

REVIEW

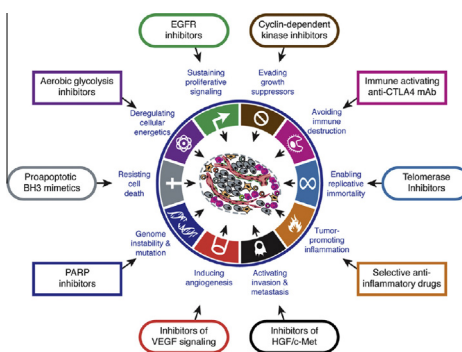
Mesothelioma treatment: Are we on target? A review



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GRAPHICAL ABSTRACT



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ABSTRACT

Targeted treatment is a therapy directed at a specific molecular target close to a hallmark of cancer. The target should be measurable with a biomarker and measurement of the target should correlate with clinical outcome when targeted treatment is administered. Current clinical guidelines do not recommend targeted or biological therapy in MPM. However, since these recommendations came out, new agents have been investigated in MPM. This review updates the use of targeted and biological treatment in patients with mesothelioma.

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Introduction

Malignant pleural mesothelioma (MPM) is a rare and aggressive neoplasm deriving from the pleural blades. More than 80% of cases are related to previous professional asbestos exposure and its worldwide incidence is expected to further increase [1]. Although the epidemic of asbestos-related disease is plateauing in most of the industrialised world, little is known about the epidemic in developing countries, where professional and environmental exposure is increasing [2]. With a natural history of 7–9 months if untreated and less than 5 per cent 5-year survivors, there is room for therapeutic improvement [2]. Disease extent and performance status at diagnosis are the clinical prognostic factors, besides epithelioid histological subtype that confers a better outcome than the less common sarcomatoid one.

The European Respiratory Society (ERS), the European Society of Thoracic Surgery (ESTS) and the European Society of Medical Oncology (ESMO) have issued recommendations regarding the management of MPM [3,4]. The only treatment with level one evidence of improvement in outcome is the administration of palliative chemotherapy consisting of 4–6 cycles of a platinum doublet with an antifolate, either pemetrexed or raltitrexed [5,6]. With this combination, good performance patients have a median overall survival (OS) of approximately 1 year and a median progression free survival (PFS) of less than 6 months. There is no standard second line treatment and targeted or biological treatment has no indication in these guidelines. This review updates the use of targeted and biological treatment in patients with advanced MPM.

Hallmarks of cancer and targeted treatment

Development of human cancers is a complex and multistep process. Organising the factors involved in the arise and growth of cancer is an important part of developing new treatment modalities. Hallmarks of cancer include biological capabilities and modulating factors to create an environment in which cancer cells can thrive [7] (Table 1). Eight biological capabilities allow cancer cells to survive, proliferate and

Hallmarks of cancer and targeted treatment

Table 1 Hallmarks of cancer and targeted treatment in MPM.

Hallmark of cancer	Mechanism of counter action	Target	Drug	Design of trial	PFS (mo)	MST (mo)	OS (mo)	Target selection	References
Sustaining proliferative signalling	EGFR inhibitors	EGFR	Gefitinib	Single arm phase II first line				Y	[12]
			Erlotinib	Single arm phase II first line	2		10	Y	[13]
	MAb against EGFR	EGFR	Cetuximab	Single arm phase II first line + platinum/pemetrexed				Y	[16]
			Imatinib	Single arm phase I first line + platinum/pemetrexed				N	[18]
	MAb against PDGFR	PDGFR	Dasatinib	Single arm phase II first line + gemcitabine				N	[19]
			Cixutumumab	Single arm phase II in pretreated patients				N	[20]
	MAb against IGFR	IGFR		Single arm phase II in pretreated patients				N	[22]
			RTK	Multiple growth factors	Sorafenib	Single arm phase II in pretreated patients	3.6		9.7
			Single arm phase II in pretreated patients		5.1			N	[24]
		Sunitinib	Single arm phase II in pretreated patients		3.5		6.1	N	[25]
Evading growth suppressors	Cyclin-dependent kinase inhibitors	RB1, TP53							
Avoiding immune destruction	Immune activating anti-CTL4 mAb	CTL4	Tremelimumab	Single arm phase II	6.2	10.7		N	[29]
				Phase II randomised				N	[30]
			Pembrolizumab Nivolumab					Y	[31]
Enabling replicative immortality	Telomerase inhibitors								
Tumour promoting inflammation	Selective anti-inflammatory drugs								
Activating invasion & metastasis	TKI c-MET	Mesothelin	Tivantinib	Phases I and II + cisplatin/pemetrexed				N	[34]
				Phase II single agent in pretreated patients				Y	[35]
	Inhibitors of HGF/c-MET	Mesothelin	Amatuximab	Single arm phase II first line + cisplatin/pemetrexed	6.1	14.8		N	[37]
			SS1P	Single arm phase II first line + cisplatin/pemetrexed				N	[38]
Inducing angiogenesis	Inhibitors of VEGF signalling	VEGFR	Cediranib	Single arm phase II first line + cisplatin/pemetrexed				N	[47]
				Single arm phase II second line	2.6		9.5	N	[85]
				Single arm phase II second line	1.8		4.4	N	[86]

Table 1 (continued)

Hallmark of cancer	Mechanism of counter action	Target	Drug	Design of trial	PFS (mo)	MST (mo)	OS (mo)	Target selection	Reference	
Genome instability and mutation	Anti-VEGF targeting ligand	VEGF ligand	Thalidomide	Phase III randomised maintenance thalidomide versus placebo				N	[41]	
			Bevacizumab	Single arm phase II first line + cisplatin/pemetrexed				N	[42]	
				Single arm phase II first line + carboplatin/pemetrexed				N	[43]	
				Phase II randomised first line + cisplatin/gemcitabine				N	[44]	
				Phase III randomised first line cisplatin/pemetrexed				N	[45]	
	Vascular disrupting agents	TNF α	NRG-hTNF		Single arm phase II in pretreated patients	4.7			N	[52]
					Randomised phase II second line versus placebo				N	[53]
					Maintenance after first line platinum/pemetrexed				N	[54]
					Single arm phase II second line	1.5	8.2			55
					Translational: prevalence of somatic and germline mutations					Y
Resisting cell death	Proapoptotic BH3 mimetics	NF- κ B		Phase II randomised first line + cisplatin/pemetrexed				N	[66]	
				Phase II randomised maintenance versus placebo				Y	[68]	
				Single arm phase II in pretreated patients				N	[62]	
				Phase III randomised second line versus placebo	1.5	7		N	[70]	
				Phase II single arm second line	2.1	5.8		N	[73]	
Deregulating cellular energetics	Aerobic glycolysis inhibitors	Arginine	ADI-PEG20	Phase II randomised						
						Phase II single arm first line + cisplatin				N
								Y	[78]	

Abbreviations: PFS: progression free survival; mo: months; MST: mean survival time; Y: yes; N: no; EGFR: epidermal growth factor receptor; Mab: monoclonal antibody; PDGFR: platelet derived growth factor receptor; IGFR: insulin-like growth factor receptor.

RTK: receptor tyrosine kinase; RB1: retinoblastoma 1; CTL4: cytotoxic T-cell lymphocytes 4; PDL1: programmed death ligand 1; PD1: programmed death 1; HGF: hepatocyte growth factor; TKI: tyrosine kinase inhibitor; VEGFR: vascular endothelial growth factor receptor.

TNF α : tumour necrosis factor α ; NRG-hTNF α : asparagine-glycine-arginine-human tumour necrosis factor α ; BAP1: BRCA1 associated protein 1; CDKN2A: cyclin-dependent kinase inhibitor 2A;

HSP90: heat shock protein 90; NF2: neurofibromatosis 2; HDAC: histone deacetylase;

NF- κ B: nuclear factor- κ B; ADI-PEG20: arginine-lowering agent pegylated arginine deiminase.

disseminate. This is possible by two modulating characteristics, genomic instability of cancer cells and the inflammatory state of malignant lesions which is driven by the immune system. Those ten hallmarks of cancer can be influenced and are subject of investigation with therapeutic purpose. In those hallmarks, pathways to modulate cancer cells can be activated or inhibited. Targeted treatment is a treatment with a specific molecular target close to those biologically important pathways [8]. New targeted drugs are currently under investigation. Their target should be measurable with a biomarker and measurement of the target should correlate with clinical outcome when targeted treatment is administered. The aim is to obtain an improved efficacy – toxicity window with a minimum of adverse effects.

This review describes the therapeutic advances made with targeted and biological agents in MPM according to the predominant hallmark which is targeted.

Sustaining proliferative signalling

An important hallmark of cancer is the ability of tumour cells to sustain proliferative signalling [7]. Cancer cells deregulate the normal production and release of endogenous growth factors to increase their cell growth. Drugs have been developed to influence those growth factors. One of the most studied growth factors is the epidermal growth factor receptor (EGFR) [9]. EGFR plays a role in cell proliferation, differentiation, migration, adhesion and survival. The EGFR-protein consists of an extracellular domain and a tyrosine kinase residue which translates the signal to downstream intracellular docking and signalling proteins. This kinase might be carrying an activating driver somatic mutation which makes the tumour addicted to growth. These activating EGFR-mutations are however, rare in MPM [10], whereas EGFR is overexpressed at protein level in more than 50–95% of the patients [11]. This overexpression has been associated with a better prognosis, probably because overexpression is more common in the epithelioid histological type compared with the sarcomatoid type. Anti-EGFR monoclonal antibodies (mAbs), target the extracellular domain of EGFR and compete with the ligand for binding. Drugs targeting the intracellular tyrosine kinase residue are small molecule tyrosine kinase inhibitors (TKI) such as gefitinib and erlotinib. Both TKI achieved disappointing results when administered as single agents in the treatment of pretreated patients [12,13]. Analysis of the target biomarker showed no difference in overall survival or response rate between high or low protein expressions. The most likely explanation for the low efficacy of EGFR TKI is the low prevalence of activating mutations. Small molecules EGFR-TKIs have hence no place in the treatment of MPM.

In first line treatment, monoclonal antibodies are typically given in combination with a standard chemotherapy backbone, typically platinum–pemetrexed. Cetuximab is a chimeric mouse–human antibody targeting the extracellular domain of EGFR. In patients with advanced non-small cell lung cancer (NSCLC) addition of cetuximab to chemotherapy significantly improved overall survival compared to chemotherapy alone [14]. Patients benefiting most from cetuximab were those with a H-score at EGFR-immunohistochemistry (IHC) of more than 200 [15]. The phase II Mesomab-trial evaluates the

activity of cetuximab in combination with 4–6 cycles of chemotherapy with maintenance thereafter until progression [16]. Patient selection is based on EGFR protein overexpression by IHC. The results of this trial are awaited.

Platelet derived growth factor (PDGF) is a growth factor inducing mesothelial cell proliferation through a similar transmembrane receptor, the platelet derived growth factor receptor (PDGFR). A high serum PDGF in patients with MPM is an independent factor of poor prognosis [17]. There seems to be no association between IHC of PDGFR and histological subtype. Imatinib is a TKI inhibiting among others, the intracellular part of PDGFR. Neither as monotherapy nor in combination with chemotherapy, a substantial activity was however found [18,19].

Dasatinib, another TKI targeting PDGFR is also not active as single agent and is associated with increased pulmonary toxicity [20].

The ligand insulin-like growth factor (IGF) helps tumour cells to grow and divide. Insulin-like growth factor receptor (IGFR) is also expressed in MPM. The antitumoural effect of cixutumumab, a monoclonal antibody against IGFR and including inhibition of the IGFR downstream signalling, is highly correlated with the number of IGFR binding sites per cell [21]. Cixutumumab is currently tested as single agent in a phase II trial in pretreated patients with mesothelioma [22]. IGFR expression will be correlated to response.

Several other growth factor receptors are involved in intracellular signal transduction, among these vascular endothelial growth factor receptor (VEGFR) and fibroblast growth factor receptor (FGFR). Serial or parallel activation of these receptors at the protein or gene level may be a possible mechanism of resistance to EGFR inhibition and a rationale for using multireceptor tyrosine kinases (RTK) as sorafenib and sunitinib. Clinical trials with these agents in pretreated but further target-unselected patients showed however a limited activity [23–25].

Evading growth suppressors

Inactivation of the tumour suppressor genes the retinoblastoma-associated (RB1) and TP53 proteins which have an effect on cell growth and proliferation, has not yet been described in MPM [26]. The cyclin-dependent kinase inhibitors that typically act on both tumour suppressor genes have not yet been tested in MPM.

Avoiding immune destruction

Tumours have evolved multiple mechanisms to evade immune destruction [7]. Both the innate and the adaptive immune systems are able to eradicate tumour. Deficiencies in cytotoxic T lymphocytes (CTLs) and natural killer (NK) cells lead to an increased tumour incidence. With a high infiltration of tumour infiltrating lymphocytes and macrophages and a T-cell inflammatory expression pattern, MPM can be considered as an ‘inflammatory’ tumour. One of the escape mechanisms to evade immune destruction is expression of T cell inhibitory ligands such as cytotoxic T-lymphocyte antigen 4 (CTLA4), the programmed death 1 ligand (PD-L1) and the programmed death 1 receptor (PD-1) [27]. PD-L1 expression in

mesothelioma was correlated with a greater extent of disease at presentation and with the sarcomatoid histological type. This possibly explains the observed associated poor survival [28].

Immune checkpoint inhibitors block these T cell inhibitory mechanisms and allow T cells to resume their cytotoxic activity on cancer cells. Examples are the monoclonal antibodies against the CTLA4 receptor, ipilimumab and tremelimumab, against the PD-L1 and PD-1 receptor pembrolizumab and nivolumab, respectively.

Tremelimumab was investigated in pretreated patients in a phase II single-arm study whereby the primary endpoint of objective response rate was not met [29]. Disease control was noted in 31% of the patients. The median progression free survival (PFS) was 6.2 months (95% CI 1.3–11.1) and the mean survival time (MST) was 10.7 months (0.0–21.9). In an ongoing randomised phase II trial pretreated patients are allocated to either single agent tremelimumab or placebo [30].

Monoclonal antibodies directed at the PD-L1 or PD1 receptors are currently being considered for phase 2 evaluation in MPM [31]. More specifically, an active treatment for the sarcomatoid subtype is unmet. This would best be studied in a randomised first line setting, with or versus a platinum pemetrexed backbone.

Enabling replicative immortality

Cancer cells require replicative potential to grow into tumours. Telomeres are protecting the end of the chromosomes to limit proliferation. Expression of telomeres is high in cancer cells. Suppression of telomerase activity leads to telomere shortening and to a proliferative barrier. Development of telomerase inhibitors is ongoing, but presently not in MPM.

Tumour promoting inflammation

Inflammation is capable of enhancing tumourigenesis by supplying growth and survival factors that lead to activation of hallmarks of cancer. Drugs with selectively anti-inflammatory capabilities might have an effect in cancer treatment. There are currently no ongoing trials in MPM targeting this hallmark.

Activating invasion and metastasis

Activating invasion and metastasis is a mechanism that was researched for over decades and is evolving very quickly. It is a process of local invasion, followed by intravasation by cancer cells to nearby blood and lymphatic vessels. Finally, extravasation leads to dissemination with growth of macroscopic tumours and metastases. Each of those steps can be influenced and act as a target for novel therapies.

MET is a receptor tyrosine kinase which is activated by binding its ligand hepatocyte growth factor (HGF) [32]. The c-MET gene is located on chromosome 7q31 but mutations in MET are rare in MPM [33]. The c-Met/HGF axis is involved in cell growth, cell survival, angiogenesis, cell motility, migration and invasion and metastasis. Expression of MET in tumour samples of MPM was increased (82%) compared to normal tissue and is associated with a poor survival

[33]. Serum circulating HGF was twice as high in mesothelioma patients compared to healthy controls. HGF expression seems correlated with the epithelial histological type. Tivantinib is a small molecule TKI that selectively blocks the MET kinase activity. In a phase I-Ib trial, tivantinib is currently evaluated in first line with carboplatin/pemetrexed chemotherapy [34]. In the phase II part, dynamic changes in blood levels of HGF, vascular endothelial growth factor (VEGF), and soluble c-Met will be evaluated.

Another phase II trial of single agent tivantinib in pretreated patients is conducted [35]. The translational part of the study includes changes in baseline levels of serum HGF and expression of MET between responders and non-responders. Also the presence of the MET gene amplification is tested, but is not a selection criterium for inclusion. Those biomarkers will be correlated to change in tumour size and PFS.

Mesothelin is a differentiation and cell adhesion antigen, whose expression is limited to the mesothelial cells lining the pleura, pericardium and peritoneum. Its biomarker correlate is serum mesothelin, a Food and Drug Administration (FDA) approved biomarker of response in MPM treatment [36].

Amatuximab is a chimeric monoclonal antibody against mesothelin. A single arm phase II study of amatuximab plus cisplatin and pemetrexed was initiated in first line setting in patients with unresectable MPM [37]. The median number of cycles was 5 (range 1–6) and 56 patients received single agent amatuximab. PFS at 6 months was 52% (95 CI: 39.5–63.5) with median of 6.1 months (5.4–6.5). OS was 14.8 months. A partial response was seen in 39% and a stable disease 51%.

SS1P is a recombinant anti-mesothelin immunotoxin consisting of a murine antimesothelin variable antibody fragment. Preclinical studies showed marked synergy between SS1P and chemotherapy, thus initiating the phase II trial adding SS1P to first line cisplatin/pemetrexed [38]. In this phase II study, after the first cycle, all but 2 of the 21 patients (90%) developed SS1P-neutralising antibodies. SS1P Cmax values of > 150 ng/mL were achieved during cycle 2 only by the 2 patients who did not develop SS1P-neutralising antibodies. To overcome this problem of neutralising antibodies, patients were pre-treated with pentostatin and cyclophosphamide that can specifically deplete T and B cells and can delay antibody formation. This treatment combination is allowing patients to receive more cycles of SS1P [39]. Of 20 evaluable patients, 12 (60%) had a partial response (PR), 3 had stable disease (SD), and 5 had progressive disease (PD). Of 13 patients who received the median toxic dose (MTD), 10 (77%) had a PR, 1 had SD, and 2 had PD. Objective radiologic responses were associated with significant decreases in serum mesothelin ($P = .0030$), megakaryocyte potentiating factor ($P = .0005$), and cancer antigen 125 ($P < 0.0001$). Grade 3 fatigue was dose-limiting in 1 patient at 55 mcg/kg. The MTD of SS1P was established as 45 mcg/kg. Other grade 3 toxicities associated with SS1P included hypoalbuminemia (21%), back pain (13%), and hypotension (8%). The authors conclude that SS1P given with pemetrexed and cisplatin is safe and well tolerated and exhibits significant antitumour activity in patients with unresectable, advanced pleural mesothelioma. The targets serum mesothelin, megakaryocyte potentiating factor, and cancer antigen 125 levels correlated with objective tumour responses.

Inducing angiogenesis

An important hallmark of cancer is induction of (neo-)angiogenesis [7]. To survive, the tumour needs a continuous supply of nutrition and oxygen. Cancer cells do so by stimulating the vascular endothelial growth factor receptor (VEGFR) on the cell surface with the ligand VEGF. A high level of VEGF is positively correlated with the microvascular density (MVD) and associated with a poor prognosis in MPM patients [40]. Both VEGF and VEGFR are highly expressed in patients with MPM and are used as target to block angiogenesis in tumours. The following classes of anti-angiogenic agents have been evaluated in MPM:

Monoclonal antibodies

Drugs targeting the ligand VEGF are thalidomide and bevacizumab. Thalidomide is an old drug with presumed anti-angiogenic properties, besides several other mechanisms of antitumoural action. The phase III randomised NVALT5/MATES (Maintenance Thalidomide in Mesothelioma Patients) study, investigated the role of maintenance thalidomide [41]. Two-hundred-and-twenty-two patients showing disease stabilisation or response after first line pemetrexed chemotherapy with or without platinum were randomised between oral thalidomide and observation. Primary endpoint was a 50% increase in time to progression, but the hazard ratio observed was 1.0 ($p = 0.71$) for time to progression and 1.2 ($p = 0.30$) for OS. The authors conclude that thalidomide for switch maintenance treatment is not effective.

The administration of bevacizumab – a monoclonal antibody against VEGF – has been evaluated in 2 phase II studies with either cisplatin or carboplatin in combination with pemetrexed and was found feasible with acceptable toxicity [42,43]. A randomised phase II trial in which bevacizumab was added to a doublet of cisplatin/gemcitabine failed to demonstrate a survival benefit [44]. In a subgroup analysis, patients with low baseline VEGF levels showed a benefit with bevacizumab. In the ongoing phase III Mesothelioma Avastin Plus Pemetrexed–cisplatin (MAPS) Study, chemotherapy-naïve patients with unresectable MPM are being treated with cisplatin/pemetrexed with or without bevacizumab [45]. Non-progressive patients in the bevacizumab arm receive bevacizumab till progression. The primary endpoint is overall survival (OS) and the secondary endpoint is progression-free survival (PFS). Patients' selection is however, not done on baseline levels of circulating VEGF or other biomarker of angiogenesis. Whilst accrual has been closed, the results are currently awaited.

Small molecules

Small molecules that inhibit the VEGF tyrosine kinase receptor more or less specifically are, vatalanib, cediranib, dovitinib, pazopanib, nintedanib and axitinib. Single agent vatalanib did not show a substantial activity in a phase II study in 47 untreated patients [46]. There was no correlation between serum levels of VEGF, PDGF, TSP-1, or mesothelin and treatment response, PFS, or survival.

Cediranib, an oral pan-inhibitor of VEGFR, c-kit and PDGFR in combination with cisplatin/pemetrexed in first

line is currently being evaluated in phase I and PFS in phase II [47].

Pazopanib was tested in a phase II study as single agent in chemo-naïf and pretreated patients. The most frequent drug-related toxicities were hypertension, proteinuria, liver enzyme elevations, myelosuppression, and fatigue, all of which were mostly grades 1–2 [48]. The primary endpoint PFS rate at six months was 47%.

Nintedanib is a small molecule inhibiting VEGFRs 1 and 2, FGFR and PDGFR. The safety and efficacy of nintedanib is currently evaluated in an exploratory randomised placebo controlled phase II study in combination with cisplatin/pemetrexed in patients with untreated MPM. Primary endpoint is PFS [49].

Axitinib is a small molecule inhibiting VEGFR, c-kit and PDGFR. A randomised phase II study of axitinib in combination with cisplatin/pemetrexed showed more grade 3/4 toxicity (neutropenia) in the axitinib group versus the chemotherapy only group [50]. The partial response was 35% in the experimental arm versus 27% in the control arm. The median PFS was 8 versus 8.3 months. Although axitinib was well tolerated in combination with cisplatin and pemetrexed, there was a lack of benefit in response rate, progression free or overall survival.

Dovitinib is a dual inhibitor of VEGF and FGF receptors and is currently tested as single agent in a phase II trial in pretreated patients [51].

Vascular disrupting agents

The primary role of tumour necrosis factor (TNF) is in the regulation of immune cells. TNF is able to induce fever, apoptotic cell death, cachexia, inflammation and to inhibit tumorigenesis. Dysregulation of TNF production has been implicated in cancer.

Asparagine–glycine–arginine–human tumour necrosis factor α (NGR-hTNF) exploits the tumour-homing peptide asparagine–glycine–arginine (NGR) for selectively targeting TNF to an aminopeptidase N/CD13 isoform overexpressed by endothelial cells in solid tumours. NGR-hTNF has been tested as second line treatment. A single agent phase II trial in 57 pemetrexed-pretreated MPM patients showed a disease control rate of 46% (95% CI: 32–59), 1 partial response, 25 stable diseases [52]. This was maintained for a median time of 4.7 months with overall survival rates at 1, 2 and 3 years of 47%, 16% and 8%, respectively. Based on these promising results, a phase III NRG-015 study was initiated randomly allocating pemetrexed-pretreated patients to investigators' choice second line chemotherapy – vinorelbine or doxorubicin associated with either weekly NGR-hTNF or placebo [53]. The trial has recently completed its accrual and results are awaited.

NGR-hTNF is presently also studied in a multicentre, double-blind, 2-arm, randomised phase 2 trial with either maintenance NGR-hTNF or placebo in patients not progressing after 6 cycles of a front-line, pemetrexed based regimen [54]. The study drug is given intravenously as 1 h infusion at 0.8 $\mu\text{g}/\text{m}^2$ weekly, is started within 3–7 weeks from the last chemotherapy cycle, until PD. Primary endpoint is PFS.

BNC105P is a tubulin polymerisation inhibitor that selectively disrupts tumour vasculature and suppresses cancer cell proliferation. In a phase II study in patients with progressive

MPM after first line chemotherapy BNC105P was given intravenously until progression or toxicity [55]. The primary endpoint was objective response rate. Although the drug was safe and tolerable, the median PFS and OS were only 1.5 months (95% CI: 1.4–2.4) and 8.2 months (95% CI: 3.8–11.9) respectively. These results did not warrant further research as a single agent.

Genome instability, mutations and epigenetic dysregulation

The recent discovery of activating driver genomic alterations has resulted in a significant breakthrough in solid cancer therapy and has changed the treatment paradigm in subsets of patients with advanced cancers, among them, gastrointestinal stromal tumour (GIST), melanoma, and non-small cell lung cancer. Recent advances in massively parallel sequencing (MPS) of tumour samples have been used to understand the genomic profile of mesothelioma. Molecular genetic analysis has revealed genetic alterations, which are considered to be associated and possibly driving the development and progression of MPM [56]. The most frequently mutated genes are the cyclin-dependent kinase inhibitor 2A/alternative reading frame (*CDKN2A/ARF*) on 9p21, neurofibromatosis type 2 (*NF2*) on 22q12 and BRCA1-associated protein 1 (*BAP1*) on 3p21 [57]. A retrospective next-generation sequencing (NGS)-analysis of MPM tissue samples showed that the most frequent altered genes are adenomatous polyposis coli (*APC*), *BAP1*, colony stimulating factor 1 receptor (*CSF1R*), fms-related tyrosine kinase 3 (*FLT3*), *NF2*, kinase insert domain receptor (*KDR*), phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha (*PIK3CA*) and *p53*. These genomic data provide a challenge to develop novel therapeutic targets in MPM [58].

BAP1-expression is required for vinorelbine activity and its expression is lost in approximately 20% in patients with MPM [59]. This hypothesis led to a study to evaluate the efficacy of second-line vinorelbine plus active symptom control (ASC), versus ASC [60]. A study to determine the prevalence of somatic and germline mutations in the *BAP1* opened recently and is recruiting patients [61].

Loss of function of phosphatase and tensin homologue (*PTEN*), a putative protein tyrosine phosphatase gene, amplifies PI3K signalling and thus promotes tumourigenesis. Activation of mTOR kinase inhibits the PI3K pathway via negative feedback. Everolimus and sirolimus are PI3K/AKT/mTOR pathway inhibitors. Phase II trials with everolimus and sirolimus have given disappointing results in the treatment of MPM [62].

Combining PI3K/AKT/mTOR pathway inhibitors with other inhibitors might improve efficacy, e.g. by the simultaneous inhibition of both mTOR and MEK with everolimus and selumetinib, currently in evaluation [63,64].

Heat shock protein 90 (HSP90) is a chaperone protein that assists other proteins to fold properly, it stabilises proteins against heat stress, and it aids in protein degradation. It also stabilises a number of proteins required for tumour growth [65]. In the ongoing MESO 2 phase II study, untreated patients are randomised between cisplatin/pemetrexed with or without the oral HSP90 inhibitor, ganetespib [66]. Patients in the ganetespib group will receive this drug in maintenance until progression.

The *NF2* tumour suppression gene encodes the protein merlin. Inactivation of somatic *NF2* occurs in around 40 per cent

of the patients with mesothelioma, leading to inactive merlin [67]. Merlin has demonstrated a role in cell adhesion, invasion and cell motility in tumour cell lines. Cells lacking expression of *NF2* (merlin) tumour suppression gene products are especially susceptible to focal adhesion kinase (FAK)-inhibition. FAK may represent an important therapeutic target for MPM, mostly in its stem cells. A new class of promising drugs are the oral FAK – inhibitors, such as defactinib. In the ongoing randomised phase II COMMAND study, patients with a partial response or stable disease after four to six cycles of first line platinum–pemetrexed chemotherapy are randomly allocated to a maintenance treatment with either defactinib or placebo and this until disease progression [68].

Epigenetic regulation of tumour suppressor genes through chromatin condensation and decondensation has emerged as an important mechanism that leads to tumourigenesis [56]. Histone deacetylase (HDAC) inhibitors target epigenetic changes, among other mechanisms of action [69].

Vorinostat is an oral inhibitor of HDAC approved for the treatment of cutaneous T-cell lymphoma. Initial studies of vorinostat demonstrated objective responses in patients with MPM. However, a recently reported phase III, randomised, double-blind, placebo-controlled VANTAGE 014 trial was negative [70]. Patients with advanced MPM who failed prior pemetrexed and either cisplatin or carboplatin therapy were randomly allocated to receive vorinostat or placebo twice per day for 3 of 7 days in a 3-week cycle. The MST in the intention-to-treat population was 31 weeks in the vorinostat arm compared to 27 weeks in the placebo arm, hazard ratio of 0.98 (95% CI 0.83–1.17). The median PFS time was disappointingly not different with 6.3 weeks in the vorinostat arm and 6.1 weeks in the placebo arm (HR = 0.75, 95% CI: 0.63–0.88). The development of vorinostat in MPM has been halted.

Resisting cell death

Programmed cell death by apoptosis is a natural mechanism in cancer [7]. The trigger to apoptosis is DNA-damage. Necrosis releases pro-inflammatory signals that recruit inflammatory cells of the immune system to remove necrotic debris. However, this inflammation can promote tumour by inducing angiogenesis and cancer cell proliferation. Nuclear Factor- κ B (NF- κ B) is activated by asbestos fibres; this causes activation of numerous NF- κ B dependent genes, including *c-myc* [71]. NF- κ B is upregulated in mesothelioma cells and plays an important role in survival of these cells. Downregulation of NF- κ B and thus increasing apoptosis is a target for drugs.

Bortezomib is a small molecule proteasome inhibitor that is blocking NF- κ B and up-regulates pro-apoptotic BH3 proteins [72]. Pre-treatment of mesothelioma cells with bortezomib shows synergistic effect in combination with cisplatin. Bortezomib was evaluated as a single agent in 23 pre-treated patients but showed limited activity in this setting [73]. Partial response was confirmed in one patient who received four cycles of bortezomib and one patient had stable disease. However, progression occurred in the majority of patients within the first two cycles with a median PFS and OS of 2.1 and 5.8 months, respectively. The EORTC Lung Cancer Group (LCG) conducted a single-arm phase II study with bortezomib and cisplatin in chemotherapy-naïve patients [74]. Primary endpoint was PFS rate at 18 weeks. Endpoint

validation showed that patients without progression at 18 weeks had a median OS of 16.9 months compared to 11.9 months in those who progressed by 18 weeks. Toxicity was comparable to other regimens. Although promising, the results of the trial did not meet the predefined rate of activity at 18 weeks which would justify a phase III trial. We can conclude that bortezomib exhibits insufficient activity to warrant further investigation in unselected patients with mesothelioma.

Deregulating cellular metabolism

As both antifolates raltitrexed and pemetrexed, the standard chemotherapeutic drugs used in the first line treatment of mesothelioma, specifically block folate-dependent enzymes in the purine and pyrimidine biosynthesis, these drugs can be considered targeted agents 'avant la lettre' [5,6]. Measurement of the expression of thymidylate synthase has been proposed as a predictive biomarker for their use [75].

Among the different biochemical and metabolic pathway, which can be targeted, we highlight a recent promising target with its biomarker.

L-arginine deprivation is a novel metabolic anticancer strategy being tested in several cancers on the basis of L-asparaginase, which is an amino acid-degrading enzyme used in the management of acute lymphoblastic leukaemia [76]. Arginine is a semi-essential amino acid in humans. Normal argininosuccinate synthetase 1 (ASS1) expressive cells are capable of forming arginine. Loss of expression of ASS1 in MPM is associated with the loss of intrinsic arginine production. Extracellular arginine deprivation can lead to apoptosis in MPM-cells. The ASS1 loss is tumour-type dependent, and in mesothelioma, is due to promoter methylation. The expression of ASS1 is low in 63% of MPM [77]. The phase II Arginine Deiminase and Mesothelioma (ADAM) randomised patients with ASS1-deficient advanced MPM between best supportive care (BSC) with or without the arginine-lowering agent pegylated arginine deiminase (ADI-PEG20) intramuscular injection 320 UI/m²/week [78]. The primary endpoint was PFS. Almost doubling of the PFS was observed, with a median PFS of 98 days for the experimental arm and 59 days for the control arm with a PFS HR 0.53 (95% CI 0.31–0.90), favouring ADI-PEG20. No objective responses were recorded. The drug was generally safe and well tolerated. However, a mechanism of resistance to ADI-PEG20 was noticed with the development of antidrug neutralising antibodies that peak by day 50 with a concomitant increase in plasma L-arginine levels [79].

The phase II TRAP (tumours requiring arginine to assess ADI-PEG20 with Pemetrexed and cisplatin) trial is combining arginine deprivation therapy with chemotherapy. ASS1 negative patients will be randomised to cisplatin/pemetrexed and ADI-PEG20 or cisplatin/pemetrexed alone [78].

Discussion and future directions

Many hallmarks are still to be explored, as enabling replicative immortality and tumour promoting inflammation and corresponding targeted new drugs to be developed. Podoplanin is highly expressed in MPM. Podoplanin antibodies inhibiting platelet aggregation and hematogenous metastasis might be useful in the future [80]. Small molecule inhibitor of CARP-1, a peri-nuclear phosphoprotein that is a regulator of cancer

cell growth and apoptosis signalling, might be effective in the treatment of MPM and is in development [81]. Cilengitide, a synthetic peptide inhibitor that induces growth inhibition is currently tested in phase III in lung carcinoma. In MPM cell lines it is currently tested preclinically [82].

Combination of therapies directed at different targets, as with EGFR and MET inhibitors, has a stronger effect than targeting either factor alone [83]. Dual targeting of pathways can be more efficacious on mesothelioma proliferation and viability than inhibition of an individual pathway [84].

Conclusions

Are we on target? Not yet! We have too many targets but few validated biomarkers. A true breakthrough has still to be obtained, as the development of targeted agents in MPM suffers from the same weaknesses as in non-small cell lung cancer: poor target definition and inappropriate trial design. Most studies were conducted in a biomarker unselected patient population, either because no valid biomarker is available – as in angiogenesis – or an inappropriate expression technique for the marker was used, as with immunohistochemistry for tyrosine kinase inhibitors. The future lies in randomised phase II trials with the targeted agent added to or compared to a standard chemotherapy backbone in first line and in proof of concept trials in pretreated patients, both stratified for or restricted to patients with a valid target expression. Referral of patients in a good performance status into these trials is highly recommended.

Conflict of interest

The authors have declared no conflict of interest.

Compliance with Ethics Requirements

This article does not contain any studies with human or animal subjects.

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