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Contents lists available at ScienceDirect

Biochemical Pharmacology

journal homepage: www.elsevier.com

Commentary

New therapeutic strategies for malignant pleural mesothelioma

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ARTICLE INFO

Article history:
Received 30 May 2016
Accepted 14 July 2016
Available online xxx

Keywords:
MPM
Target therapy
Immunotherapy

ABSTRACT

Malignant pleural mesothelioma (MPM) is a rare and aggressive malignant disease affecting the mesothelium, commonly associated to asbestos exposure. Therapeutic actions are limited due to the late stage at which most patients are diagnosed and the intrinsic chemo-resistance of the tumor. The recommended systemic therapy for MPM is cisplatin/pemetrexed regimen with a mean overall survival of about 12 months and a median progression free survival of less than 6 months. Considering that the incidence of this tumor is expected to increase in the next decade and that its prognosis is poor, novel therapeutic approaches are urgently needed. For some tumors, such as lung cancer and breast cancer, druggable oncogenic alterations have been identified and targeted therapy is an important option for these patients. For MPM, clinical guidelines do not recommend biological targeted therapy, mainly because of poor target definition or inappropriate trial design. Further studies are required for a full comprehension of the molecular pathogenesis of MPM and for the development of new target agents. This review updates pre-clinical and clinical data on the efficacy of targeted therapy and immune checkpoint inhibition in the treatment of mesothelioma. Finally, future perspectives in this deadly disease are also discussed.

1. Introduction

Malignant pleural mesothelioma (MPM) is an aggressive malignant disease affecting the surface mesothelium of the pleural cavity, primarily associated with exposure to asbestos fibers. Despite the rarity of this disease, MPM incidence is increasing worldwide, and it is estimated to peak around the next 15 years [1]. The production and the use of asbestos is forbidden in most of the industrialized countries, but in many developing countries it is still currently used and approximately 125 million people are believed to be exposed in the workplace. Based on the World Health Organization (WHO 1994–2008), age-adjusted mortality rate (AAMR) was 4.9 per million population, with an increase of 5.4% per year [2]. Considering the long latency of tumor development (30–40 years) and the late stage at which most patients are diagnosed, radical surgery is only applicable to a very few early stage fit patients and its benefit is still controversial [3].

At present the only recommended systemic therapy for MPM, based on the phase III EMPHACIS trial [4], is platinum/antifolate regimen that has extended the median overall survival (OS) of MPM patients to approximately 1 year with a median progression free survival (PFS) of less than 6 months. Due to the high chemo-resistance of the disease, systemic treatment results in only short-term regression and local tumors relapse rapidly. The management of MPM patients remains controversial. Currently, a multimodal treatment regimen of chemotherapy, surgery, and radiotherapy provides the best long-term results; however, even after such an aggressive approach, the prognosis remains poor, with mean patient survival time of just over one year. Based on the increasing incidence and on the poor prognosis, additional studies concerning the molecular pathogenesis of MPM are required to develop new therapeutic strategies.

There are three major histological types of mesothelioma. The epithelioid type, characterized by square-shaped cells with visible nucleus, is the most common (50–70%) and tends to have a much more favorable prognosis; the sarcomatoid type (10–20%) with elongated and spindle-shaped cells is the most aggressive one; the biphasic type is a mixture of epithelial cells and sarcomatoid cells (20–35%).

Genetic analyses have identified several genetic and genomic alterations in MPM. The most frequent somatic mutations and copy-number alterations affect *cyclin-dependent kinase inhibitor 2A (CDKN2A/ARF)*, *neurofibromatosis type 2 (NF2)*, *BRCA1-associated protein-1 (BAP-1)* and *Cullin 1* (*CUL1)* genes [5]. The genomic alterations in human MPM that have been previously reported include losses of chromosome arms 1p, 3p, 4q, 6q, 9p, 13q, 14q, 22q and gains of chromosome arms 1q, 5p, 7p, 8q, 17q. In addition, dysregu-

Abbreviations: AE, adverse event; CTLA-4, cytotoxic T-lymphocyte-associated antigen 4; EGFR, epidermal growth factor receptor; FAK, focal adhesion kinase; IGFR, insulin growth factor receptor; MPM, malignant pleural mesothelioma; MTD, maximum tolerated dose; mTOR, mammalian target of rapamycin; NSCLC, non-small cell lung cancer; OS, overall survival; PD, progressive disease; PFS, progression free survival; PD-1, programed death 1; PDL-1, programed death ligand 1; PI3K, phosphatidylinositol 3-kinase; PR, partial response; RB, retinoblastoma; RT, radiotherapy; RTK, receptor tyrosine kinase; SD, stable disease; TKI, tyrosine kinase inhibitor; TNFα, tumor necrosis factor-alpha; VEGFR, vascular endothelial growth factor receptor

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http://dx.doi.org/10.1016/j.bcp.2016.07.012
0006-2952/

lation in signal transduction pathways, related to cell survival and proliferation, has also been demonstrated [6].

This review updates recent advances and new therapeutic options for the treatment of advanced MPM under pre-clinical and clinical investigation, with particular emphasis to target therapies and immunotherapy (Fig. 1 and Table 1).

2. Systemic chemotherapy and trimodality therapy

Considering the controversial role of surgery the efficacy of surgery is limited and the cytotoxic chemotherapy remains one of the main therapeutic options to prolong survival and improve the quality of life. Since 2003 the systemic treatment of MPM has remained unchanged and the combination chemotherapy with a platin compound and a folate antagonist is still the standard first-line treatment for advanced MPM ineligible for surgery therapy. Two randomized phase III studies [4,7] demonstrated the survival benefit with cisplatin/anti-folate therapy over cisplatin alone. The OS observed with the combinations of cisplatin/pemetrexed and cisplatin/raltitrexed were 12.1 and 11.4 months respectively, significantly higher than the cisplatin monotherapy (9.3 and 8.8 months, respectively). On the basis of these data, the cisplatin/pemetrexed doublet has become the only first-line therapy approved by the US Food and Drug Administration (FDA) for patients with advanced unresectable MPM. Cisplatin is often substituted with carboplatin due to its lower toxicity and results of two phase II studies showed similar activity to cisplatin (time to progression 6.5–7 months and OS 12.7–14 months) [8,9].

At present, a phase II trial comparing four versus six cycles of pemetrexed/platinum in MPM is ongoing with the aim to define the best regimen of chemotherapy (NCT02497053). Another outstanding question is whether the pemetrexed maintenance therapy improves PFS of patients with MPM who have completed an initial therapy. A small study has demonstrated the safety and the feasibility of pemetrexed maintenance in 13 patients [10], and a phase II trial of pemetrexed maintenance versus observation for patients without progression after completion of first-line therapy with pemetrexed and cisplatin/carboplatin is ongoing (NCT01085630).

Several phase II studies indicate that the combination of platinum and gemcitabine is also a reasonable first-line option for the systemic therapy of MPM because of its acceptable toxicity profile, good response rate, and its clinical benefit for patients [11]. Currently, gemcitabine as a first-line therapy is not supported given the lack of phase III studies comparing the two chemotherapy regimens, however gemcitabine in combination with platinum or alone is being used in the clinic as a second-line setting.

Neoadjuvant or adjuvant chemotherapy and adjuvant radiotherapy (RT) are combined with surgery in the trimodality therapy (TMT). Surgery includes pleurectomy/decortication (P/D), extrapleural pneumonectomy (EPP) and extended pleurectomy/decortication (eP/D), that differs from P/D for the resection-reconstruction of the diaphragm. The first study was published in 2004 and reported a median survival of 23 months in patients undergoing cisplatin/gemcitabine chemotherapy followed by EPP and postoperative RT [12]. During the last decade advances in chemo/radiotherapy have been done (also, due to the development of modern RT techniques), that have improved the outcomes of patients who undergo TMT.

A recent meta-analysis evaluated the safety and the feasibility of TMT, involving EPP, neoadjuvant or adjuvant chemotherapy and RT, in 16 studies: median OS ranged from 12.8 to 46.9 months and perioperative mortality ranged from 0 to 12.5%. Among these studies, the

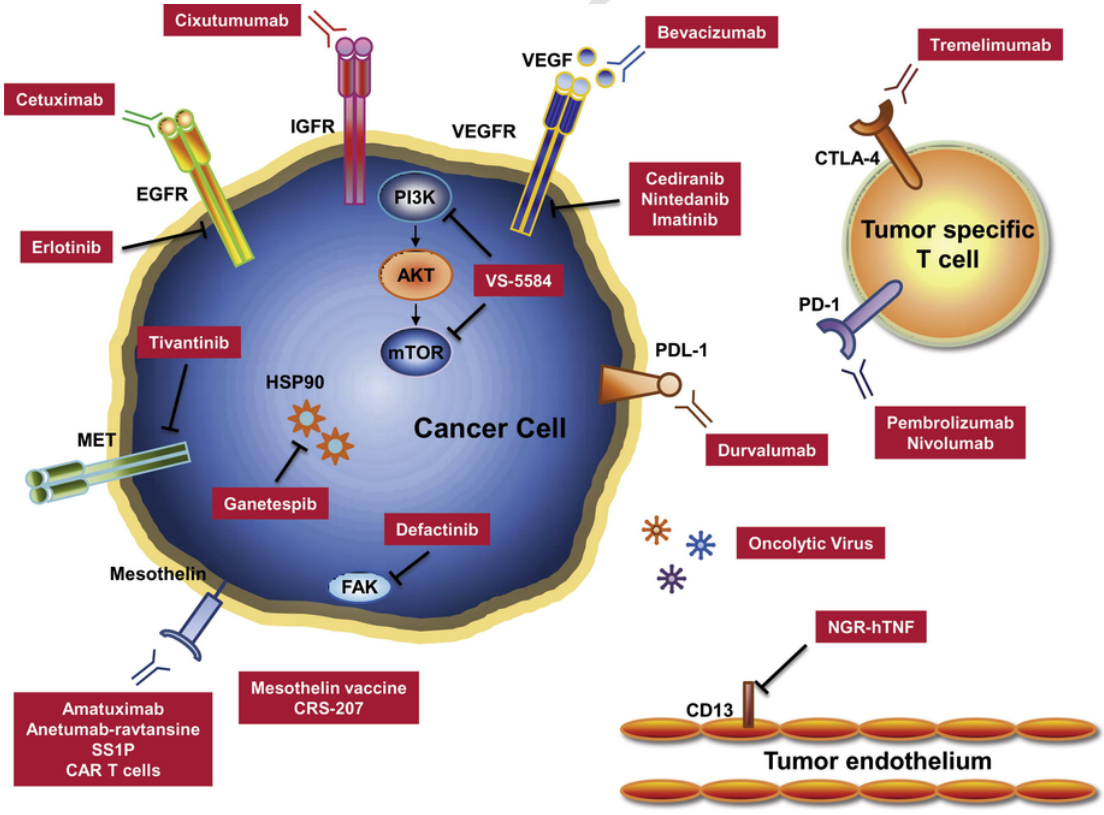

Fig. 1. Molecular targets in MPM and associated inhibitors. Different drugs targeting altered signaling in mesothelioma cancer cells and in surrounding microenvironment under clinical evaluation are shown.

Table 1
Ongoing active and recruiting trials with targeted and immune-stimulating agents in mesothelioma (as of May 2016).

Target	Drugs	In combination with	Development Phase	http://clinicaltrials.gov/
EGFR	Erlotinib	Alone	Phase II	NCT01592383**
	Cetuximab	Cisplatin-carboplatin/pemetrexed	Phase II	NCT00996567*
IGFR	Cixutumumab	Alone	Phase II	NCT01160458**
MET	Tivantinib	Carboplatin/pemetrexed	Phase I/II	NCT02049060*
PI3K/AKT/mTOR	VS-5584	Defactinib	Phase I	NCT02372227**
FAK	Defactinib	Neoadjuvant	Phase II	NCT02004028*
HSP90	Ganetespib	Cisplatinum/pemetrexed	Phase I/II	NCT01590160**
VEGF/VEGFR	Bevacizumab	Cisplatin/pemetrexed	PhaseII/III	NCT00651456**
	Cediranib	Cisplatin/pemetrexed	PhaseI/II	NCT01064648**
	Nintedanib	Cisplatin/pemetrexed	Phase II/III	NCT01907100*
	Imatinib mesylate	Gemcitabine	Phase II	NCT02303899*
CD13	NGR-hTNF	Alone	Phase II	NCT01358084*
		Gemcitabine or vinorelbine or doxorubicine	Phase III	NCT01098266**
Mesothelin	Amatuximab	Cisplatin/pemetrexed	Phase II	NCT02357147*
	Anetumab/Ravtans.	Alone	Phase II	NCT02610140*
	SS1P	Cisplatin/pemetrexed	Phase Ib	NCT02639091*
		Cisplatin/pemetrexed	Phase I	NCT01445392**
		Pentostatin/cyclophosphamide	Phase I/II	NCT01362790*
	CAR T cells	Cyclophosphamide	Phase I	NCT02414269*
	CRS-207	Pemetrexed/cisplatin ± cyclophosphamide	Phase I	NCT01675765*
CTLA-4	Tremelimumab	Alone	Phase IIb	NCT01843374**
		Durvalumab	Phase II	NCT02588131*
PD-1	Pembrolizumab	Alone	Phase I	NCT02054806**
		Alone	Phase II	NCT02399371*
		Defactinib/gemcitabine	Phase I	NCT02546531*
	Nivolumab	Alone	Phase II	NCT02497508§
		Alone or with ipilimumab	Phase II	NCT02716272*
Cancer cell (virotherapy)	Measles virus	Alone	Phase I	NCT01503177*
	Herpes virus	Alone	Phase I/II	NCT01721018*
	Vaccinia virus	Alone	Phase I	NCT01766739*
WT-1	WT-1 vaccine	Montanide + GM-CSF	Phase II	NCT01890980**
miR15-16	TARGOmiRs	Alone	Phase I	NCT02369198*

* Recruiting.

** Active, not recruiting.

§ Not yet recruiting.

randomized controlled trial (MARS 1) reported no benefit of TMT to patients, since the median OS was 14.4 months for the EPP group compared with 19.5 months for the no EPP group and the mortality rate was 18.8%. Despite the negative results of this study, the authors concluded that TMT can be performed after proper selection of patients and in specialized cancer centers [13].

3. Genetic alterations in MPM and targeted therapy

In recent years, advances in technologies for molecular genetic analyses have led to the identification of multiple genetic abnormalities that may be involved in the pathogenesis of MPM. However, many oncogenic events typical to other tumors are uncommon in MPM, and loss of tumor suppressor genes, more than activation of oncogenes, has emerged as a molecular signature of MPM.

One of the most frequently inactivated tumor suppressor gene in MPM is the *CDKN2A/ARF* gene on 9p21, deleted in ~70% cases of epithelioid histotype and in near 100% of sarcomatoid histotype [14]. This gene generates a number of transcript variants which differ in the first exons and encode for structurally related isoforms of the cyclin-dependent kinase 4 (CDK4) inhibitors $p16^{INK4a}$. In addition, the transcript contains an alternate open reading frame (ARF) that encodes for $p14^{ARF}$, that functions as a stabilizer of the tumor suppressor protein p53. Since $p14^{ARF}$ and $p16^{INK4a}$ function as cell cycle regulatory proteins in the p53 and retinoblastoma (RB) pathways, respectively, their inactivation has been suggested to accelerate asbestos-induced tumorigenesis *in vivo* [15]. In contrast, MPM shows low genetic mutation rate in RB, p53, and related signaling components such as MDM2 in comparison with other cancers [14].

NF2 gene on 22q12 has been shown to be inactivated, either by homozygous deletion or mutation, in 40–50% of MPM cases [16]. *NF2* encodes for a tumor suppressor protein, merlin (moesin-ezrin-radixin-like protein), a membrane-cytoskeleton protein with a negative regulatory function on multiple signal transduction cascades, including mammalian target of rapamycin (mTOR) and Hippo signaling pathways [17]. Merlin-Hippo signaling inactivation leads to constitutive YAP activation, which in turn is responsible for the transcription of multiple cancer-promoting genes, including *cyclin D1*, and *forkhead box M1*. YAP activation has been described in >70% of primary MPM tissues [18].

BAP1, localized to chromosome 3p21.1, encodes a nuclear ubiquitin C-terminal hydrolase involved in various cellular processes, including chromatin remodeling. Germline mutations in *BAP1* gene have been associated with familial MPMs [19]. In a recent study, 66% of biopsies from MPMs were BAP1 negative by immunohistochemistry (IHC), with BAP1 protein loss associated to homozygous deletion of the BAP1 locus in the vast majority of cases [20]. No significant association has been found between *CDKN2A/ARF* loss and loss/mutation of *BAP1* or *NF2*, suggesting these as independent genetic events in MPM [21].

To date, no oncogene driver mutation that may be responsive to targeted therapies in MPM has been discovered. Nevertheless, over the last decade a variety of biological agents have been tested both at pre-clinical and clinical level against over-expressed targets or deregulated signaling pathways.

3.1. Anti EGFR

A number of studies indicate that asbestos fibers can physically interact with epidermal growth factor receptor (EGFR), causing its autophosphorylation and activation with downstream induction of mitogen-activated protein kinases (MAPK) and/or AKT downstream signaling cascades [22]. Asbestos fibers have also been shown to upregulate EGFR mRNA and protein expression [23].

Phase II studies in advanced or recurrent MPM patients reported that erlotinib and gefitinib (ATP-competitive, small-molecule EGFR tyrosine kinase inhibitors, TKIs) were not effective as single-agents [24,25] despite overexpression of EGFR was detected in 50–95% of cases. The low prevalence of EGFR activating mutations may explain the lack of clinical efficacy. A phase II trial is currently evaluating erlotinib in peritoneal mesothelioma patients carrying EGFR mutations (NCT01592383).

Concurrent activation of multiple receptor tyrosine kinases (RTKs), constitutive activation of AKT signaling associated with phosphatase and tensin homolog (PTEN) loss, as well as epithelial-mesenchymal transition (EMT) have been proposed as additional mechanisms of resistance to EGFR inhibition in MPM. A recent study in human mesothelial cell lines has shown that EGFR is frequently coactivated with the hepatocyte growth factor receptor (MET) through a mechanism of cross-activation, and that combined inhibition of both receptors down-regulates intracellular signaling and reduces cell proliferation more effectively than single inhibition [26]. Another pre-clinical study has demonstrated the existence of a strong correlation between the EMT and the susceptibility to EGFR-TKIs, suggesting that combining EGFR-TKIs with treatments capable of reverting the EMT phenotype, by restoring E-cadherin expression, may confer cell sensitivity to EGFR blockade [27]. These pre-clinical data suggest that combined therapies with EGFR-TKIs and other targeted agents should be considered in the design of future clinical trials for MPM treatment. Less encouraging, however, are the studies on the combination with cytotoxic therapies, such as the study evaluating the combination of gefitinib with gemcitabine and cisplatin, which did not show any synergistic or additive effects *in vitro* [28].

Due to the unsatisfactory results obtained with EGFR-TKIs, recent efforts have been focused on monoclonal antibodies directed against the extracellular domain of EGFR for MPM treatment. It has been recently [29] demonstrated that EGFR and HER2 molecules are frequently coexpressed in MPM cells, and that the expression of both receptors may be further increased by the dual EGFR/HER2-TKI lapatinib, providing a rationale for combination with anti-EGFR monoclonal antibodies. Actually, lapatinib was shown to enhance trastuzumab- and cetuximab-mediated antibody-dependent cellular cytotoxicity (ADCC) in MPM cell lines and in patient-derived MPM cells, suggesting that these drug combinations may be an effective therapeutic strategy for MPM treatment.

It has been reported that low doses of IL-2 may enhance cetuximab-mediated ADCC activity against EGFR-expressing MPM cell lines independently of the level of EGFR expression. In addition, intrathoracic administration of cetuximab was shown to inhibit tumor growth and prolong survival of severe combined immunodeficient (SCID) mice bearing MPM cells [30].

A study of cetuximab combined with cisplatin or carboplatin/pemetrexed as first line treatment in patients with MPM with EGFR protein over-expression is ongoing (MesoMab NCT00996567).

3.2. Anti IGFR

Insulin growth factor receptors (IGF-1R and IGF-2R) together with insulin growth factor (IGF) are expressed in MPM cells, generating an autocrine loop that may induce distinct phenotypes depending on the downstream signal transduction adaptor molecules involved. Indeed, it has been reported that signaling mediated by insulin receptor substrate (IRS)-1 is associated with increased cellular growth, whereas signaling through IRS-2 is associated with increased cellular motility [31].

The efficacy of cixutumumab, a fully human monoclonal antibody to IGF-1R, has been investigated in relation with IGF-1R expression in a panel of established cell lines and in early passage tumor cells obtained from patients [32]. A strong correlation was found between the IGF-1R expression level and cixutumumab anti-tumor activity: both cixutumumab-mediated inhibition of cell proliferation, through downregulation of IGF-1R, AKT and ERK phosphorylation, as well as cixutumumab-induced cell death via ADCC were dependent on IGF-1R expression. The findings from this study could have implications for ongoing clinical trials of antibodies targeting IGF-1R. Actually, the evaluation of tumor IGF-1R expression and the correlation with response is among the exploratory objectives of an ongoing

phase II study (NCT01160458) testing cixutumumab as single agent in pre-treated mesothelioma patients.

3.3. Anti MET

MET is expressed in the majority of MPMs, and its activation by the related ligand (hepatocyte growth factor/scattering factor, HGF/SF) contributes to disease pathogenesis by promoting cell growth and survival, motility and invasion. Although MPM cells express HGF, the major source for its production is represented by the stroma. By releasing platelet-derived growth factor (PDGF) and fibroblast growth factor (FGF), MPM cells recruit activated HGF-secreting fibroblasts into the tumor microenvironment, thus establishing a paracrine circuit that reinforces cancer progression [33]. There is evidence that MET can be also transactivated through a ligand-independent mechanism which involves a complex cross-talk with a variety of RTKs, including EGFR, IGF-1R, and vascular endothelial growth factor receptor (VEGFR), suggesting that combinatorial approaches targeting multiple RKTs may improve the efficacy of therapies for MPM treatment. Considering that signals from MET as well as from the other RTKs, presumably active in MPM, all converge into the phosphatidylinositol 3-kinase/mTOR (PI3K/mTOR) cascade, targeting the components of this pathway together with MET may represent an alternative effective strategy in treating MPM.

Tivantinib, a selective non-ATP competitive oral inhibitor of MET, has been tested in MPM cell and mouse xenograft models in combination with GDC-0980 and NVP-BEZ235, dual inhibitors of class I isoforms of PI3K and mTOR. This combination was strongly synergic in suppressing MPM cell proliferation and tumor growth [34]. The efficacy of dual inhibition of MET and PI3K/mTOR pathway has been also demonstrated combining the MET/ALK inhibitor crizotinib with GDC-0980 or with the pan-class I PI3K inhibitor NVP-BKM120 both *in vitro* and *in vivo* [35].

A Phase I/II study to evaluate the safety and tolerability of tivantinib in combination with carboplatin and pemetrexed as first-line treatment in patients with advanced non-squamous non small cell lung cancer (NSCLC) or MPM is currently recruiting patients (NCT02049060). Interim results were presented at 2015 ASCO annual meeting [36] showing that adding tivantinib to chemotherapy is safe, with preliminary evidence of antitumor activity in NSCLC patients.

Tivantinib has been also tested in pre-treated patients in a terminated phase II trial (NCT01861301). Results from the trial, evaluating 18 patients with either pleural or peritoneal mesothelioma, indicated that 43% of all the peritoneal mesothelioma patients were stable for 9 months or more. However, MET expression or mutation did not correlate with disease control [37]. The authors recommended looking into alternative biomarkers that would be more predictive of the activity of tivantinib in mesothelioma patients.

3.4. Anti PI3K/AKT/mTOR

The constitutive activation of RTKs in MPM is associated with the upregulation of downstream signaling cascades, including the PI3K/AKT/mTOR pathway, which plays a key role in cell growth, survival and proliferation. Phosphorylation of AKT protein, indicative of activation of the PI3K pathway, has been shown in both MPM cells and primary tumors [6]. Loss of PTEN expression accounts for PI3K/AKT signaling activation in 10–62% of MPMs [38]. A global gene expression analysis performed in fresh-frozen MPM tumors demonstrated a strong correlation between overexpression of some components of this pathway, i.e. PI3K, mTOR and rapamycin-insensitive companion of mammalian target of rapamycin (RICTOR), and poor survival [39]. In a recent pre-clinical study [40] the coordinated activation of EGFR, MET, and AXL resulted in AKT but not in MAPK signaling induction. Along the AKT pathway, mTOR was shown to play a key role in the control of cell survival/proliferation, and combined targeting of PI3K and mTOR, either by NVP-BEZ235 or by the mTOR inhibitor RAD001 associated with AKT knockdown, was significantly more effective in inhibiting cell proliferation and viability than inhibition of the individual signaling intermediates. A comparable growth-inhibitory response was achieved by simultaneous EGFR, MET, and AXL inhibition. A variety of pre-clinical studies have demonstrated that inhibition of AKT/mTOR signaling may enhance MPM cell sensitivity to cytotoxic agents. A strong synergistic cytotoxicity was observed when cisplatin was combined with perifosine, a synthetic alkylphospholipid which inhibits AKT membrane recruitment and activation [41], or with the mTOR inhibitor temsirolimus [42]. Moreover, inhibition of the PI3K/mTOR pathway has been shown to sensitize MPM cells to chemotherapy through a mechanism involving the down-regulation of drug efflux mediated by the ABCG2 transporter. This finding has important therapeutic implications, considering that cytotoxic drugs currently used in MPM treatment, such as pemetrexed and doxorubicin, are well known substrates of ABCG2 [43].

The results of a phase II study investigating the clinical activity of single agent everolimus in advanced MPM patients progressed after chemotherapy with platinum compounds have been recently published [44]. This agent demonstrated no efficacy in unselected pre-treated patients. Another phase II trial (NCT01024946) with everolimus in advanced mesothelioma with merlin/*NF2* loss as sensitivity biomarker has been completed and results are warranted. An alternative combination strategy inhibiting both PI3K/mTOR (VS-5584) and focal adhesion kinase (FAK) (defactinib) is under evaluation in a phase I study (NCT02372227). Pre-clinical studies have demonstrated the efficacy of such combination both in 2D and 3D culture models as well as in 3D tumor explants *ex vivo* [45].

3.5. Anti FAK

There is evidence that loss of merlin protein expression, which occurs in 40–50% of MPM cases [16] as previously indicated, is associated with the simultaneous activation of FAK [46], a non-receptor TK downstream of integrin proteins with an important role in cellular adhesion and spreading processes. In particular, merlin-negative MPM cells, being characterized by weak cell-cell adhesions, may become more dependent on cell-extracellular matrix-induced FAK signaling for their survival/proliferation. Therefore, merlin loss confers exquisite sensitivity to FAK inhibitors, such as defactinib, and merlin IHC has been proposed as a valuable predictive biomarker for responsiveness to this drugs. Moreover, a study has demonstrated that defactinib reduces the subpopulation of cancer stem cells (CSCs) in MPM. The underlying mechanism involves not only direct inhibition of FAK on tumor cells, but also inhibition of PYK2, the other FAK family member, on tumor-associated macrophages (TAMs). In particular, PYK2 inhibition, by decreasing the number of TAMs *in vivo*, reduces the release of cytokines (IL-6 and IL-8) that stimulate CSC proliferation and survival [47]. Very recently, a reduction of macrophage tumor infiltration *in vivo* has been described as a mechanism contributing to the anti-tumor activity of VS-4718, another small molecule inhibitor of FAK/PYK2 [48].

Based on the encouraging pre-clinical results, defactinib entered into clinical evaluation. The COMMAND study (NCT01870609) was a phase II multicenter study of defactinib as maintenance therapy versus placebo in patients with partial response (PR) or stable disease

(SD) after first-line pemetrexed/platinum therapy. Prior to randomization to the study, tumor merlin status of each patient (high or low) was established by IHC performed at a central laboratory. Despite promising early results, the trial did not produce a sufficient level of efficacy to justify continuation and stopped in October 2015. Nevertheless, the clinical investigation of defactinib efficacy is still active and an open label defactinib-neoadjuvant phase II study in subjects with MPM who are eligible for surgery is currently accruing patients (NCT02004028). The purpose of this study is to assess responsiveness as function of biomarker expression in tumor tissue. The safety, pharmacokinetics, and tumor response rate to defactinib will also be assessed. Preliminary results indicate that treatment with defactinib for either 12 or 35 days is associated with tumor volume reduction and tumor immunomodulation [49].

3.6. Anti HSP90

Heat shock protein 90 (HSP90) is a molecular chaperone that assists protein folding and maturation controlling the stability of many proteins associated with cancer cell proliferation and death, including many RTKs activated in MPM. It has been demonstrated that the HSP90 inhibitor 17-AAG down-regulates multi-RTK signaling in mesothelioma cell lines and is more effective in inhibiting cell survival and proliferation than single treatments with RTKIs [50]. HSP90 inhibitors have been shown to repress MDM4 protein, a MDM2-like molecule involved in the negative regulation of p53. Taking into account this effect, two HSP90 inhibitors (17-AAG and 17-DMAG) have been tested in combination with nutlin-3a, which blocks p53 degradation by inhibiting its interaction with MDM2 protein. This combination resulted in a synergistic suppression of tumor growth in an orthotopic mouse model, offering a new therapeutic strategy for treatment of mesothelioma bearing the wild-type p53 genotype [51]. A phase I/II study of first line ganetespib (oral HSP90 inhibitor) with cisplatin/pemetrexed in patients with mesothelioma (MESO2 NCT01590160) is currently recruiting participants.

3.7. Anti-angiogenetic drugs

A number of pre-clinical and clinical evidence suggests that angiogenesis may be a critical step in the pathogenesis of MPM. Twenty to 71% of MPMs have been found to express at least one of the VEGFRs whereas PDGFRβ expression ranges from 30% to 45% of cases [52,53].

In addition, increased expression of VEGF, VEGF-C, PDGFA and B, FGF-1 and FGF-2 has been frequently detected in MPM. Circulating VEGF and FGF-2 have been inversely correlated with survival [54], and high levels of VEGF expression have been associated with increased tumor microvascular density, which has emerged as an independent predictor of poor prognosis in MPM.

Bevacizumab is a humanized monoclonal antibody neutralizing all of the isoforms of human VEGF. Treatment with bevacizumab was shown to inhibit the development of both thoracic tumors and pleural effusion in SCID mice orthotopically inoculated with VEGF-producing MPM cells [55]. Interestingly, a better efficacy was achieved when the treatment was started early after tumor inoculation, suggesting that anti-angiogenic therapies may be beneficial for controlling the clinical early stage of MPM. Finally, the combination of bevacizumab with pemetrexed prevented the production of pleural effusion and prolonged the survival of mice more effectively than single agent treatments.

Results from a phase III trial in which 448 patients with chemotherapy-naive, unresectable MPM received pemetrexed and cisplatin (PC) or pemetrexed and cisplatin plus bevacizumab (PCB) for 6 cycles (NCT00651456) have been recently published. Zalcman et al. [56] reported that the addition of bevacizumab to cisplatin and pemetrexed improved OS, the primary outcome of this study, at 18.8 months versus 16.1 months (HR 0.77 (95% CI 0.62–0.95)). Overall, 158 of 222 (71%) patients in PCB group and 139 of 224 patients (62%) in PC group had grade 3-4 adverse events (AEs). Grade 3 or higher hypertension and thrombotic events were more common in the PCB group and more patients stopped treatment (24.3% versus 6%). Although these toxicities may limit patient eligibility, this study firstly demonstrated the efficacy of a triplet regimen with an anti-angiogenic agent for MPM treatment. Median PFS and OS in this trial were longer than those reported in other two phase II studies [57,58] with gemcitabine, cisplatin and bevacizumab or pemetrexed, carboplatin, and bevacizumab, respectively. Differences in patient enrollment and study design, together with a lower efficacy of the gemcitabine-based chemotherapy could explain this discrepancy.

Additional trials are investigating new therapeutic combinations of pemetrexed and cisplatin with other anti-angiogenic drugs such as nintedanib (a small molecule TKI targeting VEGFR, PDGFR and FGFR) (phase II/III study LUME-Meso NCT01907100) and cediranib (an oral inhibitor of PDGFR and VEGF-1, -2, and -3 receptor family) (phase I/II NCT01064648). The efficacy of such combinations has been recently demonstrated for nintedanib, using an orthotopic human MPM xenograft model in SCID mice. Nintedanib was effective also as monotherapy, inhibiting angiogenesis and tumor growth and significantly prolonging the survival of mice [59]. Cediranib was tested as monotherapy in a multicenter phase II trial in patients with advanced MPM [60]. Unfortunately, the study did not meet its primary endpoint and treatment was associated with substantial toxicity.

Other studies of single drug VEGF inhibitors were disappointing: a phase II study of single agent dasatinib (a pan kinase inhibitor) in patients with previously treated MPM demonstrated lack of activity and unfavorable toxicity [61]. Two phase II trials have tested sorafenib (a pan kinase inhibitor) in pre-treated patients revealing moderate activity in advanced MPM patients [54,62]; more encouraging results, at least pre-clinically, were obtained when sorafenib was combined with everolimus, although in this context sorafenib was evaluated not for its anti-angiogenic properties but for its ability to inhibit the ezrin pathway, which appeared to play a key role in the motility and local aggressiveness of MPM cells [63].

Finally, the single-agent imatinib mesylate, a selective inhibitor of tyrosine kinases including bcr-abl, c-kit, c-fms, and PDGFRβ, largely failed to show significant response rates in MPM [64].

Considering that pre-clinical studies on mesothelioma reported synergistic benefit with the combination of imatinib mesylate and chemotherapy [53], a phase I study of cisplatin-pemetrexed-imatinib mesylate in 17 chemo-naive mesothelioma patients was conducted. Although this regimen had clinical benefit in some patients (non-sarcomatoid histology, better performance status, higher baseline tumor p-PDGFRα, and completion of 6 cycles of therapy), it was not well-tolerated [65]. A phase II study evaluating the combination of gemcitabine and imatinib mesylate in pemetrexed-pretreated patients with MPM expressing PDGFRβ and/or c-kit by IHC is recruiting patients (NCT02303899).

3.8. Anti CD13

CD13 is a membrane-bound metalloprotease, exerting an important role in chemokine processing and tumor invasion, and is considered an attractive target for inhibiting angiogenesis. CD13-null mice

had a normal development but showed a severe impairment in angiogenesis under pathological conditions [66]. It is well known that tumor necrosis factor-alpha (TNFα), besides its immunomodulatory effects, exerts a powerful antivascular activity mainly mediated by apoptosis of tumor-associated endothelial cells. By inducing death of these cells, TNFα may also increase the tumor-selective uptake of chemotherapeutic drugs.

Asparagine-glycine-arginine-human necrosis factor alpha (NGR-hTNF) consists of the N terminus of TNFα fused to the C terminus of the peptide NGR, which is able to selectively recognize a CD13 isoform over-expressed on tumor endothelium avoiding the toxicity of systemic administration of TNFα [67]. NGR-hTNF induced anti-tumor activity at least 10-fold stronger than TNFα in murine models even when delivered in the nanogram range (0.005 μg/kg) [67]. Moreover, a significant synergism was observed by combining NGR-hTNF with multiple chemotherapeutic agents in pre-clinical models [68].

In a single arm phase II trial in pemetrexed-pretreated MPM, NGR-hTNF induced a 46% disease control rate, maintained for a median of 4.7 months, and a median OS of 12.1 months [69]. Based on these results, the phase III trial NGR-015 (NCT01098266) testing NGR-hTNF plus best Investigator's choice is ongoing. Preliminary results [70] indicated that despite the primary endpoint (OS) was not met, patients with short treatment-free interval had benefit in terms of either OS and PFS. NGR-hTNF is also tested in a phase II study (NGR019 NCT01358084) as maintenance treatment.

3.9. Anti mesothelin

Mesothelin is a glycoprotein physiologically expressed on the surface of mesothelial cells and highly expressed in many cancers including MPM. For this reason it is considered a tumor antigen and an appropriate target for immunotherapy [71]. Pre-clinical studies indicate that mesothelin expression promoted cell invasion and matrix metalloproteinase secretion both *in vitro* and in an orthotopic MPM model [72].

Amatuximab is a chimeric monoclonal antibody directed against mesothelin and was tested combined with pemetrexed and cisplatin in a single-arm phase II study in 89 patients with unresectable MPM (NCT00738582). The treatment was safe and well tolerated. Although there was no improvement in PFS (6.1 months), the median OS was superior (14.8 months) to historical controls (13.3 months) [73]. A phase II double-blind, randomized multicenter study (NCT02357147) of amatuximab 5 mg/kg, administered weekly, in combination with pemetrexed and cisplatin as first line treatment in subjects with unresectable MPM is currently recruiting patients [74].

Anetumab ravtansine (BAY94-9343) is a fully human anti-mesothelin antibody conjugated to the maytansinoid tubulin inhibitor DM4 with efficacy in pre-clinical studies [75]. In a phase I study (NCT01439152), anetumab ravtansine at the maximum tolerated dose (MTD) (6.5 mg/kg) was well tolerated and showed encouraging durable tumor responses in patients with metastatic mesothelioma [76]. A phase Ib study of anetumab ravtansine in combination with pemetrexed and cisplatin in mesothelin-expressing solid tumors (NCT02639091) and a randomized phase II study of anetumab ravtansine or vinorelbine in patients with MPM overexpressing mesothelin in the second-line setting (NCT02610140) are recruiting patients.

SS1P is a recombinant anti-mesothelin immunotoxin that consists of a murine antimesothelin variable antibody fragment linked to PE38, a portion of *Pseudomonas* exotoxin A. SS1P was tested in a phase I trial (NCT01445392) in combination with pemetrexed and cisplatin in chemotherapy-naive patients. The combination of SS1P with cisplatin and pemetrexed resulted in response rates of 60% in 20 evaluable patients and 77% in 13 patients who received the MTD (45 mcg/kg) [77].

SS1P combined with pentostatin and cyclophosphamide, with the aim to minimize neutralizing antibody formation, is under evaluation in a phase I/II trial (NCT01362790).

4. Immune checkpoint inhibitors

In order to prevent autoimmunity, T cell activation and function are finely regulated at multiple steps through the control of immune checkpoint pathways. The cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4) and programed death 1 (PD-1) play a central role in this process. In particular, CTLA-4 (CD152) is an immune suppressive receptor, member of the CD28/B7 immunoglobulin superfamily, mainly expressed on CD4 T lymphocytes and to a lower extent on antigen presenting cells (APC) and granulocytes [78]. CTLA-4 engagement down-regulates the amplitude of T cell response: by binding B7-1 (CD80) and B7-2 (CD86), it competes with its costimulatory counterpart CD28. These interactions are critically important for the initial activation of naive T cells, by inhibiting T-cell function and preventing inappropriately immune responses against self-antigens in secondary lymphoid organs, and limiting the extent and duration of immune responses [79]. On the other and, the PD-1 pathway regulates effector T cells at the later stages of the immune response in peripheral tissues [80]. PD-1 is mainly expressed on activated T and B cells, but it has also been found on monocytes, natural killer cells, and tumor-infiltrating lymphocytes (TILs) [81]. PD-1 binds two inhibitory molecules, PD-L1 and PD-L2, members of the B7 family, with PD-L1 mostly expressed on leukocytes, whereas PD-L2 is limited to dendritic cells and monocytes. PD-1 engagement directly inhibits TCR-mediated effector functions, acting as a negative regulator of immune response. In addition, PD-L1 is highly expressed by most carcinomas including MPM [82]; the expression of PD-L1 in tumor cells has been demonstrated to attenuate anti-tumor immune response [83] through the inhibition of T cell activation and the increase of apoptosis of antigen-specific human T-cell clones [84]. The distinction between the secondary lymphoid organs or peripheral localization and role of CTLA-4 and PD-1 respectively is not absolute, indeed CTLA-4 plays also a relevant role in the regulation of the suppressive function of T regulatory (Treg) lymphocytes, that are typically localized in tumor tissues and are thought to locally inhibit anti-tumor immunity, by repressing effector T cell responses [85,86]. In this context, it is noteworthy that in a model of human immortalized suppressive T cells (MT-2) it has been recently demonstrated that asbestos exposure enhances Treg function with an increasing production of suppressive cytokines as IL-10 and TGFβ [87]. CTLA-4 and PD-1 inhibitory pathways are upregulated in many cancers, thus playing a critical role in cancer-associated immune suppression and evasion. Their key role in regulating the immune system have made CTLA-4 and PD-1/PDL-1 attractive therapeutic targets for cancer, and in the last few years several new drugs have been developed and have been approved in melanoma and NSCLC or are in phase II and/or III clinical trials stage development in many other cancers including MPM.

4.1. Anti CTLA-4

The therapeutic efficacy of CTLA-4 blockade in MPM in association with both chemo or radiotherapy has been demonstrated in several studies on *in vivo* murine models.

When anti-CTLA-4 monoclonal antibody was administrated between cycles of chemotherapy (cisplatin) in a murine model of mesothelioma, a great inhibition of tumor growth was observed, especially when the treatment started in the initial stage of tumor growth [88]. Moreover, CTLA-4 blockade alternated with cisplatin treatment inhibited tumor cell repopulation, whereas the number T lymphocytes infiltrating the tumor was increased. This therapeutic strategy also resulted in an increased expression of genes encoding for IL-2, IFN-γ, granzyme B and perforin, suggesting that the treatment with anti-CTLA-4 antibody between cycles of chemotherapy may enhance the antitumor immune response.

By contrast, chemotherapeutic agents have been discriminated on their ability to generate an immune response against tumor cells and it has been demonstrated that CTLA-4 blockade with an anti-CTLA-4 antibody in combination with the non-immunogenic chemotherapeutic drug cisplatin failed to induce synergistic effect in an *in vivo* model of MPM [89]. On the other hand, when anti-CTLA-4 was associated with the immunogenic cytotoxic drug gemcitabine, a clear additive effect of both treatments with a significant delay of tumor outgrowth was observed. In addition, the combination therapy induced long-lasting protective anti-tumor immunological memory, as demonstrated after the re-inoculation of tumor cells in mice that had completely rejected their tumors, with more than 90% of mice completely resistant to tumor re-challenge.

The CTLA-4 blockade was also effective when associated with local RT. RT can induce both a direct cancer cell death in the irradiated field, as well as a systemic anti-tumor effect against tumor-associated antigens released by dead tumor cells, due to the priming/amplification of cytotoxic T cells and activation of dendritic cells [90]. The combination of local radiation with CTLA-4 blockade enhanced the anti-tumor immune reaction in a murine model of MPM, not only against the primary tumors but also versus secondary tumors (abscopal effect). Further, local RT increased T cell infiltration (Treg and cytotoxic T lymphocytes) in both primary and secondary tumors, while the combination with CTLA-4 blockade augmented the fraction of effector T cells over Treg cells [91].

The MESOT-TREM-2008 study (NCT01649024) was a phase II trial with tremelimumab, a fully human anti-CTLA-4 monoclonal antibody, as monotherapy in patients with unresectable MPM progressed to a first-line platinum-based regimen [92]. Twenty-nine patients (28 MPM and 1 peritoneal) were treated with 15 mg/kg once every 3 months; no patient had a complete response, 2 patients had a long-lasting response, 9 patients (31%) achieved disease control. The median OS of 10.7 months and the survival rates at 1 and 2 years of 48.3 and 36.7% respectively are promising results even if the primary endpoint of the study was not accomplished. It is worth of note that one patient had an initial disease progression followed by a long-lasting PR (18 months); this clinical course has been also documented in melanoma patients treated with ipilimumab, another anti-CTLA-4 monoclonal antibody [93]. This result indicates that, differently from cytotoxic or targeted drugs, treatment with anti-CTLA-4 antibodies can induce a false tumor progression and a careful assessment of disease progression is mandatory before therapy is discontinued.

The MESOT-TREM-2012 study (NCT01655888) was a further phase II study which explored a more intensive schedule of tremelimumab (10 mg/kg once every 4 weeks for 6 doses, then every 12 weeks until disease progression or severe toxicity) in 29 second-line mesothelioma patients. According to irRC (immune related Response Criteria) four PRs were recorded and 2 responses occurred after initial progressive disease (PD). Fifty patients (52%) had disease control, with a median duration of 10.9 months. The most common AEs were dermatological (rash, pruritus) and gastrointestinal (colitis and diarrhea) [94].

A multicenter, international (180 centers), phase IIb, randomized double-blind placebo-controlled study (NCT01843374) in 564 patients with unresectable MPM or peritoneal mesothelioma who progressed on previous anti-folate/platinum regimens is ongoing. Recently, in a press release, AstraZeneca, the sponsor of the study, announced that the trial did not meet its primary endpoint of improving OS.

The emerging efficacy of immunomodulatory antibodies targeting the PD-1/PD-L1 axis prompted to design the phase II NIBIT-mESO-1 study aimed to investigate the efficacy of tremelimumab combined with the anti-PD-L1 MEDI4736 (durvalumab) in mesothelioma patients (NCT02588131). The study is actively recruiting and at present 10 patients have been so far enrolled and no grade 3-4 treatment-AEs have been observed so far [95].

4.2. Anti PD-1

PD-L1 expression has been reported in 40% of 106 samples of mesothelioma (21% in the epithelioid subtype, 94% in the sarcomatoid subtype and 57% in the biphasic subtype) and was significantly associated with poorer outcomes [82]. The higher expression of PD-L1 in sarcomatoid MPM and the correlation with a shorter survival have been also recently confirmed by the comparison of two commercial antibodies for the evaluation of PD-L1 expression [96].

To this regard, it is worth of note that there are no unique guidelines for the evaluation of PD-L1 expression and several PD-L1 antibodies are used in clinical trials. Also the cut off points for positive results are not well defined, with a range for positive cells detected by IHC from 1% to 50% [96,97]. Another major point to be clarified in thoracic tumors is the cell type (tumor cells or TILs) on which to evaluate PD-L1 expression. Indeed, in some thoracic tumors, as the non-small cell neuroendocrine carcinomas of the lung, the expression of PD-L1 has been shown only in TILs [98]. Recently, it has been observed in multiple cancer types (including NSCLC) that the response rate to PD-L1 antibody MPDL3280A was higher in those patients having tumor expressing high levels of PD-L1 by tumor infiltrating immune cells, suggesting that anti-PD-L1 antibodies are most effective in patients in which pre-existing immunity is suppressed by PD-L1 [99].

Preliminary results from a phase I trial (KEYNOTE-028 NCT02054806) of pembrolizumab (a humanized anti PD-1 antibody) in patients with PD-L1 positive advanced solid tumors reported that, of the 84 patients with MPM, 38 (45%) had PD-L1 positive tumors and 25 were enrolled. PD-L1 IHC positivity was defined as membrane staining in $\geqslant$1% of tumor cells with concomitant expression in the stroma. A dose of 10 mg/kg every two weeks was given. A PR was observed in 6 patients (24%) and 13 patients (52%) had SD resulting in a high disease control rate of 76%. Drug-related AEs were nausea (40%), fatigue (32%) and decreased appetite (28%). Nevertheless, PD-L1 expression failed to demonstrate a predictive role of response to pembrolizumab [100].

NCT02399371 is a phase II trial currently recruiting participants specifically designed to explore the efficacy of pembrolizumab in mesothelioma and the role of PD-L1 as a predictive biomarker.

Pembrolizumab has also been currently evaluated in a phase I trial in combination with defactinib and gemcitabine (NCT02546531).

A second anti-PD-1 antibody, nivolumab, is currently tested in patients with recurrent mesothelioma (phase II NivoMes NCT02497508) or combined with ipilimumab in unresectable MPM patients (phase II MAPS2 NCT02716272). Interim results of the

NivoMes trial indicated that among the 18 evaluated patients, 7 showed disease control (39%) (5 patients had a PR and 2 SD) and 9 patients had PD. Considering that 2 patients had pseudo-progression prior to a PR, the authors underlined that the follow up is at present too short to determine whether the sites of growth are due to real progression or pseudo-progression [101].

5. Conclusions and future directions

Novel target therapies require better knowledge of altered molecular pathways with a driver role in tumor growth. In MPM inactivation of tumor suppressor genes by genetic or epigenetic events rather than driver mutations in oncogenic genes are considered to be major causative factors and this behavior has strongly hampered the development of new therapies. Moreover, the majority of clinical trials of molecular agents targeting the classical hallmarks of cancers have yielded disappointing results. Indeed, despite multiple molecular alterations as well as deregulation of signaling pathways have been evidenced in MPM, a relevant target has not emerged, presumably due to the complex interconnection among different signaling pathways, which limits the efficacy of therapeutic approaches with single specific targeted agents. Pre-clinical studies indicate that concurrent targeting of multiple components of key signaling pathways might be a valuable therapeutic option for MPM management. This approach might also allow lower doses of individual drugs, with the advantage of reducing toxicity to the patients. In addition, both pre-clinical and clinical evidence suggest that the efficacy of targeted agents can be significantly enhanced by combination with chemotherapy. As an alternative to combination therapy, sequential therapy might be proposed to target multiple pathways and limit the development of escape pathways, with further advantages in terms of cost and toxicity exposure. Future clinical trials might be specifically designed to evaluate the efficacy of sequential versus combination therapy for MPM treatment. In tandem with investigation of new combinatorial drug therapeutic approaches for MPM management, intense research efforts are focused on identification of predictive/prognostic biomarkers of disease progression. To date, the majority of clinical trials conducted on MPM have enrolled unselected patients, due to the lack of valid biomarkers, as for anti-angiogenic drugs, or to the use of inappropriate techniques for the evaluation of biomarker expression, as for TKIs kinase inhibitors. Biomarker-driven trials are expected to improve patient outcomes and selective biomarkers of response are urgently needed, especially for those therapies, such as immunotherapy with anti-CTLA-4 and anti-PD1/PD-L1, that have been showing the most encouraging results. For example, the expression of PD-L1 on tumor biopsies, stromal cells or TILs has been proposed as a predictive biomarker in a variety of cancers such as lung cancer, however its role remains controversial. In mesothelioma, a predictive role of PD-L1 expression in tumor for response to pembrolizumab has not been demonstrated yet. The ongoing NCT02399371 trial has been specifically designed to explore the role of PD-L1 as a predictive biomarker and may clarify this important aspect.

In addition to immune check-point inhibitors, a variety of immunotherapeutic approaches are being investigated for MPM treatment. Mesothelin, being expressed at very low level on normal mesothelial cells and highly expressed in MPM, can be considered as a good tumor antigen and is an emerging attractive target for an immunotherapy based on adoptive transfer of CAR (chimeric antigen receptor) T cells. At present a phase I clinical trial (NCT02414269) is testing the efficacy of regionally administration of mesothelin-CAR T cells with cyclophosphamide in patients with mesothelioma or pleural metastases from lung or breast cancers with mesothelin expression.

An interesting new area of investigation is represented by the regional administration of oncolytic viruses (OVs). Although OVs were originally designed to induce specific lysis of tumor cells, it is becoming clear that their anti-tumor activity is also mediated by additional mechanisms involving the activation of systemic anti-tumor immune responses. Ongoing phase I, I/II clinical trials have been evaluating the intrapleural application of measles (NCT01503177), herpes (NCT01721018), and vaccinia virus (NCT01766739) in MPM patients.

Therapeutic vaccination strategies under clinical evaluation in MPM include dendritic cells vaccines, peptide vaccines such as that derived from the product of Wilms tumor suppressor gene 1 (WT1), a transcription factor highly expressed in mesothelioma (NCT01890980), and modified bacterial organisms such as *Listeria monocytogenes* expressing mesothelin (CRS-207) (NCT01675765).

At present, it is difficult to ascertain the superiority of one immunotherapeutic approach over the others in either MPM or other thoracic malignancies. Indeed, the complex interplay between the tumor and the immune system differs in each patient and the response to such therapies may be greatly affected by the state of the immune system, the individual tumor characteristics, and the stage of the tumor. Therefore, the identification of proper biomarkers of response to different forms of immunotherapy may help the advancement toward a personalized immunotherapy.

In addition, considering that the immunosuppressive activity exerted by tumor cells is high in MPM and involves multiple pathways, combination therapies targeting these inhibitory pathways at multiple level are likely to induce a stronger immune response to cancer. Studies specifically designed to evaluate the superior efficacy of these combinations are warranted.

Among the current investigational approaches for MPM treatment, it is worth mentioning the role of miRNAs both as diagnostic/prognostic markers and as tools to define novel therapeutic strategies. MesomiR 1 is the first-in-man phase I study testing the intravenous administration of TargomiRs, (EGFR-targeted EDV-packaged miR-16-based mimics) for patients with MPM and NSCLC (NCT02369198). This novel strategy using targeted EDV nanocells to restore the expression of down-regulated miRNAs may represent a valid therapeutic option for mesothelioma treatment and confirming results are eagerly awaited.

Disclosure

None of the authors have any financial conflict of interest.

Acknowledgements

We thank "Association Noi per Loro Onlus", project "Andrea Spadola".

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