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Review

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Diagnostic, prognostic, and therapeutic implications of genetic profiling in pleural mesothelioma

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Abstract

Pleural mesothelioma (PM) is an aggressive malignancy of the pleural lining that typically arises secondary to asbestos exposure. With the advent of next-generation sequencing, major progress has been made in the molecular characterization of pleural mesothelioma over the past three decades. However, these advances have been largely unable to identify effective targeted therapies for PM. Additionally, there remains an absence of accepted gold-standard consensus for staging and treatment, which partly explains the overall poor outcomes in patients with PM. In recent years, genetic profiling of PM tumors has proved to be an effective tool in the diagnosis and prognosis of PM. Genomic sequencing has identified several potential targets for the development of novel therapeutics in PM. This review summarizes the progress in diagnosis, prognosis, and therapeutics derived by genomics and tumor profiling of PM tumors.

Keywords: Pleural mesothelioma, gene-profiling, next generation sequencing, gene ratio profiling, tumor biomarkers, targeted therapies

INTRODUCTION

Pleural mesothelioma (PM) is a rare but aggressive malignancy that arises from the mesothelial cells of lung pleural lining. PM is most classically associated with prior asbestos exposure, though it has also been linked

to ionizing radiation, other non-asbestos fibers, and genetic factors[1,2]. PM affects approximately 3,200 new patients per year in the United States and all comers have a median survival of about 8 months depending on treatment, stage, and histology[2-4]. The global incidence rates have been reported to be between 1-30 cases per million persons, likely secondary to regional differences in industrialization, mining history, and asbestos use[5]. Clinical outcomes in PM are related to certain patient and tumor characteristics. Favorable patient factors for overall survival include female sex, histological subtype, early stage, smaller tumor volume, good nutritional status, and younger age at diagnosis[2,6,7]. At the molecular level, lower expression of specific genes such as BRCA1-associated protein (*BAP1)*, myosin heavy chain 10 (*MYH10)*, Ras homolog family member A (*RHOA)* were correlated with shorter overall survivals in non-epithelioid PM histology[6]. Germline mutations in BAP1 have been associated with a hereditary predisposition to the development of PM[2].

In the last decade, significant efforts have been made in the molecular characterization of PM. Next-generation sequencing technologies have been applied to improve diagnosis and identify patients who can benefit from specific treatments[3,8-11]. Herein, we describe the diagnostic, prognostic, and therapeutic implications of recent advances in PM genomics and tumor profiling.

PM ORIGIN AND CLASSIFICATION

The pathophysiological process for the development and progression of PM is thought to be due to chronic pleural inflammation (most often caused by asbestos exposure), which facilitates the malignant transformation of the pleura[10]. The asbestos fibers reach the pleural lining via lymphatics, where they remain for months or years. This leads to a chronic inflammatory state, associated with activation of the high mobility group protein B1 (HMGB1), the activating nuclear factor kappa-light-chain enhancer of activated B cells (NF-kB) and the phosphatidylinositol 3-kinase (PI3K) pathways[2]. These inflammatory pathways are thought to drive the proliferation of the pleural mesothelial cells, leading to their malignant transformation by the accumulation of genetic mutations[12].

Mesothelial tumors and proliferations are defined by their level of differentiation and invasiveness. Benign mesothelial tumors include adenomatoid and well-differentiated papillary mesothelial tumors. Recent WHO classification has included mesothelioma *in situ* within the category of benign proliferation[13]. The diagnosis of mesothelioma *in situ* requires BAP1 loss by immunohistochemistry (IHC) or homozygous cyclin-dependent kinase inhibitor 2A (CDKN2A) deletion by fluorescent *in situ* hybridization (FISH). Though progression is typically slow, it is estimated that up to 70% of mesothelioma *in situ* will progress to invasive malignant mesothelioma[13].

Malignant PM is classified into three major subtypes based on tumor histology. Epithelioid, the most common subtype (~60% of cases), contains ≥ 90% epithelial-shaped cells. Conversely, sarcomatoid, the rarest subtype (≤ 20% of cases), has ≥ 90% spindle-shaped cells arranged in haphazard patterns. Finally, the biphasic subtype consists of both epithelioid and spindle-shaped cells. Epithelioid PM has been associated with longer overall survival (12-27 months), whereas sarcomatoid with shorter overall survival(7-18 months)[10]. In 2018, the National Mesothelioma Audit showed one-year survival of 51%, 28%, and 14% that decreased to 3%, 1%, and 1% after three years for epithelioid, biphasic, and sarcomatoid subtypes, respectively[14]. Some rare histological patterns, including pleomorphic and transitional PM, had previously been grouped by pathologists as epithelioid. However, based on their structural characteristics and transcriptomic profiles, transitional PMs have recently been reclassified as non-epitheliod[13,15].

PM DIAGNOSIS

Clinically, the first symptom of the disease presentation of PM is typically dyspnea secondary to a pleural effusion or chest wall pain[16]. Other common symptoms include dry cough, chest pain, fatigue, and weight loss[2]. Diagnostic workup often starts with drainage and cytological evaluation of the pleural fluid; however, the reported sensitivity of cytological diagnosis ranges between 30%-75%[2,17]. Usually, pathological tissue diagnosis with biopsy via video-assisted thoracoscopic surgery (VATS) or core needle is needed to confirm a diagnosis[16,18]. A single small incision in the line of a potential future thoracotomy is recommended due to the propensity of the tumor to spread into any port sites[16,19]. Tumor invasion must be assessed histologically to definitively diagnose PM. Even so, histologic diagnosis can still present challenges, with multiple patterns described within each histologic subtype[17,18]. In addition, most PM tumors have several histological patterns within a single specimen, indicating an intra-tumor spatial heterogeneity that may not always be captured in the limited volume obtained by biopsy[17]. Sarcomatoid histotype can also pose diagnostic challenges as it can be similar in appearance to normal reactive stroma. The subjective judgment of the component of spindle cells as benign/reactive *vs.* malignant can make the identification of PM tumors difficult to characterize[2]. Even a surgically-obtained PM biopsy can present diagnostic challenges and require pathologic expertise to diagnose. As such, accurate, objective, and accessible diagnostic tests are still needed for PM.

GENETIC PROFILING IN PM; DIAGNOSIS AND PROGNOSIS

Genetic alterations

The advent of next-generation sequencing (NGS) has allowed for the identification of many relevant genomic, epigenomic, and transcriptomic alterations that are associated with the development of PM[20]. Many of the chromosomal abnormalities associated with PM result in aneuploidy secondary to chromosomal loss or gain mutations. Several studies have identified frequent chromosomal gains in 1q, 5p, 7p, 8q, and 17q and frequent chromosomal losses in 1p, 3p, 4q, 6q, 9p, 13q, 14q, and 22q[1,20-22]. Though nonspecific to PM, these findings have been used to differentiate benign and malignant mesothelial proliferations[20]. Common genetic alterations associated with PM are shown in Table 1.

The loss or inactivation of tumor suppression genes, such as BAP1, neurofibromatosis type 2 (NF2), cyclin-dependent kinase inhibitor 2A (CDKN2A), cyclin-dependent kinase inhibitor 2B (CDKN2B), large tumor suppressor kinase 2 (LATS2), and tumor protein 53 (TP53) has also been implicated in the pathogenesis of PM[20,21,23]. To date, there have been no identified oncogenes in the pathogenesis of PM, making it particularly difficult to identify targets for effective therapies[21].

Diagnostic BAP1 and CDKN2A testing

The clinical tests used for the diagnosis of PM include cytological evaluation and histological classification. Though useful, these tests have variable sensitivity and cannot always confirm diagnoses. Immunohistochemistry (IHC) staining has been used to detect the presence or absence of BAP1 expression and support a diagnosis of PM. BAP1 IHC use involves binary evaluation of the presence or absence of BAP1 nuclear staining (defined as positive when tumor cell nuclei show immunoreactivity). The presence of nuclear BAP1 staining is associated with wild-type *BAP1*, whereas the complete absence of cellular staining correlates with *BAP1* biallelic loss[24-26]. Intact nuclear BAP1 staining is more common in sarcomatoid PM[27-29]. The role of cytoplasmic BAP1 in the diagnosis of PM is still controversial and incompletely understood[25,28-38].

FISH has been used clinically in PM diagnosis by detecting homozygous CDKN2A deletion. The frequency of homozygous deletion of CDKN2A in PM tumors has been shown to be 74%[39]. Illei *et al.* examined

Table 1. Common genetic alterations in PM and potential therapeutic targets

Gene name	Chromosomal region	Incidence	Potential therapeutic targets
BRCA1 Associated Protein 1 (BAP1)	3p21.1	17%-45%[3,107]	EZH2; PARP
Cyclin-dependent kinase inhibitor 2A (CDKN2A)	9p21.3	48.2%-74%[39,43,107]	MDM2; p53; CDK4/6
Methylthioadenosine phosphorylase (MTAP)	9p21.3	74%[43]	Adenylosuccinate synthetase; MAT2A; PRMT
Tumor Protein P53 (TP53)	17p13.1	7%-19%[3,22,107]	G2-checkpoint; MDM2; p53
Neurofibromin 2 (NF2)	22q12.2	16%-32.8%[3,107]	YAP-TEAD; FAK; mTOR and PI3K
The large tumor suppressor kinase 2 (LATS2)	13q12.11	1%[3]	YAP-TEAD
Lysine-specific demethylase 4A (KDM4A)	1p34.2	100%[97]	H3K9me3

EZH2: Enhancer of zeste 2 polycomb repressive complex 2 subunit; PARP: poly[ADP-ribose]polymerase inhibitor; MDM2: mouse double minute 2 homolog; CDK4: cyclin-dependent kinase 4; CDK6: cyclin-dependent kinase 6; MAT2A: methionine adenosyltransferase 2A; PRMT: protein arginine methyltransferase; YAP: yes-associated protein; TEAD: TEA domain family member; H3K9me3: histone 3 lysine 9 trimethylation).

cytologically suspicious and confirmed cases of PM and found that FISH was successful in confirming the diagnosis in 12 of 13 cases[40]. In a different study, the combined approach of BAP1 IHC and CDKN2A FISH on a series of 93 PM cases yielded a diagnostic tool with high sensitivity (84%) and specificity (100%)[41]. While highly diagnostic, FISH testing presents challenges to implementation in the clinical setting. It is less widely available, more expensive, and more technically challenging than immunohistochemistry. Also, the translational product of CDKN2A (p16) is not a reliable substitute for CDKN2A FISH[42]. However, deletion of the CDKN2A gene has been correlated with deletion of the methyltioadenosine phosphorylase (MTAP) gene located ~100 kb from the CDKN2A on chromosome 9p21. Since these genes are closely located, they are often simultaneously deleted. It has been shown that IHC staining for MTAP is a reliable surrogate for CDKN2A loss with a sensitivity of 75% and specificity of 95%[42,43].

Gene ratio test in diagnosis and prognosis

Our group has developed an algorithm to translate expression profiles into simple molecular tests with clinical relevancy[44]. Gene expression profiles from a group of tissues are compared with expression profiles of another group distinguishable for a single characteristic such as histology or outcome. Ratios of gene expression are then generated using genes with significantly different levels of expression between the two tissues. This method has been used to distinguish PM from adenocarcinoma using a 6-gene 3-ratios test[45] as well as in the differential diagnosis of PM, where a sequential combination of ratio tests was able to identify PM samples from other thoracic malignancies with high sensitivity and specificity[46]. In addition, the gene ratio test was also successfully applied to determine the histological subtype of PM samples[46].

In the last decade, molecular subtype stratification has proved to be a powerful tool for tumor classification and clinical prognostication. The gene ratio technique has been used to identify patients likely to benefit from tumor resection[44,47,48]. Sixty treatment-naïve frozen PM samples from patients that underwent extrapleural pneumonectomy (EPP) were used to develop a prognostic 4-gene, 3-ratio test (TM4SF1/PKM2, TM4SF1/ARHGDIA, and COBLL1/ARHGDIA). A combined ratio > 1 predicted a good outcome, whereas a combined ratio < 1 predicted a poor outcome [Table 2][47]. A later study showed that the prognostic gene ratio test has robust predictive value and technical assay performance[48]. In addition, a predictive model, the Mesothelioma Prognostic Test (MPT), was developed by investigating the associations of the gene ratio test and pathological predictive parameters (i.e., histology and lymph node status) with survival. The model assigned values of 0 or 1 to each of the three parameters (gene ratio test, histology, and lymph node status). Specifically, values of 0 were assigned to non-epithelioid histology, presence of cancer in a lymph node, and gene ratio test associated with poor prognosis, whereas values of 1 were assigned to epithelial histology, absence of cancer in a lymph node, and gene ratio test associated with good prognosis. The MPT score was calculated for each sample by adding the sum of the three values. Using this algorithm, patients were

Table 2. Prognostic models for PM. This table summarizes the currently available clinical- and molecular-prognostic models of PM, including study cohort and size, variables analyzed, stratification of prognostic groups, and statistical outcomes

Model	Study cohort	Variables	Prognostic groups	Outcomes
Clinical models				
CALGB, 1998[7]	Treatment naïve PM Pts (*n* = 337)	ECOG performance status Age Hemoglobin WBC count Chest pain Weight loss		**Median survival (95%CI)**
			1	13.9 mo (11.1-31.4)
			2	9.5 mo (CI 6.9-14.7)
			3	9.2 mo (CI 7.5-10.5)
			4	6.5 mo (3.7-9.4)
			5	4.4 mo (3.4-5.1)
			6	1.4 mo (0.5-3.6)
Australian model, 2016[50]	All PM patients (*n* = 482)	Weight loss Hemoglobin Serum albumin Histological diagnosis ECOG performance status		**Median survival (IQR)**
			1	34.0 mo (22.9-47.0)
			2	17.7 mo (11.6-25.9)
			3	12.0 mo (6.0-20.6)
			4	7.4 mo (3.3-11.1)
Molecular models				
Gordon *et al.*, 2003[47]	Surgical EPP patients (*n* = 46)	**Gene ratios** KIAA0977/GDIA1 L6-related EST/CTHBP L6-related EST/GDIA1		**Median survival**
			Good-outcome	36 mo
			Poor-outcome	7 mo
Gordon *et al.*, 2005[108]	Surgical EPP or P/D patients (*n* = 75)	Gene ratios CD9/KIAA1199 CD9/ THBD DLG5/KIAA1199 DLG5/THBD		**Median survival**
			Good-outcome	12 mo
			Poor-outcome	5 mo
Blum *et al.*, 2019[8]	Surgical PM patients (*n* = 382)	E-score S-score		**Hazard ratio**
			E-score	1.00 (0.38, 2.64)
			S-score	5.08 (1.56, 16.55)
MESO EMT gene signature[74]	Treatment naïve PM patients (*n* = 82)	EMT-high EMT-low		**Hazard ratio**
			EMT-low	
			EMT-high	2.9 ($P = 4.4 \times 10^{-5}$)
Cioce *et al.*, 2021[76]	Treatment naïve PM patients (*n* = 84)	18 - Gene prognostic signature		**Hazard ratio**
			Signature-low	
			Signature-high	2.30 [1.71-3.09] ($P = 2.8 \times 10^{-8}$)
Combined Clinical and Molecular Models				
Mesothelioma Prognostic Test (MPT), 2009[48]	Surgical EPP or P/D patients (*n* = 120)	TM4SF1/PKM2 TM4SF1/ARHGDIA COBLL1/ARHGDIA Histological diagnosis lymph node involvement		**Median survival (95%CI)**
			Low risk	31.9 mo (21.9-41.7)
			Intermediate risk	12.9 mo (9.9-16.4)
			High risk	6.9 mo (2.6 to 8.9)
Mesothelioma risk score (MRiS), 2021[11]	Surgical EPP or P/D patients (*n* = 384)	Surgery type (P/D *vs.* EPP) Mesothelioma Prognostic test (MPT) CLDN15/VIM score Tumor volume Neutrophil-to-lymphocyte ratio serum albumin ECOG performance status		**Median survival**
			EPP	
			Low risk	39 mo
			Intermediate risk	21 mo
			High high	12 mo
			P/D	
			Low risk	36 mo
			Intermediate risk	14 mo
			High risk	9 mo

CALGB: Cancer and leukemia group B; ECOG: eastern cooperative oncology group; EMT: epithelioid to mesenchymal transition; EPP: extrapleural pneumonectomy; P/D: pleurectomy and decortication.

separated into three distinct risk groups: low risk (MPT = 3, survival 31.9 months), intermediate risk (MPT = 1-2, survival 12.9 months), and high risk (MPT = 0, survival 6.9 months) [Table 2][48]. However, as this model requires histological differentiation and lymph node status, it is applicable only to surgical patients, thus limiting its application. In 2021, Yeap *et al*. investigated the association of the validated MPT score with clinical variables previously shown to be associated with the outcome of patients with PM[11]. These variables included the claudin15/vimentin (C/V) gene ratio test (indicative of molecular subtype)[3], tumor volume (TV) (calculated via preoperative imaging), neutrophil to lymphocyte ratio (NLR), serum albumin, and eastern cooperative oncology group (ECOG) performance status[7,49-55]. It was shown that MPT, molecular subtype, TV, and NLR were independent prognostic factors in patients with PM. Therefore, a novel algorithm, the MPM Risk Score (MRiS), was developed to stratify patients and inform them about prognosis before surgery [Table 2][11].

MicroRNAs in diagnosis and prognosis

MicroRNAs (miRNAs) are a class of non-coding RNA with an average length of 22 nucleotides. MiRNAs have been shown to regulate gene expression[56]. They are critical for a variety of biological processes and their altered expression has been associated with cancer, where they can act as oncogenes or tumor suppressor genes[57]. A specific combination of miRNAs is associated with each cancer; therefore, specific miRNA signatures may be used for diagnosis, patient stratification, and prognosis[58-62].

Recent studies have found diagnostic miRNAs that are upregulated or downregulated in circulation, tumor tissue, and pleural effusion of patients with PM[63]. MiRNA were investigated as biomarkers to differentiate PM from reactive mesothelial proliferations (RMPs)[64]. Formalin-fixed, paraffin-embedded (FFPE) preoperative diagnostic biopsy tumor specimens and corresponding non-neoplastic pleural samples from 5 patients with PM were used to analyze an array of 742 miRNAs. A four-miRNA (miR-126, miR-143, miR-145, and miR-652) signature able to differentiate between PM and the non-neoplastic controls was identified and consequently validated in an independent cohort of 40 PMs[64]. Other studies have evaluated miRNA expression as a prognostic factor for overall survival and progression-free survival (PFS). Pass *et al.* found that increased hsa-miR-29c* expression predicted a favorable prognosis[65]. When examined within cell lines, they found that overexpression of hsa-miR-29c* led to decreased tumor cell characteristics associated with invasiveness and malignancy such as proliferation, migration, and colony formation[65]. Busacca *et al*. evaluated the differential expression of miRNA between normal human mesothelial cell lines and mesothelioma cell lines[66]. Sixty-five microRNAs were found dysregulated using miRNA expression profiles. Selected miRNAs were then investigated in 24 mesothelioma specimens. MiR-17-5p, miR-21, miR-29a, miR-30c, miR-30e-5p, miR-106a, and miR-143 were significantly correlated with PM histological subtype, whereas reduced expression of miR-17-5p and miR-30c was associated with longer survival[66].

"Liquid Biopsies" in diagnosis and prognosis

Due to the difficulties posed by PM diagnosis and the need for invasive procedures to acquire tissue biopsies for complete histopathologic diagnosis, recent efforts have been made to develop targets for "liquid biopsy" from serum, effusion, plasma, serum, or saliva specimens[67]. Typically, the term "liquid biopsy" refers to circulating free tumor DNA (ctDNA) that can be detected in plasma samples, though it can refer to the measurement of soluble biomarkers such as tumor markers and tumor-specific proteins[68].

MiRNA

Circulating serum miRNA in peripheral blood samples has also been evaluated as a diagnostic tool for PM. Cavalleri *et al.* evaluated circulating exosomal miRNA in peripheral blood from PM patients and found a specific miRNA signature (miR103, miR-98, miR-148b, miR-744, and miR-30e-3p) highly accurate in differentiating PM patients from asbestos-exposed, non-PM controls[69]. In another study, the TaqMan OpenArray was used to analyze 47 pleural effusion (PE) cells and supernatant from 26 patients with PM, 10 from patients with benign disease, and 11 from patients with adenocarcinoma. A three-miRNA signature (miR-143, miR-210, and miR-200c) was identified that accurately differentiated the PM samples from the others, indicating that the expression of this three-miRNA signature could be used in the diagnosis of PM[70].

Mesothelin

Serum mesothelin is a protein expressed on the cell surface of tumor cells in many malignancies, including PM[71]. Burt *et al.* explored serum mesothelin as a predictive biomarker of recurrence in PM[71]. They retrospectively reviewed outcomes data of 102 PM patients who underwent surgical resection of PM between 2014-2016 and who had documented preoperative mesothelin values. They found that in PM patients who underwent complete macroscopic resection, there was a significant decrease in postoperative values compared to preoperative values (3.4 ± 4.9 nmol/L *vs.* 0.8 ± 0.5 nmol/L). They also found that higher levels of preoperative serum mesothelin were associated with poor overall survival. Finally, they showed that in patients with epithelioid PM who underwent macroscopic complete resection, a rise in serum mesothelin was associated with the presence of recurrence (sensitivity = 90%, specificity = 93%)[71]. These findings suggest a clinical role of mesothelin surveillance in the postoperative setting as an indicator of disease progression and a possible indicator for recurrence.

Circulating tumor DNA (ctDNA)

Circulating tumor DNA has shown efficacy in detecting malignancy in both patients with lung cancer with EGFR mutations as well as patients with colorectal cancer with KRAS mutations[68]. Though ctDNA assays for other malignancies are in development, there have not been any validated ctDNA assays in PM. Preliminary work in ctDNA assays in PM has failed to show the diagnostic success of ctDNA that is seen in other malignancies. Martinson *et al.* performed whole exome sequencing (WES) on tumor tissue and matched germline DNA from 11 surgical PM patients[72]. They created a patient-specific ctDNA assay design using multiple tumor regions from each patient to identify variants to compare germline ctDNA. However, only 4 of 11 patients were positive for germline ctDNA using this approach[72]. Similarly, Hylebos *et al.* performed WES on paired tumor and germline DNA from 10 PM patients[73]. They identified patient-specific single nucleotide variants from genes previously associated with PM and found that ctDNA was present in 3 of 5 treatment-naïve patients[73]. This study showed that ctDNA can be detected in treatment naïve PM patients. However, large prospective studies need to be performed to identify ctDNA assays that are sensitive, specific, and generalizable for the diagnosis of PM.

Molecular clusters-based classification

Unsupervised consensus clustering of bulk-RNA transcriptomes from 216 PM tumors distinguished four distinct molecular clusters of PM: sarcomatoid, epithelioid, biphasic-epithelioid (biphasic-E), and biphasic-sarcomatoid (biphasic-S). These four molecular clusters are approximately correlated with the histological epithelioid to sarcomatoid histological spectrum[3]. Differential gene expression analysis between the two extreme clusters identified 189 upregulated and 241 downregulated genes related to the epithelial-to-mesenchymal transition (EMT) process[3]. In addition, the ratio of the expression of two genes, Claudin 15 and Vimentin (C/V), was able to significantly differentiate each of the four molecular clusters[3].

The Cancer Genome Atlas (TCGA) group performed comprehensive multi-omic molecular profiling on 74 primary tumor samples from treatment-naïve PM patients[22]. Four distinct prognostic clusters significantly associated with overall survival ($P = 0.008$) were identified[22]. Cluster 1 was associated with longer survival and correlated with epithelioid histology ($P = 0.002$), whereas cluster 4 was associated with short survival and with the mesenchymal part of the EMT spectrum ($P < 0.001$)[22].

Blum *et al.* conducted a comprehensive meta-analysis of mesothelioma profiles from 422 PM tumors from publicly available PM datasets, which included the previously published Bueno and TCGA datasets[8]. Through this analysis, they identified two main groups corresponding to the most extreme clusters that included epithelioid and sarcomatoid samples, respectively. The remaining tumors represented a continuum, or "histo-molecular gradient" between the two extremes. Using a deconvolution approach, they identified two molecular signatures: the E-score and the S-score, which were able to define the proportion of epithelioid-like and sarcomatoid-like components in each tumor. They determined that tumors with high S-scores were correlated with worse overall survival (HR = 6.28, $P = 0.001$)[8], and that S-score was more prognostic of poor survival than the E-score or histological subtype (HR = 5.08, $P = 0.007$) [Table 2][8]. This multi-omic meta-analysis confirmed the insights gained from the aforementioned studies, which include the presence of unique histo-molecular clusters within PM, validating the hypothesis of PM existing on an EMT spectrum[21].

Using the TCGA database, Wu *et al.* developed a 9-gene EMT gene signature to create the MESO EMT score[74]. This 9-gene score showed statistically significant survival differences between the "High" and "Low" groups (Logrank $P = 4.4 \times 10^{-5}$) [Table 2]. They then used single-cell RNA sequencing to analyze pleural effusion and tumor biopsies and found that the 9 EMT genes from their MESO EMT score were predominantly expressed specifically in cancer cells. Additionally, they found an association of high MESO EMT scores with high rates of epigenetic hypermethylation, suggesting that the mesenchymal transition that has been observed in PM may be due to epigenetic signaling[74].

Aldehyde dehydrogenase (ALDH) functions to enzymatically oxidize intracellular aldehydes in many pathological conditions. High ALDH activity has been shown to be associated with chemoresistance in MPM cell lines[75]. Using the TCGA cohort ($n = 84$), Cioce *et al.* found that patients with high ALDH1A3 (a member of the ALDH family) had significantly shorter survival compared to low ALDH1A3 patients ($P = 0.015$) [Table 2][76]. They used the TCGA cohort and differential gene expression to identify genes that were associated with ALDH1A3 expression. They then created an 18-gene signature that identified ALDH1A3-High and ALDH1A3-Low patients. This 18-gene prognostic signature showed that patients in the "Low" group ($n = 38$) survived longer than patients in the "High" ($n = 46$) group ($P = 0.000000028$). They found that chemotherapy-resistant patients trended towards high scores on the 18-gene prognostic signature though the analysis was underpowered to show significance[76]. This study showed that prognostic gene scores can be particularly useful in certain subsets of patients and potentially guide clinical management.

TREATMENT

Current clinical practice

PM is behind in terms of consensus and staging for treatments which partly explains the overall poor outcomes. Patients usually get treated regardless of staging in manners that differ among centers and specialists[19]. To define the best standards of treatment, we have identified three different categories important for treatment strategy: stage, patient's physical status, and tumor biology[19].

To simplify staging, we divide patients into three groups: patients with ipsilateral radiographically resectable tumors without localized invasion of the chest wall and/or mediastinum or mediastinal lymph node involvement; patients with localized disease with chest wall/mediastinal or lymph node involvement, and patients with distant systemic disease[19].

As for physical status, data show that nutritional status measured by albumin, frailty, age, chest pain, chest contraction, and hematological status (platelets, WBC, and neutrophil-to-lymphocyte ratios) can correlate both with stage and survival[11]. Finally, even when all of these parameters are kept constant, specific tumor biology evident by histological subtypes, sex, mutational status, and transcriptional status predict both responses to therapies and outcomes.

Current treatment strategies employ a multimodal approach, including surgery, systemic therapy, and radiotherapy[77]. Treatment is guided by staging, histological subtype, and functional status[77]. These treatments have been shown to be beneficial only to certain populations of highly selected patients[10,77,78]. Current studies have yet to stratify patients that would most benefit from various treatment strategies.

For surgical candidates, macroscopic complete resection through pleurectomy and decortication or extrapleural pneumonectomy is pursued, and in a highly selected subset of patients with early-stage disease, pleurectomy has been associated with improved 5-year survival at 22%[79]. However, these operations cannot always completely address the local microscopic disease spillage[78]. As such, some centers employ cisplatin-based intracavitary heated chemotherapy or other modalities to enhance local control at the time of surgery. Although no randomized clinical trials have been performed, several studies suggest benefits in overall survival[80].

Recently, clinical trials have shown survival benefits of immunotherapy treatment in selected patients. Programmed cell death receptor 1 (PD-1) and its ligand (PDL-1) are expressed in activated immune cells. The expression of these proteins has been shown to play an important role in immune evasion by tumor cells[81]. The phase III CheckMate 743 trial (NCT02899299) published 3-year clinical outcomes data for immunotherapy *vs.* chemotherapy[82]. They enrolled 605 previously untreated and unresectable PM patients and compared immunotherapy with nivolumab (an anti-PD-1 antibody)/ipilimumab (an anti-CTLA-4 antibody) (n = 303) *vs.* chemotherapy with cisplatin/pemetrexed (n = 302). They found that median overall survival was greater in the immunotherapy group (18.1 *vs.* 14.1 months; HR: 95%CI = 0.73 (0.61-0.87)). Subgroup analysis revealed that the survival benefit was mostly seen in the non-epithelioid histotypes[82]. The results from the PrE0505 clinical trial showed that a combination of durvalumab (an anti-PD-L1 antibody) and platinum-pemetrexed chemotherapy led to superior overall survival compared to historical controls that received cisplatin/pemetrexed alone (20.4 *vs.* 12.1 months)[83]. Recent data has indicated a potential use for immunotherapy and anti-PD-L1 immune checkpoint blockade (ICB) via durvalumab ± tremelimumab (an anti-CTLA-4 antibody) in a neoadjuvant setting[84]. In a phase II, randomized, prospective clinical trial (MPM; NCT02592551), surgically resectable PM patients were randomized to ICB combination therapy with durvalumab + tremelimumab (n = 11), monotherapy with durvalumab alone (n = 9), or no ICB (n = 4). When comparing patients treated with ICB combination therapy to ICB monotherapy, they showed greater postoperative overall survival (P = 0.041) and disease-free survival (P = 0.014) in the combination therapy group[84]. These findings suggest that ICB combination therapy may be useful not only in unresectable PM patients, but in the neoadjuvant setting as well.

The key pharmaceutical trials and their therapeutic pathways for PM are shown in Table 3 and Figure 1. Though these trials show promising progress towards novel first and second-line treatments, there remain no reliable molecular or genetic biomarkers to predict optimal treatment response[77,85].

Table 3. Key pharmaceutical trials and targeted pathways

Author	Phase	Design	Pathway	Drug [mechanism]	Results
Fennell *et al.*[90]	II	Single-arm, open-label (n = 36)	BAP1/BRCA1	Rucaparib [PARPi]	Disease control at 12 week = 58% [95%CI: 37-77]
Zauderer *et al.*[89]	II	Single-arm, open-label (n = 74)	BAP1/EZH2	Tazemetostat [EZH2i]	Disease control at 12 week = 51% [95%CI: 40-63]
Fennell *et al.*[95]	II	Randomized, double-blind (n = 344)	NF2/Merlin	Defactinib [FAKi]	Progression-free survival = 4.1 mo [95%CI: 2.9-5.6]
Fennell *et al.*[92]	II	Single-arm, open-label (n = 26)	CDKN2A	Abemaciclib [CDK4i/CDK6i]	Disease control at 12 week = 54% [95%CI: 36-71]
Ali *et al.*[91]	II	Single-arm, open label	BAP1/BRCA1	Niraparib [PARPi]	Ongoing
Lapidot *et al.*[97]	Preclinical	Xenografted mouse model of PM	KDM4A	PKF118-310 [KDM4Ai]	Preclinical

BAP1: BRCA1 associated protein 1; EZH2: enhancer of zeste 2 polycomb repressive complex 2 subunit; NF2: neurofibromin 2; CDKN2A: cyclin-dependent kinase inhibitor 2A; KDM4A: lysine-specific demethylase 4A; PARP: poly[ADP-ribose]polymerase inhibitor; FAK: focal adhesion kinase; CDK4: cyclin-dependent kinase 4; CDK6: cyclin-dependent kinase 6.

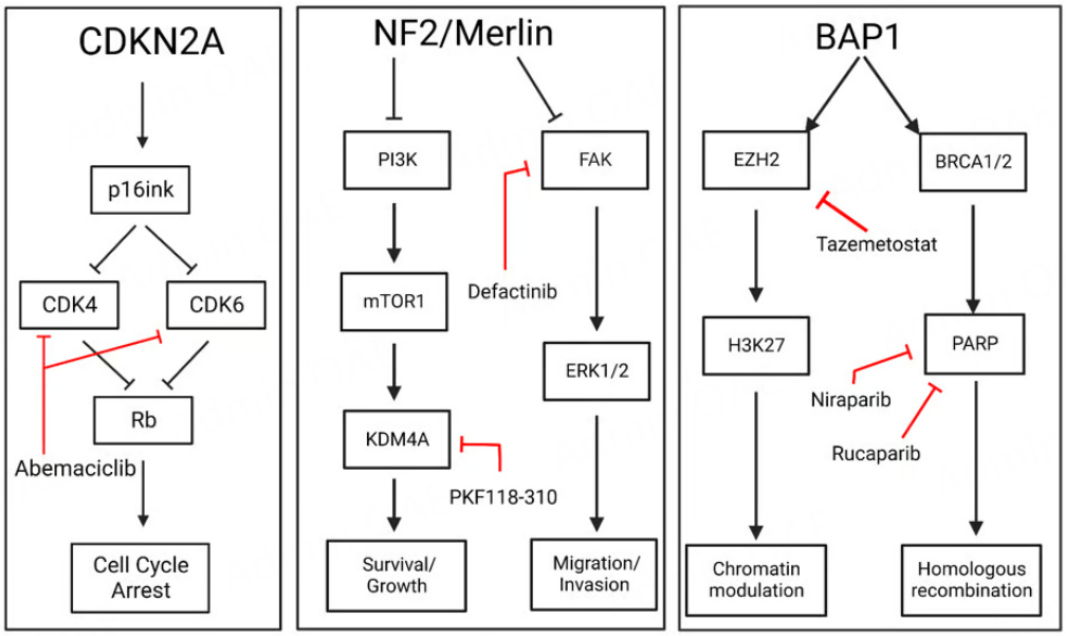

Figure 1. Commonly mutated pathways in PM and pharmaceutical targeted agents. Adapted with permission[109] and created with BioRender.com. CDKN2A: Cyclin-dependent kinase inhibitor 2A; CDK4: cyclin-dependent kinase 4; CDK6: cyclin-dependent kinase 6; Rb: retinoblastoma protein; NF2: neurofibromin 2; FAK: focal adhesion kinase; mTOR1: mammalian target of rapamycin complex 1; KDM4A: lysine-specific demethylase 4A; ERK: extracellular signal-regulated kinase; BAP1: BRCA1 associated protein 1; EZH2: enhancer of zeste 2 polycomb repressive complex 2 subunit; H3K27: lysine 27 on histone H3; PARP: poly [ADP-ribose] polymerase)

Therapeutic implications of genetic profiling

In many cancers, targeted therapy has been effective in treating highly selected patient populations by targeting known unique molecular markers. The lack of specific and feasible molecular targets presents a unique challenge for the development of targeted therapies in PM[86]. Few targeted drug trials have been conducted on genes known to be altered in PM.

BAP1 signaling pathway has been evaluated as a potential target for novel therapies. BAP1 loss has been associated with increased levels of the enhancer of zeste 2 polycomb repressive complex 2 subunit (EZH2), a methyltransferase that tri-methylates lysine 27 on histone H3 (H3K27), altering downstream gene expression[87,88]. In a phase 2 clinical trial, Zauderer *et al.* evaluated tazemetostat, an EZH2 inhibitor, in 74 PM patients refractory to first-line chemotherapy, 73 of whom had BAP1-inactivated tumors[89]. The absence of BAP1 expression was confirmed by DNA sequencing and IHC of tumor specimens. Results of the trial showed that the disease control rate was 51% at 12 weeks (95%CI: 40-63; 38 of 74 patients) and 28% at 24 weeks (18-39; 21 of 74)[89]. No patients reached complete response, and only two patients had confirmed partial response based on Response Evaluation Criteria in Solid Tumors (RECIST) criteria[89]. Currently, several PARP inhibitors are in early-phase clinical trials[87]. In a phase II single-armed study, patients with relapsed mesothelioma were treated with PARPi (rucaparib) monotherapy. Thirty-six patients were included in the study; 66% had confirmed BAP1 deficiency, 57% were BRCA1 deficiency, and 29% were double deficient in BAP1 and BRCA1. A total of 26 patients who had BAP1 and/or BRCA1 deficiency were eligible for treatment with rucaparib. The primary endpoint was disease control at 12 weeks, which was achieved in 15 patients (58% [95%CI: 37-77]). Six patients reached 24 weeks (23% [9-44]). None of the treated patients achieved a complete response, and only three patients (12%) achieved a partial response[90]. Niraparib, another PARPi, is currently in a phase II clinical trial to evaluate the response of PM patients with and without BAP1 mutations[91].

Similarly, the CDKN2A pathway has been evaluated as a potential target for novel therapies. P16ink4A is a transcriptional product of the CDKN2A pathway, which is an inhibitor of CDK4 and CDK6, which regulate cell cycle progression and promote tumor growth[92]. In a phase 2, single-armed clinical trial, Fennell *et al.* studied the effects of abemaciclib, a CDK4i/CDK6i, on p16ink4A-deficient PM patients[92]. All patients had completed at least one cycle of cisplatin-based therapy and were molecularly screened for p16ink4A deficiency prior to inclusion in this study. They found a disease control rate of 54% (14 of 26 patients) at 12 weeks (95%CI: 36-71), with 80% of patients showing a reduction in tumor volume[92].

The tumor suppressor gene NF2, located on 22q12, is a plasma membrane-associated inhibitor of proliferation[93]. Mutations in NF2 are associated with many benign and malignant tumors including PM[22,93]. Large bulk RNA-sequencing studies have shown NF2 mutation rates are particularly high in PM tumors with sarcomatoid histotype[3]. Loss of function mutations in NF2 has been shown to dysregulate the Hippo signaling pathway, which is commonly altered in PM[3,86]. NF2 encodes for moesin-ezrin-radixin-like protein (merlin), a tumor-suppressor protein that recruits LATS1 and LATS2, leading to the phosphorylation of downstream targets yes-associated protein 1 (YAP1) and WW Domain-Containing Transcription Regulator 1 (TAZ), preventing their translocation into the nucleus and, ultimately, leading to upregulation of many oncogenic genes[86,93]. The focal adhesion Kinase (FAK) is a serine/threonine of the Hippo pathway, involved in cell growth and migration. FAK is elevated in many human cancers[93]. In cell-line and *in vivo* mouse experiments, Shapiro *et al.* investigated the sensitivity of merlin-deficient PM to FAK inhibition and observed that FAK inhibition led to decreased tumor growth[94]. In the phase II double-blind placebo control COMMAND trial, the FAK inhibitor (Defactinib) was investigated as maintenance therapy to improve progression-free survival. Three hundred forty-four patients with PM who had completed one cycle of first-line chemotherapy (cisplatin/pemetrexed) were randomized to receive defactinib (n = 173,) *vs.* placebo (n = 171)[95]. No differences in median PFS were observed in the defactinib arm (4.1 months; 95%CI: 2.9-5.6) *vs.* the placebo arm (4.0 months; 95%CI: 2.9-4.2) and the trial was terminated due to treatment inefficacy[95]. In both the defactinib arm and the placebo arm, patients with merlin-low tumors showed a trend towards worse overall survival compared to patients with merlin-high PMs, but the difference did not reach significance[95]. Recently, we have found high expression of the stimulator of

interferon genes (STING) protein expression in PM by immunohistochemistry profiling of archival samples from 300 diverse thoracic malignancies. As STING activation promotes antitumor immunity, STING agonists may be used to promote antitumor activity in vivo and achieve clinical success[96].

Lysine-specific demethylase 4A (KDM4A), a gene overexpressed in PM, regulates the demethylation of histone 3 lysine 9 trimethylation (H3K9me3), a post-translational modification that is involved in several biological processes, including forming transcriptionally silent heterochromatin[97]. In a preclinical model, Lapidot *et al.* examined KDM4A as a potential target for PM treatment[97]. Using tissue microarrays from 53 PM tumors, KDM4A was found to be highly expressed in PM samples compared to normal mesothelial tissue ($P = 4.74 \times 10^{-10}$). In xenograft mouse models, intra-tumoral treatment with PKF118-310, a KDM4A inhibitor, showed a consistent reduction of tumor volume when compared to intra-tumoral injection placebo controls ($P < 0.03$), suggesting that the KDM4A pathway may be a potential target for therapeutic therapies[97].

MicroRNAs in PM treatment

As previously described, miRNAs have shown diagnostic and prognostic utility in the management of PM. However, recent studies have begun to explore their potential role as therapeutics in PM. Several studies have targeted various miRNAs in mouse models and shown that miRNA replacement can inhibit tumor growth[98-100]. In preclinical models, Tomasetti *et al.* subcutaneously xenografted mice with cell lines that were transfected with miRNA-126 plasmids (Met5A^{MiR126} and H28^{MiR126}) *vs.* empty plasmids (Met5A$^{pCMV\text{-}MiR}$ and H28$^{pCMV\text{-}MiR}$) to show that miRNA-126 played a role in mitochondrial metabolism and tumor suppression[99]. Later, Monaco *et al.* developed a strategy for targeted delivery of miRNA-126 to PM cell lines using endogenous, epithelial-derived exosomal vesicles[101]. They used fibroblast (IMR-90), endothelial (HUVECs), nonmalignant mesothelial (Met-5A), and PM (H28 and MM-B1) cell lines to create an in vitro tri-culture stromal model. They then treated these models with exosomes enriched with miRNA-126. They found stromal miRNA-126 uptake via paracrine and exocrine transport, which was dependent on the miRNA-126 levels in the cellular microenvironment (miRNA-126 sensitive *vs.* miRNA-126 resistant). Additionally, they found that treatment with miRNA-126 enriched vesicles inhibited angiogenesis in miRNA-126 sensitive PM cells[101]. Though early in the preclinical phase, these studies show examples of novel strategies for targeted therapies in the treatment of PM[100].

Similarly, miRNA-15 and miRNA-16 have been shown to be dysregulated in many solid tumors[102,103]. They target the mRNA of many genes associated with tumor progression and are thought to act as tumor suppressor genes[104]. Reid *et al.* showed that miRNA-15 and miRNA-16 are downregulated in PM, suggesting these miRNAs as potential targets for novel treatments[105]. In a single-arm phase 1 clinical trial, Van Zandwijk *et al.* intravenously treated 26 PM patients with recurrent PM with miRNA-16 - mimic minicells[106]. They evaluated disease progression using follow-up CT scans after at least 4 weeks. Of the 22 patients who had baseline CTs, 5% had partial response ($n = 1$), 68% had stable disease ($n = 15$), and 27% had disease progression ($n = 6$)[104]. Because this study was aimed to evaluate the drug-related toxicities associated with this novel PM therapy, it was not powered to accurately assess treatment efficacy as measured by disease response or overall survival. Nevertheless, this trial represents the first-in-human trial of targeted miRNA therapy for PM. As new miRNA treatments and delivery strategies continue to be explored, the ultimate role of miRNA in the treatment of PM remains to be seen.

CONCLUSION

Omics-based technologies permit the identification of gene signatures based on expression and sequencing data and are essential to classify patients for precision medicine[21]. Genetic profiling has demonstrated its

utility by selecting patients and guiding treatment in many malignancies[106]. In PM, the advances in the genomic and transcriptomic characterization have led to a better understanding of the heterogeneity and complexity of this disease, but progress in the treatments of patients with PM is limited[3,13,87]. Further improvements in the analysis of omics data, together with advances in computational technologies, will contribute to the validation of genetic profiling approaches for new applications in PM.

DECLARATIONS

Authors' contributions

Wrote and revised the text: Sadek A, De Rienzo A, Bueno R

Provided comments on the initial version and approved the final draft of the manuscript: Sadek A, De Rienzo A, Bueno R

Availability of data and materials

Not applicable.

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Conflicts of interest

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Ethical approval and consent to participate:

Not applicable.

Consent for publication

Not applicable.

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