop MM was transmitted in an autosomal dominant fashion [19]. Our findings were initially received with some skepticism, as some researchers did not believe that genetics could play a role in MM [39]. Only recently we identified germline mutations in *BAP1*, a tumor suppressor gene located on chromosome 3p21.3, as the cause of the “BAP1 cancer syndrome” [40]. This cancer syndrome is characterized by the presence of benign atypical melanocytic lesions [41, 42], known as melanocytic BAP1-mutated atypical intradermal tumors (MBAITs) [40], a very high incidence of both pleural and peritoneal MMs as well as uveal melanomas (UVMs). Carriers of germline BAP1 mutations also have an elevated risk of developing several other malignancies, such as cutaneous melanoma, clear cell renal cell carcinoma, intrahepatic cholangiocarcinoma, basal cell carcinoma, etc. [24, 43–46]. All known carriers of germline *BAP1* mutations have -so far- developed one or more malignancy by age 55 [47].

In addition to germline mutations, we recently conclusively demonstrated that the majority (63.6%) of sporadic MMs contain somatic *BAP1* mutations/inactivation [48]. Our findings confirmed previous data on *BAP1* inactivation in epithelioid-type MM [49], and are supported by two recent next generation sequencing (NGS) studies of the MPM genome [50, 51]. These two NGS studies revealed that various inactivating mutations occur randomly and are rarely shared among MPM biopsies, with the exception of BAP1 that was found mutated in 41% [50] and 58% [51] of MPMs, respectively (peritoneal MM were not studied), pointing at BAP1 as the putative driver mutation for a significant number of MMs.

It has been suggested that somatic loss of *BAP1* is associated to a slightly longer survival [52, 53], a finding probably related to the fact that most somatic mutations are detected in epithelial MM that have a better prognosis than biphasic and sarcomatoid MM.

When MM occur in a setting of germline *BAP1* mutations, their prognosis is significantly better with survivals of 5–10 or more years [54]. We are investigating how germline BAP1 mutations on one hand promote MM development, while on the other hand they are associated to reduce disease aggressiveness.

BAP1 is a member of the ubiquitin C-terminal hydrolase (UCH) subfamily of deubiquitinating enzymes (DUBs). Among UCH family members, BAP1 is unique because of its long C-terminal tail, which contains two nuclear localization signals [55]. It has been postulated that to function as a tumor suppressor BAP1 must maintain both nuclear localization and deubiquitinating activity [56]. In the nucleus, BAP1 is present in multiprotein complexes and has been functionally implicated in the regulation of various biologic processes including: cell cycle, cellular differentiation, gluconeogenesis, chromatin remodeling, gene transcription, and DNA repair [47]. Currently, BAP1 functions as a tumor suppressor have been ascribed to (i) BAP1 deubiquitination of histone H2A, leading to transcriptional activation of genes that regulate cell growth [57]; (ii) BAP1 functions as a transcriptional coregulator by interaction to host cell factor-1 (HCF1), Ying Yang 1 (YY1), and E2F1, to induce transcription of genes involved in cell cycle regulation [58–61]; and (iii) BAP1 contribution to DNA repair [62,63] (FIGURE 1).

Given the involvement of BAP1 in chromatin remodeling and its ability to deubiquitinate H2A, it is possible that BAP1 mutations confer an enhanced sensitivity to ‘epigenetic’ modulators. Epigenetic regulation of tumor suppressor genes through chromatin condensation and decondensation has emerged as an important mechanism that leads to tumorigenesis. The balance between the acetylated and deacetylated forms of histone proteins is regulated by histone acetyltransferases (HATs) and histone deacetylases (HDACs). HATs increase acetylation promoting greater chromatin accessibility for gene expression. HDAC inhibitors alter the wrapping of the DNA around histones, modify the access of transcription factors and consequently impact the expression of various genes. The effect of 4 different HDAC inhibitors, valproic acid, trichostatin A, LBH-589, and suberoylanilide hydroxamic acid (vorinostat), was analyzed in primary UVM cells and in UVM cell lines. These compounds were able to reverse the H2A hyperubiquitination caused by BAP1 loss, and induced morphologic differentiation, cell-cycle exit, and a shift to a differentiated, melanocytic gene expression profile in cultured UVM cells. Valproic acid inhibited the growth of UVM tumors *in vivo* [64]. *In vitro* data for the role of HDAC inhibition in MM showed increased apoptosis in MM cell lines after treatment with HDAC inhibitors, either alone or in combination with chemotherapy [65–70]. Complete suppression in a mouse xenograft model of a human MM line was observed after a combination of valproic acid and chemotherapy [70]. Recently, BAP1 loss was shown to alter sensitivity to HDAC inhibitors in MPM cells through regulating the transcription of HDAC2. However, it was also found that established MM cell lines with low endogenous HDAC2 were resistant to HDAC inhibition [71]. These findings suggested that HDAC inhibitors might be effective in the adjuvant therapy of patients with *BAP1* mutated MM. Vorinostat is approved by FDA for the treatment of cutaneous T-cell lymphoma. In a Phase I trial, four (30%) of 13 patients with MPM that received vorinostat had a stabilization of their disease lasting more than 4 months; in addition, two unconfirmed partial responses were observed [72]. However, in a recently completed Phase III trial (VANTAGE 014) including 660 pre-treated advanced MPM patients, vorinostat given as a second-line or third-line therapy did not improve overall survival. Therefore, was not recommended as a therapy in MPM patients [73]. Another small phase II trial (conducted on thirteen patients) with the HDAC inhibitor, belinostat, also produced negative results [74] (FIGURE 1).

The molecular mechanism of BAP1 as tumor suppressor has not been fully elucidated yet, and there is much hope for possible therapies targeting BAP1 functions or capable to restore its activity.

To date, we found that none of the MM patients carrying germline *BAP1* mutations were professionally exposed to asbestos. A possible explanation is that germline *BAP1* mutations alone can cause MM and many other tumor types. Moreover, *BAP1* mutations might influence the asbestos-induced inflammatory response that is linked to asbestos carcinogenesis, thereby increasing the risk of developing MPM after minimal exposure. Indeed, in a recent study we observed that *BAP1*^{+/-} mice [75] had a significantly higher incidence of MM after exposure to very low doses of asbestos fibers that rarely induced MM in wild-type mice [76]. This was correlated with significant alterations of the inflammatory response. We observed significantly higher levels of pro-tumorigenic alternatively polarized M2 macrophages, lower levels of anti-tumoral classically activated M1 macrophages, and therefore a significantly higher M2/M1 ratio in asbestos-exposed *BAP1*^{+/-} mice [76], that is associated with a worse prognosis [77]. *BAP1*^{+/-} mice also showed lower levels of several chemokines and cytokines, in particular monocyte chemoattractant protein-1 (MCP-1), leukemia inhibitory factor (LIF), and keratinocyte-derived chemokine (KC) [76]. Thus, since germline *BAP1* mutations lead to an altered immune response following deposition of asbestos in tissues, interfering with this immune response might help prevent or delay MM in individuals carrying *BAP1* mutations. We found that *BAP1*^{+/-} mice exposed to low doses of asbestos developed MM at a similar rate as wild-type mice exposed to ten times higher doses [76]. Therefore these findings support the hypothesis that germline *BAP1* heterozygosity increases susceptibility to the carcinogenic effects of low dose of asbestos.

In summary, appropriate genetic counseling and clinical management is important for individuals who carry germline *BAP1* mutation. Reducing exposure to even minimal sources of carcinogenic fibers, and preventive therapies that are capable to modulate asbestos-induced inflammation, such as aspirin, might prove helpful (see below).

Loss of the tumor suppressor gene NF2, encoding Merlin

The role of *NF2* in MM pathogenesis was initially supported by data showing that asbestos-treated *NF2*^{+/-} mice exhibit a markedly accelerated MM tumor formation compared to wild-type littermates [78]. Biallelic inactivation of *NF2* was observed in all asbestos-induced MMs from *NF2*^{+/-} mice, and in 50% of MMs from wild-type mice. The *NF2* gene located on chromosome 22q12 encodes the tumor suppressor merlin, whose activity is tightly regulated by its phosphorylation status [79]. Merlin can interact with various proteins, and in this way modulates multiple signal transduction cascades, including mTOR, focal adhesion kinase (FAK) and Hippo signaling pathways (FIGURE 1).

Loss of Merlin activates mTOR signaling pathway—Merlin mediates cell proliferation through inhibition of mTOR, in an AKT-independent manner [80]. Loss of merlin causes activation of mTOR signaling in MM cells [81], therefore in merlin-silenced tumors there is an upregulation of mitogenic signaling and increased cell proliferation. As expected, merlin-negative MM cells were more sensitive to the mTOR inhibitor rapamycin,

compared to merlin-positive cells [81]. This observation identifies mTOR as a therapeutic target in the large fraction of MMs that carry *NF2* mutations, and provided a rationale for studying mTOR inhibitors in MM. Unfortunately, the oral mTOR inhibitor everolimus (RAD001) had limited clinical activity when tested in a phase II trial S0722 (NCT 00770120) as second- and third-line treatment agent in unselected pre-treated MPM patients [82]. Another phase II trial tested everolimus in MPM patients with *NF2* loss, as a biomarker to predict sensitivity (NCT 01024946), but also in these patients everolimus showed limited clinical activity. It was concluded that additional studies of single-agent everolimus in advanced MPM were not warranted [82].

An aspect to take into account is that mTOR inhibition alone produces compensatory upregulation of PI3KCA, and thereby allows restoration of the downstream AKT signaling [83]. To address this mechanism of mTOR resistance, GDC-0980, a potent and selective oral dual inhibitor of class I PI3K and mTOR were tested. GDC-0980 demonstrated broad activity in various xenograft cancer models, including MPM [84], but pulmonary toxicity of this class of agents limits their application in a clinical setting. Another dual PI3K/mTOR inhibitor, LY3023414 is being tested in a phase I trial.

However, in spite of these so far disappointing results, the role of PI3K/AKT/mTOR survival pathway upregulation in MM is being further evaluated in clinical trials (NCT01655225, NCT01991938).

Loss of Merlin and FAK inhibitor sensitivity—In normal cells, Merlin negatively regulates FAK, a cytoplasmic protein kinase that integrates signals from growth factor receptors and integrins, to control cell adhesion, migration and invasion [85]. FAK expression and/or activity are up-regulated in a wide range of malignancies [86]. In *NF2*-null MM cells, stable overexpression of FAK increased their invasiveness, which was decreased significantly when merlin expression was restored [87]. This finding suggested another possible targeted approach based on patient's selection by merlin status.

The oral FAK inhibitor GSK2256098 is being tested in MM patients alone (NCT01138033) and in combination with MEK inhibitors (NCT01938443). Promising preliminary results for the monotherapy were reported at the 24th EORTC-NCI-AACR Symposium on Molecular Targets and Cancer Therapeutics, with average time before the disease progressed doubled in patients with inactive merlin (Abstract #610). Another ongoing phase I trial is testing the other FAK inhibitor defactinib (VS-6063) in MM (NCT01870609).

Loss of Merlin and disruption of the Hippo signaling pathway—Mutation or inactivation of the *NF2* gene also causes the disruption of the Hippo signaling pathway in MM. The Hippo signaling pathway is a regulator of organ size, development, differentiation, and tissue regeneration, by restricting cell growth, regulating cell division and promoting apoptosis [88]. Merlin is an upstream regulator of the Hippo signaling cascade, which controls the transcriptional co-activator Yes-associated protein (YAP). The merlin-Hippo signaling inactivation leads to constitutive YAP activation [89]. YAP activation in MMs was observed after occasional gene amplification of chromosome 11q22, which is the locus of the YAP gene [90]. YAP activation induces transcription of multiple cancer-promoting

genes, including cell cycle promoting genes like cyclin D1, forkhead box M1 and connective tissue growth factor [91].

The direct inhibition of YAP activity is another possible approach for therapeutic intervention in MM. The screening of a Johns Hopkins Drug Library identified three compounds related to porphyrin that could inhibit the transcriptional activity of YAP *in vitro* [92]. One of these, verteporfin, is in clinical use in photocoagulation therapy for macular degeneration, and it was moderately effective at blocking mouse YAP1-overexpression- or loss of NF2-driven hepatic tumorigenesis. Another possible approach is targeting molecules involved in the NF2/Hippo pathway. Hedgehog signaling has a role in maintaining YAP protein stability, and is activated in mesothelioma. Treatment of mesothelioma xenografts with the hedgehog antagonist HhAntag led to a decrease of the tumor volume accompanied by a decrease in Ki-67 labeling index [93]. These data suggest that a further investigation of these compounds in MM may be warranted.

Deficiency in the CDKN2A /ARF locus

Molecular genetic analysis has revealed several key genetic alterations in MM. Previously, *cyclin-dependent kinase inhibitor 2A (CDKN2A)/alternative reading frame (ARF)* and *neurofibromatosis type 2 (NF2)* were considered the most commonly mutated tumor suppressor genes in MM, as they are found mutated in 30% to 50% and 35% to 40% human MM biopsies, respectively [79,94,95].

The *CDKN2A/ARF* is a tumor suppressor gene located at chromosome 9p21.3 [96]. *CDKN2A* encodes p16^{INK4a}, whereas *ARF* encodes p14^{ARF}. p16^{INK4a} maintains the retinoblastoma protein (pRb) in its active hypophosphorylated form, by inhibiting the cyclin-dependent kinase (CDK)-mediate hyperphosphorylation that leads to pRb inactivation; loss of p16^{INK4a} results in inactivation of pRb and, consequently, failure of cell cycle arrest. The p14^{ARF} protein promotes degradation of the human ortholog of mouse double minute 2 (MDM2), leading to stabilization of p53. The homozygous deletion of *CDKN2A/ARF* leads to loss of function of both p53 and pRb tumor suppressors, with a consequent major impact on the cell cycle (FIGURE 1). Studies in genetically engineered mouse showed that both *CDKN2A/ARF* gene products suppress asbestos carcinogenicity. *ARF* inactivation appears to contribute to MM pathogenesis and genomic instability, and the inactivation of both genes cooperates to accelerate asbestos-induced tumorigenesis in mice [97].

Only a limited number of MM biopsies contain *TP53* mutations, the tumor suppressor gene that encodes p53. However, since genetic defects in p16^{INK4a}/p14^{ARF} are very common, and lead to loss of function of both p53 and Rb, the defective p53 pathways is a potential target for MM gene therapy [98]. Direct restoration of p16^{INK4a} using gene therapy has also been tested and it has shown some promising activity in preclinical models, but is still far from clinical development [99,100].

Receptor Tyrosine Kinase Inhibitors

Receptor tyrosine kinases (RTKs) constitute a large family of receptors that regulate the cell cycle and are often activated in MM [101]. Activation of these receptors triggers biochemical cascades that lead to the transduction of abnormal cell growth signals. This is a

crucial event in cancer initiation and progression, and inhibition of RTKs or of their ligands with specific antibodies or small molecules has been proven to be an effective and safe targeted approach in several malignancies [102].

Mesothelioma secretes pro-angiogenic factors, platelet-derived growth factor (PDGF) and vascular endothelial growth factor (VEGF), both of which are also associated with cell proliferation and pleural effusion. Inhibition of angiogenesis was shown to produce antitumor responses and decrease pleural effusion [103].

High levels of VEGF were found in the pleural effusions of MM patients and were associated with a worse patient survival [104]. Bevacizumab is a humanized monoclonal antibody against VEGF approved for use in several cancers, including metastatic colorectal and renal cancer, and non-small cell lung cancer [105]. Addition of bevacizumab to the standard of care failed to increase survival of MM patients in three independent phase II clinical trials [106–108]. However, at the most recent meeting of the American Society of Clinical Oncology, results from a randomized phase III trial (IFCT-GFPC-0701 MAPS) were presented: in this study, when bevacizumab was added to the standard of care (pemetrexed + cisplatin), patients experienced a significant longer median survival (18.82 months vs. 16.07 months, $p = 0.0127$) (2015 ASCO Meeting, Abstract #7500). These results might translate into addition of bevacizumab as part of the first line treatment for MPM. A specific RTK inhibitor targeting all three isoforms of VEGF receptors (VEGFRs) 1–3, cediranib, also showed only modest activity in unselected MM patients [109,110]. Moreover, other RTK inhibitors targeting VEGFRs and multiple other receptors, such as sunitinib, sorafenib and valatinib, also showed very limited activity in MM [111–116].

An autocrine growth stimulatory effect of PDGF via PDGF receptors (PDGFRs) plays a role in the pathogenesis of MM [117]. High serum levels of PDGF were found in MPM patients and associated with poor prognosis [118]. Expression patterns of PDGF α - and β -receptors (PDGFR α and PDGFR β) differ between normal and malignant mesothelial cell lines. Normal mesothelial cells predominantly express PDGFR α subunit and less PDGFR β mRNA and protein, whereas most MM cell lines produce PDGFR β mRNA and protein [119–121]. Therefore, two PDGFR β inhibitors, imatinib and dasatinib, were tested in clinical trials; however, their use did not result in improved survival in MM patients [122–125].

Erlotinib and gefitinib, inhibitors of epithelial growth factor receptor (EGFR), also failed in clinical trials, despite high expression of EGFR in MM specimens [126,127]. Negative results were obtained also using erlotinib in combination with bevacizumab after platinum-based chemotherapy [128].

Several fibroblast growth factors (FGFs) and FGF receptors (FGFRs) are expressed in MM tissue and cell lines, and a correlation of FGF2 levels with tumor aggressiveness and reduced patient survival has been observed [129,130]. Down-regulation of FGF2 was shown to suppress proliferation of MM cells but did not influence nonmalignant cells [131]. Moreover, inhibition of FGFR1 impairs MM cell growth and migration *in vitro* and *in vivo*, and potentiates the effect of chemotherapeutic drug or ionizing irradiation [130]. Thus,

inhibition of FGF signals appears encouraging and may warrant further evaluation of FGFR targeting strategies as potential anti-MM therapies.

Also the hepatocyte growth factor (HGF) and the receptor c-Met are activated in MM. The HGF/c-Met pathway has a crucial role in tumor invasion and metastasis, and it has been demonstrated that inhibition of this pathway suppresses tumor infiltration into neighboring tissues [132]. An inhibitor for the c-Met kinase is under investigation for clinical efficacy. Also preclinical studies to inhibit the HGF/c-Met pathways were conducted for MM [84, 133].

It appears to us worthwhile to note that in several of the mentioned clinical trials, a small percentages of patients (usually ~1–5%), did experience partial benefits from the therapy with RTK inhibitors, highlighting the need to identify predictive biomarkers to select likely responders (FIGURE 1).

Arginosuccinate synthetase-1 (ASS1) deficiency: synthetic lethal approaches using the arginine depletor ADI-PEG20

Arginine is an amino acid that modulates a diverse array of metabolic pathways. Reduced expression of arginosuccinate synthetase-1 (ASS1), the rate-limiting enzyme for arginine biosynthesis, is observed in 63% of MPM. ASS1-deficient MPM cells are auxotrophic for arginine (i.e., unable to synthesize it) and consequently susceptible to arginine deprivation, a novel antimetabolite strategy [134].

A randomized phase II study examined the efficacy and safety of the arginine-lowering agent pegylated arginine deiminase (ADI-PEG20) in patients with ASS1-deficient MPM; compared to best supportive care, ADI-PEG20 almost doubled progression-free survival and was generally safe. The main grade 3–4 toxicities were neutropenia, fatigue and anaphylactic reactions (7%) [NCT01279967]. Therefore ADI-PEG20 should be further examined either alone or in combination with selected therapies.

Deregulation of microRNAs

Deregulation of microRNAs (miRNAs) expression is generating strong interest in cancer research [135]. Specific miRNA expression profiles have been found in MM biopsies compared to normal tissue [136]. miRNAs have been proposed as possible diagnostic tools [136], prognostic markers [137], and treatment option for MM [138]. To overcome the difficulties of directly delivering miRNA mimics, minicells composed of achromosomal bacterial cells and targeted by specific antibodies have been developed. EGFR-targeted minicells have been used to restore miR16 and induce growth arrest in mesothelioma xenografts [138]. Minicells can be given safely to patients with advanced cancer [139], and a clinical trial has started in MM (ACTRN12614001248651).

Other molecular alterations

The proteasome inhibitor bortezomib showed significant preclinical activity, mainly via inhibition of NF- κ B activity [140]. However, in a recent phase II study, bortezomib failed to show the hypothesized efficacy as a first-line treatment in combination with cisplatin [141].

There are no current registered trials investigating the use of bortezomib in MM. Inhibition of NF- κ B activity is also one of the activities of ranpirnase, a ribonuclease enzyme with activity against MM cells [142]. Ranpirnase has been tested in MM patients in combination with doxorubicin (NCT00003034). However, it did not meet statistical significance for the primary endpoint of survival in MM in a confirmatory phase IIIb clinical trial according to the manufacturer's annual report [143]. Other less-investigated targets for MM therapy include the Ras/MAPK pathway [144, 145]; ii) calretinin [146]; iii) Wnt2 [147]; iv) HSP90 (Heat shock protein S90) [148]. More experimental data are required to determine whether these molecules may be realistic therapeutic target.

In summary, so far, molecular therapies have not positively influenced the average survival of MM patients. Therefore, and considering the intertumor and intratumor genetic heterogeneity of MMs, the challenge ahead of us is to identify the subset of patients who will respond to a given type of therapy. Various studies have proposed that future treatment strategies should not be based on monotherapy (i.e., targeting one gene), but should comprise multimodal treatment targeting 2 or more genes/pathways. The problem is that such combined molecular therapies have been shown some promising results in mice but are, so far, quite toxic, and this toxicity precludes their use in clinic [149].

Immunotherapy

Immune checkpoint inhibitors

Avoiding immune destruction has been proposed as one of the new hallmarks of cancer [150]. One way to evade immune destruction is by expression of endogenous immune checkpoints that normally terminate immune responses after antigen activation (FIGURE 2). This state of tumor-induced immunological anergy is associated to up-regulation in tumor-infiltrating T cells of immune checkpoint molecules, such as cytotoxic T-lymphocyte-associated protein 4 (CTLA-4), and programmed cell death protein 1 (PD-1) [151]. Alternatively tumors can block immune activation by upregulating PD-1 ligands. Like many others tumors, MM express high levels of the immunosuppressive PD-1 ligand 1 (PD-L1) [152, 153]. Monoclonal antibodies against CTLA4 (tremelimumab, ipilimumab), PD-1 (nivolumab, pembrolizumab), and PD-L1 (avelumab, MPDL3280A) can reactivate the immune response against cancer cells, and have shown promising clinical results in melanoma and some other cancer types [151].

The CTLA4 inhibitor, tremelimumab, at a dose of 15 mg/kg once every 12 weeks, showed clinical activity in 38% of advanced MM patients in a phase II study [154]. An intensified regimen, i.e., 10 mg/kg once every 4 weeks for six doses, followed by 10 mg/kg every 12 weeks, showed a good safety profile, and clinical and immunological activity in patients with advanced MM, with more than 40% of the patients achieving disease control with a median duration of response of almost 11 months [155]. The same intensified schedule is now being investigated in an ongoing randomized, double-blind, placebo-controlled, phase 2b study (NCT01843374). Other clinical trials with PD-1 inhibitor pembrolizumab (NCT02399371) and PD-L1 inhibitor avelumab (NCT01772004) are currently ongoing. Recently, results from the KEYNOTE-028 trial showed that pembrolizumab is well tolerated and improves clinical outcomes for patients with MM; response rate to pembrolizumab was

28%, and another 48% of patients achieved stable disease. Compared with second-line chemotherapy, the response rate achieved with this immune checkpoint inhibitor is considerably higher [156].

Overall, immune checkpoint inhibitors (FIGURE 2) represent one of the most promising class of new molecules for cancer therapy, and results from the trials in MM are eagerly expected.

Anti-transforming growth factor- β

Transforming growth factor β (TGF- β) is a pleiotropic cytokine and a potent growth inhibitor in normal conditions. However, advanced tumors frequently do not respond to TGF- β , and often produce large amounts of this cytokine. In several malignancies, TGF- β promotes remodeling of the microenvironment to support tumor growth, facilitate metastases and attenuates host antitumor immune responses. Elevated TGF- β plasma levels correlate with advanced tumor stage, metastases, and poor survival. GC1008 (fresolimumab), a humanized monoclonal antibody that neutralizes all three human isoforms of TGF- β (FIGURE 2), has been tested in a phase I trial in 13 patients with MPM. No objective response was seen; however, three patients had stable disease at 3 months and five patients developed an enhanced immune response to MM [157].

Mesothelin-targeting agents

Mesothelin is expressed at low levels in most normal tissues, and it is expressed at higher levels by many solid tumors including MM, pancreatic cancer, ovarian cancer, and non-small cell lung cancer [158]. For this reason, mesothelin has been considered a potential therapeutical target in all of these malignancies. The biologic function of mesothelin overexpression in MM is unknown, but preclinical data suggest that it promotes MM cell invasion and matrix metalloproteinase secretion [159]. Other studies supported the hypothesis that mesothelin plays a role in cell adhesion by showing that it is the receptor for cancer antigen-125 (CA-125, also known as mucin 16 or MUC16), and the interaction between mesothelin and CA-125 leads to heterotypic adhesion [160,161].

Mesothelin-targeting agents (FIGURE 2) can be divided into three categories: (i) anticancer antibodies/antibodies-drug conjugates; (ii) mesothelin-targeting vaccines; (iii) mesothelin-targeting recombinant T cells.

Antibodies and antibody-conjugates—Amatuximab (MORAb-009) is a humanized antibody that showed strong pre-clinical activity in combination with chemotherapy against mesothelin-expressing tumors, via inhibition of the interaction of mesothelin with CA-125 [162,163]. In a phase I clinical trial, amatuximab showed an acceptable toxicity profile and promising activity, with 11/24 (46%) patients showing sustained stable disease [164]. A phase II trial of amatuximab in combination with cisplatin and pemetrexed showed that for treated patients, compared to historical controls, the progression-free survival was not significantly different. However, the median overall survival of 14.8 months with a third of patients alive and 5 continuing to receive amatuximab at the time of analysis was superior to historical controls [165]. A phase III study is planned to further investigate these findings.

Antibodies against specific molecules have been recently engineered to carry cytotoxic drugs specifically to cancer sites [166]. SS1P is an immunotoxin consisting of the variable fragment of amatuximab linked to the cytotoxic bacterial toxin *Pseudomonas* exotoxin A that showed strong preclinical activity against mesothelin-expressing cancers [167,168]. Two phase I trials of SS1P monotherapy with different treatment schedules showed modest activity and minor responses [169,170]. In another phase I trial, SS1P was co-administered with pemetrexed and cisplatin in chemonaïve patients, and resulted in partial responses in 77% of the patients [171]. Decrease in serum mesothelin, megakaryocyte potentiating factor, and CA-125 correlated with tumor responses [171]. The relatively modest results obtained in these trials were attributed to the formation of neutralizing antibodies against SS1P after the first cycle of treatment. Induction of an immunosuppressive state with pentostatin and cyclophosphamide effectively prevented the formation of anti-SS1P antibodies, allowed the infusion of more cycles of SS1P therapy and resulted in durable partial responses in 30% of the patients [172]. This combination is currently being explored in a phase I/II trial (NCT01362790). To overcome the limitations due to SS1P immunogenicity, a low-immunogenic engineered version (RG7787) has been developed and tested preclinically in mesothelin-expressing malignancies [173,174]. Interestingly, a recent RNA interference whole-genome screen identified components of the endoplasmic reticulum/Golgi as potential targets for chemical intervention that could increase SS1P killing of cancer cells [175], supporting rationally designed combination therapies of immunotoxins and chemical drugs. Similar to SS1P, αMSLN-MMAE is a novel antibody-drug conjugate formed by a humanized anti-mesothelin antibody and microtubule-disrupting agent monomethyl auristatin E, which showed significant preclinical activity [176] that is being translated in a phase I clinical trial.

BAY 94–9343 (ametuman ravnansine) is a novel, potent, antibody-drug conjugate consisting of a human anti-mesothelin antibody conjugated to the maytansinoid tubulin inhibitor DM4 via a disulfide-containing linker [177]. Preclinical evidences showed superiority to standard chemotherapy, and have led to clinical testing in an ongoing phase I trial (NCT01439152).

Also, Bristol-Myers Squibb has developed a mesothelin-targeted antibody drug conjugate (BMS-986148) that will be tested in a phase I trial (NCT02341625).

Mesothelin-targeted immunoactivators represent a novel class of emerging compounds. A mesothelin-targeted interleukin-12 conjugate showed some promising preclinical efficacy, and represented the first reported immunocytokine for mesothelin-positive tumors [178]. Moreover, a mesothelin-targeted *Mycobacterium tuberculosis* heat shock protein 70 promoted T cells activity against mesothelin-expressing cancers [179].

Mesothelin-targeting vaccines—Stimulating MM patients' immunity against mesothelin is an additional line of investigation. CRS-207 is a genetically modified *Listeria monocytogenes* attenuated vaccine expressing mesothelin, which stimulates activation of T cells against mesothelin-expressing cancer cells [180]. An ongoing Phase I trial is evaluating the combination of CRS-207 with chemotherapy in MM patients (NCT01675765). In pancreatic cancer, priming of the immune response with GVAX (granulocyte-macrophage colony-stimulating factor-secreting allogeneic pancreatic tumor cells) followed by CRS-207

treatment resulted in extension of survival, supporting the hypothesis that CRS-207 can synergize with other immune-stimulating approaches with minimal toxicity [181]. Combination of GVAX/CRS-207 with PD-1 inhibitor nivolumab is being tested in pancreatic cancer (NCT02243371) and, if successful, could be studied also in MM.

Other vectors are also being considered besides *Listeria*. Recently, preclinical evidences of anti-cancer activity in a pancreatic model have been shown for a mesothelin vaccine based on human adenovirus 40 [182]. Further development of mesothelin-targeting vaccines is expected in the coming years.

Mesothelin-targeting recombinant T cells—Redirecting T lymphocyte antigen specificity by gene transfer can provide large numbers of tumor-reactive T lymphocytes for adoptive immunotherapy. After gene transfer, autologous T cells express a chimeric antigen receptor (CAR), which enables the T cell to destroy target cells. CAR T cells are engineered cells expressing artificial receptors used to graft the specificity of monoclonal antibodies onto T cells. Mesothelin-targeted CAR T cells (CARTmeso) were pioneered and improved at the University of Pennsylvania, and have shown very robust pre-clinical activity, both *in vitro* and *in vivo* [183–187]. CARTmeso can be however reversibly inactivated within the solid tumor microenvironment, and inhibition of the PD-1 pathway could augment human CAR T cell function [188]. Also, the route of administration of CARTmeso might influence the efficacy of therapy [189]. Phase I studies are currently ongoing (NCT02159716, NCT01583686).

Other immunotherapies

Besides immune checkpoint inhibitors and mesothelin-targeting agents, several other approaches are currently being tested in phase I clinical trials after promising pre-clinical results.

Wilms tumor protein 1 (WT1) is transcription factor highly expressed in MM and ovarian cancers that has gained researchers' attention as a potential immunotherapeutic target. WT1 peptides are immunogenic and induce T-cell responses against MM cell lines [190]. In fact, WT1 peptide vaccines have been developed [190,191] which showed immune responses in patients with MM [192]. Phase I clinical trials have been recently completed or are still ongoing to test the safety of WT1-based vaccines (NCT00398138, NCT01265433, NCT01890980). Similar to mesothelin, CAR T cells against WT1 have been tested for anti-cancer activity [193,194]. A phase I clinical trial is ongoing (NCT02408016).

A novel target for engineered CAR T cells is fibroblast activation protein (FAP), a protein expressed by cancer-associated fibroblast of most solid tumors [195]. In addition, MM cells also express FAP, making FAP even more attractive as a target [196], and preclinical evidences strongly support FAP's potential role in MM therapy [197]. A phase I clinical trials with FAP-CAR T cells is currently recruiting patients (NCT01722149).

Preclinical eradication of MM was achieved with a PD-1 based DNA vaccine, which simultaneously exploits the immune stimulating functions of the vaccination and those of PD-1 inhibition [198].

Vaccination with MM tumor cell lysate is another strategy being used to induce an antitumoral response. A phase I trial is testing an autologous tumor cell vaccine with an adjuvant (ISCOMATRIX) and celecoxib to augment antigen presentation (NCT01258868). Another phase I trial is evaluating an allogeneic tumor cell vaccine (K526-GM) in combination with cyclophosphamide and celecoxib (NCT01143545).

Other possible targets under investigation are survivin and membrane chondroitin sulphate proteoglycan 4 (CSPG4). Survivin is member of the inhibitory apoptotic proteins (IAPs) family and plays an important role in the control of apoptosis, cell division and cell migration/metastasis. Survivin is an attractive candidate for cancer immunotherapy since it is abundantly expressed in most human cancers, including MM, and it is largely absent in normal adult tissues. A vaccine based on Fowlpox vector expressing survivin was found to successfully trigger an immune response in a MM mouse model [199]. The response resulted in delayed tumor growth and improved survival, indicating that the vaccine could serve as the basis for the development of clinically relevant MM immune-based treatments and/or prevention strategies [199]. Recently, highly immunogenic vaccines against human survivin have been developed and tested preclinically [200], confirming their potential role in human MM therapy.

CSPG4, which has been successfully targeted in melanoma and breast cancer, is highly expressed in MM, but not in normal mesothelium. Treatment with CSPG4 mAb TP41.2 reduced MM cells adhesion, motility, migration, invasiveness and growth in soft agar. It also inhibited MM growth *in vivo* and significantly increased the survival of xenografted SCID mice, which suggest that CSPG4 mAb-based immunotherapy may represent a novel approach for the treatment of MM [201].

Finally, other immunotherapeutic approaches investigated for MM involved the use of an IL4-toxin conjugate [202], gene therapy, and oncolytic viral therapy. These therapies, whose results have been significant in preclinical models but have not been successfully translated to the clinics, have been extensively reviewed elsewhere [98,203].

Oncolytic viruses

Viruses are strong stimulants to the immune system by the activation of the innate as well as the adaptive responses. Oncolytic viruses can induce host cell death and antigen release. Intrapleural administration of viral vectors is technically feasible, and has several advantages in MPM treatments because MPM develops in a closed cavity and is localized within the cavity in its early stage. Besides direct lysis, oncolytic viruses also stimulate an anti-cancer immune response. Combination of oncolytic virotherapy and immunotherapy is under investigation [204,205].

Significant evidences of preclinical activity have been obtained in MM in particular for measles virus (MV) [206,207]. MV-NIS is an attenuated MV expressing the human thyroidal sodium iodide symporter (NIS), which allows radiologic detection of the virus distribution and combination with radiometabolic therapy with iodine [206]. A phase I trial with MV-NIS is currently ongoing [NCT01503177]. Also adenovirus [208], retrovirus [209], and herpes simplex virus [210] have been examined for the therapeutic effects in preclinical

settings, and are progressing to early human experimentation [NCT01721018, NCT01212367].

Targeting asbestos-induced inflammation in both MM treatment and prevention

Inflammation is the hallmark of asbestos deposition in tissue and contributes to asbestos carcinogenesis [211]. Novel strategies that interfere with asbestos- and other carcinogenic mineral fibers- mediated inflammation might prevent or delay the onset of MM in high-risk cohorts, including genetically predisposed individuals, and/or inhibit tumor growth. HMGB1, NOD-like receptor family pyrin domain containing 3 (NALP3) inflammasome, TNF- α , and Interleukin-1 β (IL-1 β), can all serve as potential targets for inhibiting chronic inflammation induced by asbestos. Early inflammatory reactions triggered by asbestos are NLRP3-dependent, but NLRP3 is not critical in the chronic development of asbestos-induced MM; indeed, after the administration of asbestos, acute IL-1 β production and recruitment of immune cells into peritoneal cavity were shown to be significantly decreased in the NLRP3-deficient mice, but NLRP3-deficient mice still displayed a similar incidence of MM and survival times as wild-type mice [212].

Asbestos induces necrotic cell death of mesothelial cells and therefore causes the release of HMGB1 into the extracellular space [15, 16]. HMGB1 is a damage-associated molecular pattern (DAMP) and a key mediator of inflammation [213]. In healthy cells, HMGB1 is found primarily in the nucleus, where it stabilizes chromatin and plays multiple roles in DNA transcription, replication, and recombination. During programmed cell necrosis, HMGB1 translocates from the nucleus to the cytosol and is then passively released to extracellular space, where it binds several pro-inflammatory molecules and triggers the inflammatory responses that distinguish this type of cell death from apoptosis. Secreted HMGB1 stimulates receptor for advanced glycation end products (RAGE), Toll-like receptor 2 (TLR2), and TLR4, expressed on neighboring inflammatory cells, mostly macrophages. HMGB1 induces the secretion of TNF- α by macrophages and activation of nuclear factor κ B (NF- κ B) [214]. Activation of NF- κ B promotes cell proliferation and inhibits cell death, leading to enhanced survival of human mesothelial cells that have accumulated DNA alterations following asbestos exposure, thus facilitating their malignant transformation [18] (FIGURE 3). Moreover, we found that most MM cells actively secrete high levels of HMGB1, and that HMGB1 plays a critical role in MM growth and progression. Therefore, targeting HMGB1 may help to prevent or even treat MM. Therapies that seek to block HMGB1 signaling have been investigated; specific molecules that target the activities of HMGB1 are: anti-HMGB1 and anti-RAGE antibodies, HMGB1 antagonist Box A, and ethyl pyruvate that inhibits HMGB1 secretion [214].

Since chronic inflammation has been associated with an increased risk of developing numerous types of cancers, many anti-inflammatory agents have been studied. For example, celecoxib, a nonsteroidal anti-inflammatory drug targeting COX-2, was shown to have antitumor properties in MM and it was able to inhibit the *in vitro* and *in vivo* tumorigenic potential of MM cells [215]. Aspirin is a widely used anti-inflammatory drug. It has been

found that daily treatment with aspirin for 5 years can reduce tumor burden in colon cancer and other common malignancies [216]. We recently discovered that aspirin and its metabolite salicylic acid inhibit HMGB1 and reduce MM cell migration and growth *in vitro* and *in vivo*, providing a novel rationale to the known anti-cancer effects of aspirin [217]. Also the HMGB1 specific inhibitor BoxA [218], markedly reduced MM growth in xenograft mice and significantly improved survival of treated animals, in a way not additive to aspirin, consistent with our hypothesis that both act via inhibition of HMGB1 activity [217]. Our data [217] suggest that aspirin treatment, and treatment with specific anti-HMGB1 antagonists, might be beneficial to MM patients. Although to the best of our knowledge the possible therapeutic effects of aspirin in MM have not been studied, we found some evidence supporting a role of aspirin in preventing or possibly delaying the growth, and thus the diagnosis, of MM. Specifically, the Physician's Health Study suggests a possible association between aspirin use and reduced MM incidence (*Anonymous (1989) Final report on the aspirin component of the ongoing Physicians' Health Study. Steering Committee of the Physicians' Health Study Research Group. The New England journal of medicine 321(3):129–135.*). In this study, 22,067 physicians were followed for 24 years with 17 reported cases of MM. An intention to treat analysis comparing aspirin (325 mg on alternate days) and placebo treatment revealed a relative risk of 0.7 (0.3–1.9, 95% confidence interval), indicating a 30% reduction in MM risk. Although the small number of MM cases resulted in insufficient power for a statistically significant analysis, these research findings together with our recent data suggest that aspirin may have potential as an effective drug for MM.

Finally, specific FDA-approved reagents that inhibit molecules involved in asbestos-induced inflammation are available: anakinra, an IL-1 β receptor antagonist; infliximab, a chimeric human-mouse anti-TNF- α ; etanercept, a soluble TNF receptor fusion protein; glyburide, that inhibits the NALP3 inflammasome [219]. All these molecules should be further investigated for MM treatment (FIGURE 3).

Moreover, preliminary evidence from our laboratory suggests that serum levels of HMGB1 might increase following asbestos exposure and MM [16,220,221]. Therefore, in addition to the therapeutic potential of abrogating HMGB1 functions [217], HMGB1 is a possible biomarker for asbestos exposure and early detection of MM [15,16]. Since early detection of MM improves overall patient survival, the availability of biomarkers for early detection of MM would greatly facilitate the follow up of individuals at high-risk for developing MM. Several others MM biomarkers have been investigated, including mesothelin [222,223], osteopontin [224] and fibulin-3 [225]. Availability of reliable biomarkers is extremely important in MM, because given the long latency between initial exposure to mineral fibers and the development of MM [1], there is a large window of time that provide the opportunity not only for early detection, but also for prevention of disease.

Some chemopreventative approaches have been tested in the past to reduce MM incidence. However, most of these studies were conducted when the molecular pathogenesis of MM was mostly unknown, and were based on the efficacy of chemopreventive agents in reducing incidence and mortality of other malignancies, particularly colorectal cancer. Antioxidant vitamins (e.g., isoforms of vitamin A and E) showed contradictory results in reducing MM

incidence, with early reports of efficacy [226] not confirmed after a longer follow-up [227]. Recently, it has been reported that antioxidant vitamins do not reduce MM incidence in a murine model of asbestos-induced MM [228]. More recently, prevention of MM with anti-inflammatory drugs (NSAIDs and COX-2 inhibitors) has been also proposed, however evidences from both animal models and a human cohort do not support this hypothesis [229]. Similarly, statins, which like some NSAIDs have been shown to reduce the chances of developing colorectal cancer, do not alter the incidence of MM in asbestos-exposed mice or humans [230]. Other experimental approaches involving iron reduction also showed limited efficacy in animal models [231]. So far, chemoprevention of MM has been as desirable as elusive. With increased understanding of MM pathogenesis, targeted strategies aimed to prevent MM are being developed. For example, we are currently testing the hypothesis that HMGB1 inhibition with aspirin might reduce or delay the onset of MM.

Expert commentary

Despite progress in several other solid tumor types, MM is still associated with a very poor overall survival, and limited treatment options with no targeted therapies.

So far, all attempts to treat MM using gene therapy have failed. The polyclonal origin of MM [38] and the lack of driver mutations, with the possible exception of *BAP1* [48,50,51], may be largely responsible for this failure. Accordingly, a small subset of patients (~5%) enrolled in clinical trials responded to genetic therapy; these might be the same patients in which most tumor cells harbored the targeted gene alteration.

Combination therapies are often proposed as the way to address the failure of current genetic therapies to treat cancer patients. However, combination therapies have caused serious toxic effects, and their toxicity limited clinical use [149]. An apparent exception was presented this year at the ASCO meeting: when bevacizumab was added to the standard of care (pemetrexed + cisplatin), patients experienced a significant longer median survival (18.82 months vs. 16.07 months, $p = 0.0127$). These results might translate into addition of bevacizumab as part of the first line treatment for MPM.

BAP1 is the most promising driver gene in MM pathogenesis, because more than 60% of MM biopsies contain *BAP1* mutations/inactivation [48,49] and germline *BAP1* mutations cause a cancer syndrome with high incidence of MM [24,40]. Future studies will address whether targeting BAP1 will represent the possible exception to the failure, to this day, of genetic therapy in MM treatment. The study of pathways directly or indirectly affected by the BAP1 loss of function might be a promising strategy to develop targeted therapies in a large set of *BAP1* mutant MM patients.

Given the disappointing results obtained so far in molecular therapy in MM, an increasing number of investigators are shifting their efforts to immunotherapies. Immune based approaches can target the inflammatory response caused by asbestos deposits that over years leads to MM, or might target MM tumor cells or the tumor cell stroma.

Finally, the lack of chemoprevention trials for individuals at risk of MM because of exposure to carcinogenic mineral fibers, and/or *BAP1* germline mutations, highlights the urgent need

for research in this field: we will test the hypothesis that aspirin by targeting HMGB1 and influencing the chronic inflammatory process that drives mineral fiber carcinogenesis might, similarly to what observed in colon cancer, reduce or delay the onset of MM.

Five-year view

MM incidence is still increasing worldwide and it is not expected to decrease in the next two decades. MPM is commonly associated with asbestos deposition in tissues, which causes a chronic inflammatory reaction and contributes to carcinogenesis. Because the latency period from initial asbestos exposure to disease progression is decades long, therapies aimed at decreasing chronic inflammation, such as aspirin intake [232], could prevent or delay carcinogenesis in exposed individuals and lead to a substantial decrease in MM mortality.

Also genetic testing for *BAP1* mutations in exposed cohorts will help identify genetically susceptible individuals who have the highest risk of developing MM.

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* = of interest

** = of considerable interest

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Key issues

- MM is a very aggressive tumor that arises from mesothelial cells of the pleural, pericardial, and peritoneal cavities. Patients are usually diagnosed at advanced stages and the prognosis is very poor, with a median survival of 6–12 months and a 5-year survival of <5% [211].
- MM incidence it is still increasing worldwide and it is not expected to fall off in the next two decades [1,10].
- Most MPM patients have unresectable disease and are treated with palliative combination chemotherapy (cisplatin and pemetrexed is the first-line treatment approved by the FDA). However, MPM is largely unresponsive to conventional therapy and the minority of patients that initially respond to therapy eventually become resistant [36].
- MPM is often associated with asbestos exposure. Asbestos carcinogenesis is linked to chronic inflammation that may lead to malignant mesothelial cell transformation after decades long latency [15,18,211].
- Novel strategies that interfere with asbestos-mediated inflammation might prevent or delay the onset of MM. HMGB1 is a candidate target for anti-inflammatory therapy, and a potential marker of exposure to carcinogenic mineral fibers [16]. Aspirin is an FDA-approved candidate drug that targets HMGB1 and this drug taken in daily amounts of 85–375 mg has conclusively shown the ability to decrease the incidence of colon cancer and of other inflammation-related malignancies. Similarly, individuals exposed to asbestos and at risk of developing MM may benefit from daily doses of aspirin [232]. Also reactivation of the immune system against cancer cells via immune checkpoint inhibitors has shown significant clinical results.
- People have different genetic susceptibilities to asbestos and to MM development. Germline *BAP1* mutations are present in families with a high incidence of MM [24]. *BAP1* somatic mutations have also been found in >60% of sporadic MM, pointing at *BAP1* as the most commonly mutated gene in this malignancy [48]. Genetic testing for *BAP1* mutations should help in the identification of genetically susceptible individuals who have the highest risk of developing MM.
- Germline *BAP1* mutations predispose to the tumorigenic effect of asbestos, possibly because of a deregulated inflammatory response. Care of individuals carrying germline *BAP1* mutations should consider reducing exposure of to even minimal sources of carcinogenic fibers and a preventive therapy to target the inflammatory response [233].
- The polyclonal origin of MM complicates attempts to develop molecular therapies [38]. Future treatment strategies should not be based on monotherapy,

but should comprise multisite and multimodal treatment, if such treatment can be developed without the present toxic effects.

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a. Key genetic alterations

b. RTKs inhibitors

Figure 1. Molecular alterations involved in the development of MM and possible strategies for therapeutic intervention

(a) Key genetic alterations. The *BAP1* gene encodes BRCA1 associated protein-1 (BAP1), which plays a role in the regulation of gene transcription, chromatin remodeling and DNA repair, by forming multi-protein complexes with several nuclear proteins, including host cell factor-1 (HCF1), Ying Yang 1 (YY1) and histone H2A. The *NF2* gene encodes the protein merlin, which acts as an upstream regulator of the mammalian target of rapamycin (mTOR), focal adhesion kinase (FAK) and Hippo pathways. The merlin-Hippo signaling inactivation leads to constitutive Yes-associated protein (YAP) activation. The *CDKN2A/ARF* gene

encodes p16^{INK4a} and p14^{ARF}, respectively. The p16^{INK4a} protein activates the retinoblastoma protein (pRb) pathway by inhibiting the cyclin-dependent kinase (CDK)-mediate hyperphosphorylation of pRb. The p14^{ARF} protein mediates p53 stabilization by promoting the degradation of the human ortholog of mouse double minute 2 (MDM2). **(b)** Receptor tyrosine kinases (RTKs) are frequently activated in MM. Inhibition of RTKs or their ligands with specific antibodies or small molecules has been tested as targeted approach in MM. Strategies for therapeutic intervention are summarized in this and in the following figures, accordingly to results obtained in past and ongoing clinical trials, or to possible future directions. The color code defines drugs that have not been tested (blue), drugs that gave positive results and are under further evaluation (green), and drugs that gave negative results (red). For further detail refer to the relative section in the main text.

Figure 2. Anti-tumor immune response and mechanisms of MM immune escape with possible strategies for immunotherapeutic interventions

Malignant mesothelioma (MM) cells express mesothelin and other tumor specific antigens. During the anti-tumor immune response (displayed on the left), antigen-presenting cells (APCs) display tumor antigens represented by major histocompatibility complexes (MHCs) to naïve T cells, inducing T cell activation and subsequent immune destruction of cancer cells via T cell receptor (TCR). However, tumors can activate several immune escape (displayed on the right) mechanisms to evade immune destruction. Molecules like transforming growth factor β (TGF- β) can block T cell function. T cell function can be also

abrogated by activation of cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) and programmed cell death protein 1 (PD-1) on activated T cells. CTLA-4 binding to B7, and the interaction of PD-1 ligand 1 (PD-L1) with PD-1, are crucial to inhibit immune response. MM cells can also block immune activation by upregulating PD-L1. Potential targets and strategies for MM immunotherapy are summarized in the figure. Color code: drugs that have not been tested (blue), drugs that gave positive results and are under further evaluation (green), and drugs that gave negative results (red).

Figure 3. Targeting asbestos-induced inflammation to prevent malignant transformation of mesothelial cells

Asbestos is very cytotoxic to human primary mesothelial (HM) cells and causes DNA damage, as well as extensive necrotic cell death leading to the release of high-mobility group box 1 (HMGB1) into the extra cellular space. Secreted HMGB1 stimulates receptor for advanced glycation end products (RAGE), Toll-like receptor 2 (TLR2), and TLR4, expressed on neighboring inflammatory cells, mostly macrophages. HMGB1 release elicits macrophage accumulation and triggers the inflammatory response, with secretion of Interleukin-1 β (IL-1 β) and especially tumor-necrosis factor- α (TNF- α). HMGB1 and TNF- α cooperate to promote the activation of nuclear factor κ B (NF- κ B) pathway, which increases HM survival after asbestos exposure. This allows HM with asbestos-induced DNA damage to divide rather than die and, if key genetic alterations accumulate, to eventually develop into malignant mesothelioma (MM). HMGB1 is also highly expressed and secreted by MM cells, establishing an autocrine circuit that further influences their proliferation and survival. Potential targets and strategies for MM therapy that have been proposed based on recent studies are summarized in the figure. Color code: drugs that have not been tested (blue), drugs that gave positive results and are under further evaluation (green), and drugs that gave negative results (red).