Targeted Therapies in Mesothelioma

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17.1 Introduction

Malignant Pleural Mesothelioma (MPM) is fatal disease characterized by chemoresistance and poor prognosis [1]. Since 2003, when a platinum-based chemotherapy *plus* pemetrexed was introduced as standard first-line therapy [2], no significant improvements in MPM management have been done. To date, no indications for second-line therapies after first-line failure are available [3]. In the last years, many efforts have been directed to the identification of anticancer therapies able to target tumor-related molecular changes. Targeted therapies may improve cancer management in terms of both patients' prognosis and quality of life, because of the higher specificity and the lower toxicity profile compared to most cytotoxic drugs. The identification of key molecular targets in MPM represents a hard challenge because MPM pathogenesis is not completely known. This neoplasia is characterized by low mutational load, but recurrent somatic mutations in tumor suppressor genes [4]. Moreover, the three histologic subtypes are characterized by different biological and clinical behaviors, increasing the need to develop personalized therapeutic approaches. Here, we focus on potential molecular targets and specific targeted therapies under clinical investigation in MPM.

17.1.1 NF2/Merlin

NF2 is a tumor suppressor gene frequently altered in MPM [5–7]. Recent studies performed in a large series of MPM specimens using high-throughput technologies (whole-exome sequencing, RNA-seq) confirmed high frequency of *NF2* alteration including mutations and copy number variations [8–10]. Of note, sarcomatoid subtypes carried higher rate of *NF2* mutation compared to epithelioid ones [9].

NF2 gene encodes for merlin protein, a tumor suppressor blocking several signal transduction pathways involved in cell proliferation, survival, and metabolism. Wild-type merlin is regulated by post-translational modifications defining its conformational status and activity. It is inactivated through the phosphorylation at Serine 518 by cAMP-dependent kinase (PKA) and activated by the myosin phosphatase MYPT1-PP1 [11]. As a consequence, deregulation of merlin can occur in the absence of *NF2* gene mutation [12]. Indeed, mRNA overexpression of CPI-17 (phosphatase inhibitor of 17 kDa), a cellular inhibitor of MYPT1-PP1, has been detected in mesothelioma tumor samples carried wild-type *NF2*, suggesting that merlin is completely inactivated in MPM [13].

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17.1.1.1 NF2/Merlin and Hippo Pathway

Merlin controls cell proliferation and viability through the regulation of the Hippo pathway, a signal transduction cascade including the proteins: MST1/2 Kinases (Mammalian STE20-like protein kinase 1/2), MST1/2 coactivator SAV1(Salvador1), LATS1/2 Kinases (Large Tumor Suppressor Kinases 1/2), and LATS1/2 coactivator MOB1 (Mps one-binder 1) [14]. Merlin-dependent activation of the Hippo pathway results in the phosphorylation and inactivation of YAP (Yes associated protein), a cofactor essential for TEAD (TEA domain family member) transcriptional activity. YAP/TEAD complex activates the transcription of genes involved in cell proliferation, cell growth, and inhibition of apoptosis [15] (Fig. 17.1). In MPM, Hippo pathway deregulation seems to be related mainly to merlin loss of function [16, 17], although concomitant mutations of *NF2* and *LATS2* genes have been reported [9, 18]. Immunohistochemistry analysis performed on MPM cell lines and tumor tissues revealed strong nuclear localization of YAP in a high percentage of samples [16, 19, 20] and YAP knockdown in MPM cells resulted in the inhibition of cell growth, motility, and invasive abilities [21]. Altogether, these observations highlight the strong link existing between YAP hyper-activation and MPM uncontrolled growth, suggesting that YAP may be a potential candidate for MPM-targeted therapies. A drug screening performed using the Johns Hopkins library identified the small-molecule Verteporfin (VP) (Visudyne, Novartis) as a YAP inhibitor [22]. VP is an FDA (Food and Drug Administration)-approved photosensitizer drug used for the treatment of neovascular macular degeneration. In addition to its photosensitizer properties, VP has light-independent ability in inducing YAP conformational change and in blocking YAP/TEAD interaction [23]. The potential of VP as anticancer drug is under investigation in phase I/II clinical trials in different human cancers, including

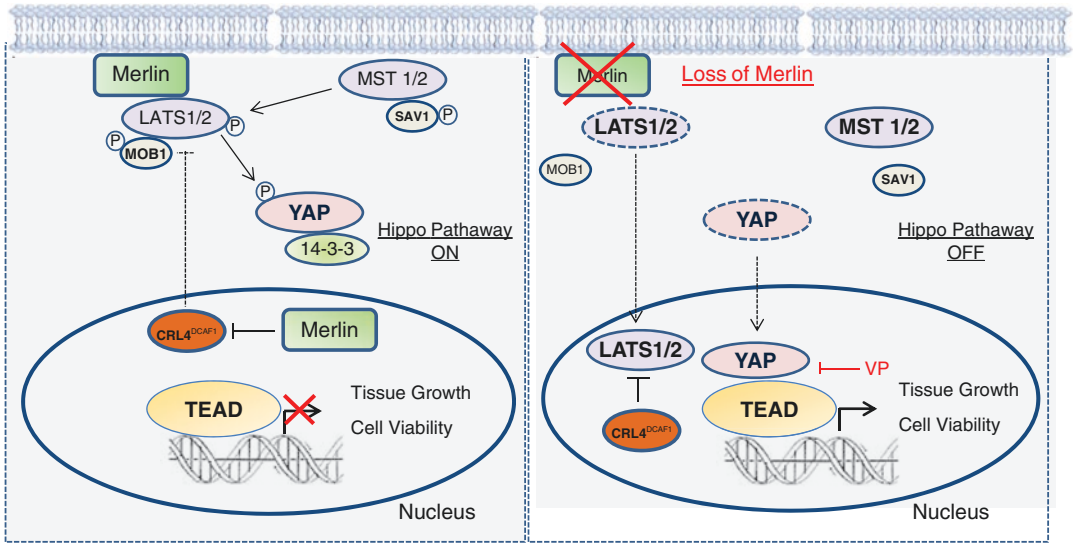

Fig. 17.1 *Merlin regulates Hippo pathway activation.* Merlin blocks TEAD transcriptional activity (left panel): following growth arrest signals, merlin recruits LATS1/2 and MOB1 in the cytoplasm at the membrane level. MST1/2 phosphorylates LATS1/2 and MOB1 activating LATS1/2 that, in turn, phosphorylates YAP; phospho-YAP binds 14-3-3 and is retained into the cytoplasm. Into the nucleus, merlin inhibits CRL4 ^{DCAF1}, the E3 ubiquitin ligase implied in LATS1/2 degradation. Loss of merlin (right panel) results in YAP/TEAD association and activation of transcription. Verteporfin induces YAP conformational change inhibiting YAP/TEAD interaction. *LATS* Large Tumor Suppressor Kinases 1/2, *MOB* Mps one-binder 1, *MST 1/2* Mammalian STE20-like protein kinase1/2, *SAV* salvador, *YAP* Yes-associated protein, *TEAD* TEA domain family member, *CRL4* cullin4A-RING E3 ubiquitin ligase, *DCAF1* DDB1- and CUL4-associated factor1, *VP* Verteporfin

breast and pancreatic cancers, brain tumors, and pleural malignancies (www.clinicaltrials.gov, NCT02939274, NCT03067051, NCT00002647; NCT02702700). As regard MPM, encouraging results have been obtained in in vitro studies demonstrating VP-dependent reduction of cell proliferation, cell viability, and cell invasion in MPM cell lines [18, 20].

17.1.1.2 NF2/Merlin and mTOR Pathway

PI3K-AKT-mTOR is a signal transduction pathway involved in cell proliferation, protein synthesis, glucose metabolism, apoptosis resistance, angiogenesis, and invasion. Activation of PI3K-AKT-mTOR passes through RTKs (Tyrosine Kinase Receptors) activation or G-Protein Coupled Receptors (GPCRs)-dependent RAS induction [24]. mTOR (mammalian target of rapamycin) is a serine/threonine kinase included in two protein complexes: the rapamycin-sensitive mTORC1 (mammalian target of rapamycin complex 1) and the rapamycin-insensitive mTORC2. mTORC1 induces mRNA translation, protein synthesis, and nucleotide production and negatively regulates autophagy and mTORC2 [25]; mTORC2 regulates protein kinases activity including AKT [26]. Physiological inhibitors of PI3K-AKT-mTOR pathway are the phosphatase and tensin homolog PTEN and merlin [27] (Fig. 17.2).

Aberrant activation of PI3K/AKT/mTOR pathway is a hallmark of many cancers including MPM [28, 29]. In MPM, recurrent *NF2* mutations [8–10], loss of PTEN [30], or gain of function mutations of PI3K or AKT [8] are reported to be responsible for mTOR pathway activation. In recent years, rapamycin or rapamycin-derived (rapalog) inhibitors have been used to inhibit mTORC1; among them, the most studied were sirolimus (rapamycin), temsirolimus (CCI-779), and everolimus (RAD001, Novartis Pharmaceuticals). Preclinical studies strongly encouraged the use of rapalogs in MPM. Indeed, Lopez-Lagos et al. [31] demonstrated that merlin null cells showed mTORC1 activation and higher sensitivity to rapamycin treatment compared to merlin-expressing cells. Moreover, Pignochino and coworkers observed anticancer activity of everolimus in MPM cell lines and mouse xenograft models. Of note, everolimus strongly synergized with sorafenib (a multi-kinase inhibitor) [32]. Unfortunately, phase II trials evaluating everolimus activity in unselected MPM patients (www.clinicaltrials.gov; NCT00770120; NCT01024946) showed no clinical efficacy [33]. Probably, the lack of efficacy of everolimus-based therapy in MPM was due to the wide spectrum of PI3K/AKT activities as well as the loss of mTORC1 negative regulation of mTORC2. To overcome low efficacy of mTORC1 inhibitors, the dual PI3K and mTORC1/2 inhibitor apitolisib (Genentech) was assessed in clinical trials. Although the promising response rate of MPM patients is in phase I trial (www.clinicaltrials.gov, NCT00854152; [34]), the drug revealed high toxicity profile in metastatic renal cell carcinoma phase II trials (www.clinicaltrials.gov, NCT01442090; [35] (Table 17.1). Encouraging results were obtained with another AKT inhibitor: Afuresertib (Novartis, Pharmaceuticals). In vitro preclinical study demonstrated that afuresertib strongly inhibited cell growth and clonogenic activity of MPM cell lines, induced cell cycle arrest, and acted in cooperation with cisplatin in inducing MPM apoptosis [36]. Of note, phase I clinical trial of Afuresertib in multiple myeloma showed promising results [37], encouraging further assessment of this drug for the treatment of other cancers including MPM.

17.1.1.3 NF2/Merlin and FAK

Cell anchorage to Extracellular Matrix (ECM) triggers signal transduction pathways involved in cell growth, survival, motility, and invasiveness [38]. A central role in transducing these signals is carried out by the Focal Adhesion Kinase (FAK). FAK is a non-receptor cytoplasmic tyrosine kinase consisting of four domains: N-terminal FERM domain (regulatory domain), catalytic domain, proline-rich domain, and C-terminal focal adhesion domain. It is activated by Integrin Receptors, Growth Factor and Cytokine Receptors [38] (Fig. 17.3). FAK overexpression and deregulation has been described in several types of cancers, and it was linked to uncontrolled

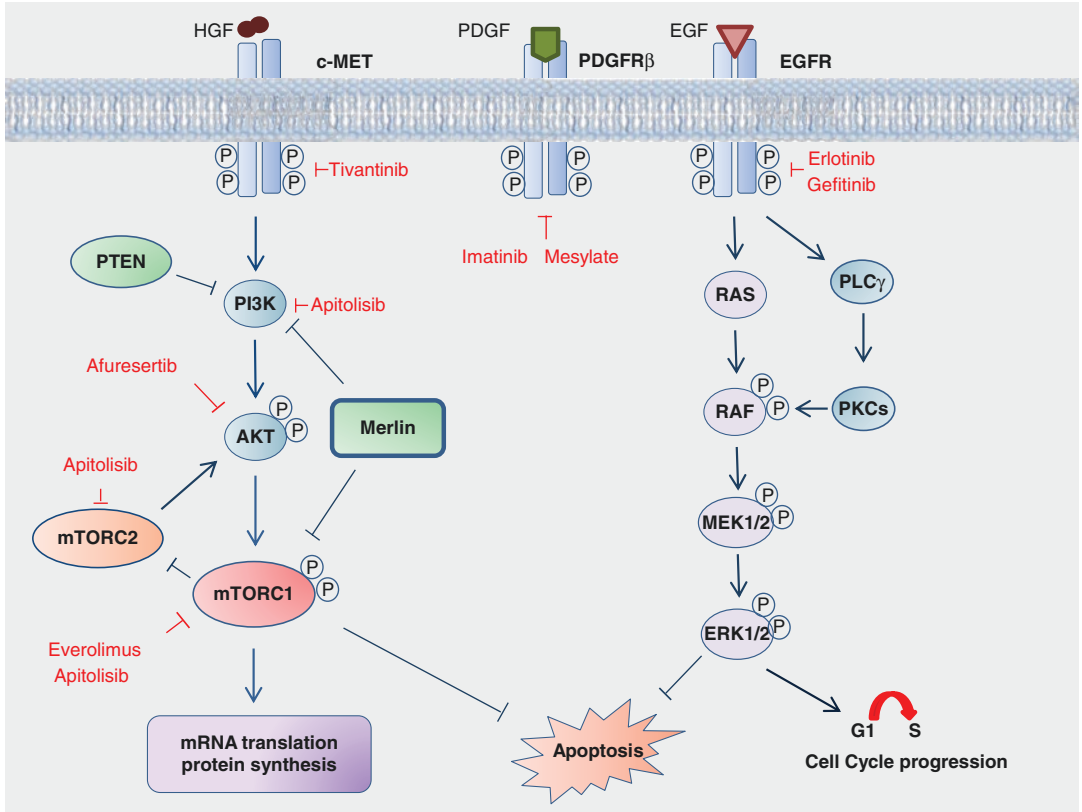

Fig. 17.2 *Schematic representation of TKRs-induced pathways*. Growth factors binding to their specific receptors induce intracytoplasmanic phosphorylation and activation of TKRs. TKRs transduce their signals mainly through PI3K-AKT-mTOR pathway and MAPK (Mitogen-Activated Protein Kinase) pathway and are mainly implicated in cell proliferation and survival. *PI3K-AKT-mTOR pathway*: activated PI3K induces AKT phosphorylation and activation. AKT activates mTORC1 that in turn induces mRNA translation and protein synthesis. mTORC1 inhibits mTORC2. Activated mTORC2 regulates the activity of several protein kinases including AKT. Merlin and PTEN are negative regulators of PI3K-AKT-mTOR pathway. Tivantinib inhibits the kinase domain of c-MET receptor; imatinib mesylate inhibits PDGFR; erlotinib and gefitinib inhibit EGFR. Everolimus inhibits mTORC1; apitolisib inhibits mTORC1, mTORC2, and PI3K; Afuresertib inhibits AKT. *HGF* Hepatocyte Growth Factor, *c-MET* mesenchymal–epithelial transition protein, *PGF* platelet-derived growth factor, *PDGFR* platelet-derived growth factor receptor, *EGF* epithermal growth factor, *EGFR* epithermal growth factor receptor, *PTEN* phosphatase and tensin homolog, *PI3K* phosphatidylinositide 3 kinase, *mTORC1/2* mammalian target of rapamycin complex 1/2, *RAS* rat sarcoma (small GTPase), *RAF* rapidly accelerated fibrosarcoma, *MEK* mitogen-activated protein kinase kinase, *ERK* extracellular signal-regulated kinase

tumor growth and metastasis [38]. FAK acts mainly at the membrane levels, but Nuclear Localization Sequence (NLS) in the FERM domain has also been described [39], supporting the hypothesis that FAK may have a role in genes regulation. Small-molecule FAK inhibitors were extensively used both in preclinical studies and in clinical trials. These drugs consist mainly of selective ATP-competitive inhibitors of FAK (e.g., VS-4718, GSK2256098), although some of them target both FAK and its homolog PYK2 (e.g., VS-6062, VS-6063). In vitro results obtained using VS-4718 (Verastem) and VS-6062 (Verastem) in several types of cancer showed a strong activity of FAK inhibitors in reducing cell growth, motility, invasiveness, and metastatic potential [40]. Moreover, VS-4718 was able to deplete tumor suppressive microenvironment [41], while VS-6062 blocked TGF-β-dependent epithelial-to-mesenchymal transition and showed

Table 17.1 Clinical trials with targeted therapies in MPM patients

Targets	Drugs	Phase	Setting	Biomarkers	Primary end points	Clinical trial ID	References
mTORC1	Everolimus	II	Second line		PFS	NCT00770120	[33]
	Everolimus	II	Second line	Merlin/NF2 loss	RR	NCT01024946	
PI3K; mTORC1/2	Apitolisib	I	First/ Second line		Safety, MTD, PK	NCT00854152	[34]
FAK	Defactinib	II	Second line	Merlin status	OS, PFS	NCT01870609	
	GSK2256098	I	First/ Second line	pFAK expression; merlin status	Safety, MTD	NCT01138033	[47]
	GSK2256098 *plus* Trametinib	I	First/ Second line	pFAK; pERK expression	Safety, MTD	NCT01938443	[48]
EGFR	Erlotinib	II	First line	pEGFR; pERK; pAKT; pmTOR; PTEN expression	RR, correlation with EGFR pathway activation	NCT00039182	[51]
	Erlotinib *plus* Bevacizumab	II	Second line		RR	NCT00137826	[103]
	Gefitinib	II	First line	EGFR expression	RR	NCT00025207	[49]
	Gefitinib	II	First line		RR, safety	NCT00787410	
c-MET	Tivantinib	II	Second line	MET status; HGF serum levels	RR	NCT01861301	[59]
	Tivantinib *plus* carbo/pem	I-Ib	First line	HGF, MET and VEGF serum levels, pMET, MET expression	DLT	NCT02049060	
PDGFR, c-Kit, BCR-ABL	Imatinib mesylate	II	First/ Second line		Effect on life-threatening rare diseases associated with imatinib mesylate-sensitive tyrosine kinases	NCT00154388	[61]
	Imatinib *plus* cis/ pem	I	First line	PDGFRα; PDGFRβ; pPDGFRβ expression	MTD	NCT00402766	[62]
	Imatinib *plus* Gemcitabine	II	Second line		PFS	NCT02303899	

(continued)

Table 17.1 (continued)

Targets	Drugs	Phase	Setting	Biomarkers	Primary end points	Clinical trial ID	References
EGFR, VEGFR, RET	Vandetanib	II	Second line		DC	NCT00597116	
PDGFR, BCR-ABL, Src family non-receptor TK	Dasatinib	II	Second line	EphA2 and PDGFRβ expression; plasma levels of VEGF and PDGFRβ	PFS	NCT00509041	[67]
	Dasatinib	I	First line	pSrc and pPDGFR expression	Modulation of pSrc	NCT00652574	[68]
HDACs	Vorinostat	III	Second line		OS, toxicity	NCT00128102	[78]
	Belinostat	II	Second line	Fetal hemoglobin	RR	NCT00365053	[79]
	Valproate *plus* Doxorubicin	II	Second line		Response rate	NCT00634205	[80]
Proteasome	Bortezomib	II	First/ Second line		RR	NCT00513877	[84]
	Bortezomib *plus* Cisplatin	II	First line		PFS	NCT00458913	[85]
microRNA	TargomiRs	I	Second/ Third line		MTD, DLT	NCT02369198	[89]
p16	Ribociclib	II	Second line	CDK4/6, CyclinD1/3, p16 status	Clinical benefit rate	NCT02187783	

PFS progression-free survival, *RR* response rate, *OS* overall survival, *MTD* maximum-tolerated dose, *DLT* dose-limiting toxicities, *PK* pharmacokinetic

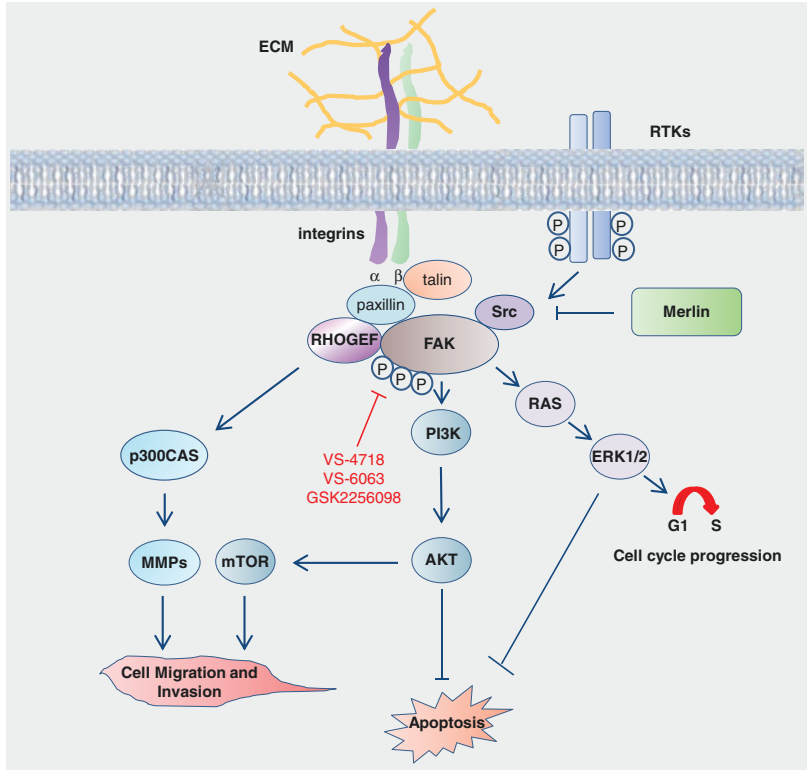

Fig. 17.3 *FAK pathway*: integrin or RTK-mediated activation of FAK involves recruitment of different proteins including talin, paxillin RHOGEF, and Src. Activated FAK induces cell cycle progression through RAS/ERK1/2 pathway, inhibits apoptosis through RAS/ERK1/2 and PI3K/AKT pathways, promotes cell migration and invasion through PI3K/AKT/mTOR pathway and activation of p300CAS. VS-4718, VS-6063, and GSK2256098 are ATP-competitive FAK inhibitors that block FAK autophosphorylation. *ECM* extracellular matrix, *RTKs* Tyrosine Kinase Receptors, *FAK* Focal Adhesion Kinase, *RHOGEF* Rho guanine nucleotide exchange factor, *ERK1/2* extracellular signal-regulated kinase 1/2, *PI3K* phosphatidylinositide 3 kinase, *mTOR* mammalian target of rapamycin, *p130Cas* p130 Crk-associated substrate, *MMPs* matrix metalloproteases

antiangiogenic effects [40]. As regard MPM, in vitro studies using FAK inhibitors revealed a link between merlin expression and anti-FAK therapy sensitivity. Indeed, MPM cell lines expressing merlin were more resistant to VS-4718 in respect of MPM cells characterized by loss of merlin. Shapiro et al. hypothesized that in merlin null cells, the loss of merlin-dependent signals derived from cell-to-cell contact may increase signals derived from cell-to-ECM contact, resulting in a hyper-activation of FAK [42]. In line with this hypothesis, reintroduction of merlin, in merlin null MPM cells, decreased FAK expression levels, FAK phosphorylation, and consequently cell invasiveness [43]. Although the strong preclinical evidence supporting the role of merlin as predictive biomarker for anti-FAK therapy, a phase II, double-blind, randomized, placebo-controlled trial aimed at determining the activity of VS-6063 (Defactinib, Verastem) in MPM, based on merlin status, showed no efficacy and was stopped (www.clinicaltrials.gov; COMMAND NCT01870609). A possible explanation of this failure was provided by Kato et al. that in their work identified E-cadherin as additional predictive biomarker for anti-FAK therapy in merlin null MPM. Using a large panel of MPM cell lines, they demonstrated that the expression levels of E-cadherin mRNA in merlin null cells significantly correlated with VS-4718 resistance, suggesting that evaluation of both markers may be useful for the selection of MPM patients

suitable for anti-FAK therapy. Importantly, they also demonstrated that MPM patients characterized by low expression levels of merlin and E-cadherin mRNA showed the poorest overall survival [44]. An additional small-molecule FAK inhibitor tested in clinical trial was GSK2256098 (GlaxoSmithKline). GSK2256098 showed strong efficacy in reducing cell growth, anchorage-independent cell growth, survival, motility, and invasiveness both in vitro and in vivo [45, 46]. The first pharmacokinetic and pharmacodynamic study of GSK2256098 administered as single agent in advanced solid tumors included 29 MPM patients (46% of total patients enrolled) (www.clinicaltrials.gov, NCT01138033). Preliminary results showed a tolerable safety profile and antitumor activity in both merlin null and merlin-expressing MPM. Evaluation of PFS (progression free survival) revealed a greater efficacy in those patients characterized by merlin null status (23.4 weeks merlin null vs 10.9 merlin-positive patients), encouraging the stratification of patients based on merlin expression [47]. Finally, a clinical trial evaluating the efficacy of combined therapy using GSK2256098 and trametinib (a MAPK pathway inhibitor) in MPM is ongoing and preliminary results are promising (www.clinicaltrials.gov, NCT01938443) [48] (Table 17.1).

17.1.2 Tyrosine Kinase Receptors

Tyrosine Kinase Receptors (TKRs) are important class of transmembrane receptors transducing growth factor signals. The binding of growth factors with specific TKRs activates transduction pathways such as MAPKs (Mitogen Activated Protein Kinases), PI3K/AKT, Phospholipase Cγ (PLCγ), and Protein Kinases C (PKC), and regulates cell proliferation, survival, migration, invasion, and angiogenesis (Fig. 17.2). Oncogenic role of gain of function TKRs mutations or TKRs overexpression has been described in several types of cancers, and an important role in MPM carcinogenesis has been shown for c-MET (mesenchymal–epithelial transition protein), Platelet-Derived Growth Factor Receptor (PDGF), and Epidermal Growth Factor Receptors (EGFRs) (Fig. 17.2) [28].

EGFR overexpression has been detected in about 50% of MPM patients [49, 50]. Erlotinib (Tarceva, Genentech Inc.) and gefitinib (Iressa, Astra Zeneca Pharmaceuticals) are Tyrosine Kinase Inhibitors (TKIs) targeting specifically the intracytoplasmic catalytic domain of EGFR. These drugs have been successfully introduced in the treatment of NSCLC, where the response is strictly related to the presence of gain of function mutations in exons 19 and 21 of the EGFR gene [51]. Despite this, phase II clinical trials conducted in untreated mesothelioma patients failed to show activity with both erlotinib and gefitinib [50, 52], probably because EGFR mutations in MPM are infrequent [53] (Table 17.1).

c-MET is a tyrosine kinase receptor activated by the binding with Hepatocyte Growth Factor (HGF). HGF/MET signaling involved mainly the activation of PI3K/AKT pathway [54]. Overexpression of c-MET in mesothelioma tumors has been described, especially in epithelioid subtypes [55], and seemed to be related to mir-34 b/c silencing [56]. Moreover, mesothelioma patients expressed higher serum levels of HGF compared to healthy subjects [57]. These results encouraged the investigation of c-MET inhibitors in mesothelioma clinical trials. Tivantinib (ARQ 197), an orally bioavailable small-molecule c-MET inhibitor, was tested in phase II trial for the treatment of malignant mesothelioma previously treated (www.clinicaltrials.gov, NCT01861301). While in hepatocellular carcinoma the anticancer activity of tivantinib was related to c-MET overexpression [58], results of this trial showed disease control only in peritoneal mesothelioma group and no correlation with c-MET expression or mutation [59]. On the other hand, in MPM preclinical models, tivantinib showed low activity used as single agent, but synergistic antitumor activity in association with pemetrexed [60] or PI3K/mTOR inhibitors [61]. To date, phase I-Ib trial testing the efficacy of tivantinib plus carboplatin/pemetrexed as first-line therapy for malignant pleural mesothelioma and non-small cell lung cancer is ongoing (www.clinicaltrials.gov, NCT02049060) and results are awaited (Table 17.1).

PDGF is a growth factor inducing proliferation of mesothelioma cells. Its receptor is expressed in two different isoforms (PDGFRα and PDGFRβ). Normal mesothelial cells express PDGFRα, while mesothelioma tumors express high levels of PDGFRβ [51]. Imatinib mesylate (STU 571, Gleevec, Novartis), an inhibitor of tyrosine kinase associated with PDGFR, c-Kit and BCR-ABL fusion protein, was tested in several trials both as single-agent and combined therapies. Phase II trials showed no results when imatinib was administered as single agent [62, 63]; in a phase I study designed to determine the maximum-tolerated dose of imatinib mesylate in association to cisplatin and pemetrexed on 17 MPM patients, the combination was not well tolerated discouraging further examination [64]; finally, phase II trial aimed at assessing the antitumoral activity of a combination of imatinib mesylate and gemcitabine in patients with unresectable malignant mesothelioma expressing either PDGFR or c-Kit is ongoing (www.clinicaltrials.gov, NCT02303899) (Table 17.1).

Failure of TKIs in MPM treatment can be caused by the concomitant activation of different TKRs (MET; EGFR; PDGFR). For example, high percentage of MPM tumors and cell lines (70%) showed simultaneous overexpression of c-MET and EGFR and preclinical models revealed a synergistic antitumor activity using crizotinib (c-MET kinase inhibitor) and afatinib (EGFR inhibitor) [65]. Multi-targeted TKIs have been developed. Vandetanib (ZD6474, Zactima, Astra Zeneca Pharmaceuticals), an oral inhibitor of EGFR, VEGFR and RET tyrosine kinases, showed strong anticancer activity in MPM cell lines acting both inhibiting RET-dependent cell survival and VEGFR-dependent angiogenesis [66], and strongly enhancing carboplatin/pemetrexed cytotoxicity [67]. Despite this, its efficacy as single agent in vandetanib versus vinorelbine randomized phase II trial in 25 patients with inoperable or relapsed malignant mesothelioma showed disappointing results (www.clinicaltrials.gov; NCT00597116). Dasatinib (BMS354825, Sprycel, Bristol-Myers) targets BCR-ABL fusion protein and inhibits signals derived from PDGFR and Src family of non-receptor tyrosine kinase [68]. Single-agent dasatinib assessed in second-line or neoadjuvant setting showed high toxicity profile without anticancer efficacy [69, 70] (Table 17.1). These negative results highlight the need to test further TKI combinations and to identify reliable predictive biomarkers to select those patients suitable for specific therapies.

17.1.3 Apoptosis Dysregulation

Dysregulation of apoptotic pathway is a feature of MPM. O'kane et al., analyzing 54 MPM tumor samples that consist of both sarcomatoid and epithelioid subtypes, revealed overexpression of the antiapoptotic proteins BCL-2, BCL-XL, and Mcl-1 and downregulation of the proapoptotic Bad, Bax, and Bid. Most important, percentage of patients overexpressing BCL-XL and underexpressing Bad and Bid was significantly higher in sarcomatoid than in epithelioid subtypes [71]. Overexpression of caspase inhibitors XIAP (X-Linked Inhibitor Of Apoptosis) and survivin in MPM specimens has also been reported [72].

17.1.3.1 Apoptosis Dysregulation and HDAC Inhibitors

Histone deacetylases are 18 different enzymes divided into four classes based on functional criteria [73]. They control a plethora of cellular function including cell cycle arrest, apoptosis, angiogenesis, and immunomodulation regulating the activity of both histones and nonhistone proteins, such as p53, NF-kB, HSP90, and HIF-1alpha [74]. HDAC inhibitors include a wide spectrum of natural and synthetic compounds [75], and are classified as pan-deacetylase inhibitors, including vorinostat (Suberoylanilide Hydroxamic Acid-SAHA: Zolinza, Merck), panobinostat (LBH589; Farydak, Novartis), belinostat (PXD101; Beleodaq, Spectrum Pharmaceuticals), and trichostatin A, and class-specific inhibitors such as butyrate and valproate (inhibit class I and IIa HDACs) and SBHA (suberohydroxamic acid) (inhibits HADC 1 and 3) [73]. In MPM cell lines, downregulation of BCL-XL was implicated in butyrate-induced apoptosis [76], and in SBHA sensitization to TNF-Related

Apoptosis-Inducing Ligand (TRAIL) [77]. Sensitization to TRAIL treatment was also obtained with panobinostat that acted inhibiting the expression of XIAP and increasing caspases' activation [78]. Vandermeers et al. demonstrated increased apoptosis induction combining cisplatinum and pemetrexed treatment with both valproate and SAHA. Anticancer efficacy of valproate *plus* cisplatinum/pemetrexed therapy was also validated in an epithelioid in vivo model [79]. In MPM, HDAC inhibitors have been tested in clinical trials both as single agent and combined therapy (Table 17.1). Oral vorinostat, an FDA-approved drug for the treatment of cutaneous T-cell lymphoma, was tested in a phase III, double-blind, randomized, placebo-controlled trial (www.clinicaltrials.gov; NCT00128102). Six hundred and sixty-one mesothelioma patients progressed after platinum *plus* pemetrexed treatment were included in the study. Results of this phase III study showed no improvement in Overall Survival (OS) in vorinostat versus placebo-treated group [80]. Negative results were also obtained with belinostat in a phase II study in which 13 MPM patients were included for second-line treatment and received intravenous infusion of the drug. The study was stopped for lack of efficacy [81]. On the contrary, a phase II trial aimed at evaluating oral valproate administration plus doxorubicin for refractory or recurrent mesothelioma after platinum-based first-line therapy showed encouraging response rate (16%) and disease control (36%). Among 45 MPM patients enrolled into the study, the best response was observed in those patients with good performance status at the time of protocol inclusion [82].

17.1.3.2 Apoptosis Dysregulation and Proteasome Inhibitors

Proteasome is a multiprotein complex responsible for proteins degradation and homeostasis. Bortezomib (Velcade) is a potent proteasome inhibitor, approved by FDA for multiple myeloma treatments. It is able to activate intrinsic apoptosis mainly blocking the degradation of IKB (Inhibitor KB) and consequently the activation of the pro-survival NF-kB (nuclear factor kappa-light-chain-enhancer of activated B cells) pathway [83].

In MPM preclinical studies, the ability of bortezomib to induce apoptosis was confirmed [84]. Of note, a strong synergizing activity was reported when bortezomib was administrated in combination with carboplatin/pemetrexed therapy [85]. Despite this, clinical evaluation of bortezomib in MPM patients failed to reach satisfactory results. Phase II trial designed to evaluate the efficacy of bortezomib as single agent in first- and second-line setting showed no activity, discouraging further evaluations [86]; phase II study aimed at evaluating the efficacy of first-line therapy combining cisplatin and bortezomib did not fulfill the primary endpoint (progression-free survival rate at 18 weeks >67.5%) and showed higher toxicity than cisplatin/pemetrexed (or raltitrexed) therapy [87] (Table 17.1).

17.1.3.3 Apoptosis Dysregulation and MicroRNAs

MicroRNAs (miRNAs) are short, noncoding, RNAs that targeting sequence-specific mRNAs are implied in post-transcriptional regulation of genes expression. Based on their target mRNAs, microRNAs can act as oncogenes or tumor suppressor genes. Dysregulation of miRNAs expression has been described in many malignancies, including cancers. In MPM mir-34b/c, mir-16 and mir-193a-3p are downregulated. These miRNAs are implied in the regulation of pro-survival and antiapoptotic pathways [56, 88–90]. TargomiRs are minicells loaded with specific microRNAs (EDVs—EnGeneIC Dream Vector) representing a reliable delivery system for in vivo administration. Mir-16 mimic encapsulated in an EGFR-targeted EDVs was successfully tested in MPM xenograft model [89] paving the way for clinical assessment. Van Zandwijk et al. conducted a phase I, open-label, dose-escalation study aimed at testing safety and activity of mir-16-loaded minicells in patients with recurrent pleural mesothelioma previously treated (Table 17.1). Twenty-six MPM patients were enrolled into the study. 5×10^9 TargomiRs per week were well tolerated and revealed early signs of antitumor activity detected by CT and PET-CT (5% of patients had partial response and 68% of patients had stable disease). However, Targomir

activity could not be clearly attributed to mir-16 targeting because the evaluation of mir-16 silencing on post-treatment biopsies has not been performed [91]. Nevertheless, results of the study are encouraging and warrant further clinical investigations.

17.1.4 Cell Cycle Regulation

Molecular pathogenesis of MPM is characterized by frequent deletion of CDKN2A gene. CDKN2A encodes p14/ARF and p16/INK4A proteins. p16/INK4a plays an important role in the regulation of the G1/S cell cycle checkpoint; it inhibits the activity of Cyclin-Dependent Kinases (CDKs) 4/6 preventing the phosphorylation of RB (Retinoblastoma protein) and thus G1/S cell cycle progression [51] (Fig. 17.4). Low expression of p16/INK4a significantly correlated with chemotherapy resistance and worse survival of MPM patients [92], suggesting that MPM patients carrying p16 deletion may benefit from CDK inhibitor-based therapy. CDK4/6 inhibitors

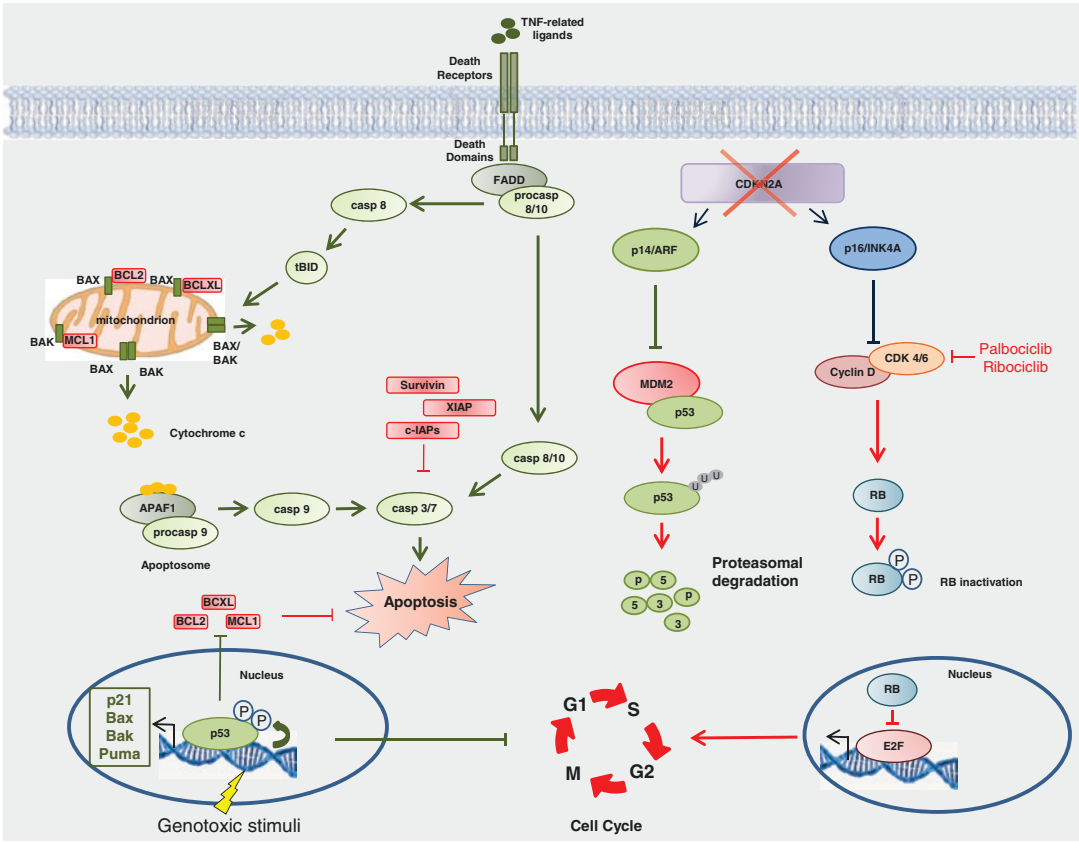

Fig. 17.4 *Apoptotic pathways are represented on the left.* TNF (Tumor Necrosis Factor)-related ligands trigger extrinsic apoptosis. Genotoxic agents induce mitochondrial intrinsic apoptosis through p53 phosphorylation and activation. Proapoptotic proteins are represented in green. Antiapoptotic proteins are represented in red. *The role of p14ARF and p16INK4a proteins in the cell cycle regulation is represented on the right.* p14ARF inhibits MDM2 and subsequently induces p53 accumulation and activation. p16INK4a inactivates cyclinD/CDK4/6 complex preventing the phosphorylation and inactivation of Rb, thus inducing G1/S cell cycle arrest. Palbociclib and ribociclib inhibit CDK4/6. *FADD* Fas-associated protein death domain, *procasp/casp* procaspase/caspase, *BCL2* B-cell lymphoma 2, *BCLXL* B-cell lymphoma extra-large, *MCL1* myeloid cell leukemia 1, *BAX* BCL2-associated X protein, *BAK* BCL2 Antagonist Killer, *APAF1* apoptotic protease activating factor-1, *c-IAP* cellular inhibitor of apoptosis, *XIAP* X-linked inhibitor of apoptosis, *MDM2* mouse double minute 2, *CDK4/6* cyclin-dependent kinase 4/6, *RB* retinoblastoma protein

(such as palbociclib and ribociclib) are under investigation in several tumors. These drugs mimic p16 activity preventing RB phosphorylation [93]. Palbociclib (PD-033299; Pfizer) is an oral available, potent CDK4/6 inhibitor characterized by a mild toxicity profile. It was approved by FDA for the treatment of estrogen-positive metastatic breast cancer. Of note, palbociclib showed efficacy in MPM cell lines when associated with PI3K inhibitors [94], but clinical trials aimed at testing its efficacy in MPM patients has not been performed yet.

The efficacy of ribociclib (LEE01; Novartis) is under evaluation in solid tumor, including MPM. Phase II open-label, nonrandomized clinical trial including patients characterized by aberrant expression of CDK4/6, cyclin D1/3, or p16 is ongoing (www.clinicaltrials.gov; NCT02187783) (Table 17.1).

p14/ARF controls both cell cycle progression and apoptosis activation inhibiting MDM2 (Mouse Double Minute 2), the E3 ubiquitin ligase responsible for p53 degradation (Fig. 17.4). In p53 wild-type tumors, p14/ARF activity can be bypassed using small-molecule p53 activators such as Nutlin 3a, an inhibitor of MDM2-p53 interaction [95]. Nutlin 3a showed greater activity in those tumor characterized by over-activation of MDM2 [96]. This is of particular interest in MPM because MDM2 overexpression was reported in tumor samples, especially in sarcomatoid and biphasic subtypes [97]. In MPM preclinical studies, Nutlin 3a caused p53-dependent G1/S cell cycle arrest inducing p21 increase [98] also in ZL34 and MSTO-211H cell lines not expressing p14/ARF [92, 99]. Moreover, p53 activation was able to decrease the antiapoptotic protein survivin. However, in the absence of strong apoptotic stimuli, Nutlin 3a did not induce MPM cell death but strongly synergized with rhTRAIL-dependent apoptosis [98]. Clinical trial aimed at testing the activity of RG7112 (Roche), a Nutlin 3a analog optimized for clinical use, showed promising activity in leukemias [100] but modest responses and high toxicity in solid tumors [101]. A more potent nutlin analog, RG7388 (Roche) (idasanutlin) [102], is in phase III trial in relapsed/refractory AML (Acute Myeloblastic Leukemia) (www.clinicaltrials.gov; NCT02545283) encouraging future assessment in MPM both as single agent and combined therapy.

17.2 Conclusions

Although clinical evaluation of targeted therapies in MPM found a strong rationale in several molecular alterations characterizing this neoplasia, clinical trials aimed at evaluating the efficacy of biologic agents targeting key oncogenic pathways did not achieve the expected results [28]. A possible explanation of this failure may lie in the lack of driver mutations, which instead characterize other types of cancers. Indeed, while TKIs are ineffective in MPM, EGFR-mutated NSCLC is particularly suited to anti-EGFR therapies, so that these treatments entered in clinical practice. Loss of tumor suppressor genes results in the simultaneous dysregulation of different downstream pathways. For example, loss of *NF2*/merlin triggers cell proliferation through Hippo, PI3K-ATK-mTOR, and FAK pathways. In this context, targeting a single transduction pathway has shown to be ineffective to abrogate the proliferative pressure of cancer cells. These negative results highlight the need to better understand MPM biology. A comprehensive evaluation of cellular features, their interconnections, and their relationships with tumor microenvironment may help to develop novel therapeutic approaches aimed at targeting multiple key signals simultaneously.

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