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Published in London, United Kingdom







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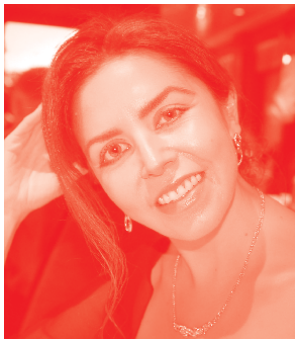

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http://dx.doi.org/10.5772/intechopen.89396
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First published in London, United Kingdom, 2020 by IntechOpen
IntechOpen is the global imprint of INTECHOPEN LIMITED, registered in England and Wales, registration number: 11086078, 5 Princes Gate Court, London, SW7 2QJ, United Kingdom
Printed in Croatia

British Library Cataloguing-in-Publication Data
A catalogue record for this book is available from the British Library

Additional hard and PDF copies can be obtained from orders@intechopen.com

Mesothelioma
Edited by Sonia Maciá
p. cm.
Print ISBN 978-1-83968-154-7
Online ISBN 978-1-83968-155-4
eBook (PDF) ISBN 978-1-83968-156-1

Meet the editor

Sonia Maciá, MD, is an ESMO-certified Medical Oncologist. She obtained a master's degree in Pharmacoeconomy in 2018 from Universitat Pompeu Fabra Barcelona and a PhD in Lung Cancer from the Department of Clinical Medicine, University Miguel Hernández. Dr. Maciá worked as a practicing Medical Oncologist for nine years and then decided to devote her career to clinical research. In 2012 she joined Pivotal SL, a European CRO, as Medical Manager, being thereafter promoted to Medical Director. After a few months in a similar role at ICON Plc, she joined Highlight Therapeutics to work on clinical development, providing medical support to clinical trials in solid tumors. Dr. Maciá has submitted more than forty abstracts to international congresses, published more than thirty papers in peer-reviewed journals, and authored six book chapters.

Contents

Preface XIII

Section 1
Introduction 1

Chapter 1 3
Mesothelioma, a Review of Current Guidelines
by Sonia Maciá

Section 2
Clinical and Therapeutics 15

Chapter 2 17
Peritoneal Mesothelioma: Clinical and Therapeutic Aspects
by Cristian Mesina, Mihaela-Iustina Mesina-Botoran, Theodor Viorel Dumitrescu, Mihai Calin Ciorbagiu and Cosmin Vasile Obleaga

Chapter 3 31
Current Mesothelioma Treatment and Future Perspectives
by Danijela Štrbac, Katja Goričar, Viljem Kovač and Vita Dolžan

Section 3
Emerging Drugs 43

Chapter 4 45
Emerging Drug Therapies for Mesothelioma
by Derek B. Oien, Jeremy Chien, Julian Molina and Viji Shridhar

Section 4
Predictive and Prognostic Biomarkers 59

Chapter 5 61
Predictive and Prognosis Factors of Clinical Utility in Mesothelioma
by Rodríguez-Cid Jeronimo Rafael and Flores-Mariñelarena Rodrigo Rafael

Chapter 6 79
Biomarkers Progress and Therapeutic Implications in Malignant Mesothelioma
by Jordyn Feinstein and Muaiad Kittaneh

Chapter 7 91
Genetic Alterations of Malignant Pleural Mesothelima
by Benjamin Wadowski, David T. Severson, Raphael Bueno and Assunta De Rienzo

Preface

Mesothelioma is a rare type of cancer belonging to those types of tumors for which there has been a lack of treatment advances in recent years. Overall, it is considered an extended disease with very limited aggressive treatment approaches.

This book presents a description of the most relevant topics on diagnosis, biomarkers, and treatment updates, including interesting discussion on prognostic features and novel therapies that are either approved or under clinical development.

Chapters provide insight into the current challenges in assessing predictive biomarkers and targeted therapies.

I thank the chapter authors for their valuable contributions and commitment to provide a clear and succinct overview of these topics.

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Section 1

Introduction

Chapter 1

Mesothelioma, a Review of Current Guidelines

Sonia Maciá

Abstract

Mesothelioma is considered as a rare tumor originating in the mesothelial surfaces of pleura or, more rarely, in other sites such as peritoneum, which harbors a very poor prognosis. Despite clinical research efforts, lack of available therapies remains clear. Standard of care treatments and guidelines have not been evolved much along recent years. In this chapter, main guidelines will be reviewed, besides a systematic Pubmed review, with a focus on epidemiology, diagnosis tests, and approved local and systemic treatments, including most important advances. Searched terms included "mesothelioma," "ESMO and NCCN guidelines," "diagnosis," "surgery," "targeted therapy," "clinical trials," "palliative treatment," and "meta-analysis." First-line regimen recommendations have not evolved since the phase III pivotal study of cisplatin-pemetrexed was published, and this combination became the standard of care. Targeted therapies have brought disappointing results. However, recent clinical trial data with immunotherapies are bringing some light and may become a new paradigm in the following years.

Keywords: malignant mesothelioma, chemotherapy, pemetrexed, immunotherapy, clinical trials, nivolumab, pembrolizumab, targeted therapy

1. Introduction

Malignant mesothelioma (MM) is a fatal disease which originates in the mesothelial surfaces of pleura or, more rarely, in other sites such as peritoneum. Most cases have been classically linked to asbestos exposure; however, ionizing radiation may also increase the risk of mesothelioma [1].

Its prognosis is very poor and it is difficult to treat, mainly because most patients are diagnosed with advanced disease [1–3]. Despite clinical research efforts, lack of available therapies remains clear and median overall survival is still approximately 1 year, with only 10% patients alive 5 years after diagnosis. Standard of care treatments and guidelines have not been evolved much along recent years. In this chapter, NCCN and ESMO guidelines have been reviewed, besides an electronic search of the Pubmed database, with a focus on the phase II and III clinical trials, guidelines, meta-analysis, and systematic reviews regarding epidemiology, diagnosis tests, surgical approach, and approved local and systemic treatments, including most important advances. Searched terms included "mesothelioma," "ESMO and NCCN guidelines," "diagnosis," "surgery," "targeted therapy," "clinical trials," "palliative treatment," and "meta-analysis." First-line regimen recommendations have not evolved since the phase III pivotal study of cisplatin-pemetrexed was published, and this combination became the standard of care despite its modest benefit

in survival. Pemetrexed seems to be the most active drug, but its use in the first-line setting limits its administration in further lines. However, a rechallenge may be done in responder patients, who might still get benefit [4].

Only few drugs have demonstrated a mild activity in refractory MM, and targeted therapies have provided disappointing results so far. However, recent clinical trial data with immunotherapies are bringing some light and may become a new paradigm in the following years.

2. Epidemiology

Malignant mesothelioma (MM) is a rare tumor, with an incidence of less than 5 out of 100,000 inhabitants in Europe [1]. Diagnosis is usually done when disease is well advanced, and patients have a high symptom burden [3]. Incidence has decreased along the last decades globally worldwide. Mesothelioma has been typically related to asbestos exposure, which is the most well-known risk factor, although the latency period can be long, with a latency period being approximately 40 years, although in some cases, it may be as long as 60–70 years. Recent reports have suggested that also ionizing radiation may have a role, such as in patients previously treated with radiotherapy (RT). Other studies also suggest that erionite (which may be found in travel roads) increases the risk of MM. Smoking is not a risk factor. There may be a genetic risk in patients with BRCA-1 mutation [5–7].

The most common type of mesothelioma is malignant pleural mesothelioma, being up to 70% cases, followed by peritoneal (30%) and pericardial mesothelioma (1–2%) [2]. According to histology, there are three subtypes: epithelial, sarcomatoid, and biphasic [3], with epithelial subtype having a better prognosis.

Prevalence is highly linked to mortality, and mesothelioma is an unmet medical need due to its very poor prognosis, having a median overall survival of approximately 9–12 months, with only very modest improvements in survival over time [8].

3. Diagnosis

Most common symptoms include dyspnea, thoracic pain, and weight loss. Usually unilateral effusions are observed. A detailed occupational history is key, checking asbestos exposure among other previously exposed potential risk factors. Patients often present with advanced disease, but without distant metastases, as local implants or effusion cause pain and/or dyspnea. Brain metastases are rare [3].

Diagnosis assessments include chest X-ray, computed tomography (CT) scan of chest and upper abdomen, and thoracentesis, with examination of the pleural effusion and general laboratory blood tests [1]. Citology samples from pleural effusion are frequently negative or inconclusive, hence, histology may bring some further light for a more accurate diagnosis. Some biomarkers may be helpful, including calretinin, WT-1, D2-40, and citokeratyn 5/6, being negative in mesothelioma and positive in lung adenocarcinoma [9]. In order to obtain adequate histology, a thoracoscopy is highly recommended to optimally stage and to allow pleural fluid evacuation (with or without pleurodesis) [9, 10]. Mesothelioma can be difficult to identify and distinguish from benign pleural lesions and from other malignancies; it is therefore recommended to obtain biopsies from the tissue of both abnormal and normal appearance. When a thoracoscopy is not feasible or contraindicated, ultrasound-guided true-cut biopsies are a good alternative [10].

4. Pathology

MM comprises a heterogeneous group of tumors, which are mainly classified as three subtypes (epithelioid, biphasic, and sarcomatoid), despite the numerous variants that are described in the 2004 WHO classification [9].

Diagnosis samples may be obtained from pleural effusions, pleural biopsies, and surgical samples [1, 8–10]. Cytological diagnosis from effusion samples may be feasible, but sensitivity is highly variant, with variable atypia (usually low grade). Therefore, usually tissue biopsies with immunohistochemistry analysis are pivotal for confirmatory diagnosis.

Standardly used and most recommended biomarkers for diagnosis include calretinin, cytokeratin 5/6, WT1, and podoplanin (D240). For non-small cell adcenocarcinoma, the most useful markers are TTF1, CEA, and EP4 [8].

5. Staging

Staging procedures are aimed to describe anatomical extent correlating with prognostic features, which is key in order to make treatment decisions. Standard procedures for staging include chest and abdomen CT with contrast and PET/CT (for those patients who may undergo surgery). Video-assisted thoracoscopy (VATS) is recommended if contralateral disease is suspected [3].

Patients should be evaluated by a multidisciplinary committee, including oncologist, radiation oncologist, pathologist, pulmonologist, diagnostic imaging specialist, and surgeon.

The limitation of most classifications is their inaccuracy in describing tumor (T-) and node (N-) extent. The most recent staging system was presented by the International Mesothelioma Interest Group (IMIG) [11]. However, it failed to be an independent prognostic factor when analyzed in the clinical setting using multivariate analysis [11–14]. Hence, further workup is needed in order to get an accurate and prognostic staging system.

If a surgical resection is planned, either mediastinoscopy or endobronchial ultrasound of mediastinal lymph nodes are recommended [15]. Besides, two additional tests may be useful if suggested by imaging: laparoscopy in order to rule out any transdiaphragmatic extension and chest MRI to check vascular involvement [14–17].

6. Treatment for mesothelioma

6.1 First-line therapy for mesothelioma

Chemotherapy is recommended as the sole therapy for patients with ECOG 0–2 who are not amenable for surgery. For patients with ECOG 3–4, best supportive care is strongly recommended.

Chemotherapy has a role in the palliative treatment of advanced mesothelioma, getting an improvement of symptoms and modest benefit in survival. Standard first-line treatment is based on platinum doublets, with either pemetrexed or raltitrexed [18, 19], being cisplatin/pemetrexed the only FDA-approved regimen. This combination was investigated in a phase III trial comparing cisplatin/pemetrexed vs. cisplatin monotherapy, getting a benefit in survival by 2.8 months (12.1 vs. 9.3 months, $P = 0.02$) [18].

Carboplatin may be used as an alternative to cisplatin, particularly in fragile patients, with no significant differences in survival and a better safety profile [20, 21].

Clinical research has been trying to look for an improvement with the addition of several agents; however, several phase II trials have failed to demonstrate improvement over standard treatment with the addition of antioangiogenics such as bevacizumab or sunitinib [22, 23]. However, a phase III trial compared cisplatin/pemetrexed with or without bevacizumab in patients who were suitable for receiving bevacizumab (ECOG 0–2 with no history of bleeding or thrombosis). Experimental arm was better in terms of survival, with a benefit by 2.7 months (18.8 vs. 16.1 months, P = 0.0167). Grade 3–4 adverse events were more common in the experimental arm, 71 vs. 62%, with more cases of hypertension, grade 3 proteinuria and grade 3–4 thromboembolic events in the bevacizumab arm. The NCCN guidelines then recommends cisplatin/pemetrexed plus bevacizumab followed by maintenance bevacizumab in patients without contraindications [24].

6.2 Second-line therapy for mesothelioma

There is a lack of treatment options in the second line and beyond setting, this being an important medical need with no standard of care yet. Pemetrexed as single agent when compared with the best supportive care was not able to provide an improvement in survival [25]. Vinorelbine showed a benefit in terms of responses in several small phase II trials [26].

Both immunotherapies and targeted therapies are under evaluation as well, but they have not been yielded into approval [27, 28]. In the absence of the standard second-line or further-line therapy, it is recommended that patients are enrolled into clinical trials. Recent data suggest that checkpoint inhibitors may have a role in this setting, with a response rate slightly higher than that previously obtained by other agents [3].

Checkpoint inhibitors target the programmed death-1 (PD-1) receptor, which improves tumor immunity. Both nivolumab and pembrolizumab target PD-1 receptors, but testing this receptor is not required [29].

6.3 Immunotherapy and targeted therapies

Some immunotherapies have been tested or are under clinical development for MPM, including antibodies blocking immune checkpoints that function as negative regulators of T-cell function, cytotoxic T-lymphocyte-associated antigen 4 (CTLA4), programmed death 1 (PD-1), and programmed death ligand 1 (PD-L1). However, there is still a lack of strong support for their use.

In two nonrandomized studies, the anti-CTLA4 antibody tremelimumab showed preliminary evidence of activity in patients with previously treated mesothelioma [28, 30]. Thereafter, a randomized, placebo-controlled study investigated tremelimumab in patients with mesothelioma (the DETERMINE trial). This trial did not meet the primary end point of OS, as we did not find statistically significant differences in OS between the tremelimumab group [median OS 7.7 months (95% CI: 6.8–8.9)] and the placebo group [median OS 7.3 months (95% CI: 5.9–8.7)] [31].

In the KEYNOTE-028 trial, previously treated patients with PD-L1-positive MPM received pembrolizumab 10 mg/kg every 2 weeks for up to 2 years or until confirmed progression or unacceptable toxicity. Five of 25 patients (20%) had a partial response (objective response rate of 20%) and 13 (52%) patients had stable disease. Additionally, there was a maintained clinical benefit, with a median duration of response 12.0 months (95% CI: 3.7 not reached) [32, 33]. The NivoMes

study, which evaluated nivolumab in unselected patients with previously treated mesothelioma reported response rates of 28%. The JAVELIN study of the anti-PDL-1 antibody avelumab in unselected patients with previously treated mesothelioma reported a response rate of 9.4% with a median PFS of 17.1 weeks. Subgroup analysis in the PD-L1-positive population (cutoff > 5%) showed a response rate of 14% [34]. Novel vaccine approaches using MPM neoantigens identified by gene sequencing are also entering clinical trial on the basis of early animal studies [33].

As a summary, preliminary data on PD-1- and PD-L1-targeting monoclonal antibodies in MPM suggest that immunotherapy with single agents may have some benefit, possibly because of its complex biology.

6.4 Radiotherapy

Administering RT to the entire pleural surface without damaging radiosensitive sites and keeping a good safety profile is very challenging. Radiotherapy (RT) is used in different settings as treatment for MM: palliative, adjuvant, and as part of a multimodality treatment.

As palliative treatment for pain relief bronchial obstruction or other disease related symptoms, there is no strong evidence to support its use; however, it may be recommended in cases of infiltration of the chest wall, administered in short courses such as 1 × 10 or 3 × 8 Gy [35], always understanding that dose of radiation should be based on its purpose.

6.4.1 Pre- and postoperative RT

Limited evidence is available, extracted from retrospective studies only. In general results are poor, in terms of disease control rate, because of the complex growth patterns of the disease. Furthermore, its safety profile is poor due to the wide field size and neighboring vital organs. The introduction of intensity-modulated RT (IMRT) seem to overcome most of these issues and allow the remaining tumor tissue to be properly irradiated. Preliminary results adjuvant IMRT seemed particularly promising. Further studies are needed to better establish the role of RT. Recent studies have underlined the importance of RT technique, both in terms of local control and toxicity. It is therefore recommended that RT is delivered in specialized centers (expert advice) [36, 37].

6.5 Surgery

Surgery may be recommended for patients with stage I to IIIA disease who are in good conditions and are medically operable. A careful assessment before proceeding to surgery is strongly recommended [1, 3].

Objectives of surgery are staging, palliative, and, more uncommonly, curative intent.

6.5.1 Surgery with radical intent

It cannot be considered to have a real radical intention, as its objective is actually obtaining a macroscopic resection removing as much tumor as possible since it is virtually impossible to obtain free resection margins [1]. It can include pleurectomy/decortication (complete removal of involved pleural and all gross tumor) or extrapleural pneumonectomy, including in bloc resection of pleura, lung diaphragm, and often also part of pericardium [38].

Some studies assessed a second-step surgery, following an induction chemotherapy, which is reported as a trimodality approach. Different combined modality regimens have been investigated.

The European Organization for Research and Treatment of Cancer (EORTC) analyzed trimodality therapy in a phase II trial (EORTC 08031). Patients with MM (up to stage cT3N1M0) received induction chemotherapy (cisplatin and pemetrexed × 3) followed by surgery within 21–56 days. Forty-two out of 57 (73.7%) included patients could undergo surgery. Survival figures were positive, with an overall survival of 18.4 months and 13.9 months progression-free survival. Operative mortality was 6.4% [39].

Other phase II trial with a similar design was performed in the USA and included 77 patients, achieving an overall survival of 16.8 months, with an operative mortality of 7% [40].

Although trimodal therapy seemed feasible in selected patients with promising results, it was further evaluated in a phase III trial in the UK with negative results (MARS1 study). In this trial, mortality was as high as 18.8%, with only 45% patients undergoing surgery after induction treatment, and with a lower survival for patients undergoing surgery compared to the control arm where patients received only the induction therapy (14 vs. 19 months) [41].

However, a systematic review performed afterward, including 34 studies from 26 institutions, found highly variant results, with the median survival ranging from 9.4 to 27.5 months and surgical morbidity from 22 to 82%. Probably, it may be explained by different surgical approaches, variability in terms of surgeon's prior experience, and heterogeneity of included patients, but some patients may get benefit from this treatment [42]. A multidisciplinary team with sufficient experience should provide recommendations on the suitability of patients for trimodality therapy.

6.5.2 Surgery for staging and palliation

Control pleural effusion, talc poudrage, or even decortication in a captured lung may be performed through surgery. One study compared VATS (partial) pleurectomy vs. standard talc poudrage in 196 patients. There was no benefit in terms of survival, but control of pleural effusion and quality of life were significantly better for experimental arm at 6 and 12 months [43].

7. Conclusions

This chapter shows a review of both NCCN and ESMO guidelines besides PubMed available literature. Mesothelioma is one of those tumors with less advanced in the recent years, probably due to its aggressive nature and the limited incidence, which makes clinical research more time consuming. This is considered still as a medical need due to the lack of treatment options beyond the second line. However, research is improving and some immunooncology agents have started to show a small but significant benefit in terms of survival.

Conflict of interest

The author declares no conflict of interest.

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Section 2

Clinical and Therapeutics

Chapter 2

Peritoneal Mesothelioma: Clinical and Therapeutic Aspects

Cristian Mesina, Mihaela-Iustina Mesina-Botoran, Theodor Viorel Dumitrescu, Mihai Calin Ciorbagiu and Cosmin Vasile Obleaga

Abstract

Mesothelioma is a very rare malignant disease that originates from mesothelial cells that line the serosa: pleura, peritoneum, pericardium, or testicular vaginal tunic. Peritoneal mesothelioma accounts for 7–10% of all mesotheliomas diagnosed, and ranks second after pleural localization of mesothelioma. The incidence of peritoneal mesothelioma is 0.5–3 cases per million in men and 0.2–2 cases per million in women. Diagnosis of peritoneal mesothelioma is difficult due to nonspecific symptoms and because of this patients present in advanced stages of the disease. Histologically there are three major categories of malignant peritoneal mesothelioma: epithelioid, sarcomatoid, and biphasic. The differential diagnosis of peritoneal mesothelioma is made with peritoneal pseudomyxoma, ovarian tumors, and peritoneal metastases from colorectal cancer. An important role in differential diagnosis, in addition to immunohistochemistry, is played by various tumor markers and genetic tests. The treatment of peritoneal mesothelioma is performed by cytoreductive surgery (CRS) with hyperthermic intraperitoneal chemotherapy (HIPEC), with good results for patients in the early stages of the disease. For patients with advanced disease, a new treatment has been proposed: pressurized intraperitoneal aerosol chemotherapy (PIPAC). For patients who cannot use CRS and HIPEC, the only therapeutic option remains chemotherapy (systemic + intraperitoneal).

Keywords: peritoneal mesothelioma, cytoreductive surgery, hyperthermic intraperitoneal chemotherapy

1. Introduction

Mesotheliomas arise from cells lining the serosa: pleural, pericardial, peritoneal, and testicular vaginal tunic. Mesothelial tumors range from localized malignant mesothelioma to aggressive diffuse malignancies that invade the anatomical structures of the neighborhood and can give distant metastases. Rare mesothelial tumors that represent less than 1% of all diagnosed mesothelial tumors are paratesticular mesothelioma and pericardial mesothelioma. The peritoneal localization of mesothelioma is on the second place after the pleural localization. Peritoneal mesothelioma (PM) is a rare disease with an incidence of 0.6–3 per million in men and 0.2–2 per million in women [1]. Diffuse malignant

peritoneal mesothelioma (DMPM), which accounts for 30% of all malignant mesotheliomas, is characterized by symptomatic polymorphism and difficulty in establishing a positive diagnosis. In this sense, the immunohistochemical examination has a very important role in differentiating this disease from peritoneal carcinomatosis [2].

2. Peritoneal mesothelioma: Symptomatology, histopathology, differential diagnosis, and treatment

2.1 Symptomatology of PM

The vast majority of patients are asymptomatic. The most common signs appear when the tumor mass compresses the neighboring organs or the rupture of cystic tumor formations mimicking the symptoms of acute peritonitis, as happened in the case operated and treated in our surgery clinic. Thus, the most common symptoms are abdominal pain, ascites, anorexia, weight loss, palpable tumor formation, and localized or generalized muscle defense [3].

Due to the more frequent localization of peritoneal mesothelioma on the pelvic peritoneum, peritoneal adhesions appear on the rectum, uterus, and bladder, causing the appearance of other symptoms such as dysuria, urinary symptoms, intestinal obstruction, and dyspareunia.

Peritoneal mesothelioma should be differentiated from multicystic peritoneal mesothelioma (MCPM) which is a benign, multicystic abdominal tumor such as cystic lymphangioma, endometriosis, cystic adenomatoid tumor, pseudomyxoma peritonei, and malignant peritoneal mesothelioma. For the positive diagnosis of MCPM, it is necessary to perform an immunohistochemical examination [3].

Benign multicystic peritoneal mesothelioma (BMPM) known as multilocular peritoneal cysts is an extremely rare disease which has the peritoneal mesothelium as a starting point. Although this disease is considered benign, relapse after surgery is reported in over 50% of cases [4] and two cases of malignant transformation have been reported [5]. Pathogenesis of the disease is unknown. There is a discussion of a possible etiopathogenicity related to pelvic inflammatory disease, Mediterranean fever, endometriosis, and a history of abdominal surgery. Three hypotheses have been proposed in the etiology of BMPM disease. One hypothesis argues that BMPM arises from an inflammatory process involving peritoneum, which results in hyperplastic and dysplastic reactive transformation of peritoneal mesothelial cells. Another theory supports the primary neoplastic origin without the involvement of a chronic inflammatory process. Other authors support the hormonal theory in which the development and progression of BMPM is closely related to sensitivity to sexual hormones. This theory is supported by the fact that BMPM has a higher incidence in women during the reproductive period and that BMPM responds to tamoxifen and gonadotropin-releasing hormone analogs [6]. Most authors agree on the fact that chronic peritoneal inflammatory process causes proliferation and migration of peripheral mesothelial cells often associated with metaplasia of the underlying connective tissue [6–8]. Transition between multicystic mesothelioma and adenomatoid tumor has been observed on several occasions [9, 10].

The symptoms of BMPM are insignificant but become apparent when the cystic tumors are large enough to produce mass effect on surrounding organs, or if the cysts break and produce an acute peritonitis-like reaction, as we have shown. Symptoms may be chronic abdominal and/or pelvic pain, abdominal distension, intestinal obstruction, and intestinal transit disorders [1, 11–13].

The physical examination may reveal muscle defense, abdominal distension, or acute appendicitis-like symptoms [14].

There are benign or malignant diseases that can mime BMPM. These diseases are intestinal lymphangioma and malignant peritoneal mesothelioma. Lymphangioma can be diagnosed when the cysts contain predominantly chyli fluid [15] and when the presence of lymphoid aggregates, smooth muscle cells, and D2-40-positive immunoexpression is discovered in the immunohistochemical examination. Malignant peritoneal mesothelioma has a history of asbestos exposure, abdominal pain, and weight loss.

2.2 Histopathology of PM

Three histological types of peritoneal mesothelioma have been described: epithelioid, sarcomatoid, and biphasic. Patients with sarcomatoid and biphasic subtypes have a more reserved prognosis than patients with the epithelioid subtype. Multicystic mesothelioma and well-differentiated papillary mesothelioma are forms of peritoneal mesothelioma that have a favorable prognosis.

2.2.1 Benign mesothelioma

Benign mesothelioma is a term applied to solitary lesions of peritoneum. Two types of benign mesothelial proliferation in the peritoneal cavity are benign multicystic peritoneal mesothelioma (MCPM) and adenomatoid tumor.

2.2.2 Malignant mesothelioma

Malignant mesothelioma is commonly found in adults and serum levels of osteopontin and mesothelin are serum biomarkers used for diagnosis.

Well-differentiated papillary mesothelioma of peritoneum is multicentric, extensive, and is characterized by prominent formation of papillae lined by bland mesothelial cell with minimal or no invasion. These are associated with an evolution without clinical symptoms, and people with this clinical form of mesothelioma have a long survival.

Deciduoid mesothelioma is characterized by the presence of large tumor cells with an abundant ground-glass cytoplasm that simulates the appearance of decidual cells. This histological form has been described in young women, located not only in the pelviperitoneal cavity but also in the pleural cavity in patients of both sexes. It is characterized by a short survival.

Mesothelioma with clear cell features can be confused with metastatic carcinoma from the kidney. The cytoplasmic clearing is due to the accumulation of glycogen in which case the alternative term glycogen-rich mesothelioma has been used.

Malignant mesothelioma with small cell is characterized by the presence of small cells. Most of reported cases have been immunoreactive for keratin and mesothelial markers including calretinin, CK 5/6, WT1, and podoplanin; some cases also stained for neuron-specific enolase and occasionally CD 57.

Lymphohistiocytoid mesothelioma is characterized microscopically by a diffuse proliferation of atypical histiocyte-like malignant mesothelial cells admixed with numerous lymphocytes (T-cell type) and lesser number of plasma cells. The phenotype of the histiocyte-like elements reflects their mesothelial nature and the behavior of this tumor is aggressive.

Pleomorphic mesothelioma in the WHO classification scheme is considered a variant of epithelioid mesothelioma and is characterized by pleomorphic large cells with abundant eosinophilic cytoplasm and single or multiple nuclei with

marked variation in size and large nucleoli. The staining for traditional markers of mesothelioma-like calretinin, CK 5/6, and WT1 is variable but they are intense positive for pankeratin and cytokeratin 7. These tumors are a variant of sarcomatous tumors rather than epithelioid mesothelioma, being characterized by an aggressive behavior characteristic of sarcomatous tumors.

Desmoplastic mesothelioma is a subtype of sarcomatoid epithelioma, characterized by abundant deposition of fibrous tissue demonstrating a storiform arrangement of neoplastic spindle cells. The main differential diagnosis is with benign fibrous proliferations. Immunohistochemical receptors for keratin, calretinin, and WT1 is in favor of desmoplastic mesothelioma.

2.3 Role of immunohistochemistry, electron microscopy, and molecular testing in differential diagnosis of mesothelioma

2.3.1 Immunohistochemistry and electron microscopy

The diagnosis of malignant mesothelioma in the absence of detectable invasion is problematic in the absence of invasive disease. Homozygous deletion of $p16^{INK4a}$ (CDKN2A) detected using a fluorescent in situ hybridization (FISH) assay and loss of BAP1 expression by immunohistochemistry may be helpful in separating benign from malignant mesothelial proliferations including desmoplastic mesothelioma.

Other immunostains such as epithelial membrane antigen (EMA), p53, GLUT1, and IMP3 are proposed for separating benign from malignant mesothelial proliferations. Malignant epithelioid mesotheliomas need to be distinguished from metastatic carcinoma, specially adenocarcinomas with pseudo-mesotheliomatous growth pattern [3, 16].

The role of immunohistochemistry is in separating sarcomatoid mesotheliomas from sarcomatoid carcinomas and soft tissue sarcomas [2]. Mesotheliomas usually produce large amounts of hyaluronic acid, which can be demonstrated with the alcian blue or colloidal iron stains. The presence of obvious droplets of mucicarmine-positive or periodic acid-Schiff (PAS)-positive material in the cytoplasm of the tumor cell makes the diagnosis of mesothelioma very unlikely, although it does not rule it out completely inasmuch as the existence of rare mucin-positive mesotheliomas has been demonstrated.

Electron microscopy played an important role in the differential diagnosis between mesothelioma and metastatic carcinoma. This was primarily based on the appearance of the microvilli in the apical surface of the tumor cells, which in mesothelioma are longer and more slender than those in adenocarcinoma.

Many metastatic adenocarcinomas likely to be confused with mesothelioma are positive for cytokeratin 7, as are epithelioid mesotheliomas, making cytokeratin 7, as are epithelioid mesotheliomas, making cytokeratin 7 and 20 of limited value except in very specific context of metastases from the gastrointestinal tract.

The following immunostains are most commonly available and utilized in differential diagnosis of mesothelioma:

1. Epithelial markers that are usually present in both tumors (mesothelioma and metastatic carcinoma): pankeratins, EMA, and basement membrane components;

2. Organ-associated and lineage-specific markers that are often expressed in metastatic carcinoma but not mesothelioma: napsin A (lung and kidney), PAX8 (kidney, mullerian, thymus), CDX2 (gastrointestinal tract, pancreatobiliary),

p63/p40 (squamous cell, urothelial), and GATA3 (breast, urothelial, squamous cell);

3. Markers that are usually expressed in metastatic carcinoma but not mesothelioma: MOC-31, Ber-EP4, carcinoembryonic antigen (CEA), B72.3, BG8, CD15, MUC4, and claudiu-4;

4. Markers that are usually expressed in mesothelioma but not in carcinoma: calretinin (breast, mullerian serous), WT1 (breast, mullerian serous), keratin 5/6 (urothelial, squamous cell), D2-40/podoplanin (mullerian serous, squamous cell), and thrombomodulin (squamous cell).

2.3.2 Molecular genetic features

Mutations in the TP 53 gene are uncommon. In 60–80% of mesothelioma cases, homozygous deletion of $p16^{INK4a}$ (CDKN2A) is found, which is an investigation used to differentiate benign mesothelial disorders from malignant mesothelial proliferations. CDKN2A deletion is a potential biomarker for a more aggressive course in some cases of mesothelioma. The most common recurrent somatic mutations in malignant mesothelioma target three genes functioning as tumor suppressors: cyclin-dependent kinase inhibitor 2A (CDKN2A), BCRA1-associated protein 1 (BAP1), and neurofibromin 2 (merlin) (NF2).

2.3.3 Differential radiological and histopathological diagnosis

Differential diagnosis is made with other peritoneal malignancies such as peritoneal pseudomyxoma, ovarian tumors, and peritoneal metastases from colorectal cancer. Peritoneal pseudomyxoma is a rare disease characterized by multifocal epithelial deposits in the peritoneal cavity, secreted by mucin, with or without gelatinous ascites, in the absence of extraperitoneal involvement [17]. It was first described by Werth and later by Rokitansky in 1942, being considered a fatal condition, with unexplained etiology. It predominates in women, the ovarian tumor pathology being incriminated as responsible in a significant percentage in the etiopathogenesis of peritoneal pseudomyxoma. In men, adenoma (mucocele) appendicular tumors and appendicular adenocarcinoma are the main cause described [17]. Virtually any primary solid tumor is the epicenter of the malignancy. In the case of peritoneal pseudomixoma, the predominant tumor volume is in the peritoneum, and the primary tumor is insignificant, whether it is appendicular, ovarian, or in other organs [18]. Pseudomyxoma peritonei involves the presence of mucinous, gelatinous deposits in the peritoneum, deposits that can reach impressive sizes. Thus, death can be caused by respiratory failure. It seems that the basis of this condition is a certain type of mucous cells that have a special pattern—the presence of MUC2 [18]. Removal of the tumor and gelatinous material is the purpose of treatment.

Peritoneal pseudomixoma is the most serious complication of the appendicular mucocele and develops as a result of spontaneous or iatrogenic implantation of the tumor into the peritoneal cavity [19]. The peritoneal and occasionally pleural pseudomixoma, which appeared as a result of the evolution of the appendicular mucocele, is rare and constitutes 6–8.8% [19–21]. Pseudomucinous cysts of the ovary, usually associated with appendicular mucocele, are the predominant cause of peritoneal pseudomixoma in older women and in men; the origin of peritoneal pseudomixoma is usually the vermicular appendix [21]. The pathology has a slow

evolution through the loss of intestinal function, fistula formation, and eventual death. The most common complications are occlusion and intestinal bleeding.

Extra-abdominal eruption of appendicular cystadenocarcinoma with spontaneous cutaneous fistula formation is extremely rare, being published only four cases in the world literature [22–25]. The pathogenetic mechanism of spontaneous skin fistula formation in patients with mucinous cystadenocarcinoma of the appendix is enigmatic, but we assume that the occurrence of this complication depends on the malignant nature of the tumor.

Patients with appendicular mucocele are asymptomatic in about 25% of cases; even in the case of large lesions, the most common complaints are pain in the right iliac fossa, similar to acute appendicitis and palpable tumor formation in 50% of cases [20, 21].

2.4 Treatment

Malignant peritoneal mesothelioma (MPM) is a rare disease with a recurrence rate of 40–50% after surgical debulking. Identifying the histological type of peritoneal mesothelioma, the number of invaded lymph nodes, and the Ki-67 proliferation marker are very important parameters for surgical treatment, but this is possible in most cases after laparotomy and cytoreductive surgery (CRS). The preoperative CT scan, performed by an experienced radiologist, can help us identify anatomical sites unfavorable for surgical treatment such as intestinal serosa and/or porta hepatis [26].

2.4.1 Cytoreductive surgery (CRS)

For the selection of patients benefiting from CRS, the peritoneal cancer index (PCI) is used, which consists of combining a score [27] given by 13 abdominopelvic regions (central, right upper, epigastrium, upper left, left flank, left lower, pelvis, right lower, right flank, upper jejunum, lower jejunum, upper ileum, lower ileum) to which lesion size score is added (LS 0—no tumor seen; LS 1—tumor up to 0.5 cm; LS 2—tumor up to 5 cm, and LS 3—tumor >5 cm or confluence).

In MPM, there is an intraoperative extensive invasion at the level of the parietal and visceral peritoneum on the surface of the small and large intestines but also in the mesentery and mesocolon. Lymph nodes will be removed whenever there is a suspicion of invasion, but a complete CRS may require resections of the small and large intestines (especially the splenic angle of the colon or the sigmoid colon). In order to achieve HIPEC, a complete hemostasis is needed; otherwise, intraperitoneal hemorrhage occurs during the procedure. Before HIPEC, an extensive intraoperative peritoneal toilet will be performed either with distilled water or with diluted hydrogen peroxide (0.25%) or povidone iodine, which aim at the mechanical cleansing of possible cancer cells.

Recently, the use of cytoreductive surgery (CRS) in the treatment of peritoneal mesothelioma with hyperthermic intraperitoneal chemotherapy (HIPEC) has been discussed [28]. Median overall survival for patients with peritoneal mesothelioma treated by CRS and HIPEC ranges from 29 to 95 months [29–32].

Research [29] on 405 patients with peritoneal mesothelioma from 29 centers in Europe and the US reported that after treatment of peritoneal mesothelioma with CRS and HIPEC, a median survival of 53 months and 5-year overall survival rate of 47%. Overall survival of patients with peritoneal mesothelioma treated with chemotherapy alone (pemetrexed + cisplatin) was poor (approximately 13 months).

A study [33] of 1514 patients with peritoneal mesothelioma who were treated with CRS, CRS and HIPEC, and chemotherapy alone showed a survival

of 52 months for CRS, 61 months for CRS and HIPEC, and 17 months after chemotherapy.

The reduction of the MPM recurrence rate was obtained by combining CRS with HIPEC. The study conducted by Nizri and colleagues [34] on 19 patients with MPM who underwent CRS combined with HIPEC showed that after a median follow-up of 69 months, all patients were alive and only 4 of the 19 patients had recurrences (21%).

2.4.2 Hyperthermic intraperitoneal chemotherapy (HIPEC), early postoperative intraperitoneal chemotherapy (EPIC), pressurized intraperitoneal aerosol chemotherapy (PIPAC), and normothermic intraperitoneal chemotherapy (NIPEC) in treatment of MPM

Additional chemotherapy was used to treat patients with MPM according to three therapeutic protocols as follows:

1. HIPEC with doxorubicin and cisplatin

2. Early postoperative intraperitoneal chemotherapy (EPIC) with paclitaxel that was added intraperitoneally in the first 5 days after CRS.

3. HIPEC then EPIC and then long-term intraperitoneal paclitaxel or pemetrexed intraperitoneally to which cisplatin is added intravenously as an adjunct to normothermic intraperitoneal chemotherapy (NIPEC).

In the absence of CRS and HIPEC, the median survival of patients with MPM is approximately 1 year. Aggressively applied surgical treatment along with additional chemotherapy increased the median survival of patients with MPM over 5 years.

The standard recommendations for HIPEC are cisplatin if renal function is good (250 mg/m^2), cisplatin plus doxorubicin, cisplatin plus mitomycin, or mitomycin only. There are also authors who use bidirectional chemotherapy by adding systemic ifosfamide plus mesna disulfide by continuing the 90-minute infusion of HIPEC with doxorubicin and cisplatin.

Survival in patients with MPM is improved in patients who used CRS plus HIPEC compared to patients who used CRS plus hyperthermic perioperative chemotherapy.

Recent studies [35] suggest a new therapeutic modality for patients with peritoneal mesothelioma: pressurized intraperitoneal aerosol chemotherapy (PIPAC). This new therapeutic modality, combined with systemic chemotherapy, may be an option for patients to whom CRS and HIPEC cannot be applied.

A retrospective study [36] of 29 patients with peritoneal mesothelioma treated with PIPAC (doxorubicin + cisplatin) showed encouraging results. Many patients with advanced peritoneal mesothelioma do not benefit from CRS and HIPEC, where chemotherapy (systemic + intraperitoneal) remains the only therapeutic option.

2.4.3 Molecular therapy and immunotherapy

One hope for molecular therapy in patients with MPM was the identification of ALK rearrangements that would be present in 3% of patients with MPM. This has been shown to be present in patients <40 years of age who have not been exposed to asbestos fibers. It is hoped that these patients will benefit from ALK inhibitors.

Gefitinib and erlotinib, which are tyrosine kinase inhibitors, acting on the epidermal growth factor receptor (EGFR), have been shown to have no significant action in MPM. By contrast, angiokinase inhibitors (nintedanib) acting on VEGF receptors, platelet-derived-growth factors, fibroblastic growth factors, and Src and Abl kinase signaling improved progression-free survival in patients with MPM when co-administered with pemetrexed and cisplatin [37].

Bevacizumab, which is an anti-VEGF antibody [38] in combination with cisplatin and pemetrexed, significantly increased overall survival in patients with MPM. Immune checkpoint inhibitors such as anti-CTLA 4 (tremelimumab and iplimumab) and anti-PD1 antibodies (avelumab and durvalumab) are under investigation.

2.4.4 Recommendations in the treatment of MPM

The recommendations discussed at the Washington DC 2016 meeting by the Peritoneal Surface Oncology Group International (PSOGI) regarding therapeutic strategies [39] in patients with MPM were the following:

1. Patients with MPM who are operable will be given CRS and HIPEC. The applied surgical treatment will include peritonectomy procedures (there are still controversies related to parietal peritonectomy: selective parietal peritonectomy vs. complete parietal peritonectomy). During the surgical treatment, it will be taken into account that the preservation of the viscera is preferred and the invaded retroperitoneal lymph nodes will be removed. Optimal cytoreduction will be assessed by validated peritoneal staging scoring systems: CC or R-score, in which the CRS objectives are to achieve a CC-0 or CC-1 score, in which the peritoneal nodules have a diameter of less than 2.5 mm.
   HIPEC will be used with cisplatin and carboplatin, either alone or in combination with doxorubicin, pemetrexed, ifosfamide, and mitomycin. Mitomycin has also been used as the only chemotherapeutic agent but with a slight decrease in survival. Normothermic intraperitoneal chemotherapy with pemetrexed and other chemotherapeutic agents has also been used with a slight increase in the survival of patients with MPM.

2. Patients with well-differentiated papillary and multicystic mesothelioma will be treated with either CRS alone or HIPEC-associated CRS depending on the stage of the disease. The benefit of combining HIPEC therapy is unknown.

3. Patients with biphasic, sarcomatoid, or unresectable PMP will only be treated by systemic chemotherapy. New chemotherapeutic agents are being tested, especially for patients who have seen an increase in Ki67, seen in immunohistochemical studies.

4. The contribution of adjuvant chemotherapy to the treatment of patients with PMP is unknown. The study conducted by Sugarbaker and colleagues in 2017 [27] on long-term adjuvant combined intraperitoneal and systemic chemotherapy showed promising results.
   It has been shown in published studies that the response rate of malignant epithelioid mesothelioma to systemic chemotherapy is around 20%. The chemotherapeutic agents used are pemetrexed, carboplatin, cisplatin, and bevacizumab.

5. New chemotherapeutic agents such as anti-mesothelin antibody (anetumumab), anti-PDL-1 (pembrolizumab), CAR T cells, and *Listeria*-based immunotherapy can improve the survival of patients with PMP.

There are still no clear recommendations in the follow-up of patients with MPM after radical excision surgery [40]. There is a follow-up guide developed by the European Society for Medical Oncology for pleural mesothelioma, but no frequency or methods of investigation used in the postoperative period (CT, MRI, or ultrasonography) are specified. Serum follow-up markers are conventional: CA125 and mesothelin.

2.5 Conclusions

Patients with peritoneal mesothelioma, due to nonspecific symptoms, present in advanced stages of the disease. An important role in determining the histological subtype of peritoneal mesothelioma is played by immunohistochemistry. Multidisciplinary management is preferred for patients with MPM. CRS and HIPEC appear to be the most effective therapeutic modalities in the treatment of MPM. Bidirectional chemotherapy is able to increase the resectability rate in patients with diffuse MPM, initially considered unresectable. Modern therapies such as molecular therapy and immunotherapy can increase the overall survival of patients with MPM. New therapeutic approaches have improved the prognosis only for patients in the early stages of the disease.

Conflict of interest

The authors declare no conflict of interest.

Abbreviations

DMPM	diffuse malignant peritoneal mesothelioma
MCPM	multicystic peritoneal mesothelioma
BMPM	benign multicystic peritoneal mesothelioma
PM	peritoneal mesothelioma
MPM	malignant peritoneal mesothelioma
CRS	cytoreductive surgery
PSOGI	Peritoneal Surface Oncology Group International
HIPEC	hyperthermic intraperitoneal chemotherapy
PIPAC	pressurized intraperitoneal aerosol chemotherapy
EPIC	early postoperative intraperitoneal chemotherapy
NIPEC	normothermic intraperitoneal chemotherapy
PCI	peritoneal cancer index
EGFR	epidermal growth factor receptor

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Chapter 3

Current Mesothelioma Treatment and Future Perspectives

Danijela Štrbac, Katja Goričar, Viljem Kovač and Vita Dolžan

Abstract

The established treatments in malignant mesothelioma are based on trimodality approach including surgery, radiation and chemotherapy. Such approach has proved to clinically benefit mesothelioma patients, however the current treatments seem to have reached a limit regarding the survival and disease control. One approach to overcome the limitations of current treatments is focused on finding appropriate serum or genetic biomarkers that could support personalized medicine and improve outcomes with established treatment modalities in mesothelioma patients. The other approach is exploiting better understanding of molecular and genetic characteristics of mesothelioma to search for new treatment modalities. Immunotherapy with anti PD-1, PD-L1 and CTLA-4 agents is a new frontier in mesothelioma treatment. As in many solid tumors, CAR-T cell therapy is emerging from the field of hematological malignancies. Immunomodulatory approaches seem to be a new perspective in treatment of malignant mesothelioma. This chapter aims to explore possible new therapeutic approaches in mesothelioma.

Keywords: mesothelioma treatment, genetic biomarkers, patient based therapy, gene therapy, immunomodulation

1. Introduction: trimodality approach to mesothelioma treatment

The established treatments in mesothelioma are based on trimodality approach including surgery, radiation and chemotherapy. Such concept for MM was introduced in the late 1990s by Sugarbaker et al. It was proposed that the treatment of mesothelioma should start with extrapleural pneumonectomy (EPP) and followed by chemoradiation [1]. A study of 120 patients concluded that a 40% survival rate was feasible in patients with epithelial histology and negative nodes. A need for a more precise staging and more effective management strategies was stated [1].

Two and a half decades after the trimodality approach was introduced, little has changed in the treatment of mesothelioma. According to the National Comprehensive Cancer Network (NCCN) guidelines, in stages I to III of surgically operable mesothelioma, a chemotherapy regimen of pemetrexed with cisplatin or carboplatin is proposed in either preoperative or postoperative setting. For patients who received the entire trimodality approach, a median survival of 20 to 29 months has been reported [2, 3].

However, the majority of mesothelioma patients are diagnosed in advanced stages, are inoperable and/or have a poor performance (WHO performance status (PS) of 2 or above). Treatment with systemic chemotherapy significantly improves survival of MM patients and patients are usually treated with a platinum agent in

combination with either pemetrexed or gemcitabine [4, 5]. Studies have shown that both chemotherapy regimens have comparable results [4, 6, 7]. The only FDA approved treatment for advanced stages of mesothelioma is pemeterexed/cisplatin with possible options of vinorelbine or gemcitabine.

The combination with pemetrexed has become standard treatment in various clinical guidelines such as the NCCN, the European Society of Medical Oncology (ESMO) and American Society of Clinical Oncology (ASCO) [3, 8, 9]. In a Slovenian clinical study, gemcitabine in a prolonged infusion with cisplatin was shown as one of the most successful systemic treatments [4, 6]. Although current treatments clinically benefit mesothelioma patients, they seem to have reached a limit regarding the survival and disease control. One approach to overcome the limitations of current treatments is focused on finding appropriate serum or genetic biomarkers that could support personalized medicine and improve outcomes with established treatment modalities in mesothelioma patients [10].

A deeper understanding of tumor biology has also enabled the development of target drugs. These drugs target and inhibit the molecular signaling pathways along which a tumor develops, grows, and spreads. Several target drugs have been tested in the treatment of MM in the last few years, but so far no targeted treatment has shown sufficient results to allow patients to be treated outside of clinical trials. Slovenian researchers also participated in one of these clinical trials with target drugs, focusing on bortezomib and cisplatin treatment [11]. The addition of bevacizumab to gemcitabine and cisplatin or pemetrexed and cisplatin has shown slightly better results. An addition of bevacizumab to the pemetrexed/cisplatin doublet has increased overall survival for up to 2.7 months, but it is suitable only for patients that do not have bleeding tendencies or a risk of thrombosis. Bevacizumab treatment has shown sufficiently promising results that it has come into routine use in the United States [12].

Among the novel treatment approaches, immunotherapy is becoming the most promising, especially with immune checkpoint inhibitors such as inhibitors of programmed cell death 1 (PD-1, *PDCD1*) and programmed cell death 1 ligand 1 (PD-L1, *CD274*) [13]. Based on the results of clinical trials, it is currently estimated that 20–25% of patients with MM may benefit from treatment with immune checkpoint inhibitors [14].

Subsequent treatment lines are less effective in mesothelioma. Novel second line treatment approaches include immunotherapy with PD-1 inhibitors, such as pembrolizumab or nivolumab. Nivolumab can be used in a combination with CTLA-4 inhibitor, ipilimumab [15, 16]. However, if immunotherapy is not accessible or has a high toxicity such as pneumonitis, a chemotherapy regimen with gemcitabine or vinorelbine is a valid option.

The aim of this chapter is to explore possible new therapeutic approaches in mesothelioma.

2. Biomarker guided chemotherapy treatment in malignant mesothelioma

Research of biomarkers in malignant mesothelioma has been ongoing for the last twenty years. Predictive and prognostic biomarkers are also needed to support the treatment and follow up of patients with MM [17]. It has been shown that apart from clinical characteristics such as C-reactive protein or tumor stage, serum and genetic markers may be associated with treatment outcome in MM [10, 18–29]. Traditional research in mesothelioma biomarkers involves soluble molecules, such as mesothelin, fibulin and survivin [18, 20, 30], but novel serum biomarkers for

disease risk, diagnosis and treatment are also emerging [31]. Mesothelin is the only clinically validated biomarker in the diagnosis of mesothelioma. However, there are no predictive biomarkers that would allow patient stratification and a more personalized treatment approach. Studies have shown that patient stratification based on genetic biomarkers could improve chemotherapy outcome, but these approaches are not routinely used in the clinic yet [32, 33]. It is becoming more and more widely accepted that pharmacogenomics is enabling personalized medicine by testing for genetic variability in drug metabolizing enzymes, transporters, and drug targets thus accounting for interindividual variability in drug levels (pharmacokinetics), drug response (pharmacodynamics) and adverse events. Using pharmacogenomics approach, the treatment of malignant mesothelioma could perhaps be tailored also to individual's genetic make-up, thereby promising safer and also more effective drug treatment [34–38].

2.1 Pharmacogenomics of cisplatin treatment

Cytotoxic activity of cisplatin and other platinum analogues is based on their ability to covalently bind to DNA, form intrastrand DNA adducts or interstrand cross-links, and lead to replication and transcription arrest. DNA adducts are recognized and repaired by nucleotide excision repair (NER) mechanisms. Genetic variability in NER genes such as ERCC excision repair 2 (*ERCC2*) and ERCC excision repair 1 (*ERCC1*) was associated with malignant mesothelioma treatment outcomes [23, 39]. In particular, *ERCC1* rs3212986 (c.*197G > T) wild-type genotype was significantly associated with better progression-free survival (PFS), but also with increased odds of treatment-related toxicities. The risk for cisplatin toxicity was also increased in patients with wild type genotype of *ERCC2* rs1799793 (p.Asn312Asp) polymorphism [23].

Interstrand crosslinks are among the most detrimental forms of DNA damage because both DNA strands are affected. As translesion DNA polymerases are needed to bypass these crosslinks and restore one of the two DNA strands in order for repair mechanisms to proceed, they may also contribute to response to cisplatin treatment [40]. Studies have shown that disruption or suppression of expression of two genes participating in translesion repair, *REV3L* and *REV1* modifies sensitivity to cisplatin [41, 42]. Similarly, *REV3L* polymorphisms rs465646 (c.*461C > T) and rs462779 (p. Thr1224Ile) were significantly associated with longer overall survival in MM patients treated with cisplatin based doublet chemotherapy, while *REV1* rs3087403 (p. Val138Met) allele and *REV1* TGT haplotype were associated with increased risk for leukopenia and neutropenia [43].

2.2 Pharmacogenomics of pemetrexed treatment

Only a few studies investigated the influence of genetic polymorphism in the folate metabolic pathways on treatment outcome in MM patients that received antifolate chemotherapeutic pemetrexed [22, 44, 45]. MM patients with at least one polymorphic *MTHFD1* rs2236225 (p.Arg653Gln) allele had a lower response rate and shorter PFS than carriers of two wild-type alleles. Furthermore, polymorphisms in pemetrexed transporter genes, such as *ABCC2* and *SLCO1B1* influenced the risk for toxicity in patients receiving antifolates [22]. Another study investigating 5,10-methylenetetrahydrofolate reductase (*MTHFR*) and *ERCC1* gene polymorphisms failed to prove an association between the selected polymorphisms and treatment outcome, but did show that a 6-base pair insertion/deletion in the 3′ untranslated region of the thymidylate synthase *TS* gene was associated with differences in disease control rate and PFS in MM [44].

2.3 Pharmacogenomics of gemcitabine treatment

Because gemcitabine is frequently used in combination with cisplatin in Slovenian mesothelioma patients, a study investigating pharmacogenomics factors that may influence the response to gemcitabine has also been performed. Deoxycytidine kinase and ribonucleotide reductase M1 (*RRM1*) were investigated as the main metabolic and target enzymes, respectively. The study indicated that the *RRM1* rs1042927 (c.*316C > A) polymorphism significantly decreased overall survival. Two promoter polymorphisms, *RRM1* rs11030918 (c.-524 T > C) and rs12806698 (c.-37C > A), decreased the odds of nausea and vomiting, while the *RRM1* TTCCA haplotype was associated with worse tumor response and worse overall survival [25]. DNA repair gene polymorphisms, particularly *XRCC1* rs25487 (p.Arg399Gln), may also modify the response to gemcitabine/platinum combination chemotherapy and effect overall survival in mesothelioma patients [24].

2.4 Clinical-pharmacogenomic models predicting outcome of malignant mesothelioma treatment

Pharmacogenomic findings motivated further research into developing a clinical-pharmacogenomic model combining clinical and genetic data and an algorithm that would enable treatment stratification in MM. The clinical-pharmacogenomic model that could help predict response to gemcitabine/cisplatin combination and survival of MM patients included C-reactive protein, histological type, performance status, *RRM1* rs1042927, *ERCC2* rs13181, *ERCC1* rs3212986, and *XRCC1* rs25487. The clinical-pharmacogenomic model that could help predict response to pemetrexed/cisplatin combination included C-reactive protein, *MTHFD1* rs2236225, and *ABCC2* rs2273697 [10]. An algorithm for treatment stratification was proposed based on both clinical-pharmacogenomic models, where a more favorable chemotherapy regimen could be recommended in 64.2% of patients: pemetrexed/cisplatin in 35.9% and gemcitabine/cisplatin in 28.3%. The algorithm predicted that 21.4% of patients would respond equally well to both treatments, but 14.5% of patients would probably not respond well to either [10]. The algorithm requires further independent validation, before it could be used in the clinical decision making, but is nevertheless proof that a tailored treatment could be applied in mesothelioma chemotherapy.

3. Future perspectives in the treatment of mesothelioma

3.1 Immunotherapy in mesothelioma

Immunotherapeutic approach is proposed as second line treatment in mesothelioma. It entails three basic immunological targets as either anti-PD-1 (nivolumab, pembrolizumab), anti-PD-L1 (atezolizumab, durvalumab) or anti-CTLA-4 (ipilimumab) or in combination, such as nivolumab/ipilimumab. The most promising trial data come from a combination of ipilimumab and nivolumab with median survival of 15.9 months. However, there is 94% rate of treatment related adverse events with combination immunotherapy [15].

Therefore, monotherapy approaches have been proposed in second line setting. Pembrolizumab in monotherapy is promising with a 20% partial response rate with a median response duration of one year. Grade 3 or 4 toxicity rate is reported at 20% [46, 47].

These data, however promising, present a high rate of toxicity and rather limited response and survival rates. With analogy to the genetic biomarkers for cytotoxic chemotherapy, further research should be done to determine genetic biomarkers in immunotherapy [48].

3.2 Gene therapy in mesothelioma

The principle of gene therapy is to infiltrate tumor cells and deactivate genes involved in tumor growth and progression. Classical example of gene therapy is to target p53 expression and induce apoptosis in mesothelioma cells. Several clinical trials targeted crucial pathways in mesothelioma cells that would ultimately lead to cell death using oncolytic viruses as vectors. The genes injected in these trials were interleukin-2, interferon α2b, herpes simplex virus thymidine kinase, and interferon β. The response was achieved mostly around the injected site in the pleural cavity, however some clinical response was noted months after injection into tumor site. The direct cell death that was the goal of this gene therapy was limited, however a delayed immune response was proposed since several antibodies were found in patients with response to treatment [49].

While gene therapy with oncolytic viruses as vectors of injection has been tested as monotherapy, combination with chemotherapy has been proposed to achieve a dual effect of local and systemic disease control [50–53].

3.3 CAR-T cells in mesothelioma

Chimeric antigen receptors (CARs) are genetically encoded artificial fusion molecules that can re-program the specificity of peripheral blood polyclonal T-cells against a selected cell surface target. The overall structure of a CAR consists of four domains joined in series, namely: an antigen recognition domain (targeting moiety), a hinge/spacer, a transmembrane element and a signaling endodomain. The CAR ectodomain determines target specificity and, most commonly, contains elements derived from a monoclonal antibody [54].

Unparalleled clinical efficacy has recently been demonstrated using this approach to treat patients with refractory B-cell malignancy, such as lymphomas. Solid tumors were the next to be included in CAR T cell (CAR-T) immunotherapy, but have posed certain toxicity challenges, such as on target off tumor toxicity. A fatal toxicity was noted in human epidermal growth factor receptor 2 (HER-2) CAR-T cells which led to respiratory and multi organ failure with cytokine release syndrome [55].

Also mesothelioma has been studied in the setting of CAR-T therapy. An *in vitro* study of MET receptor tyrosine kinase specific CAR-T cells was designed to target MET expressing mesothelioma cells. The data from the *in vivo* animal models showed that this type of CAR therapy can be safe and effective in MET expressing mesothelioma [56]. A small study reported two patients treated with mesothelin targeting CAR-T cells (CAR-T meso cells). The investigators in this study used a novel approach of mRNA engineered CAR-T cells to overcome the off- tumor on target toxicity. They concluded that the treatment with CAR-T meso cells is feasible in pretreated patients with progressive disease, since they reported partial tumor response [57].

4. Conclusions

The treatment of mesothelioma presents a clinical challenge, especially in the second and further lines of treatment. There is still place for improvement of

current treatment strategies, in particular the response to chemotherapy, by enabling pharmacogenomics based informed selection of patients who would benefit most from a particular treatment regimen. Based on our previous studies, clinical-pharmacogenomic prediction models and algorithms could facilitate treatment stratification and contribute to improved treatment outcome in MM. The future of mesothelioma treatment seems to involve immunologically based treatment with either the already present immunotherapy or the evolving CAR-T therapy. The innovation of the decades old principles of CAR-T cell therapy has proven to be successful in hematological malignancies and mesothelioma seems to be on the forefront of research in solid tumors with such innovations as are the mRNA CAR-T meso cells.

Acknowledgements

This work was financially supported by the Slovenian Research Agency (ARRS Grants No. P1-0170, P3-0307, L3-8203 and L3-2622).

Conflict of interest

The authors declare no conflict of interest.

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Section 3

Emerging Drugs

Chapter 4

Emerging Drug Therapies for Mesothelioma

Derek B. Oien, Jeremy Chien, Julian Molina and Viji Shridhar

Abstract

The systemic chemotherapy combination of cisplatin and pemetrexed has been the mesothelioma standard of care for well over a decade. This regimen has only achieved a disappointing overall median survival of about 1 year. Improved survival has been reported when systemic chemotherapy is combined with surgery and radiotherapy, and for using localized chemotherapy in some cases. The choice of mesothelioma treatment often depends on the anatomical location, histologic subtype, and disease progression. Several experimental drugs have also been investigated in mesothelioma, often with limited positive results that maintain the reputation of mesothelioma as a graveyard for drug development. This chapter will review the use of drug treatment in mesothelioma and highlight emerging experimental drug therapies in clinical trials. Experimental drugs for mesothelioma include inhibitors for checkpoints, epidermal growth factor, AXL, focal adhesion kinase, vascular endothelial growth factor, poly-ADP-ribose-polymerase, and hippo signaling.

Keywords: targeted drugs, experimental therapeutics, molecular therapies, drug combinations, NF2 mutations, BAP1 mutations

1. Introduction

The treatment of mesothelioma currently varies by primary origin of the tumor, histologic subtype, and disease progression. The most common mesothelioma is malignant pleural mesothelioma (about 80% of cases) [1]. Research for new drug treatments are often investigated in pleural mesothelioma and later extrapolated to less common types such as peritoneal mesothelioma (about 10% of cases). Both of these mesothelioma types have the same three subtypes of epithelioid, sarcomatoid, and biphasic histology. Biphasic mesothelioma is a combination of epithelioid and sarcomatoid histology, each contributing to at least 10% of the tissue [2]. Mesothelioma tends to spread regionally, then into the alternate thoracic lobe for pleural mesothelioma or across the abdomen for peritoneal mesothelioma, and can metastasize across the diaphragm or as distant metastases [2, 3]. Distant metastases were found in a postmortem study in over half of the 318 pleural mesothelioma patients examined, while distant metastasizes of peritoneal mesothelioma are not as common [2, 4]. Surgery is more common when disease is diagnosed early and tumors are resectable, but most patients are diagnosed at later stages of disease when they are not candidates. For pleural mesothelioma, extrapleural pneumonectomy and pleurectomy/decortication are the most common nonpalliative procedures for tumors that are confined to the excised region [5]. Some of these patients

will be treated with postoperative radiation and systemic chemotherapy, while the benefits of preoperative treatment are still being investigated. For epithelioid peritoneal mesothelioma, cytoreductive surgery is often combined with perioperative chemotherapy [2]. Cytoreductive surgery has been found to have minimal benefit for sarcomatoid and biphasic peritoneal mesothelioma, and systemic chemotherapy is often the first line treatment for these patients [6]. Treatment for relapsed and treatment-refractory mesothelioma is generally palliative or experimental. Currently, there are about 200 initiated and active clinical trials for mesothelioma listed at clinicaltrials.gov (U.S. National Library of Medicine), and the majority of these are drug-based interventions.

There are no targeted therapies currently approved for mesothelioma. Many ongoing research studies and clinical trials are investigating receptor tyrosine kinase inhibitors and checkpoint inhibitors of the immune system. Surprisingly, very few studies are being done that specifically target frequent genetic alterations in mesothelioma. In this review, we discuss the current chemotherapy and highlight emerging experimental drugs for mesothelioma treatment.

2. Systemic and localized chemotherapy

The current chemotherapy standard of care for mesothelioma is a systemic combination of cisplatin and pemetrexed. Adding pemetrexed with cisplatin improved overall median survival of pleural mesothelioma patients from 9.3 months with cisplatin alone to 12.1 months for the combination, which was determined by a phase III clinical trial of the combination in 2003 [7]. Second-line treatments include cisplatin combined with gemcitabine or irinotecan [8–10], and vinorelbine monotherapy [11]. Depending on the disease progression, systemic chemotherapy is often combined with surgery or radiation. The prediction of which late-stage patients will benefit from surgery has proven to be difficult [5]. Radiotherapy alone has not been shown to improve overall survival, but this method is used in combination with surgery or systemic chemotherapy and for palliative purposes. Systemic cisplatin and pemetrexed therapy also remains the standard of care for peritoneal mesothelioma, and this regimen is often used for sarcomatoid and biphasic histologic subtypes [6]. Combining gemcitabine with cisplatin was reported to achieve an overall median survival of about 27 months for patients with unresectable peritoneal mesothelioma, but this combination has also shown considerable toxicity [9]. Similar to several other abdominal cancers, many epithelioid peritoneal mesothelioma patients benefit from intraperitoneal chemotherapy administration.

Cytoreductive surgery followed by perioperative hyperthermic (or heated, hot) intraperitoneal chemotherapy for epithelial peritoneal mesothelioma patients (about 75% of peritoneal mesothelioma patients [2]) has extended overall median survival, which was reported as 53 months [12] and 38 months [13] in two separate multi-institutional studies. The drugs are heated to 42°C and administered to the peritoneal cavity for hours, often while rocking the patient to improve drug dissemination [2]. Intraperitoneal administration of chemotherapy gained attention in the 1980s when this route was shown to have a superior pharmacokinetic profile for cisplatin over intravenous injection in canines [14]. With intraperitoneal administration, most of the chemotherapy remained in the peritoneal cavity and therefore much higher concentrations of drugs could be used, which were up to 30 times greater than common doses for intravenous injection [2, 15]. The effectiveness of hyperthermic intraperitoneal chemotherapy is based on the limits of drug penetration depth and correlates to the ability for achieving complete or near-complete cytoreduction [2, 16]. The drugs used are often varied combinations of cisplatin,

mitomycin-C, and doxirubicin [6]. A significant proportion of patients have also benefited from additional long-term normothermic intraperitoneal chemotherapy following the hyperthermic perioperative dosing [6].

Overall, the main chemotherapy drugs for mesothelioma have led to unsatisfactory overall median survival percentages even when combined with radiation and surgical methods. Many mesothelioma patients try experimental drugs as part of clinical trials or compassionate-use programs. Unfortunately, mesothelioma has gained a reputation as a graveyard for drug development based on the minimal successes and modest extensions of overall survival from experimental drugs. Clinical trials to evaluate targeted drugs in mesothelioma tumors with specific genetic alterations have only recently increased to a relatively small number.

3. Frequent genetic alterations

The most well-known and frequent genetic alterations in mesothelioma are mutations in *BAP1*, *NF2*, and *TP53* genes and deletion of the *CDKN2A* gene. These mutations, along with mutations in *LATS2* and *SETD2*, were reported as the most frequent in two independent sequencing studies of mesothelioma tissues [17, 18]. Activation of the LATS2 kinase is regulated by NF2, and the SET2D protein is an H3 histone methyltransferase associated with tumor suppressor activity [19]. While the high frequency of some mutations in mesothelioma have been known for decades (e.g. *NF2*) and others have been discovered within the last decade (e.g. *BAP1*), there are still no targeted therapies approved for mesothelioma. Clinical trials requiring genetic testing for inclusion will be discussed in the next section.

Mutations in *BAP1*, the gene for the BRCA1-associated protein-1 deubiquitinating enzyme, were initially associated with mesothelioma as germline hereditary mutations [20], but it is now estimated that about 60% of mesothelioma tumors contain a mutation in *BAP1* (the majority being somatic acquired mutations) [5, 21–23]. It has been demonstrated that BAP1 regulates the DNA repair and apoptotic signaling in response to asbestos exposure [24, 25], which is the most common cause of mesothelioma. BAP1 loss also has been correlated to elevated trimethylation of H3 lysine 27 in mice, which recently lead to targeting the enhancer of zeste homolog 2 (EZH2) methyltransferase as a potential mesothelioma treatment strategy [26]. Germline *BAP1* mutations have been found in over 200 families across the globe, and about a third of cancer diagnoses in carriers of *BAP1* mutations are types of mesothelioma [5, 27]. BAP1-negative mesothelioma tumors mainly consist of the epithelioid histologic subtype [5].

The most unique frequent mutations for mesothelioma are that of the *NF2* gene. The *NF2* gene encodes the merlin protein (also known as neurofibromin 2), which has tumor suppressor activity and is associated with cell cycle/growth control through the hippo pathway [28]. Canonical hippo signaling controls the yes-associated protein (YAP), a transcription regulator for many cell cycle-associated genes. Verteporfin is a small molecule with YAP inhibitor activity that is approved for macular degeneration and has recently shown activity against *in vitro* mesothelioma models [29, 30]. We have found that mesothelioma cells are very sensitive to the antimalarial drug quinacrine *in vitro* when inactivating *NF2* mutations are present (*unpublished data*). While there are no clinical trials for mesothelioma involving these molecules, both of these drugs have potential to be repurposed for NF2-negative mesothelioma. Outside of mesothelioma, *NF2* mutations are only frequently found in a few rare neurological cancers and the inherited neurofibromatosis type II syndrome. It is estimated that about 40% of mesothelioma tumors have *NF2* mutations, although there are many other hippo-related genes found mutated in mesothelioma tumors at lower frequencies [31, 32].

Inactivation of the *TP53* and *CDKN2A* genes are not unique to mesothelioma, and these genes are known to be the first- and second-most common mutations in all cancer, respectively. The *TP53* gene is only mutated in about 15% of mesothelioma tumors [18], far below the *TP53* mutation rate for most other cancer types. Deletion of the *CDKN2A* gene is found in about 45% of all mesothelioma tumors [18]. The *CDKN2A* (cyclin-dependent kinase inhibitor 2A) gene encodes for p14arf and p16INK4a tumor suppressor proteins that regulate cell cycle activities.

Mansfield and colleagues recently used mate-pair sequencing analyses to show most mesothelioma tumors contain several chromosomal rearrangements [33]. In 22 mesothelioma patient samples examined, 13 samples contained *CDKN2A* deletions and 14 samples had *NF2* deletions. This suggests the genetics of mesothelioma cancer cells may be altered more than previously detected in several studies that used next generation sequencing methods.

4. Emerging molecular therapies

Pemetrexed was the last drug to be approved by the FDA for mesothelioma in 2004, and now several novel molecular therapies which have had success in other cancers are now being tried in mesothelioma. Among the long list, angiogenesis inhibitors and immune checkpoint inhibitors have arguably made the most progress in clinical trials.

In a recent phase III clinical trial, the vascular endothelial growth factor (VEGF) inhibitor bevacizumab was added to cisplatin and pemetrexed combination therapy for patients with unresectable mesothelioma (**Table 1**, NCT00651456) [34]. This three-drug combination resulted in significant improvement for overall survival to 18.8 months without a significant negative impact for health-related quality-of-life in patients with advanced pleural mesothelioma [35]. This combination has not yet been approved by the FDA. Another VEGF inhibitor, cediranib, was evaluated in combination with cisplatin and pemetrexed in a phase II trial for unresectable, chemotherapy naïve pleural mesothelioma (NCT01064648). This study reported improved progression-free survival and response rate, but further development has been halted based on the toxicity profile obtained during the trial [36]. Two other multitarget drugs that inhibit VEGF receptors, axitinib and nintedanib, did not meet clinical benefit goals when combined with cisplatin and pemetrexed [37]. Axitinib was unsuccessful when evaluated in a phase II trial for chemotherapy naïve, unresectable epithelioid pleural mesothelioma (NCT01211275). Combining nintedanib with pemetrexed and cisplatin did not meet the primary progression-free survival goals in a phase III clinical trial for advanced pleural mesothelioma [38]. The European-based BEAT-mesophase III trial is in the early stages and adds atezolizumab to the cisplatin, pemetrexed, and bevacizumab combination for advanced pleural mesothelioma (NCT03762018). Atezolizumab is a monoclonal antibody against programmed cell death-ligand 1 (PD-L1). The MiST phase II trial also has an arm for evaluating atezolizumab and bevacizumab in relapsed mesothelioma that has positive PD-L1 expression (NCT03654833). It is estimated that up to 25% of mesothelioma patients may benefit from immune checkpoint inhibitors [5].

Interest in PD-L1 inhibitors for mesothelioma is based on prior success of these inhibitors in other cancer types and a study showing about 40% of the 212 mesothelioma patient samples examined express PD-L1 [17]. It was also shown in the latter study that high PD-L1 expression correlated with poor survival for the mesothelioma patients. In addition to the BEAT-meso clinical trial, atezolizumab is also being evaluated in a phase II trial on unresectable or advanced pleural mesothelioma (NCT03786419). The combination of PD-L1 inhibitor durvalumab with cisplatin

Study title	Drug interventions	Phase	NCT number
Mesothelioma Avastin Plus Pemetrexed-cisplatin Study*	Bevacizumab, pemetrexed, cisplatin	2/3	NCT00651456
Pemetrexed Disodium and Cisplatin With or Without Cediranib Maleate in Treating Patients With Malignant Pleural Mesothelioma	Cediranib, pemetrexed, cisplatin	2	NCT01064648
Standard Chemotherapy With or Without Axitinib in Malignant Mesothelioma (N08CPA)*	Axitinib, pemetrexed, cisplatin	2	NCT01211275
Nintedanib (BIBF 1120) in Mesothelioma**	Nintedanib, pemetrexed, cisplatin	2/3	NCT01907100
Bevacizumab and Atezolizumab in Malignant Pleural Mesothelioma (BEAT-meso)	Bevacizumab, atezolizumab, cisplatin, pemetrexed	3	NCT03762018
Mesothelioma Stratified Therapy (MiST): A Multi-drug Phase II Trial in Malignant Mesothelioma	Bemcentinib & pembrolizumab, atezolizumab & bevacizumab, rucaparib, abemaciclib	2	NCT03654833
A Study of Atezolizumab in Unresectable or Advanced Malignant Pleural Mesothelioma	Atezolizumab	2	NCT03786419
Pembrolizumab in Patients With Advanced Malignant Pleural Mesothelioma	Pembrolizumab, pemetrexed, cisplatin	2/3	NCT02784171
CheckpOiNt Blockade For Inhibition of Relapsed Mesothelioma	Nivolumab	3	NCT03063450
Study of Nivolumab Combined With Ipilimumab Versus Pemetrexed and Cisplatin or Carboplatin as First Line Therapy in Unresectable Pleural Mesothelioma Patients	Nivolumab, ipilimumab, pemetrexed, cisplatin, carboplatin	3	NCT02899299
Randomized, Double-blind Study Comparing Tremelimumab to Placebo in Subjects With Unresectable Malignant Mesothelioma	Tremelimumab	2	NCT01843374
A Phase 2 Study of Durvalumab in Combination With Tremelimumab in Malignant Pleural Mesothelioma**	Tremelimumab, durvalumab	2	NCT03075527
Pembrolizumab + Defactinib In Pleural Mesothelioma	Pembrolizumab, defactinib	1	NCT04201145
Targeted Therapy Directed by Genetic Testing in Treating Patients With Advanced Refractory Solid Tumors, Lymphomas, or Multiple Myeloma (The MATCH Screening Trial)	Several targeted drugs including defactinib for tumors with NF2 inactivating mutations	2	NCT02465060
Everolimus (RAD001) for the Treatment of Malignant Pleural Mesothelioma With Merlin/NF2 Loss as a Biomarker to Predict Sensitivity*	Everolimus	2	NCT01024946
Study of the EZH2 Inhibitor Tazemetostat in Malignant Mesothelioma*	Tazemetostat	2	NCT02860286
A Trial of Niraparib in BAP1 and Other DNA Damage Response (DDR) Deficient Neoplasms (UF-STO-ETI-001)	Niraparib	2	NCT03207347
Olaparib in People With Malignant Mesothelioma	Olaparib	2	NCT03531840

Study title	Drug interventions	Phase	NCT number
Anti-Mesothelin Immunotoxin LMB-100 Followed by Pembrolizumab in Malignant Mesothelioma	LMB-100, Pembrolizumab	2	NCT03644550

**Completed.*
***Suspended/terminated.*

Table 1.
Highlighted drug-based clinical trials for mesothelioma from clinicaltrials.gov (U.S. National Library of Medicine).

and pemetrexed as a first-line treatment for unresectable pleural mesothelioma has also been reported to be advancing to a larger randomized phase III trial [5, 39]. Pembrolizumab is a PD-1 (which binds to PD-L1) inhibitor currently being used for a phase II/III trial (NCT02784171) for advanced pleural mesothelioma both as a monotherapy (phase II) and in combination with cisplatin and pemetrexed (phase III). Nivolumab is a PD-1 inhibitor in two phase III clinical trials, which are for relapsed mesothelioma (NCT03063450) and as a first-line treatment when combined with ipilimumab for unresectable pleural mesothelioma (NCT02899299). Ipilimumab is a cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) inhibitor that has showed encouraging results when previously combined with nivolumab in two separate phase II trials for mesothelioma [40, 41]. The CTLA-4 inhibitor tremelimumab was reported to be unsuccessful as a second-line treatment in two phase II clinical trials. As a monotherapy, it did not prolong overall survival for both unresectable pleural and peritoneal mesothelioma (NCT01843374) [42] and the primary endpoint for overall response rate was not met when tested in combination with durvalumab for pleural mesothelioma (NCT03075527) [43].

The PD-1 inhibitor pembrolizumab is also being combined with the focal adhesion kinase inhibitor defactinib in a phase 1 clinical trial (NCT04201145). There had previously been a lot of interest in the ability of focal adhesion kinase inhibition to selectively eliminate mesothelioma cells, but enthusiasm significantly decreased after defactinib failed to improve progression-free and overall survival in prior mesothelioma clinical trials (NCT02004028, NCT01870609) [44]. However, defactinib is now also in the MATCH screening phase II trial for patients with advanced refractory solid tumors containing *NF2* inactivating mutations as a second-line treatment (NCT02465060, subprotocol U). This is the only current clinical trial (to the best of our knowledge) that may potentially address inactivating *NF2* mutations in mesothelioma (note that the trial is not specific to mesothelioma and does not guarantee mesothelioma patient enrollment). The mTOR inhibitor everolimus had been previously studied in a second-line mesothelioma phase II trial that also evaluated NF2 loss as a biomarker of sensitivity (NCT01024946), but this trial resulted in limited clinical activity and everolimus did not progress as a monotherapy agent for mesothelioma [45]. In preclinical studies, we have found that repurposing the antimalarial drug quinacrine may be particularly effective against cells with inactivating *NF2* mutations by disrupting hippo signaling (*unpublished data*). Quinacrine is unique as an anticancer agent in that it has an excellent safety profile from almost a century of use for malaria prophylaxis/treatment [46]. Further mechanistic and clinical studies are needed to fully understand the potential of quinacrine for mesothelioma treatment. Moreover, verteporfin has also been preclinically evaluated as a YAP inhibitor for mesothelioma, but has not progressed to clinical trials yet [29, 30]. To address BAP1 inactivation, a phase II trial testing the EZH2 inhibitor tazemetostat with relapsed/refractory mesothelioma patients as a monotherapy (NCT02860286) recently concluded with encouraging preliminary data, specifically

benefiting long-term disease control [47]. Targeting *BAP1*-mutated mesothelioma tumors with poly-ADP-ribose-polymerase (PARP) inhibitors has been promising based on preclinical studies [48, 49]. The PARP inhibitor niraparib is being evaluated as a second-line treatment in a phase II trial for tumors with DNA damage response mutations including *BAP1* (NCT03207347). More recently, a phase II trial to evaluate the PARP inhibitor olaparib as a second-line treatment specifically for mesothelioma has started with arms to include *BAP1* somatic mutations and germline DNA damage repair mutations (NCT03531840). The MiST phase II trial also has an arm for investigating the PARP inhibitor rucaparib in BRCA1/BAP1-negative mesothelioma patients. Furthermore, the MiST trial has a third arm to study the CDK4/6 inhibitor abemaciclib for mesothelioma patients with p16INK4A negative (*CDKN2A* deletion) tumors. The fourth MiST arm evaluates AXL inhibitor bemcentinib in combination with pembrolizumab for relapsed mesothelioma patients without specific biomarker requirements. We have previously shown that AXL has relatively high expression in pleural mesothelioma compared to other cancer types, and that bemcentinib can selectively kill mesothelioma cells [50]. In pleural mesothelioma, a phase II trial with epidermal growth factor receptor (EGFR) inhibitor gefitinib was not successful [51]. However, peritoneal mesothelioma often has higher EGFR expression compared to pleural mesothelioma and may benefit from EGFR inhibitor therapy pending more clinical studies that are specific for this indication [2, 52].

Mesothelin and other biomarkers of mesothelioma have gained recent interest as targets for immunotoxins and chimeric antigen receptor-T (CAR-T) cells. Mesothelin has been used for diagnostic purposes in algorithms with other biomarkers as well as occasionally used for tumor surveillance [2, 5]. As a therapy target, the immunotoxin LMB-100 has been recently developed to bind mesothelin [53]. In 2018, a phase II trial started with LMB-100 followed by pembrolizumab for pleural and peritoneal mesothelioma cohorts (NCT03644550). CAR-T cells are also being developed to target mesothelin as a potential mesothelioma treatment [54].

5. Conclusions

Most mesothelioma patients have chemotherapy or experimental drugs as a major part of their treatment plan, but there have been very few highlights and minimal significant advancements for mesothelioma drugs over the last couple decades. Targeting specific types and characteristics of mesothelioma may have the most potential in the near future. It is surprising that targeted drugs as a whole have not progressed to end stages already either because of slower development pipelines or failure to hit endpoints for mesothelioma. There may also be an orphan drug clout that prevents development of drugs to target tumors with *BAP1* and *NF2* mutations. Proteomic characteristics of mesothelioma, specifically biomarkers currently used for diagnostic and tumor surveillance purposes, may also prove useful for novel chimeric therapies (e.g. protac and chimeric antigen receptor T cells), which are currently being developed for mesothelin. These and emerging targeted drugs such as AXL inhibitors, EGFR inhibitors for peritoneal mesothelioma, PARP inhibitors for *BAP1*-mutated tumors, and quinacrine for *NF2*-mutated tumors all have potential to finally kill the reputation of mesothelioma as a drug development graveyard.

Acknowledgements

This work was supported in part by grants from the Department of Laboratory Medicine and Pathology at the Mayo Clinic (Molina and Shridhar) and the

American Cancer Society—Kirby Foundation Postdoctoral Fellowship (Oien, PF-17-241-01-CCG). These sponsors had no involvement in any of the design or writing of this article. The authors thank Caleb Swalve for assistance in preparing this manuscript.

Conflict of interest

The authors have no conflicts of interest to declare.

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Section 4

Predictive and Prognostic Biomarkers

Chapter 5

Predictive and Prognosis Factors of Clinical Utility in Mesothelioma

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Abstract

The constant research in therapeutics for mesothelioma has been improving their tumor response and overall survival, generating the need to propose markers that guide the doctor's therapeutic approach in a more precise way. Recently, different predictive factors have been proposed, such as mesothelin-related peptides, fibulin-3, and osteopontin associated with an image giving information about the probability of tumor response to a therapeutic agent or a combination of agents. As is well known, the importance of prognostic markers of utility lies in providing prospective information on the evolution of the patient and thus their ability to guide therapeutic decisions. Although the clinical stage and histology are currently the most described prognostic factors, recent studies have shown interest in the expression of estrogen receptor beta and calretinin, among other promising factors. Given the heterogeneity of this broad field of research in mesothelioma, it is necessary to objectively present the prognostic and predictive factors of greater clinical utility.

Keywords: prognosis factors, predictive factors, response to treatment, clinical factors, histopathology factors, biological factors, clinical scores

1. Introduction

The prognosis of patients with mesothelioma is unfavorable, with a median survival of approximately 12 months from diagnosis [1–5]; this makes a clear need to improve the effectiveness of multimodality approaches and to define in a better way the subgroups' prognosis [6–9]. One way to achieve this objective is the use of prognostic and predictive factors; a prognostic factor provides prospective information on the evolution of the patient being able to guide therapeutic decisions, while a predictive factor gives us information on the probability of tumor response to a therapeutic agent.

The characteristics that a prognostic factor must meet are: (a) simple prediction method, (b) wide availability, (c) sensitivity, and (d) reproducibility in any clinical situation. The purpose of these markers is to help define the individual prognosis of clinical groups, select patients who may need other treatments, and assign the most effective treatments to improve survival and quality of life.

Although currently the therapeutic decisions are still based on the classic clinical and pathological prognostic factors already known, such as age, functional status, sex, chest pain, weight loss, thrombocytosis, leukocytosis, anemia, and histological type [3, 10], biological and genetic factors may soon be excellent options as prognostic and predictive factors.

2. Clinical factors

Multiple mesothelioma series have validated advanced TNM stage, age ≥ 50 years, male gender, poor performance status, weight loss, platelet counts ≥400,000, white blood cell counts ≥15.5, low hemoglobin level, low albumin levels, and high serum lactate dehydrogenase levels, among others, as poor predictive and prognosis factors [11–21].

TNM stage is one of the most studied prognosis factors describing a poor survival prognosis for those with advanced or metastatic stage, however, in the same stage of the disease, patients' survival varies widely suggesting that TNM staging is not completely precise to predict a survival outcome [16]. Moreover, with the new changes applied since the release of the eighth edition of the TNM Classification for Lung and Pleural Tumors where all patients N0M0 malignant pleural mesothelioma as stage IA or IB, differing from the seventh edition classification, in which N0 also was listed within the classifications for stages II and III. These changes reclassified as stage I many patients who were formerly considered as stage II or III since some patients at stage IB experienced poorer prognosis than those at stage III [22, 23]. Identifying prognostic factors based on the new classification should help to identify the patients with a poor prognosis who may benefit from multimodality treatments. Additional to the TNM staging system, the true tumor volume was independently associated with overall survival and response to treatment; however, more studies need to be done to validate this variable [24–27].

Previous studies have suggested that females with mesothelioma experience longer survival compared to males [6, 28–33] with possible suggested explications like those they present at earlier stage [34], tumors with more favorable histology [30], different asbestos exposure responsible for a more indolent tumor biology [35], and a protective effect of circulating estrogen interacting with estrogen receptors present in their tumors, [32, 36, 37] however, only more indolent tumor biology associated to higher frequency of germline mutations in DNA repair genes [38–41] and interaction of estrogens with estrogen receptor beta [36, 37, 42, 43], other theories still controversial [15].

Platelet count is a practical and easy blood test in clinical practice that has been studied for its role as a prognosis factor due to the interaction of platelets with tumor cells contributing to tumor progression, invasion, metastasis, and angiogenesis [44]. This interaction could be explained by five possible pathways: the first one refers to the release of growth factor by the platelets, including transforming growth factor β and fibroblast growth factor enhancing cancer cell proliferation [45]. Second, platelet membranes are rich in many adhesin molecules like selectins, integrins, immunoglobulin superfamily proteins, and leucine-rich glycoproteins stabilizing the cancer cell arrest in the vasculature, increasing potential of metastasis [46]. Third, platelets could mediate the invasive potential of cancer cells by the release of thromboxane A2, 12-hydroxyeicosatetranoic acid, and matrix metalloproteinases [47–49]. Fourth, platelets release a large number of pro-angiogenic mediators such as vascular endothelial growth factor and basic fibroblast growth factor influencing the tumor angiogenesis and consequently tumor growth [50–52]. Fifth, some studies have demonstrated that platelets facilitate the immune escape of cancer cells by surrounding tumor cells and protecting them from the cytotoxic effect of natural killer cells [53, 54]. Several studies concluded that thrombocytosis is correlated with worse overall survival in patients with mesothelioma, indicating that pretreatment could be an adequate and useful factor of prognosis [18].

Recently, many people have focused on the role of inflammation in cancer due to its contribution to tumor initiation and malignant progression. More specifically in mesothelioma, inflammation becomes relevant since most patients have a history of asbestos exposure, and this mineral can skewer cells and set off chemical reactions

that lead to inflammation, DNA damage, and cell death [20]. Leukocyte blood count reflects a degree of the systemic inflammatory response in tumor patients, being a valuable and simple indicator [55]. Blood neutrophil-to-lymphocyte ratio is a systemic marker for inflammation closely related to the mortality rate and response to the treatment is useful as a predictive and prognostic factor, taking 3 as a dividing point [20, 56–60]. In the same way, serum c-protein can reflect an inflammatory environment; although its usefulness as a prognostic and predictive factor has been demonstrated in limited studies, more research is needed to validate its utility [61–63].

Malnutrition has been related to adverse outcomes in overall survival, quality of life, and increased mortality of malignant tumors [64–66]. Serum albumin level is a simple and objective indicator to evaluate malnutrition. Multiple studies have demonstrated hypoalbuminemia as an adverse independent prognostic factor for mesothelioma [19, 20, 67].

It is well known that cancer cells tend to employ alternate metabolic pathways, generating adenosine triphosphate through anaerobic glycolysis regulated by lactate dehydrogenase [68, 69]. Several studies assessed the value of high pretreatment lactate dehydrogenase levels for the prediction of a worse survival outcome in mesothelioma [10, 61, 62, 70–75]. The association between high lactate dehydrogenase levels and poor prognosis on malignancies has tried to be explained in multiple ways. The first theory implies that the production of lactate acid could be up-regulated by lactate dehydrogenase, generating an acidic environment activating metalloproteases, macrophage-mediated angiogenesis and protecting mitochondria from oxidative stress, which induces resistance to hypoxia-induced apoptosis of tumor cells [76–80]. The second theory explains a strong correlation between elevated lactate dehydrogenase levels and an up-regulation of the hypoxia-inducible factor pathway resulting in a host immunological function attenuation, and enhanced tumor angiogenesis, which has an adverse impact on prognosis in malignant tumors [81]. Despite the great evidence of the utility of lactate dehydrogenase as a convenient and cost-effective indicator for predicting overall survival outcome, cut-off values of lactate dehydrogenase reported on the literature are inconsistent, and it is important to standardize the cut-off value in future studies.

3. Histopathology factors

Together with the TNM stage, the histological type is one of the strongest prognostic factors among patients with mesothelioma. However, with the support of immunohistochemistry markers, not only has diagnosis been improved, but also new markers have appeared for a more accurate prediction of response to treatment, overall survival, and developing better therapeutic approaches.

The most significant prognostic factor until now remains histology with a better prognosis for epithelioid type than sarcomatoid or biphasic type mesothelioma [10, 12, 82, 83]. In addition to histologic subtyping (with solid growth pattern being associated with a poor outcome), nuclear atypia, mitotic count, and the presence of necrosis were found to be independent prognostic factors in epithelioid malignant pleural mesothelioma [84–86].

Ki67 antigen is used for the assessment of growth fraction of cell populations, due to it being exclusively expressed in proliferating cells; cell cycle analysis showed that Ki67 is detectable in G1, G2, S, and mitosis phases but absent in quiescent cells [87, 88]. Despite most studies indicating that high expression of Ki67 leads to a poor prognosis, some malignancies showing high Ki67 levels actually show a better response to treatment, which could be explained by the fact that cells with high proliferation are susceptible to cytotoxic agents [89–93]. The detection of Ki67 is not a routine procedure for mesothelioma's diagnosis and treatment; however,

a group has suggested to consider it due to its utility as a possible prognostic marker in epithelioid mesothelioma with a better prognosis outcome in those with low expression levels [94–98].

Calretinin is a calcium-binding protein that has been established as a useful marker in distinguishing mesothelioma from adenocarcinomas with pleural metastases [99]; Additionally, interest in using higher calretinin scores as favorable prognostic factors has been growing, although further investigation is needed [100–104].

As mentioned above in the section of clinical factors, estrogen receptor beta expressed on mesothelial tumor cells has become a promising prognostic factor and a possible future therapeutic target [36, 37, 42, 43].

4. Biological factors

Several biomarkers are selectively elevated in patients with mesothelioma. However, further study and validation are required before they are recommended as routine predictive or prognosis factors and they should be adjunct to a radiological assessment. With considerable variation in response to treatment, the emergence of promising biomarkers that could select responders from non-responders at baseline or during treatment would guide to a better therapeutic approach, prevent patients from getting ineffective treatments, and improve cost-effectiveness.

The most researched biomarker until now is the mesothelin; soluble mesothelin is a circulating form of a membrane-bound glycoprotein highly expressed by mesothelial cells in mesothelioma (predominantly epithelioid type) and other malignancies [105]. Despite the controversial evidence reported in the literature [106–114], a meta-analysis conducted by Tian et al. [115] concluded that a high soluble mesothelin level may lead to a poor prognosis for patients with mesothelioma, it being appropriate to consider mesothelin level as an independent prognostic marker.

Human fibulin-3 is a secreted glycoprotein that plays an essential role in the regulation of cell proliferation and migration [116, 117]. Recent findings have documented altered levels on patients with mesothelioma, highlighting them as a novel biomarker for this malignancy; however, as most studies have been done with limited sample size [114, 118–120], and the results may not completely mirror the actual value of fibulin-3 for prognosis, further studies are needed for a more comprehensive prognostic role of human fibulin-3 in mesothelioma.

Osteopontin is a glycoprotein that mediates cell-matrix interactions with adverse outcomes for mesothelioma [98, 121, 122]; however, its utility is limited because of the significant variability in the cut-offs used between studies. In order to be validated in the future, a consensus approach is required for sampling and analysis [122].

CA 125 is a transmembrane glycoprotein that can be detected in the fallopian tube, endometrium, endocervix, and mesothelial surface of the peritoneum, pleura, and pericardium [98]. Some cases with non-gynecological cancer showed positive immunohistochemical staining for CA125 in tumor tissue and elevated CA 125 levels in serum [123–125]. The baseline levels of serum CA125 accompanied by the stage of the disease could be used as independent prognostic factors for patients with mesothelioma; the change in serum CA125 levels can predict overall survival and response to systemic treatments [126–128].

5. Clinical scores

The best-known clinical prognostic scoring systems for mesothelioma until now derive from the Cancer and Leukemia Group B (CALGB) and the European

Organization for Research and Treatment of Cancer (EORTC), both scores have been widely used to better select patients who have a favorable prognosis and could tolerate and potentially benefit from a more aggressive combined modality treatment [3, 10].

The CALGB index was validated by examining the survival of a wide cohort dividing patients into six patient subgroups with different survival rates. The CALGB study considered extent pleural disease, lactate dehydrogenase >500 UI/L, poor performance status, platelets >400,000, non-epithelial histology, and >75 years as negative prognostic factors for survival. The most favorable characteristics were a performance status of 0, age < 49, and hemoglobin ≥14.6/μl [10].

The EORTC score has been validated in 523 patients included in 10 mesothelioma trials with the analysis suggesting that performance status >0, stage IV disease, and biphasic or sarcomatous histologies are associated with a worse outcome [129]. Additional reports confirmed that male sex, older age, and abnormal hematological values also give a poor prognosis [13, 130].

Despite both studies identifying performance status and histology as two main prognostic factors, these analyses included patients with heterogeneous tumor stages at diagnosis, the majority of whom underwent major surgery and whose treatment predated the use of pemetrexed as first-line treatment. Since the positioning of pemetrexed as a first-line treatment, no validated prognostic score has appeared, resulting in the need to generate new studies with the aforementioned scores [131].

6. Promising factors

Although there are multiple prognostic and predictive factors that are currently validated, many others have generated great interest for their potential as a therapeutic target in the future.

There is an increasing interest in the use of semi-quantitative ^{18}F-FDG PET/CT parameters, like metabolic tumor volume and total lesion glycolysis to measure the metabolic activity in the entire tumor volume with great potential to predict response to treatment [119, 132–144]; however further investigation is needed in mesothelioma patients.

Despite the wide utility of the tissue biopsy, the invasive nature limits their application, especially when repeated biopsies are needed. Given the aforementioned, liquid biopsy has gained interest from oncologists and basic researchers [145]. Although liquid biopsy is still far from replacing tissue biopsy for mesothelioma, plasma and serum samples represent minimally invasive, low-risk, and easily obtained biological fluids that many studies have indicated as potentially interesting prognosis biomarkers as mentioned in the section "Biological factors" [146].

Nowadays, immunotherapy is gaining great relevance in cancer therapeutics. Soon, oncologists will routinely ask for programmed death-ligand 1 (PD-L1) status that has been correlated with better treatment response to anti-PD-L1 antibodies and overall survival outcomes [147–151]. However, different PD-L1 antibodies coupled with specific staining platforms and scoring criteria may be necessary since finding a suitable cut-off point remains a current challenge [151, 152].

A wide number of molecular prognostic markers for mesothelioma have been investigated. The number of tumor-infiltrating myeloid cells, c-MET expression, thymidylate synthase expression, among others, represent promising biomarkers associated with strong prognostic significance. c-MET is a tyrosine kinase receptor, its overexpression was associated with longer overall survival in patients with mesothelioma [98, 153]. Thymidylate synthase expression may predict pemetrexed

efficacy, a certain correlation has also been found with overall survival and progression-free survival [154].

Dysregulated genes play a critical role in the development and progression of mesothelioma, making them future diagnosis and prognosis biomarkers [155]. Recently, Zhou et al. obtained an RNA-Seq count quantified by RSEM for RNA expression profiles of a large cohort of patients with mesothelioma according to The Cancer Genome Atlas guidelines. After a time-dependent receiver operated a characteristic curve to evaluate the prognostic performance of survival prediction, three genes (LSM6, GZMB, and HJURP) were found with a strong statistically significant prognostic association; this prognostic signature could be a clinically useful tool that in the future could be incorporated into a clinical sequencing program to individualize therapy [156].

7. Conclusion

Despite the wide variety of predictive and prognostic factors that exist, just a few are replicable worldwide. Furthermore, only pathological type and performance status are the grade-A recommendations of prognostic factors in pretreatment assessment, as well as the nodal stage, residual disease, and histology during treatment [16].

Although there is currently no validated prognostic approach, according to individual evidence, availability, and cost-benefit, it is recommended to pay special attention to the TNM classification, histological type, and serum CA125 in the decision for multimodal therapy. Despite the practicality of the prognostic scoring systems, further investigations are needed to validate the known scores or generated new ones that fit the new existing therapeutic modalities for mesothelioma.

In the near future, many other prognostic and predictive factors may be introduced in clinical practice making a selection of mesothelioma subgroups to improve the benefit achievable by currently available treatment strategies, and relentless efforts will have to be focused on designing innovative compounds selectively targeting the existing (or additional) markers to improve the grim prognosis of the disease.

Conflict of interest

Dr. Jeronimo Rafael Rodríguez-Cid has educational, investigational and advice relations with MSD, Bristol Myers, Roche, Takeda, Amgen, Abvie, Aztra Zeneca, Boehringer Ingelheim, Pfizer, Celgen, Novartis, and Bayer.

Dr. Rodrigo Rafael Flores-Mariñelarena have no conflicts of interest to declare.

Notes/Thanks/Other declarations

None to declare.

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Chapter 6

Biomarkers Progress and Therapeutic Implications in Malignant Mesothelioma

Jordyn Feinstein and Muaiad Kittaneh

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.

Acknowledgements

There is no funding involved in this review. We would like to acknowledge the mesothelioma researchers and physicians who dedicated their career to treating mesothelioma and advance the discoveries in this field.

Conflict of interest

The authors declare no conflict of interest.

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Chapter 7

Genetic Alterations of Malignant Pleural Mesothelima

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Abstract

Malignant pleural mesothelioma (MPM) is a highly aggressive tumor that arises from the mesothelial cells lining the pleural cavity. Asbestos is considered the major factor in the pathogenesis of this malignancy, with more than 80% of patients with a history of asbestos exposure. MPM is characterized by a long latency period, typically 20–40 years from the time of asbestos exposure to diagnosis, suggesting that multiple somatic genetic alterations are required for the tumorigenic conversion of a mesothelial cell. In the last few years, advancements in next-generation sequencing and "–omics" technologies have revolutionized the field of genomics and medical diagnosis. The focus of this chapter is to summarize recent studies which explore the molecular mechanisms underlying this disease and identify potential therapeutic targets in MPM.

Keywords: pleural mesothelioma, next-generation sequencing, transcriptome, exome sequencing, tumor suppressor gene

1. Introduction

Malignant pleural mesothelioma (MPM) is a lethal cancer of the mesothelial cells lining the pleural cavity and, less frequently, the pericardium, peritoneum, and tunica vaginalis [1]. Many years after the peak of asbestos use in United States, 3200 cases of MPM continue to be diagnosed annually, indicating that the U.S. population remains at risk of exposure to asbestos and development of mesothelioma [2]. There are two major histological variants: epithelioid, which accounts for about 60% of cases and has the more favorable prognosis, and sarcomatoid, whose incidence is 10%. The remaining cases demonstrate histologic characteristics of both types and are classified as biphasic [3]. The prognosis for patients with MPM is poor, with a median survival of 5–15 months [3]. However, some patients with early MPM who undergo multimodality therapy including surgical resection and chemotherapy demonstrate longer-term survival of up to 25% at 5 years [4].

Many studies have shown a causal relationship between exposure to asbestos and mesothelioma (reviewed by Bianche et al. [5]). Although it has been suggested that brief asbestos exposure is sufficient to induce disease, MPM is the consequence of prolonged exposure in most cases. However, only a small percentage of individuals exposed to asbestos develop MPM, suggesting that genetic predisposition may modulate the effect of exposure to asbestos. In addition, 20% of MPM cases with unknown asbestos exposure have been related to other risk factors such as radiation therapy and thorotrast [6].

Studies conducted on large numbers of patients indicate that the time between asbestos exposure and diagnosis of MPM is generally more than 20 years. The molecular mechanisms for the transformation of mesothelial cells are unknown; it has been suggested that asbestos induces multiple chromosomal aberrations, particularly deletions, facilitating oncogenesis [7].

Investigations prior to the advent of next-generation sequencing (NGS) revealed the complexity of the genetic alterations observed in MPM tumors by using karyotypic and comparative genomic hybridization (CGH) analyses [8, 9]. Chromosomal losses were found to be more frequent than gains and particular chromosomal regions (1p22, 3p21, 4q, 6q, 9p21, 13q13–14, 15q11–15, and 22q12) were deleted at higher frequency in MPM tissues and cell lines [10–12]. Two tumor suppressor genes (TSGs) were identified by positional cloning approaches: *CDKN2A* at 9p21 and *NF2* at 22q12. In the last few years, the genetic landscape of MPM has been characterized using high-throughput technologies [13–15]. The focus of this chapter is to summarize the major genetic changes occurring in MPM as identified by high-throughput sequencing and to describe the novel insights obtained through transcriptomic studies.

2. Exome sequencing studies

NGS technologies have allowed the sequencing of DNA and RNA at unprecedented speed, uncovering potential driver genes and creating novel biological applications [16]. In the last decade, NGS has been used to detect driver genetic mutations in cancer and provide new insights into tumorigenesis.

Shotgun pyrosequencing was used to characterize RNA expression levels and mutations of four patients in the first effort to investigate MPM by NGS. Several different mutations were found in the four transcriptomes. In addition, RNA editing gene deletions and gene silencing were identified [17].

In 2010, the first whole genome sequence of one MPM tumor and matching normal tissue was conducted using a combination of sequencing-by-synthesis and pyrosequencing methodologies [18]. This study showed that aneuploidy and chromosomal rearrangements were more numerous than point mutations in this tumor. One large deletion in the dipeptidyl peptidase like 10 (*DPP10*) gene, altering the expression of the corresponding transcript, was further investigated in 53 additional MPM tumors. Patients expressing *DPP10* had statistically longer survival compared to patients lacking *DPP10* expression [18].

In 2016, Bueno et al. conducted an extensive analysis of the mutational landscape of MPM. Ninety-nine MPM tumors were examined by whole exome sequencing, whereas additional 103 samples were characterized by targeted exome sequencing [13]. *BAP1*, *NF2*, *TP53*, *SETD2*, *DDX3X*, *ULK2*, *RYR2*, *CFAP45*, *SETDB1* and *DDX51* were found to be significantly mutated (q-score ≥ 0.8), and recurrent mutations were found in *SF3B1* (2%) and *TRAF7* (2%).

In 2018, The Cancer Genome Atlas (TCGA) program performed a comprehensive molecular profiling of 74 primary MPM samples including exome sequencing, copy-number arrays, mRNA sequencing, noncoding RNA profiling, DNA methylation, and reverse-phase protein arrays [15]. The significantly mutated genes in this study were *BAP1*, *NF2*, *TP53*, *LATS2*, and *SETD2*. Furthermore, this study identified a new near-haploid molecular MPM subtype.

The TCGA study performed a comparison of the significantly mutated genes between the Bueno and TCGA cohorts [15]. This analysis identified five genes that were frequently mutated in both studies: BRCA1-associated protein-1 (*BAP1*), neurofibromin 2 (*NF2*), tumor protein P53 (*TP53*), SET domain containing 2, histone

lysine methyltransferase (*SETD2)*, and SET domain bifurcated histone lysine methyltransferase 1 (*SETDB1*). The large tumor suppressor kinase 2 (*LATS2*) gene was found frequently altered in the TCGA cohort alone, whereas four additional genes, DEAD-box helicase 3 X-linked (*DDX3X*), Unc-51-like autophagy-activating kinase 2 (*ULK2*), ryanodine receptor 2 (*RYR2*), and DEAD-box helicase 51 (*DDX51*) were identified as commonly mutated in the series from Bueno et al. (**Table 1**).

2.1 BAP1

BAP1 is located on the short (p) arm of chromosome 3, at position 21.1., a region frequently deleted in MPM [9]. This gene encodes for a deubiquitinase involved in cell cycle regulation, modulation of gene transcription, cellular differentiation, and DNA repair [19]. *BAP1* is one of the most commonly mutated genes in MPM [13, 15, 20, 21]. Germline *BAP1* mutations have been linked to the development of *BAP1* tumor predisposition syndrome, which includes uveal and cutaneous melanoma, atypical Spitz tumors, renal cell carcinoma, and MPM. In all these malignancies but MPM, *BAP1* mutations are associated with poor prognosis [22, 23]. In contrast, some studies have shown that patients with MPM carrying *BAP1* mutations have longer overall survival compared to patients with wild-type *BAP1* [24, 25]. In one study, BAP1 immunohistochemistry (IHC) was performed using tissue microarray including 229 MPM tumors. The results showed that loss of BAP1 nuclear staining was associated with longer median survival of 16.11 months (95% CI: 12.16–20.06) versus 6.34 months for patients with nuclear BAP1 staining (95% CI: 5.34–7.34) ($P < 0.01$) [24]. Baumann et al. compared the survival in 23 patients with MPM carrying germline mutations in *BAP1* with a control group of MPM patients from the Surveillance, Epidemiology, and End Results (SEER) database and found a 7-fold increase in long-term survival in patients with BAP1 mutation [25].

Given its prevalence in MPM, loss of nuclear BAP1 expression by IHC is commonly used as a diagnostic marker in MPM [26, 27].

Recently, *BAP1* status has been associated with drug response [28, 29]. I*n vitro* studies showed MPM cell lines carrying *BAP1* mutations were significantly less sensitive to gemcitabine compared to wild-type cells. Silencing of *BAP1* in MPM

Gene symbol	Gene ID	Chromosomal location	Number of mutations in Bueno's cohort	Number of mutations in Hmeljak's cohort	Total
BAP1	ENSG00000163930	3p21.1	55	17	72
NF2	ENSG00000186575	22q12.2	39	19	58
TP53	ENSG00000141510	17p31.1	17	10	27
SETD2	ENSG00000181555	3p21.31	18	8	26
SETDB1	ENSG00000143379	1q21	7	3	10
LATS2	ENSG00000150457	13q12.11	2	9	11
DDX3X	ENSG00000215301	Xp11.4	8	0	8
RYR2	ENSG00000198626	1q43	4	1	5
ULK2	ENSG00000083290	17p11.2	4	0	4
DDX51	ENSG00000185163	12q24.33	3	0	3
Total			157	67	224

Table 1.
Number of mutations in each gene in the two studies.

wild-type cells significantly increased resistance to gemcitabine, suggesting a role of *BAP1* in drug response [28]. Kumar et al. performed a retrospective study analyzing presence or absence of nuclear BAP1 by IHC in MPM tumors from 60 patients in the MS01 trial (NCT00075699) [29]. Nuclear BAP1 expression was associated with a small but statistically nonsignificant decrease in survival in patients treated with vinorelbine.

2.2 NF2

NF2 is located on the long (q) arm of chromosome 22 at position 12.2. Loss of chromosome 22 is a common alteration in MPM [9]. This gene codes for a protein known as merlin (moesinezrin-radixin-like protein) or schwannomin, which regulates key signaling pathways involved in cell growth, adhesion, and microtubule stabilization [30]. Germline mutation or chromosomal deletion of *NF2* causes the neurofibromatosis type 2 syndrome, which is associated with tumors of the cranial and peripheral nerves as well as meningioma and ependymoma [31]. Germline mutations in *NF2* have also been linked to MPM; however, patients with both neurofibromatosis type 2 syndrome and MPM are extremely rare [32]. Recent studies have shown that *NF2* mutations occur in 14–19% of MPM [13–15, 20]. In addition, karyotype and/or FISH analyses demonstrated that 56% MPMs have shown loss of chromosome 22q. Deletions of 22q are more frequently associated with epithelioid than non-epithelioid MPM ($p = 0.037$) [20].

In 2009, a study suggested that *NF2* may be inactivated by upstream regulators in MPM tumors where no *NF2* aberration can be detected [33]. In an investigation of 204 MPM patients, low cytoplasmic merlin expression was found to predict shorter recurrence interval and shorter overall survival [34]. Lopez-Lago et al. investigated the association between loss of merlin and mTORC1 activation in MPM cell lines and found that merlin-negative or merlin-depleted cell lines were more sensitive to the growth-inhibitory effect of rapamycin [35]. In 2014, low merlin expression was found to be associated to increased sensitivity of MPM cell lines to a FAK inhibitor, VS-471 [36]. However, in clinical trials, the FAK inhibitor defactinib did not improve progression free or overall survival in patients with MPM after first-line chemotherapy [37].

2.3 TP53

Located at 17p31.1, *TP53* codes for tumor protein p53 (p53), which is a sequence-specific DNA binding protein that regulates transcription and has a tumor suppressor function controlling cell apoptosis in presence of DNA damage [38]. Named "the guardian of the genome," p53 is involved in many cellular processes such as checkpoint control, cellular senescence, and BCL-2 mediated apoptosis [39]. *TP53* is, overall, the most frequently altered gene in human cancer [40]. The frequency of *TP53* mutations in MPM across different studies is variable, but overall it is much lower than in other solid tumors [13–15, 20]. *TP53* was significantly more frequently mutated in women (10/40; 25%) compared to men (17/169, 10%) (Fisher's exact $P = 0.044$) when all samples included in two large MPM studies [13, 15] were analyzed. In addition, Bueno et al. reported that MPM patients with mutations in *TP53* had shorter overall survival than those with wild-type *TP53* ($p = 0.0167$) [13].

2.4 SETD2

SETD2 maps to 3p21.31. It encodes a histone methyltransferase specific for lysine-36 of histone H3 which regulates transcription through epigenetic

mechanisms [41]. Inactivating *SETD2* mutations have been identified in multiple cancers [42]. In particular, targeted sequencing revealed *SETD2* bi-allelic inactivation in clear cell renal cell carcinoma tumors suggesting for the first time that *SETD2* may contribute to tumor formation [43]. In MPM, single nucleotide mutations in *SETD2* as well as 3p losses are frequently observed [13, 15, 44]. In the last few years, *SETD2* alterations have been linked to mechanisms of resistance to DNA-damaging chemotherapy in several cancers [45, 46].

2.5 SETDB1

SETDB1 is positioned at 1q21, another region frequently deleted in MPM [9], and codes for histone-lysine N-methyltransferase *SETDB1* which trimethylates Lys-9 of histone H3 [47]. As an epigenetic modulator, *SETDB1* has a critical role in several biological processes such as embryonic development, adipocyte differentiation, and inflammation, as well as providing regulation of several signaling pathways including the P13K-AKT axis, p53, the STAT1-CCND1/CDK6 axis, and gene promoter methylation [48].

Targeted deep sequencing has revealed somatic *SETDB1* mutations in 10% (7/69) patients with MPM [49]. No significant correlation between mutation in *SETDB1* and survival was found ($p = 0.351$). Mutations in *SETDB1* were also identified in 3% (7/202) of MPMs in a different cohort [13]. Hmeljak et al. found that *SETDB1* mutations were present together with *TP53* and extensive loss of heterozygosity in 3% of MPM. This rare genomic subtype was associated with female sex and younger age at diagnosis [15].

2.6 LATS2

LATS2, located on 13q12.11, encodes for a serine/threonine kinase which is involved in a broad array of programs such as cell cycle regulation, cell motility, and differentiation [50]. Loss of *LATS2*, either through copy number alteration or mutation, has been identified in several different cancer types [51], as well as in MPM [15, 52]. In a cohort of 266 MPM samples, mutations in *LATS2* were observed in 5% of the samples, with lower frequency in epithelioid compared to non-epithelioid samples. In addition, *LATS2* mutations were more frequent in patients without asbestos exposure (7%) than those exposed (2%) [53]. Another study identified a new molecular subgroup of MPM characterized by a co-occurring mutation in *LATS2* and *NF2*. MPM patients in this subgroup had poor prognosis compared to the cohort at large [54].

Several investigations have linked *LATS2* to the transcription regulator YAP involved in the Hippo pathways. Mizuno et al. found that inactivation of *LATS2* leads to YAP overexpression, which, when knocked down, inhibits cell motility and invasion *in vitro* [55]. Another study demonstrated that *LATS2* is a key binding partner of AJUBA, which suppresses YAP activity in mesothelioma [56].

2.7 DDX3X

DDX3X resides on Xp11.4 and encodes an ATP-dependent RNA helicase with RNA-independent ATPase activity stimulated by either DNA or RNA [57]. DDX3X has both cytoplasmic and nuclear functions including translation, regulation of transcription, pre-mRNA splicing, and mRNA export [58]. Its functions are complex and varied: *DDX3X* has been recognized as both an oncogene and a tumor suppressor, sometimes within the context of a single type of cancer [59]. An analysis of the COSMIC database found that 12% of genetic abnormalities in *DDX3* are typical for tumor suppressors, while 81% are more typical for gain of function [59].

2.8 RYR2

RYR2 is located at 1q43. It encodes a member of the ryanodine receptor family of calcium channels, highly expressed in cardiac muscle but also found in smooth muscle and the nervous system [60]. The release of calcium from the sarcoplasmic reticulum into the cytoplasm via RyR2 triggers contraction in myocytes, whereas in the brain, it aids in functions related to learning and memory [60]. Although mutations in *RYR2* have been reported in other cancers [61], *RYR2* mutations in MPM have been identified only in one study [13].

2.9 ULK2

ULK2 maps on 17p11.2. It codes for an Atg1 homolog and serine/threonine kinase which normally localizes to the membrane of autophagosomes and plays a key role in autophagy, particularly in the setting of nutrient deprivation or mTOR inhibition [62]. *ULK2* has been linked to the development of astrocytoma [63], and colorectal cancer [64]. Rare *ULK2* mutations have been identified in MPM [13]. In spheroid models of MPM, autophagy was successfully inhibited by the ULK1/2 inhibitor MRT 68921 [65].

2.10 DDX51

DDX51 resides on 12q24.33. It is a ribosome synthesis factor required for the formation of the 3′ end of 28S rRNA [66]. Abnormal function of *DDX51* has been linked to NSCLC, leukemia, and breast cancer [67–69]. Few *DDX51* mutations have been found in MPM [13].

3. Transcriptome sequencing studies

Since gene expression is linked to tumor behavior, bulk expression profiling of tumors has revolutionized our understanding of cancer by giving insight into the expression levels of thousands of genes measured at once. In addition, the allocation of cancer specimens into molecular clusters having similar biological and clinical characteristics has improved the understanding of the molecular biology of tumors and identified both actionable targets for therapies as well as biomarkers for prediction of response [70].

In 2005, Gordon et al. profiled 40 MPM tumors using microarray technologies [71]. Four normal pleura specimens and four normal lung tissues were included in the analysis as controls because MPM arises from mesothelial cells of the pleura and often involves the lung parenchyma [71]. Unsupervised cluster analysis revealed four distinct subclasses with two, named C1 and C2, consisting only of MPM samples. These two clusters had epithelial (88%) and mixed (78%) subtypes, respectively, showing a partial correlation with tumor histology. Differential gene expression analysis demonstrated genes related to cytoskeletal/support, such as keratins, cadherins, and other proteoglycans, were over-expressed in cluster C1, whereas genes associated with extracellular matrix and structural proteins such as collagen, actin, biglycan, and fibronectin were highly expressed in subclass C2 [71].

In 2014, a study from de Reynies et al. generated a transcriptomic classification of MPM using 38 primary cultures [72]. Consensus clustering of the expression profiles identified two groups of MPM, C1 and C2, which are partially related to histology. Epithelioid MPM were found in both clusters, whereas sarcomatoid

tumors clustered only in C2. In addition, tumor samples in C1 tended to have more frequent mutations in *BAP1* ($P = 0.09$) and deletions of the chromosomal region 3p21 ($P < 0.01$), where *BAP1* is located. Furthermore, 40 genes that discriminated the two groups were used to validate the molecular classification in 108 MPM tumors. Survival analyses showed that patients in C2 had shorter survival compared to the survival of patients in cluster C1 ($P = 0.02$). This difference persisted when only epithelioid samples were included ($P < 0.01$) [72]. Pathway analyses revealed that the most deregulated pathways were those related to the epithelial-to-mesenchymal transition (EMT) process [72].

In 2016, a seminal publication on genomics in MPM described unsupervised consensus clustering of RNA sequencing data from 211 MPM tumors. This analysis classified the samples into four distinct molecular clusters: epithelioid, biphasic-epithelioid (biphasic-E), biphasic-sarcomatoid (biphasic-S), and sarcomatoid [13]. The clusters were loosely associated with the spectrum from epithelioid to sarcomatoid histology. Epithelioid and biphasic samples were distributed in all four subgroups, whereas sarcomatoid tumors were only in one cluster. Biphasic samples clustered according to the proportion of epithelioid and sarcomatoid cells contained in the specimen; biphasic tumors with the highest portion of sarcomatoid cells grouped with the sarcomatoid samples. Notably, patients in the epithelioid cluster had longer overall survival compared to the survival of patients in the other three groups. Differential expression analysis of the sarcomatoid and epithelioid clusters revealed that genes related to the EMT process were differently expressed between the two groups, and that ratio of two genes *CLDN15* and *VIM* (C/V score) significantly differentiated the four clusters [13].

A different approach to classify MPM tumors was used by Hmeljak et al. [15]. To determine whether a multi-platform molecular profiling may offer additional power to identify subsets of MPM, two clustering algorithms, iCluster [73] and PARADIGM [74] were used to integrate somatic copy-number alteration, gene expression, and epigenetic data from 74 MPM samples. Both algorithms grouped the samples into four distinct clusters with high concordance between the two methods in the assignment of the sample into the groups. Survival analyses showed significant differences in survival across the four groups. In addition, the four clusters were significantly associated with histology: cluster 1 contained many epithelioid samples, whereas cluster 4 was enriched for sarcomatoid tumors as found in previous studies [13, 71, 72]. This study, using a small number of samples, mostly epithelial, confirmed that genes related to the EMT process were differentially expressed between the two most extreme clusters [15].

In 2019, unsupervised clustering of microarray profiles assigned 63 primary MPMs into four groups (C1A, C1B, C2A, and C2B) [75]. Then, a meta-analysis of mesothelioma expression profiles was conducted to compare these clusters with the groups from previous classifications [13, 15, 71, 72, 75, 76]. This analysis identified two highly correlated MPM clusters present in all expression profiles, which corresponded to the extreme epithelioid and the sarcomatoid phenotypes. The remaining groups did not associate closely suggesting that they may represent different points of a continuum or "histo-molecular gradient" of epithelioid and sarcomatoid components. A deconvolution approach was used to identify novel insights into the intra-tumor heterogeneity of MPM by dissecting whole tissue RNA-sequencing signatures into biologically relevant components. This analysis produced two molecular signatures of 150 genes, E-score and S-score, which were related to histology and recapitulated the molecular classification. These signatures reflected the proportion of epithelioid-like and sarcomatoid-like components within each MPM tumor. In addition, the proportions of these cellular components were significantly associated with prognosis [75].

Regardless of the metric used, the whole transcriptome studies indicate that MPM is characterized by a molecular gradient associated with the EMT process. Most recently, the relationship between the C/V score [18] and other published metrics [75, 77] associated with the EMT process has been investigated [78] demonstrating a significant correlation of the C/V score with other molecular signatures. These results indicate that the ratio of just two genes can be sufficient to determine the "EMT-component" in each MPM [78].

4. Clinical significance

While further work is needed before these data can be applied directly to patient care, an understanding of the molecular heterogeneity of MPM and the mutations that contribute to different subtypes can have a meaningful impact on the direction of clinical research in this field. In 2014, *in vitro* and tumor xenograft experiments suggested that low Merlin (NF2 protein) expression may predict increased sensitivity of MPM cells to a FAK inhibitor, VS-4718 [36]. Subsequently, the use of defactinib, a FAK inhibitor, was investigated in the neoadjuvant setting for surgically resectable disease (a "window of opportunity" study). The treatment was well tolerated and resulted in successful inhibition of FAK, as well as inhibition of multiple cancer stem cell markers such as CD133 and SOX2 (Bueno et al., 2018 personal communication, International Mesothelioma Interest Group (IMIG) Conference, 2016 Birmingham UK). The use of defactinib as maintenance therapy following first-line chemotherapy in advanced MPM was also assessed in the COMMAND trial, a phase II randomized placebo-controlled study. Three hundred forty-four patients were stratified by merlin expression and randomized; however, there was no significant improvement in progression-free survival (4.1 [95% CI: 2.9–5.6] versus 4 [95% CI: 2.9–4.2] months) or overall survival (12.7 [95% CI: 9.1–21] versus 13.6 [95% CI: 9.6 to 21.2] months) of patients treated with defactinib compared to placebo [37].

Knowledge of key mutations in MPM has guided investigations into other forms of targeted therapy, although many are still at the preclinical stage. For example, LaFave and colleagues found evidence that loss of Bap1 expression increases Ezh2 expression in xenograft and *Bap1* knock-out mice and enhances sensitivity to EZH2 inhibition *in vitro*. Szlosarek and colleagues studied arginine deprivation in 68 patients with advanced ASS1-deficient malignant pleural mesothelioma (defined by >50% low expressor cells on immunohistochemical analysis) [79]. Treatment with the deprivation agent ADI-PEG20 improved progression-free survival (3.2 vs. 2 months, $p = 0.03$) with no significant difference in life expectancy or adverse events.

Beyond identifying therapeutic targets, multi-omic data have enhanced the understanding of tumor biology, providing novel ways to stratify patients, determining prognosis and predicting sensitivity to existing treatments (reviewed in [80]).

We have developed a gene expression ratio-based method to translate expression profiling data into clinical tests based on the expression levels of a small number of genes [81]. This method uses standard supervised methods for microarray analysis to compare gene expression in two types of tissues differing by a single clinical parameter such as histology or outcome. Genes with the most significant difference in expression are selected and used in combination to calculate ratios of gene expression able to predict the clinical parameter associated with a random patient sample.

Using this method, a 6-gene 3-ratio test has been developed to distinguish MPM from adenocarcinoma using resection specimens and fine needle biopsies [81, 82]. A similar approach was used to generate a 4-gene 3-ratio prognostic test to identify

patients likely to benefit from tumor resection in the preoperative setting [83, 84], as well as a 4-gene 3-ratio signature to distinguish the epithelioid from the sarcomatoid MPM subtype [85].

Despite rapidly decreasing sequencing costs [86], there remain several barriers to introducing the use of NGS technology in clinical practice, especially in MPM. In many solid tumors, the development of targeted sequencing panels has led to targeted therapies and prediction of survival of cancer patients. MPM is rare, making large-scale validation studies difficult to perform, and heterogeneous, characterized by mutations highly variable among tumors. In addition, loss of TSGs is a common feature of MPM making potential treatments associated with these genes difficult to be applied to real life treatment. Clinical trials focused on specific mutated genes [29, 37] have been infrequent and the results never translated to practice. Transcriptome analyses have classified MPM patients into several groups stratifying patients into categories of risk; however, a substantial margin of error in these predictions persists because the sensitivity and specificity of these tests are difficult to define [87]. Precision medicine based on cancer genomics is still far from being applied in clinical practice in MPM. Nevertheless, we are confident in the value of NGS for personalized medicine and believe additional efforts are needed for the implementation of NGS in identifying patients who might benefit from targeted treatments.

5. Conclusions

NGS has revolutionized the study of human genetics by transforming our ability to analyze the causes of disease, develop new diagnostics, and identify potential therapeutic targets. NGS studies have led to the discovery of several commonly mutated genes in MPM [13, 15]. Although analyses of transcriptome data have contributed to the understanding of the molecular biology of MPM subtypes, these studies were based on bulk profiling where tumors were profiled as a single entity averaging the gene expression of all the cells in the specimen and ignoring the intra-tumor heterogeneity that regulates many critical aspects of tumor biology [88]. The importance of intra-tumor heterogeneity in MPM is becoming evident. Future single-cell RNA sequencing work will be able to elucidate molecular roles of immune infiltrates and stroma in MPM as well as to clarify whether the molecular mechanisms associated with the genetic heterogeneity are due to subclonal mutations, epigenetic programs, or other environmental factors such as cell-cell interaction or nutrient availability.

Acknowledgements

This work was supported by grants to RB from the National Cancer Institute (NCI 2 R01 CA120528-11A1) and the International Mesothelioma Program at Brigham and Women's Hospital. The study sponsors played no role in the study design, collection, analysis, interpretation of data, writing of the report, or decision to submit the chapter for publication.

Conflict of interest

The authors disclose no potential conflicts of interest. Dr. Bueno reports grants from Medgenome, grants from Roche, grants from Verastem, grants from Merck, grants from Gristone, grants from Epizyme, grants from Siemens, grants from

NCI, grants from DoD, and grants from NIH. In addition, Dr. Bueno has a patent 7,622,260 licensed to BWH, a patent 8,450,057 licensed to BWH, a patent 8,551,700 licensed to BWH, and a patent 9,446,050 licensed to BWH, and Patents/Equity in Navigation Sciences.

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Edited by Sonia Maciá

Mesothelioma is an aggressive cancer with very poor survival and lack of treatment options. This book, written by a diverse panel of experts, presents a description of the most relevant topics on epidemiology, diagnosis, biomarkers, and treatment updates, including interesting discussion on molecular mechanisms, prognostic features, and novel therapies that are either approved or under clinical development for this challenging disease. It also discusses and explains genetic biomarkers, as there may be a role for some nuclear regulatory genes and proteins in development of mesothelioma, although targeted therapies so far have had limited impact.

Published in London, UK

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ISBN 978-1-83968-156-1