Chapter

Biomarkers Progress and Therapeutic Implications in Malignant Mesothelioma

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Abstract

We are witnessing enormous efforts to identify prognostic and predictive biomarkers to inform treatment decisions in malignant mesothelioma. In this chapter, we will review and discuss the current literature and supportive evidence for the progress in development and use of biomarkers in malignant mesothelioma. There are currently several clinical trials evaluating treatment options in mesothelioma, and this will be an up-to-date review of these trials from published literature.

Keywords: mesothelioma, biomarkers, ASS1, BAP1, CDKN2A, mesothelin, NF-2, PDL-1, VEGF, WT-1

1. Epidemiology of mesothelioma

Malignant mesothelioma (MM) is an aggressive, rare cancer of pleural (80%), and peritoneal cells and less frequently in the pericardium and tunica vaginalis of the testis. MM has historically been linked to mineral fiber exposure. Asbestos is a collective term given to six mineral fibers including actinolite, amosite, anthophyllite, chrysotile, crocidolite, and tremolite [1]. Exposure to other non-asbestos mineral fibers including erionite and fluoro-edenite has also been linked to MM [2]. However, cases of MM have been found in patients who were not exposed to these mineral fibers. This led researchers to discover other epidemiologies of mesothelioma heavily linked to genetic mutations, including tumor suppressors like BRCA1-associated protein (BAP1) [3].

2. Biomarkers in mesothelioma

Recent research has been aimed at studying various biomarkers in malignant mesothelioma. Researchers hope that by identifying and studying specific biomarkers, new therapies can be developed that better target the unique pathways of malignant mesothelioma pathogenesis.

2.1 Vascular endothelial growth factor

The VEGF pathway is believed to play a critical role in angiogenesis and lymphangiogenesis in human malignant mesothelioma tumors [4]. In one study, more

than 95% of malignant pleural mesothelioma (MPM) samples stained positive for VEGF [5]. An increase expression of VEGF was specifically observed in the epithelioid histology, more than biphasic and sarcomatoid. VEGF was not felt to have any prognostic significance in this study [5]. In another study, VEGF was found to be an independent, poor prognostic factor in MPM [6]. The phase III MAPS study showed that the addition of bevacizumab, a humanized anti-VEGF monoclonal IgG1 antibody, to frontline cisplatin/pemetrexed in unresectable malignant pleural mesothelioma improves overall survival (18.8 vs. 16.1; hazard ratio 0.77 [0.62–0.95]; p = 0.0167) compared to cisplatin/pemetrexed alone regardless of tumor histology [7]. Analysis from the MAPS study showed that high VEGF concentrations were associated with worse progression free survival and overall survival but VEGF did not have a clinically meaningful predictive significance of response to bevacizumab [8]. Other antiangiogenic agents like Sorafenib and axitinib have showed limited activity in malignant mesotheliomas [9, 10]. Ramucirumab is a recombinant human immunoglobulin G1 monoclonal antibody that binds to the extracellular domain of VEGFR-2 and prevents the binding of VEGFR ligands: VEGF-A, VEGF-C, and VEGF-D. A recently published Phase II abstract showed that the addition of Ramucirumab to gemcitabine significantly improved the overall survival in advanced MPM patients who progressed on first-line platinum-pemetrexed chemotherapy. This was observed regardless of patient age, tumor stage (locally advanced vs. metastatic), histotype (epithelioid vs. non-epithelioid), and time to progression at the first-line treatment [11].

2.2 Arginosuccinate synthetase

Certain cancer cells have a higher nutritional demand compared to normal cells. Arginine is an amino acid that plays an important role in biological and signaling pathways [12]. Arginine is either synthesized in the body or consumed in the diet. Normal cells synthesize arginine through the urea cycle. Research suggests that certain cancer cells cannot internally make arginine because they lack the urea cycle enzyme argininosuccinate synthetase 1 (ASS1) which ultimately makes them dependent on exogenous supplies of arginine, an important amino acid for cancer survival and growth [13]. ASS is a key enzyme that converts citrulline to arginine. This has led scientists to hypothesize that targeting the arginine synthesis pathway may be an effective therapeutic approach that targets cancer cells and spares normal cells.

Mesothelioma is one of the tumors that usually does not express ASS [14]. Arginine degradation is dependent on different enzymes, including an enzyme called arginine deiminase (ADI) that degrades arginine to citrulline. In turn, citrulline can be recycled back to arginine in normal cells through ASS [14]. A pegylated arginine deiminase (ADI-PEG 20) has been developed as an arginine depleting agent and is currently being tested in a randomized, double-blind, phase 2/3 study in subjects with malignant pleural mesothelioma with low argininosuccinate synthetase 1 expression to assess ADI-PEG 20 with pemetrexed and cisplatin (Clinicaltrials.gov ID NCT02709512).

2.3 Aurora kinase

Aurora kinase gene expression is upregulated in mesothelioma tumor tissue and is considered a negative prognostic factor [15–17]. The Aurora proteins are serine/threonine kinases that function in various stages of mitosis. Aurora kinase proteins A/B play an important role in mitosis, monopolar spindles formation, chromosomal segregation cytokinesis, and polyploidy. These proteins are overexpressed in mesothelioma [18]. Aurora kinase inhibitors, like ZM447439, are able to inhibit cell

growth in all mesothelioma cell lines [18]. Alisertib (MLN8237) is a selective aurora kinase A inhibitor that is currently being evaluated in pretreated patients with unresectable MPM (Clinicaltrials.gov NCT02293005).

2.4 Wilms' tumor protein

WT-1 is a zinc finger transcription factor protein that is responsible for controlling the expression of genes involved in cellular growth, differentiation, and/or apoptosis [19]. WT1 is a nuclear protein that is processed and highly overexpressed on the cell surface of MPM. Immunohistochemical (IHC) staining for WT1 is routinely used in establishing the diagnosis of mesothelioma. WT-1 protein expression is detected by IHC in 78.1% of MPM and associated with improved overall survival and prognosis [20]. Although WT1 protein is expressed on the cell surface in the context of MHC molecules, which makes it a target for T-cell based immunotherapeutic approach [21]. A randomized phase II trial of adjuvant galinpepimut-S, WT-1 analogue peptide vaccine, after multimodality therapy for patients with WT-1 + MPM showed that a favorable safety profile with suggested improvement in progression-free survival and overall survival and a larger randomized trial is planned [22].

2.5 Mesothelin

Mesothelin is a tumor differentiation protein that is normally expressed in low amounts on the pleural, peritoneal, and pericardial mesothelial cells. Mesothelin is highly expressed in malignant mesothelioma as well as other cancers like pancreatic, ovarian, and lung adenocarcinoma [23–25]. The differential expression of mesothelin between normal tissues and malignant cells made it an attractive candidate for cancer therapy. Mesothelin targeting agents including chimeric antigen receptor (CAR) T cells and vaccination strategies are currently in development for the treatment of MPM. CRS-207 is a live-attenuated strain of the bacterium *Listeria monocytogenes* that is engineered to express mesothelin. CRS-207 induces antitumor immune responses and increase the susceptibility of neoplastic cells to immune-mediated killing. A phase I study combining CRS-207 and pemetrexed/cisplatin chemotherapy induced significant changes in the local tumor microenvironment and objective tumor responses in a majority of treated patients [26].

2.6 Programmed death-receptor ligand

PD-L1 is overexpressed in 40–50% of mesothelioma and associated with poor outcome. In one study, high PD-L1 expression was associated with non-epithelioid MM, poor clinical outcome, and increased immunological infiltrates [27]. Several PD-L1 and PD1 targeting agents have been studied in mesothelioma with modest activity. Pembrolizumab, nivolumab, and ipilimumab are routinely used in the second-line therapy of malignant mesothelioma. PD-L1 testing is not required for prescribing pembrolizumab or nivolumab in the second-line therapy for patients with PMP [28]. Limited data suggests that high PD-L1 expression (≥25% positive tumor cells) seems to be a predictor of higher overall response rate to nivolumab on nivolumab plus ipilimumab and even better objective response rate when the PD-L1 expression is >50% [29]. Real-world data suggests that the high PD-L1 expression (≥50%) and non-epithelioid histology are associated with an improved objective response rate to pembrolizumab compared to intermediate (5–49%) and negative PD-L1 expression (<5%) in the second-line therapy of MPM [30].

2.7 BRCA1-associated protein

BRCA1-associated protein (BAP1) is a powerful deubiquitylating enzyme that acts to suppress the tumor growth. This means that it removes ubiquitin tags from specific proteins to modify and regulate their function or interaction with other molecules. BAP1 has been shown to have different tumor-suppressing functions when localized to the nucleus vs. cytoplasm. In the nucleus, it is promoted to double-stranded DNA break sites to aid in repair via homologous recombination, therefore inhibiting the growth of the damaged, mutated DNA [31, 32]. In the cytoplasm, BAP1 deubiquitylates type-3 inositol-1,4,5-trisphosphate-receptor (IP3R3) on the endoplasmic reticulum (ER). Once stabilized, IP3R3 allows the efflux of calcium (Ca^{2+}) from the ER into the cytoplasm. This increase in Ca^{2+} promotes cytochrome c activation and induces cell apoptosis [32, 33]. More recently, it has been proposed that BAP1 also regulates ferroptosis, an iron-dependent programmed cell death via the repression of cystine transporter SLC7A11 [34].

Somatic inactivating mutations in *BAP1* have been associated with numerous malignancies including female reproductive cancers, uveal melanoma, renal cell carcinoma, pancreatic cancer, and leukemia [35–41]. Somatic mutations in *BAP1* were also initially reported in up to 23% of MPM [42]. These results were reproduced in various studies with *BAP1* loss ranging from 20 to 60% in MM, further exemplifying its major role in the development of malignancy [35, 43–45].

Germline mutations in *BAP1* are associated with a novel cancer syndrome named "BAP1 Cancer Syndrome." This syndrome infers increased susceptibility to a variety of malignancies including mesothelioma, uveal and skin melanoma, cholangiocarcinoma, renal cell, basal cell, and squamous cell carcinomas, among others [32]. Malignant mesotheliomas that develop in BAP1 germline mutation carriers tend to be less aggressive with better prognosis and improved survival compared to sporadic mesothelioma [46].

There are currently no standard therapeutic approach for *BAP1* loss in mesothelioma. Histone deacetylase (HDAC) inhibitors reversed the H2A hyperubiquitination caused by *BAP1* loss, and they shift the gene expression profile of class 2 cells toward a class 1 profile in a UVM cell line [47, 48]. A phase 3 study comparing vorinostat (an HDAC inhibitor) with placebo in relapsed or refractory MPM concluded vorinostat did not improve overall survival compared to placebo and led to a statistically significant but not clinically relevant improvement in PFS [48, 49]. Molecular analysis to detect *BAP1* mutations in patients treated on this study has not been reported [48, 49].

BAP1 loss leads to increased expression of enhancer of zeste homolog 2 (EZH2) protein [50]. EZH2 is a protein component of the polycomb repressive complex 2 (PRC2) enzyme involved in chromatin modification [51]. Analysis of The Cancer Genome Atlas (TCGA) data revealed that EZH2 mRNA expression was increased in mesothelioma tumor samples [50]. Silencing EZH2 induced the apoptosis in *BAP1*-mutant mesothelioma cell lines [50]. EZH2 inhibition also reduced the mesothelioma tumor size in *BAP1*-mutant mice [50]. By contrast, Schoumacher and colleagues showed that EZH2 was not overexpressed in UM cases, and subsequently, UM cases with BAP1 loss were insensitive to the EZH2 inhibitor, EPZ-6438 [52]. These findings highlight the tissue-dependent expression of epigenetic regulators and differing roles in carcinogenesis. Tazemetostat (an EZH2 inhibitor) has been tested in mesothelioma patients with BAP1 loss-of-function and showed some promising activity. The disease control of tazemetostat was 47% at 12 weeks and 25% of patients-maintained disease control at 24 weeks [53].

PARP inhibition is another potential targeted therapy option in patients with somatic or germline *BAP1* mutations. Clinical trials are underway to

investigate the role of PARP inhibitors in patients with DNA-repair protein defects, including BAP1. Currently there is a trial investigating niraparib (PARP inhibitor) (Clinicaltrials.gov ID NCT03207347) and three trials investigating olaparib (another PARP inhibitor) in BAP1 and other DDR deficient neoplasms (Clinicaltrials.gov ID NCT03786796, NCT03531840, NCT03375307). Combination therapies using nivolumab in combination with talazoparib in unresectable or metastatic melanoma patients with mutations in BRCA or BRCAness are also underway (NCT03531840).

A recent study published by Hassan et al. suggested that patients with pleural mesothelioma with loss-of-function mutations in *BAP1* and other DNA repair genes appeared to benefit from platinum chemotherapy compared with patients without inherited mutations [54].

2.8 Neurofibromatosis type 2

Neurofibromatosis type 2 (NF2) is another tumor suppressor gene most commonly associated with the disorder Neurofibromatosis 2, in which malignancies including vestibular schwannomas and meningiomas are common. However, in more recent years, the somatic mutations of NF2 have been linked to malignant mesothelioma, in addition to multiple other organ systems [55–57]. *NF2* gene is somatically mutated in 40–50% of MPM [57–59]. *NF2* encodes for a multifunctional protein named merlin which regulates the hippo signaling pathway among other pathways related to tumor progression and oncogenic activity [56, 60]. Disruption of the *NF2* tumor suppressor gene by mutation and/or deletion results in lack of expression of the functional merlin protein [61]. Merlin is a protein that regulates cellular cytoskeleton dynamic through its function as a linker between membrane proteins and the actin cytoskeleton. Merlin is involved in cell communication, adhesion, and motility, which are functions that are related to the invasive properties of malignant cells [62]. Merlin exerts its effect through forming a complex with the cytoplasmic kinase protein focal adhesion kinase (FAK) controlling cell adhesion, migration, and invasion through integrating signals from growth factor receptors and integrins [63–65]. Merlin inactivation is a critical step in MM pathogenesis and is related, at least in part, to upregulation of FAK activity. Merlin attenuates FAK phosphorylation and disrupt the interaction of FAK with its binding partners Src and p85, the regulatory subunit of Pi3K [58]. FAK expression and/or activity are reported to be upregulated in a wide range of malignancies including mesothelioma [62].

Loss of merlin, a product of the neurofibromatosis 2 tumor suppressor gene is being evaluated as a biomarker for FAK inhibitor sensitivity in mesothelioma. When NF2 is absent or inactivated, these regulation pathways are disrupted which result in the constitutive activation of oncogenesis [56]. Interestingly, when NF2 is reactivated and expressed in mesothelioma cells, invasiveness regresses [62]. Targeting NF2 or downstream proteins like FAK has become an attractive therapeutic strategy in mesothelioma. Defactinib (VS-6063) is a FAK inhibitor. Merlin-low mesothelioma cell lines are more sensitive to defactinib than merlin-high cell lines in vitro and in vivo [62].

Defactinib (VS-6063) has been evaluated as a single agent in MPM. The phase II COMMAND trial was a randomized, placebo-controlled phase II study of defactinib in patients with unresectable mesothelioma who had had a stable disease or a PR following at least 4 cycles of platinum-based pemetrexed. Patients were randomized to receive maintenance defactinib or placebo. Patients were stratified by tumor merlin immunohistochemistry status (high vs. low) prior to randomization, and the study aimed to measure the effect of treatment allocation on the overall survival and progression-free survival. The study showed no difference in the progression-free survival or overall survival between the two treatment arms

in the intent-to-treat population or in patients who had merlin-low tumors [66]. Defactinib is currently being evaluated in combination with pembrolizumab in patients with pleural mesothelioma (Clinicaltrials.gov NCT04201145).

Another therapeutic approach that is currently being evaluated in NF2 mutant MM is NEDD8 activating enzyme (NAE) inhibition. Merlin is a negative regulator of mTORC1 and the loss of Merlin results in constitutive activation of the mTORC pathway [67, 68]. The exact mechanism by which Merlin suppresses mTOR signaling is unknown.

Merlin also suppresses tumorigenesis by accumulating in the nucleus and binding to the cullin E3 ubiquitin ligase CRL4(DCAF1) which suppresses its ubiquitination activity [69]. Merlin loss drives tumorigenesis by activating the E3 ubiquitin ligase CRL4(DCAF1), thereby inhibiting the Hippo pathway component Lats [70]. MLN4924, a NEDD8 activating enzyme (NAE) inhibitor that suppresses CRL4(DCAF1), attenuates the activation of YAP in NF2-mutant tumor cells [70]. A phase I/II clinical trial is investigating MLN4924 (Pevonedistat) alone and in combination with chemotherapy in patients with mesothelioma. MLN4924 (Pevonedistat) is a NAE inhibitor that suppresses CRL4DCAF1 and attenuates the activation of YAP in NF2-mutant tumor cells.

2.9 Cyclin-dependent kinase inhibitor 2A (CDKN2A)

Cyclin-dependent kinase inhibitor 2A is a tumor suppressor gene that is commonly mutated in MM. It encodes both proteins INK4A and ARF [71]. INK4A inhibits critical cell cycle regulators cyclin-dependent kinase 4 (CDK4) and cyclin-dependent kinase 6 (CDK6) [72]. These two kinases function to activate retinoblastoma protein (RB) and allow for cell cycle progression [72]. Without INK4A, cell cycle progression remains unchecked and allows for continuation and possible proliferation of damaged DNA. ARF acts by promoting MDM2 degradation; this degradation is necessary for the activation of p53, a widely studied tumor suppressor [73]. With p53 activated, the cell cycle is arrested and growth is suppressed. Without ARF, p53 activation is limited and cell cycle progression can continue unchecked.

Mutations in CDKN2A have been shown to be induced by environmental toxins like asbestos [74]. Furthermore, the loss of CDKN2A in MM is associated with worse prognosis and decreased survival [15, 75].

3. Multi-biomarker-driven clinical trials

The Mesothelioma Stratified Therapy Trial (MiST) is a large multi-drug phase II clinical trial evaluating the use of different biomarkers for the treatment selection in relapsed mesothelioma. BRCA1/BAP1-mutated mesothelioma treatment is being studied with Rucarapib, a PARP inhibitor. PARP enzymes are critical for cell function; they aid in DNA transcription, repair, and cell cycle regulation [76]. It is believed that by inhibiting these critical enzymes, damage will accumulate within the cell and apoptosis will be induced. In patients with absent INK4A genes, apamaciclib is being studied. Apamaciclib is a selective CDK4/6 inhibitor, theoretically working to "replace" the function of INK4A in these mutated cells to stop the cell cycle progression and tumor growth [77]. Patients with PDL1 positive mesothelioma are being treated with Atezolizumab and Bevacizumab. Atezolizumab is an anti-PDL1 antibody that selectively binds to PDL1 and prevents its interaction with B7.1 on the antigen-presenting cell (APC). This inhibits the cancer cell from utilizing PDL1 to evade the immune system [78, 79]. Lastly, for patients with no biomarkers,

pembrolizumab and bemcentinib are being studied. Pembrolizumab is a monoclonal antibody against PD-1 and functions by binding PD-1 receptor on T-cells, inhibiting their binding with PDL1 [80, 81]. Bemcentinib is an AXL receptor tyrosine kinase inhibitor, a regulator of various critical cell functions including proliferation and motility, among others [82].

4. Conclusions

Over the last two decades, we have witnessed enormous efforts to identify prognostic and predictive biomarkers to inform treatment decisions in malignant mesothelioma. The medical and scientific community continue to search for optimal biomarkers to advance the field of precision medicine. Advances in molecular and diagnostic testing have not changed the current landscape of mesothelioma treatment. More biomarker-driven clinical trials are underway. The rarity of the disease makes it difficult to move these advances at a faster pace. Different pathways continue to be under investigation. These include: BAP1, NF2, CDKN2A, PD-L1, VEGF, WT-1, mesothelin, ASS, and aurora kinases. Biomarker-driven clinical trials, access to real-world data, and collaborative efforts should continue to move the field forward and help finding clinically actionable biomarkers.

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Conflict of interest

The authors declare no conflict of interest.

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References

[1] Carbone M et al. Mesothelioma: Scientific clues for prevention, diagnosis, and therapy. CA: A Cancer Journal for Clinicians. 2019;**69**(5):402-429

[2] Attanoos RL et al. Malignant mesothelioma and its non-asbestos causes. Archives of Pathology & Laboratory Medicine. 2018;**142**(6):753-760

[3] Testa JR et al. Germline BAP1 mutations predispose to malignant mesothelioma. Nature Genetics. 2011;**43**(10):1022-1025

[4] Ohta Y et al. VEGF and VEGF type C play an important role in angiogenesis and lymphangiogenesis in human malignant mesothelioma tumours. British Journal of Cancer. 1999;**81**(1):54-61

[5] Aoe K et al. Expression of vascular endothelial growth factor in malignant mesothelioma. Anticancer Research. 2006;**26**(6C):4833-4836

[6] Demirag F et al. Prognostic significance of vascular endothelial growth factor, tumor necrosis, and mitotic activity index in malignant pleural mesothelioma. Chest. 2005;**128**(5):3382-3387

[7] Zalcman G et al. Bevacizumab for newly diagnosed pleural mesothelioma in the Mesothelioma Avastin Cisplatin Pemetrexed Study (MAPS): A randomised, controlled, open-label, phase 3 trial. Lancet. 2016;**387**(10026):1405-1414

[8] Zauderer MG. A new standard for malignant pleural mesothelioma. Lancet. 2016;**387**(10026):1352-1354

[9] Dubey S et al. A phase II study of sorafenib in malignant mesothelioma: Results of Cancer and leukemia group B 30307. Journal of Thoracic Oncology. 2010;**5**(10):1655-1661

[10] Buikhuisen WA et al. A randomized phase II study adding axitinib to pemetrexed-cisplatin in patients with malignant pleural mesothelioma: A single-center trial combining clinical and translational outcomes. Journal of Thoracic Oncology. 2016;**11**(5):758-768

[11] Pagano M et al. Randomized phase II study on gemcitabine with or without ramucirumab as second-line treatment for advanced malignant pleural mesothelioma (MPM): Results of Italian Rames study. Journal of Clinical Oncology. 2020;**15**:9004

[12] Zou S et al. Arginine metabolism and deprivation in cancer therapy. Biomedicine & Pharmacotherapy. 2019;**118**:109210

[13] Fultang L et al. Molecular basis and current strategies of therapeutic arginine depletion for cancer. International Journal of Cancer. 2016;**139**(3):501-509

[14] Feun L et al. Arginine deprivation as a targeted therapy for cancer. Current Pharmaceutical Design. 2008;**14**(11):1049-1057

[15] López-Ríos F et al. Global gene expression profiling of pleural mesotheliomas: Overexpression of aurora kinases and P16/CDKN2A deletion as prognostic factors and critical evaluation of microarray-based prognostic prediction. Cancer Research. 2006;**66**(6):2970-2979

[16] Romagnoli S et al. Identification of potential therapeutic targets in malignant mesothelioma using cell-cycle gene expression analysis. The American Journal of Pathology. 2009;**174**(3):762-770

[17] Suraokar MB et al. Expression profiling stratifies mesothelioma tumors and signifies deregulation of spindle checkpoint pathway and microtubule network with therapeutic implications. Annals of Oncology. 2014;**25**(6):1184-1192

[18] Crispi S et al. Antiproliferative effect of Aurora kinase targeting in mesothelioma. Lung Cancer. 2010;**70**(3):271-279

[19] Cassandri M et al. Zinc-finger proteins in health and disease. Cell Death Discovery. 2017;**3**:17071

[20] Cedrés S et al. Expression of Wilms' tumor gene (WT1) is associated with survival in malignant pleural mesothelioma. Clinical & Translational Oncology. 2014;**16**(9):776-782

[21] Keilholz U et al. Wilms' tumour gene 1 (WT1) in human neoplasia. Leukemia. 2005;**19**(8):1318-1323

[22] Zauderer MG et al. A randomized phase II trial of adjuvant galinpepimut-S, WT-1 analogue peptide vaccine, after multimodality therapy for patients with malignant pleural mesothelioma. Clinical Cancer Research. 2017;**23**(24):7483-7489

[23] Hassan R et al. Localization of mesothelin in epithelial ovarian cancer. Applied Immunohistochemistry & Molecular Morphology. 2005;**13**(3):243-247

[24] Argani P et al. Mesothelin is overexpressed in the vast majority of ductal adenocarcinomas of the pancreas: Identification of a new pancreatic cancer marker by serial analysis of gene expression (SAGE). Clinical Cancer Research. 2001;**7**(12):3862-3868

[25] Thomas A et al. High mesothelin expression in advanced lung adenocarcinoma is associated with KRAS mutations and a poor prognosis. Oncotarget. 2015;**6**(13):11694-11703

[26] Hassan R et al. Clinical response of live-attenuated. Clinical Cancer Research. 2019;**25**(19):5787-5798

[27] Thapa B et al. Correlation of PD-L1 expression with immune cell infiltrates, genome-wide copy number aberrations and survival in mesothelioma. Journal of Clinical Oncology. 2016;**34**(15):8518-8518

[28] National Comprehensive Cancer Center Network, NCCN, Malignant Pleural Mesothelioma (Version 1.2020). Available from: https://www.nccn.org/. [Accessed: 03 September 2020]

[29] Scherpereel A et al. Nivolumab or nivolumab plus ipilimumab in patients with relapsed malignant pleural mesothelioma (IFCT-1501 MAPS2): A multicentre, open-label, randomised, non-comparative, phase 2 trial. The Lancet Oncology. 2019;**20**(2):239-253

[30] Metaxas Y et al. Pembrolizumab as palliative immunotherapy in malignant pleural mesothelioma. Journal of Thoracic Oncology. 2018;**13**(11):1784-1791

[31] Yu H et al. Tumor suppressor and deubiquitinase BAP1 promotes DNA double-strand break repair. Proceedings of the National Academy of Sciences of the United States of America. 2014;**111**(1):285-290

[32] Kittaneh M, Berkelhammer C. Detecting germline BAP1 mutations in patients with peritoneal mesothelioma: Benefits to patient and family members. Journal of Translational Medicine. 2018;**16**(1):194

[33] Bononi A et al. BAP1 regulates IP3R3-mediated Ca. Nature. 2017;**546**(7659):549-553

[34] Zhang Y et al. BAP1 links metabolic regulation of ferroptosis to tumour suppression. Nature Cell Biology. 2018;**20**(10):1181-1192

[35] Zauderer MG et al. Clinical characteristics of patients with malignant pleural mesothelioma harboring somatic BAP1 mutations. Journal of Thoracic Oncology. 2013;**8**(11):1430-1433

[36] Abdel-Rahman MH et al. Germline BAP1 mutation predisposes to uveal melanoma, lung adenocarcinoma, meningioma, and other cancers. Journal of Medical Genetics. 2011;**48**(12):856-859

[37] Carbone M et al. BAP1 cancer syndrome: Malignant mesothelioma, uveal and cutaneous melanoma, and MBAITs. Journal of Translational Medicine. 2012;**10**:179

[38] Dey A et al. Loss of the tumor suppressor BAP1 causes myeloid transformation. Science. 2012;**337**(6101):1541-1546

[39] Fan LH et al. BAP1 is a good prognostic factor in advanced non-small cell lung cancer. Clinical and Investigative Medicine. 2012;**35**(4):E182-E189

[40] Njauw CN et al. Germline BAP1 inactivation is preferentially associated with metastatic ocular melanoma and cutaneous-ocular melanoma families. PLOS One. 2012;**7**(4):e35295

[41] Wadt K et al. A cryptic BAP1 splice mutation in a family with uveal and cutaneous melanoma, and paraganglioma. Pigment Cell & Melanoma Research. 2012;**25**(6):815-818

[42] Bott M et al. The nuclear deubiquitinase BAP1 is commonly inactivated by somatic mutations and 3p21.1 losses in malignant pleural mesothelioma. Nature Genetics. 2011;**43**(7):668-672

[43] Arzt L et al. BAP1 protein is a progression factor in malignant pleural mesothelioma. Pathology Oncology Research. 2014;**20**(1):145-151

[44] Nasu M et al. High incidence of somatic BAP1 alterations in sporadic malignant mesothelioma. Journal of Thoracic Oncology. 2015;**10**(4):565-576

[45] Yoshikawa Y et al. Frequent inactivation of the BAP1 gene in epithelioid-type malignant mesothelioma. Cancer Science. 2012;**103**(5):868-874

[46] Baumann F et al. Mesothelioma patients with germline BAP1 mutations have 7-fold improved long-term survival. Carcinogenesis. 2015;**36**(1):76-81

[47] Landreville S et al. Histone deacetylase inhibitors induce growth arrest and differentiation in uveal melanoma. Clinical Cancer Research. 2012;**18**(2):408-416

[48] Kobrinski DA, Yang H, Kittaneh M. BAP1: Role in carcinogenesis and clinical implications. Translational Lung Cancer Research. 2020;**9**(Suppl 1):S60-S66

[49] Krug LM et al. Vorinostat in patients with advanced malignant pleural mesothelioma who have progressed on previous chemotherapy (VANTAGE-014): A phase 3, double-blind, randomised, placebo-controlled trial. The Lancet Oncology. 2015;**16**(4):447-456

[50] LaFave LM et al. Loss of BAP1 function leads to EZH2-dependent transformation. Nature Medicine. 2015;**21**(11):1344-1349

[51] Yamagishi M, Uchimaru K. Targeting EZH2 in cancer therapy. Current Opinion in Oncology. 2017;**29**(5):375-381

[52] Schoumacher M et al. Uveal melanoma cells are resistant to EZH2

inhibition regardless of BAP1 status. Nature Medicine. 2016;**22**(6):577-578

[53] Zauderer MG et al. Safety and efficacy of tazemetostat, an enhancer of zeste-homolog 2 inhibitor, in patients with relapsed or refractory malignant mesothelioma. Journal of Clinical Oncology. 2020;**38**(15):9058

[54] Hassan R et al. Inherited predisposition to malignant mesothelioma and overall survival following platinum chemotherapy. Proceedings of the National Academy of Sciences of the United States of America. 2019;**116**(18):9008-9013

[55] Petrilli AM, Fernández-Valle C. Role of merlin/NF2 inactivation in tumor biology. Oncogene. 2016;**35**(5):537-548

[56] Sato T, Sekido Y. NF2/merlin inactivation and potential therapeutic targets in mesothelioma. International Journal of Molecular Sciences. 2018;**19**(4):988

[57] Sekido Y et al. Neurofibromatosis type 2 (NF2) gene is somatically mutated in mesothelioma but not in lung cancer. Cancer Research. 1995;**55**(6):1227-1231

[58] Thurneysen C et al. Functional inactivation of NF2/merlin in human mesothelioma. Lung Cancer. 2009;**64**(2):140-147

[59] Bianchi AB et al. High frequency of inactivating mutations in the neurofibromatosis type 2 gene (NF2) in primary malignant mesotheliomas. Proceedings of the National Academy of Sciences of the United States of America. 1995;**92**(24):10854-10858

[60] Pan D. The hippo signaling pathway in development and cancer. Developmental Cell. 2010;**19**(4):491-505

[61] Butt Z et al. Pain and other symptoms in patients with hepatocellular carcinoma (HCC): A qualitative analysis. Journal of Clinical Oncology. 2013;**31**(15):e15187

[62] Poulikakos PI et al. Re-expression of the tumor suppressor NF2/merlin inhibits invasiveness in mesothelioma cells and negatively regulates FAK. Oncogene. 2006;**25**(44):5960-5968

[63] James MF et al. A NHERF binding site links the betaPDGFR to the cytoskeleton and regulates cell spreading and migration. Journal of Cell Science. 2004;**117**(Pt 14):2951-2961

[64] Schlaepfer DD, Mitra SK. Multiple connections link FAK to cell motility and invasion. Current Opinion in Genetics & Development. 2004;**14**(1):92-101

[65] Mitra SK, Hanson DA, Schlaepfer DD. Focal adhesion kinase: In command and control of cell motility. Nature Reviews. Molecular Cell Biology. 2005;**6**(1):56-68

[66] Fennell DA et al. Maintenance defactinib versus placebo after first-line chemotherapy in patients with merlin-stratified pleural mesothelioma: COMMAND—A double-blind, randomized, phase II study. Journal of Clinical Oncolog. 2019;**37**(10):790-798

[67] James MF et al. NF2/merlin is a novel negative regulator of mTOR complex 1, and activation of mTORC1 is associated with meningioma and schwannoma growth. Molecular and Cellular Biology. 2009;**29**(15):4250-4261

[68] López-Lago MA et al. Loss of the tumor suppressor gene NF2, encoding merlin, constitutively activates integrin-dependent mTORC1 signaling. Molecular and Cellular Biology. 2009;**29**(15):4235-4249

[69] Li W et al. Merlin/NF2 suppresses tumorigenesis by inhibiting the E3 ubiquitin ligase CRL4(DCAF1) in the nucleus. Cell. 2010;**140**(4):477-490

[70] Cooper J et al. Combined inhibition of NEDD8-activating enzyme and mTOR suppresses. Molecular Cancer Therapeutics. 2017;**16**(8):1693-1704

[71] Serrano M et al. Role of the INK4a locus in tumor suppression and cell mortality. Cell. 1996;**85**(1):27-37

[72] Serrano M, Hannon GJ, Beach D. A new regulatory motif in cell-cycle control causing specific inhibition of cyclin D/CDK4. Nature. 1993;**366**(6456):704-707

[73] Zhang Y, Xiong Y, Yarbrough WG. ARF promotes MDM2 degradation and stabilizes p53: ARF-INK4a locus deletion impairs both the Rb and p53 tumor suppression pathways. Cell. 1998;**92**(6):725-734

[74] Testa JR, Berns A. Preclinical models of malignant mesothelioma. Frontiers in Oncology. 2020;**10**:101

[75] Jennings CJ et al. Differential p16/INK4A cyclin-dependent kinase inhibitor expression correlates with chemotherapy efficacy in a cohort of 88 malignant pleural mesothelioma patients. British Journal of Cancer. 2015;**113**(1):69-75

[76] Morales J et al. Review of poly (ADP-ribose) polymerase (PARP) mechanisms of action and rationale for targeting in cancer and other diseases. Critical Reviews in Eukaryotic Gene Expression. 2014;**24**(1):15-28

[77] Sledge GW et al. MONARCH 2: Abemaciclib in combination with fulvestrant in women with HR+/ HER2– advanced breast cancer who had progressed while receiving endocrine therapy. Journal of Clinical Oncology. 2017;**35**(25):2875-2884

[78] Fehrenbacher L et al. Atezolizumab versus docetaxel for patients with previously treated non-small-cell lung cancer (POPLAR): A multicentre, open-label, phase 2 randomised controlled trial. Lancet. 2016;**387**(10030):1837-1846

[79] Rosenberg JE et al. Atezolizumab in patients with locally advanced and metastatic urothelial carcinoma who have progressed following treatment with platinum-based chemotherapy: A single-arm, multicentre, phase 2 trial. Lancet. 2016;**387**(10031):1909-1920

[80] Robert C et al. Anti-programmed-death-receptor-1 treatment with pembrolizumab in ipilimumab-refractory advanced melanoma: A randomised dose-comparison cohort of a phase 1 trial. Lancet. 2014;**384**(9948):1109-1117

[81] Hamid O et al. Safety and tumor responses with lambrolizumab (anti-PD-1) in melanoma. The New England Journal of Medicine. 2013;**369**(2):134-144

[82] Zhu C, Wei Y, Wei X. AXL receptor tyrosine kinase as a promising anti-cancer approach: Functions, molecular mechanisms and clinical applications. Molecular Cancer. 2019;**18**(1):153