TISSUE AND CIRCULATING PROGNOSTIC BIOMARKERS IN MALIGNANT PLEURAL MESOTHELIOMA

PhD thesis

Thomas Klikovits, MD

Doctoral School of Clinical Medicine

Semmelweis University

Consultant: Ferenc Renyi-Vamos, MD, Ph.D

Official reviewers: Erika Hitre MD, Ph.D
Levente Fazekas MD, Ph.D

Head of the Final Examination Committee: Nándor Ács MD, Ph.D

Members of the Final Examination Committee: Marcell Szász MD, Ph.D
Zoltán Heiler MD, Ph.D

Budapest
2017

Table of contents

1 Abbreviations

ActA *Activin A*

AMIG *Austrian Mesothelioma Interest Group*

AQP1 *Aquaporin 1*

AUVA *Allgemeine Unfallversicherungs Anstalt*

BAL *Bronchoalveolar lavage*

BAP1 *BRCA1(breast cancer 1)-associated protein1*

Bcl-2 *B-cell lymphoma-2*

Bg8 *Blood group 8*

BSC *Best supportive care*

C4d *Complement component 4d*

CDH8 *Cadherin 8*

CDKN2A–ARF Cyclin-dependent kinase inhibitor 2A

CFAP45 *Cilia and Flagella Associated Protein 45*

CHT *Chemotherapy*

CI *Confidence interval*

CNV *Copy number variations*

COX-2 C*yclooxygenase-2*

CRO *Croatia*

CRP *C-reactive protein*

CT *Computed tomography*

DDX3X *DEAD-box helicase 3*

DDX51 *DEAD-Box Helicase 51*

DFS *Disease-free survival*

DHFR *Dihydrofolate reductase*

DPP10 *Dipeptidyl-peptidase 10*

EBUS *Endobronchial ultrasound*

EE *Environmental exposure*

EGFR *Epidermal growth factor receptor*

ELISA *Enzyme-linked immunosorbent assay*

EMT *Epithelial-to-mesenchymal transition*

EORTC *European Organization for Research and Treatment of Cancer*

EPP *Extrapleural pneumonectomy*

ERCC1 *Excision repair cross-complementation group 1*

ERS *European Respiratory Society*

ESTS *European Society of Thoracic Surgeons*

EUS *Esophageal ultrasound*

FDG-PET *Fluorodeoxyglucose positron emission tomography*

FFPE *Formalin fixed paraffin embedded*

FNA *Fine-needle aspiration*

FSH *Follicle-stimulating hormone*

Gas-6 *Growth arrest signal-6*

GPS *Glasgow Prognostic Score*

Gy *Gray*

HE *Hematoxylin/eosin*

HIOC *Hyperthermic intraoperative chemotherapy*

HR *Hazard ratio*

HUN *Hungary*

HV *Healthy volunteers*

IARC *International Agency for Research on Cancer*

IASLC *International Association for the Study of Lung Cancer*

IgM *Immunoglobulin M*

IHC *Immunohistochemistry*

IMIG *International Mesothelioma Interest Group*

IMRT *Intensity modulated radiotherapy*

IRR *Incidence rate ratio*

MAC *Membrane attack complex*

MAP2K6 *Mitogen-activated protein kinase kinase 6*

MAPK *Mitogen-activated protein kinase*

MAPS *Mesothelioma Avastin Cisplatin Pemetrexed Study*

MARS *Mesothelioma and Radical Surgery*

MASP *MBL-associated serine protease*

MBL *Mannan-binding lectin*

MCR *Macroscopic complete resection*

MIF *Migration inhibitory factor*

MMT *Multimodal treatment*

MOC-31 *Mouse Monoclonal Primary Antibody 31*

MPM *Malignant pleural mesothelioma*

mTOR *Mechanistic target of rapamycin*

NA *Not available*

NCCN *National Comprehensive Cancer Network*

NF2 Neurofibromin type 2

NFRKB *Nuclear factor related to kappa B binding protein*

NKX6-2 *NK6 homeobox 2*

NLR *Neutrophil-to-lymphocyte ratio*

NLRP3 *NOD-like receptor family, pyrin domain containing 3*

NMPD *Non-malignant pleural diseases*

NSCLC *Non-small cell lung cancer*

$nTiO_2$ *Nano size titanium dioxide particles*

NTS *Neurotensin*

NTSR1 *Neurotensin receptor 1*

OPN *Osteopontin*

OR *Odds ratio*

OS *Overall survival*

P/D *Pleurectomy/decortication*

PCBD2 *Pterin-4-alpha-carbinolamine dehydratase 2*

PDT *Intracavitary photodynamic therapy*

PFS *Progression free survival*

PLR *Platelet-to-lymphocyte ratio*

PS *Performance status*

RNS *Reactive nitrogen species*

ROS *Reactive oxygen species*

RT *Radiotherapy*

RTK *Receptor-tyrosine kinase*

RYR2 *Ryanodine receptor 2*

SCLC *Small cell lung cancer*

SD *Standard deviation*

SETD2 *SET domain containing 2*

SETDB1 *SET Domain Bifurcated 1*

SMART *Surgery for Mesothelioma After Radiation Therapy*

SMRP *Serum-soluble mesothelin family proteins, soluble mesothelin-related peptide*

SV40 *Simian virus 40*

TBNA *Transbronchial needle aspiration*

TKI *Tyrosine-kinase-inhibitor*

TNM *Tumor node metastasis*

TS *Thymidylate synthase*

ULK2 *Unc-51 Like Autophagy Activating Kinase 2*

US *United States*

VATS *Video-assisted thoracic surgery*

VATS-PP *VATS partial pleurectomy*

VEGF *Vascular endothelial growth factor*

WHO *World Health Organization*

WT-1 *Wilms-Tumor Protein 1*

2 Introduction

2.1 Malignant pleural mesothelioma

2.1.1 Epidemiology

In 2012, 14.1 million new cancer cases and 8.2 million cancer deaths occurred worldwide. Among these, lung and breast cancer are diagnosed most frequently and represent the leading causes of cancer death in men and women, overall and in less developed countries. However, in more developed countries, prostate cancer accounts for the most diagnosed malignancy and lung cancer represents the leading cause of cancer death in women [1]. In general, global cancer burden will shift to less developed countries within the next decades due to an increasing prevalence of risk factors and growing and aging populations [2]. By 2012, less developed countries accounted for only 57% of global cancer cases and 65% of cancers deaths, due to more frequent other causes of death, such as infection, and the younger age structure [1, 2].

Worldwide, liver, stomach and colorectal cancers are additionally frequently diagnosed among men, whereas stomach, cervical and colorectal cancer are frequent among women [1]. In more developed countries, prostate, colorectal, breast and lung cancer incidences tend to be higher, whereas liver, stomach and cervical cancer are more frequently diagnosed in less developed countries. These trends are predominantly attributable to infectious diseases, being more prevalent in less developed countries [3]. Additional risk factors for most frequently diagnosed cancers worldwide include smoking (lung, colorectal, stomach, liver cancer), overweight and physical inactivity (breast, colorectal). By applying effective risk factor prevention strategies (i.e. tobacco control, vaccination) and the broad use of early detection tests, a substantial proportion of cancer cases could be effectively prevented [1]. Besides highly prevalent cancer types, some less frequently diagnoses malignancies are gradually on rise and research must focus on prevention, early detection and new therapeutic targets in these diseases as well.

Malignant pleural mesothelioma (MPM) is a rare but devastating malignancy, arising from the pleural space. The tumor is known to be a rare disease; however, its incidence is increasing worldwide, probably as a result of widespread exposure to asbestos, known to be the main risk factor for MPM development. There is a significant variation

of MPM incidence among different areas worldwide. It ranges between 7 per million inhabitants in Japan and 40 per million in Australia [4]. The highest mesothelioma incidence rates are reported from some countries in Europe (UK, Italy, The Netherlands, Malta, Belgium) and in Oceania (Australia, New Zealand). In the UK for instance, the annual number of deaths from MPM increased continuously, being 153 in 1968 and 2.360 in 2010. In 2011, the numbers of deaths were 2.291. In the period of 2000-2011, incidence rates in the UK were 3.3-3.6 per 100,000 among men, and 0.5-0.7 among women [5]. In Italy, incidence in 2011 among men was 3.5 and 1.25 per 100,000 in men and women, respectively, and wide differences are noted among different geographic areas within the country [6]. Intermediate incidence rates are reported from a group of countries including large parts of Europe and the United States (US) [7, 8]. In Germany, 7.547 malignant mesotheliomas were reported to cancer registries diagnosed between 2009 and 2013, 90% of those being located in the chest. On average, 1.198 men and 312 women were affected each year. Regional clusters were predominantly located to the seaports of West Germany [9]. In the US, between 2003 and 2008 over 3000 cases were diagnosed each year, with a maximum of 3284 in 2005. In this period, the incidence was 1.93 per 100,000 among men and 0.41 among women [7]. Low incidence/mortality rates are reported from various countries of Central Europe, Ireland, Spain, and from several countries of Asia [10-12]. In Austria, there have been 276 cases of MPM approved by the "Allgemeine Unfallversicherungsanstalt – General accident insurance company" (AUVA) as being caused occupationally between 2010 and 2015. Of these, 53 were approved in 2014 only. In contrast to this, ten asbestos-related MPM cases were documented in 1995 and 41 in 2005. However, there is still uncertainty about the number of MPM cases not being reported to the AUVA and currently, there is no register on non-occupational MPM in Austria [12]. A comparison of the incidence of MPM worldwide and in Austria is depicted in Figure 1.

In Europe, the average incidence is 2 per 100.000 inhabitants. The frequency is highly dependent on the amount of asbestos removal, asbestos import and industrialization and the peak incidence is to be expected around 2020 due to the long latency period [4, 13]. In the US, MPM peaked in the 1980s to 1990s and is now plateauing. In men, incidence has been stable at 1.8 cases per 100.000 for the past 10 years, with peak values in the early 1990s (2.5 cases per 100,000 people), while in women, rates were 0.4 cases per

100.000 people, and did not change substantially over time [14]. Due to the long latency period and recent asbestos banning efforts, a possible reduction in the MPM health burden and a reduction in the number of newly diagnosed cases is expected in the near future, at least in developed countries [15].

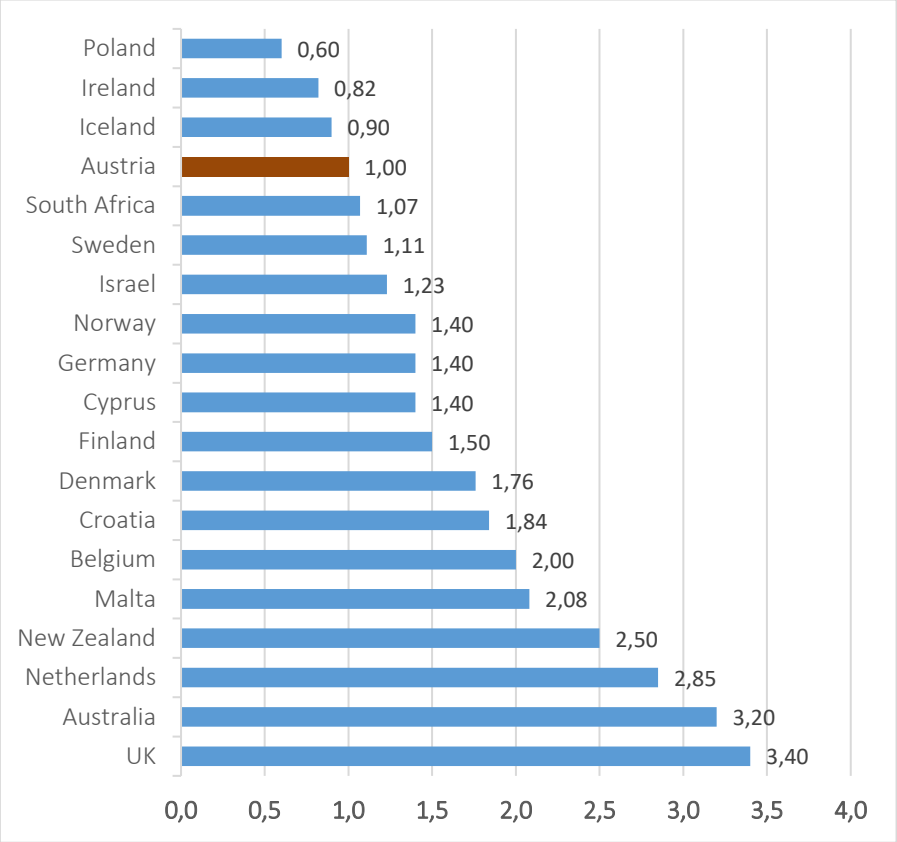

Figure 1: Incidence of MPM in Austria compared to worldwide MPM incidence ([8]). Data are given as age-standardized rates per 100.000 in men.

Currently, however, MPM incidence is still increasing in most countries of the world, and a decrease can only be seen in countries where asbestos control measures were taken [5, 14]. Thus, the overall worldwide epidemic is still increasing and in countries that still produce and/or commercially use asbestos, such as China, India, Russia, a sharp rise in incidence might be expected in the future [16-18].

2.1.2 Etiology

2.1.2.1 Asbestos-related MPM development

Previous asbestos exposure is known to be the main risk factor for the development of MPM [19, 20]. Asbestos is classified into two main groups, the serpentines and the

amphiboles. The serpentines consist of one type, chrysotile (95% of asbestos in commercial use), with a characteristic short and curly fiber, also referred to as “white asbestos” due to its white color. The amphiboles, with straight, longer fibers, include crocidolite or “blue asbestos”, amosite, tremolite, actinolite and anthophyllite. These six fibers are collectively summarized as “asbestos” due to regulatory issues and their common known health risk [21, 22]. The risk of MPM development has shown to be dependent on the fiber type, as shorter fibers are assumed to be less carcinogenic [23]. Even though some studies claimed that chrysotile could generate mesothelioma only if it was contaminated with amphiboles [24], it has been clearly shown that chrysotile is an important carcinogen and risk factor for MPM development, and also for lung cancer [25]. Thus, in the current international perception, all types of asbestos are classified as class I carcinogens, according to the World Health Organization (WHO) and the International Agency for Research on Cancer (IARC). Furthermore, it is well recognized that exposure to asbestos is the major cause of both pleural and peritoneal mesothelioma, which resulted in the banning of asbestos production and import in several European countries at various time-points after 1970, and in the European Union as late as 2005 [4, 22].

Asbestos exposure is typically labor-dependent and is recognized as an occupational disease in many countries [8]. The mean latency period between exposure to asbestos and the onset of symptoms has been reported to be up to 40 years, and 99 % of cases show a latency of more than 15 years [26]. In most epidemiological studies, MPM is more common in men and some studies have claimed that its occurrence is correlated with sex. However, other studies have shown that MPM development is to be related to asbestos exposure and, typically, there is low asbestos exposure in women because the occupations associated with exposure are traditionally carried out by men [27]. Additionally, women with MPM have shown to exhibit a threefold better overall survival (OS) than men [14]. More recently, a shift has been observed from asbestos-removal workers to professionals involved in post-construction work, e. g., electricians, plumbers, or heat protection technicians [19].

There is a clear correlation between the amount of asbestos exposure and the incidence of MPM, however, no safe lower threshold has been identified. Furthermore, there is an increasing incidence of non-occupational asbestos diseases among housewives and

family members of asbestos workers, due to cleaning of contaminated work clothes, as well as a high environmental impact in the vicinity of mining and processing facilities [8, 28, 29]. In a certain region of Italy, an epidemic of MPM was registered among inhabitants who were never exposed to local asbestos factories, whereas a high proportion of cases had only one risk factor: living close to an asbestos cement factory. The calculated risk was very high for those living <500 m from the factory and the fiber burden in the lungs of deceased cases was 10-fold that in those from other areas [30]. Another recent study reported a mesothelioma incidence rate ratio (IRR) of 12.92 for those living <500 m from an asbestos cement plant in Barcelona, Spain, while the ratios decreased to 0.70 and 0.23 for those living 500-2000 m and >2000-10000 m from the plant, respectively [31]. Accordingly, a dose dependent relation between asbestos exposure and risk for MPM development is well perceived.

Despite worldwide efforts to ban asbestos production and commercial use, there is still the additional risk for environmental exposure, whose impact on MPM development is not well studied. The quantification of environmental associated MPM occurrence is furthermore limited mainly by the lack of reliable assessment of type and amount of exposure [15, 32]. One major feature of environmental exposure associated MPM is a general higher disease burden in women than in men. The ratio (male:female) in several studies is often close or less than 1 (Figure 2).

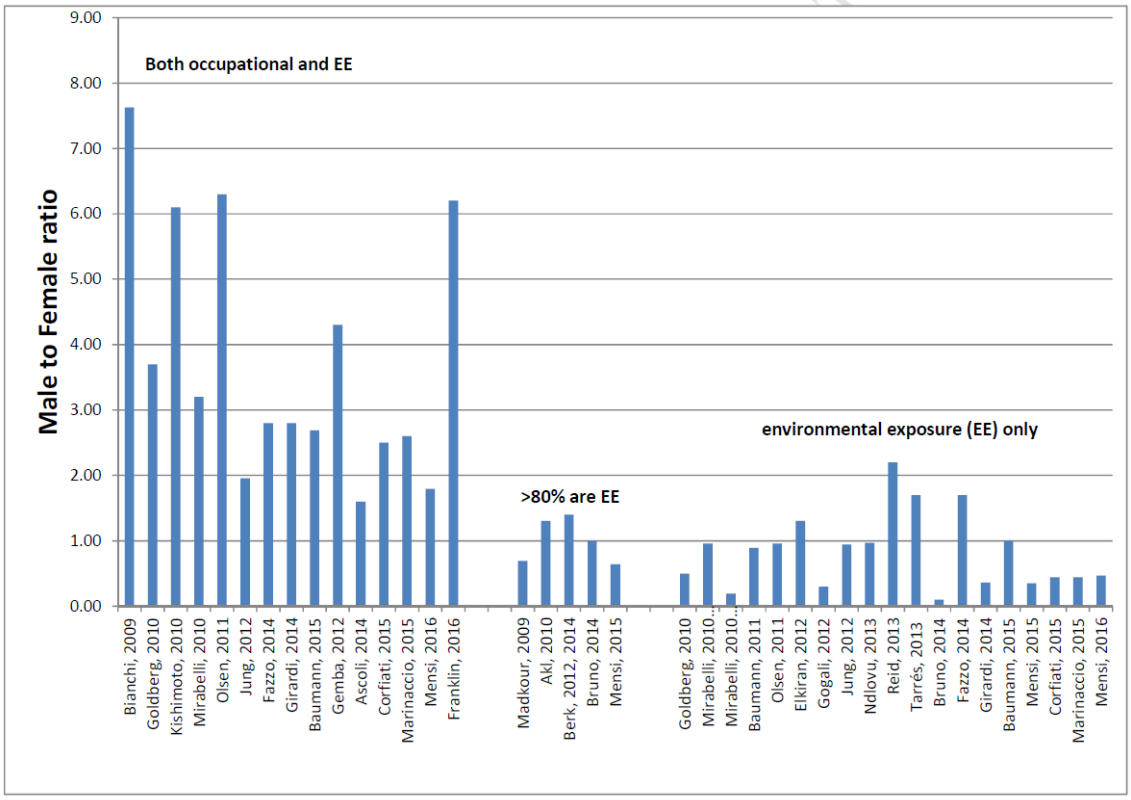

Figure 2: Male to female ratios among malignant mesothelioma cases reporting overall exposure (occupational and environmental) and environmental exposure (EE) to asbestos [15]. EE, environmental exposure

Environmental occurrence of mesothelioma has been found in people growing up near natural asbestos resources (Turkey, Corsica, Cyprus) or in areas where asbestos was used for the whitening of house walls. In this regard, erionite, an asbestos-like mineral from the soil, was revealed as the main factor of mesothelioma in young people in some villages in Turkey, where more than 50% of inhabitants died from MPM [33]. In this area, the average annual mesothelioma incidence rates are 114.8 per 100,000 for men and 159.8 per 100,000 for women, or 88.3 times greater in men and 799 times greater in women, respectively, in comparison to world background incidence rates [34, 35]. Another study revealed that whitewashing the houses with soft tremolite in Metsovo, Greece, was the reason for a high number of mesothelioma cases in young women, as they used to do this work [36].

Inhaled asbestos fibers enter the pleural space through the alveoli or retrograde through the lymphatic vessels, causing cytotoxicity, DNA damage, frustrated phagocytosis and chronic inflammation (Figure 3) [37, 38]. Important key mechanisms of the mesothelial

cells, such as chromosomal aberrations and epigenetic changes, result in cellular dysfunction at gene, microRNA and protein expression levels [39, 40].

Asbestos fibers are usually detected and entrapped by alveolar macrophages into lysosomes. The NOD-like receptor family, pyrin domain containing 3 (NLRP3) inflammasome gets activated to cleave procaspase 1 to an active form. Fibrotic nodules are formed by the release of cleaved and activated prointerleukin-1-beta. Consequently, reactive oxygen species (ROS) and reactive nitrogen species (RNS) are produced within the macrophages which ultimately cause cellular and tissue damage. Apoptosis of alveolar macrophages lead to the production of various cytokines such as IL-1β tumor necrosis factor (TNF)-α, macrophage inflammatory protein (MIP)-1/2, monocyte-chemoattractant protein-1, and IL-8 to cause chronic inflammation and proliferation of collagenic fibers. Asbestos particles are then again released and newly recognized by nearby macrophages leading to the repetition of similar cellular response mechanisms. Ultimately, partially cleaved asbestos fibers are transferred to regional lymph nodes, particularly at the opening of lymphatic vessels. Circulating and local immunocompetent cells will recognize these fibers repeatedly, recurrently and continuously, encountering for chronic local inflammation [41-43].

However, the question if the MPM progenitor cell arises from a highly differentiated mesothelial cell or a submesothelial multipotential cell is still unresolved yet. Normal mesothelial cells may regenerate from normal mesothelium and by the development of submesothelial multipotential stem cells [44]. Moreover, recent studies showed that also adipocytes, circulating multipotential fibrocytes and adult bone marrow-derived stem cells can differentiate into both epithelial and mesenchymal cells [45]. Despite this, it is still further unclear, if this MPM progenitor cell might arise from the parietal pleura more likely than from the visceral. It has been previously hypothesized that a parietal origin might be more likely since cases with only parietal affection (previously staged as T1a (TNM-7)) have a worse survival compared to cases in stage T1b (TNM-7), which involves the parietal and visceral pleura, indicating that the parietal lesion is an earlier event [46, 47].

2.1.2.2 Non-asbestos-related MPM development

In contrast to asbestos-related MPM development, the incidence of mesothelioma without asbestos contact is extremely low (<1: 1 million). Potential cofactors for MPM occurrence besides asbestos are synthetic materials (ceramics, nanoparticles), ionizing radiation, and SV-40 virus infections [48]. Exposure to nanoparticles such as carbon nanotubes has been shown to cause MPM similar to that caused by asbestos, and thus has become an environmental health issue [49]. In this regard, fiber-length is an important parameter in triggering chronic pleural inflammation. Recently, it has been demonstrated that nanofibers beyond 4 µm in length are pathogenic to the pleura and shorter nanofibers might exhibit a potentially lower risk of cellular damage [50].

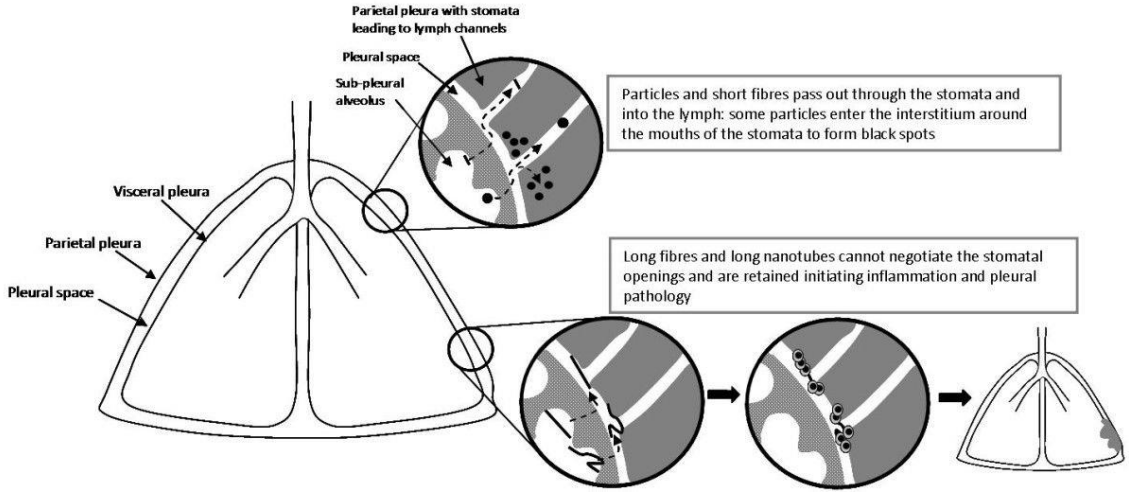

Figure 3: Hypothesized sequence of events leading to pleural responses as a consequence of long fiber retention of asbestos and nanofibers at the parietal pleural stomatal openings leading to chronic inflammation and tumor induction [51].

Among nanofibers, nano size titanium dioxide particles ($nTiO_2$) are one of the most commonly used metal nanoparticles in commercial products, such as cosmetics, sunscreens, food products, paints and drugs. $nTiO_2$ have been shown to cause reactive oxygen species (ROS) leading to toxicity [52]. A recent study has shown that with respect to the toxicity of $nTiO_2$ on human-derived mesothelial cells, the crystal form rather than the particle size has a greater effect on cellular absorption, and thus causes more cellular damage [53]. It is hypothesized that long fiber retention of nanofibers at the parietal pleural stomatal openings may lead to chronic inflammation and tumor induction eventually, similar to inflammation caused by asbestos exposure (Figure 3)

[51]. However, further studies shedding light on nanoparticles uptake and MPM induction are to be awaited.

Long-term effects of ionizing radiation have been made responsible for MPM development, however in a much smaller population than in asbestos exposed individuals [54]. Especially, individuals exposed to α-particle-emitting agents are at higher risk for MPM. Moreover, several studies showed a higher incidence of mesothelioma in patients treated with external beam radiotherapy for testicular cancer or lymphoma [55, 56].

Simian virus (SV) 40 was discovered in 1959 as a virus being endemic in rhesus monkeys, whose kidneys were used for primary cell cultures for the preparation of inactivated poliovirus vaccine (IPV) and live oral poliovirus vaccine. SV40 was shown to be oncogenic in rodents, causing mesothelioma, ependymoma, osteosarcoma and non-Hodgkin's lymphoma [57]. Formaldehyde was used at the 1950s to inactivate the poliovirus, but it did not completely turn down SV40 and thus there have been estimates that 30–100 million people in the USA and many more worldwide received potentially contaminated vaccines prepared during the years 1955–1963 [58]. In a meta-analysis including 528 MPM cases from 15 studies, the risk for the presence of SV40 DNA sequences in MPM tumor tissue was very high compared to controls (OR 17, 95% CI 10–28) [59]. Moreover, recent studies showed that rodents infected with SV40 were highly susceptible to asbestos-related carcinogenesis [60]. However, the link between SV40 infection and human cancer development is still controversial.

2.1.3 Molecular background of MPM

Because MPM is rare, genomic studies are limited and typically involve a small number of samples [61]. Nevertheless, due to the use of high-throughput analyses and a revolution in molecular characterization, the knowledge of cytogenetic and molecular changes in MPM has substantially increased in recent years [22]. Recent analyses have demonstrated frequent gained chromosomal regions in 5p, 7p, 7q, 8q, and 17q as well as frequent deletions of specific sites within chromosome arms 1p, 3p, 6q, 9p, 13q, 15q and 22q. Two of these regions are most frequently altered, the tumor suppressors cyclin-dependent kinase inhibitor 2A (CDKN2A–ARF) at 9p21 and neurofibromin type 2 (NF2) at 22q12 [62]. These have been known for a long time, but recurrent somatic

mutations in BRCA1 (breast cancer 1)-associated protein1 (BAP1) gene were more recently identified. BAP1 is a tumor suppressor gene located on 3p21, a chromosome region frequently lost in mesothelioma [61]. In contrast to many other solid tumor types, MPM has been reported to be rarely mutated in TP53 (Figure 4) [63].

Only limited data are available on whole genome sequencing of MPM cases. A recent study reported several chromosomal copy number variations (CNV) in a single MPM tumor sample [64]. In some chromosomes, copy number losses were detected (4, 14, 18, 19, and 10), others showed gains (5) or a combination of both (1, 8, 9, 11, 15, 16, 17, 21, and 22). Seventeen tumor-specific genes were identified, some of which are considered to be candidates for further investigation regarding future therapeutic options. Among genes of interest were mitogen-activated protein kinase kinase 6 gene (MAP2K6), dipeptidyl-peptidase 10 (DPP10), pterin-4-alpha-carbinolamine dehydratase 2 (PCBD2) and dihydrofolate reductase (DHFR). DHFR is of specific interest since it encodes an enzyme being important in the folate metabolism, which might be linked to reduced antifolate (ie. pemetrexed) treatment response in some MPM patients [65].

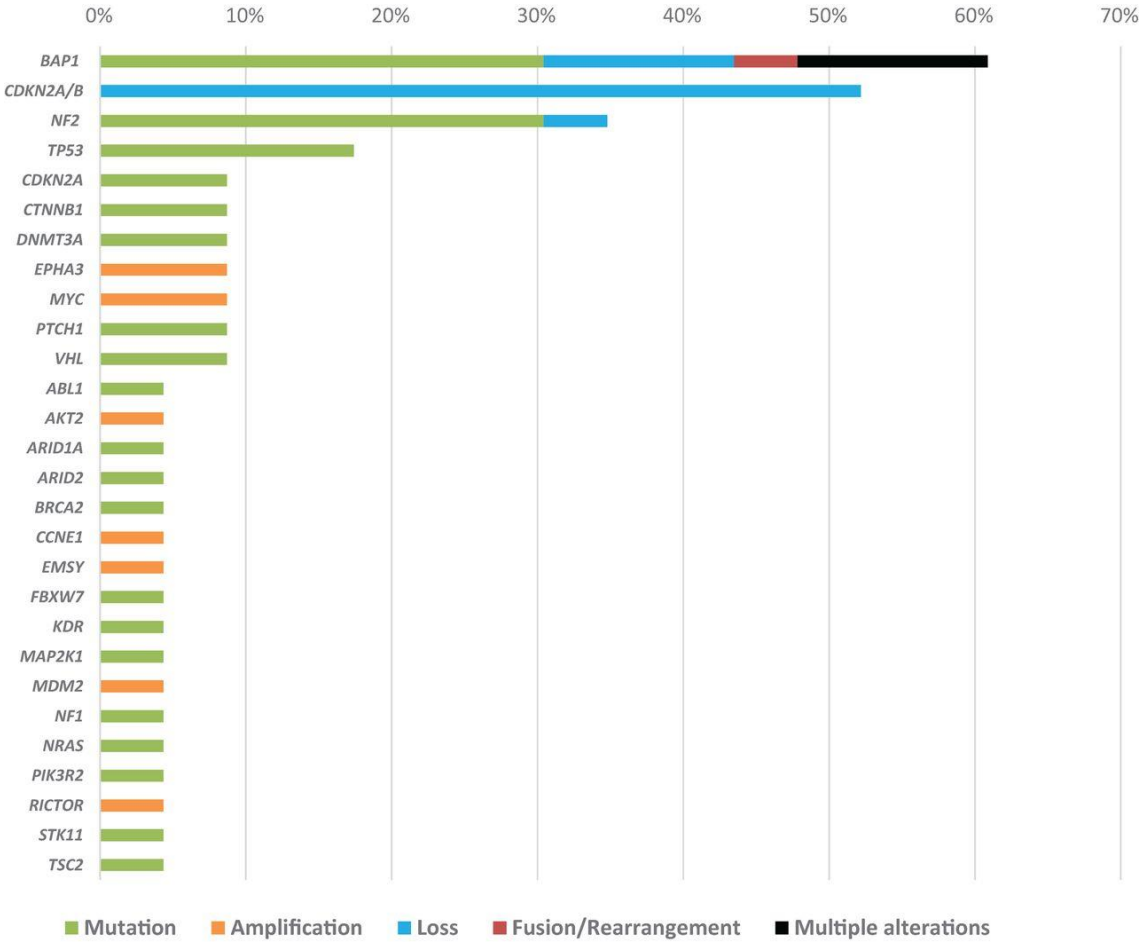

Figure 4: Frequency and types of genetic aberrations (mutation, amplification, loss, fusion/rearrangement or multiple alteration) among 23 pleural mesothelioma cases [63].

In this study, 3 further heterozygous point mutations were identified apart from CNVs. These were noted in NK6 homeobox 2 gene (NKX6-2), cadherin 8 gene (CDH8), and nuclear factor related to kappa B binding protein gene (NFRKB).

Genetic alterations can be identified at DNA level (whole genome or exome sequencing) or at the mRNA level (i.e. transcriptome sequencing). Several reports are already available with these techniques in human MPM. To date, the largest and most comprehensive analysis has been reported by Bueno and colleagues [66]. In their study, transcriptomes (n=211), whole exomes (n=99) and targeted exomes (n=103) from 216 MPM patients were analyzed. By whole exome analysis, the following genes were found to be significantly mutated: BAP1, NF2, TP53, SET domain containing 2 (SETD2), DEAD-box helicase 3 (DDX3X), Unc-51 Like Autophagy Activating Kinase 2 (ULK2), Ryanodine receptor 2 (RYR2), Cilia and Flagella Associated Protein 45 (CFAP45), SET Domain Bifurcated 1 (SETDB1) and DEAD-Box Helicase 51

(DDX51). Furthermore, recurrent gene fusions and splice alterations were identified, being frequent inactivation mechanisms for NF2, BAP1 and SETD2. Through integrated analyses, alterations in Hippo, mTOR, histone methylation, RNA helicase and p53 signaling pathways in human MPM were identified. The authors concluded that incorporating genomic analysis for the detection of actionable alterations as part of new treatment strategies will help in developing rational individualized therapy in MPM patients.

Based on recent knowledge, it is likely that several mutations need to eventually accumulate for MPM development. The long latency period between asbestos exposure and MPM formation could support this hypothesis. However, most of the aforementioned mutations can be clustered in 4 main intracellular pathways: TP53/DNA repair, cell cycle regulation, mitogen-activated protein kinase (MAPK) and PI3K/AKT [67].

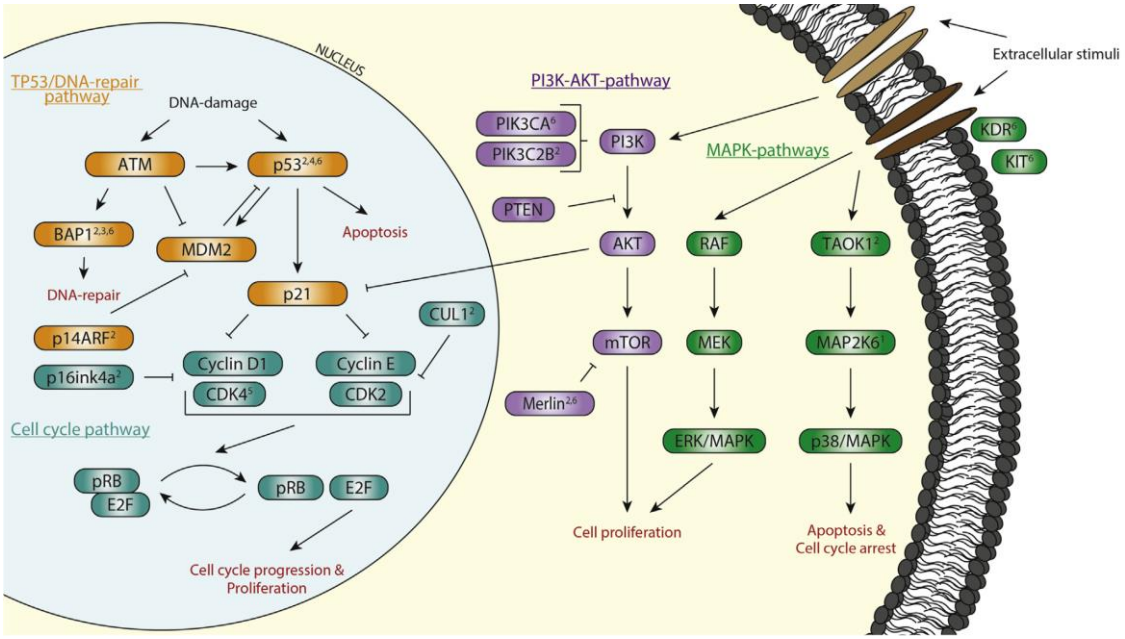

Figure 5: Affected pathways in MPM. Observed mutations cluster in four main pathways: the tumor protein p53 (TP53)/DNA-repair pathway (orange), the cell cycle pathway (blue), the mitogen-activated protein kinase (MAPK) pathway (green), and the phophatidylinositol-3 kinase (PI3K)-AKT pathway (purple) [67]

Each of these pathways is known to be important in cell growth, proliferation, and survival, processes that are all altered during tumor development [67].

Apart from somatic mutations in MPM, germline BAP1 mutation is the first gene reported to predispose for MPM development [68]. BAP1 is a nuclear protein, involved in transcriptional regulation, chromatin regulation, and forming part of multiprotein complexes that regulate cellular differentiation, gluconeogenesis, cell cycle checkpoints, transcription and apoptosis. The BAP1 gene is located on chromosome 3p21, a region that shows loss or deletion in 30–60% of mesotheliomas [68]. In families carrying BAP1 mutation, there is a dramatically increased incidence of malignant tumors, often diagnosed at earlier age compared to the general population [115]. In consequence, a "BAP1 cancer syndrome" has been proposed, including mesothelioma, uveal and cutaneous melanoma and possibly other malignant tumors [69]. Moreover, germ-line BAP1 mutations have been described in families with extraordinarily high incidence of mesothelioma [69].

2.1.4 Histology

In 2015, the most recent World Health Organization (WHO) classification of tumors of the pleura has been published. While the histologic classification of MPM remains the same in the 2015 version as it was in the 2004 WHO classification, some new observations have been reported [70-72]. MPM derives from pleural stem cells, exhibiting both epithelioid and sarcomatoid growing patterns at the same time. Depending on which component is predominant, three histological types of MPM can be distinguished: epithelioid (50–70 %), sarcomatoid (7–20 %) and a mixed or biphasic form (Figure 6) [73, 74]. Within the category of epithelioid MPM, a variety of morphologic subtypes are defined, including tubulopapillary, papillary, micro-papillary, trabecular, solid, and pleomorphic (Table 1) [71]. Especially the pleomorphic subtype of epithelioid MPM has been demonstrated to be associated with significantly poorer outcome, similar to that of patients with biphasic or sarcomatoid subtype [75].

Since there are morphological similarities of MPM to LADC, the histological diagnosis of epithelioid MPM can be challenging. However, besides hematoxylin and eosin (H&E) staining, additional immunohistochemistry can be a useful in distinguishing MPM from secondary malignances (including lung carcinomas) spreading to the pleura. Specificity and sensitivity considerations support the use of calretinin, cytokeratins 5/6, WT-1, and podoplanin (D2-40) as positive mesothelial markers, and carcinoembryonic

antigen (CEA), B72.3 (recognizing tumor-associated glyocoprotein 72), Bg8 (blood group 8, detecting the Lewis Y antigen, type 2 chain), BerEP4 (differentiating glandular epithelium from mesothelium) and MOC-31 as positive carcinoma markers [71].

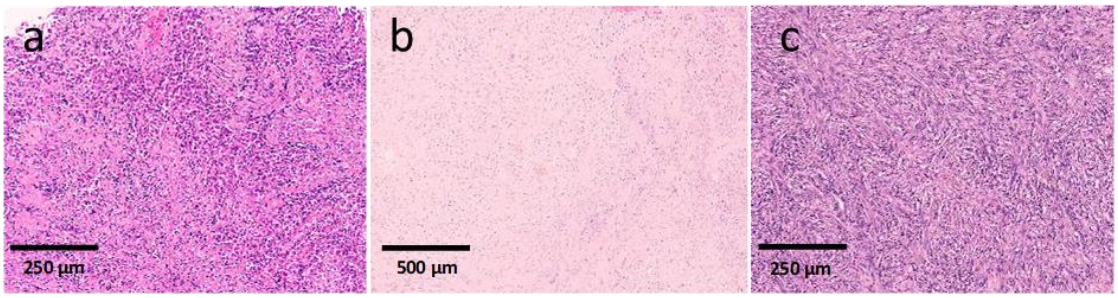

Figure 6: Examples of epithelioid a), biphasic (b) and sarcomatoid (c) MPM [73].

Table 1: Histological specification of malignant pleural mesothelioma [70, 72]

Epithelioid	Sarcomatoid	Biphasic mixed
– Tubulopapillary – Acinar – Glandular – Adenomatoid – Solid epithelioid patterns – Small cell – Oat cell – Pleomorphic Differential diagnosis: metastatic carcinomas and other epithelioid tumors	Mimic malignant mesenchymal tumors: leiomyosarcoma synovial sarcoma Desmoplastic mesothelioma bland tumor cells Differential diagnosis: sarcomatoid carcinoma and other sarcomas	Combination of all epithelioid and sarcomatoid features Differential diagnosis: Synovial sarcoma, other mixed or biphasic tumors

Sarcomatoid MPM consists of irregularly arranged elongated spindle cells, which show no uniformity in shape. The biphasic subtype shows a mixture of both, epithelioid and sarcomatoid elements.

The pathological diagnosis and differential diagnosis of MPM can be very challenging. In a French study, the initial diagnosis of MPM was revised as false positive in 13 % of cases [76]. This can be explained in part by the fact that MPM can present in very heterogeneous forms on the one hand and must be distinguished from benign processes and other tumors. Such a differential diagnosis can be particularly difficult since mesothelioma-like features can also be found in some lymphomas, thymomas, and carcinomas, etc. However, Brcic and colleagues found a substantial interobserver reproducibility among two observers in the histological subtyping of MPM [74].

In small tissue biopsies, early invasive MPM is particularly difficult to be diagnosed, often disguised by cutting artefacts or the malorientation of sections, but may be suspected if there is nodular mesothelial cell proliferation. If definitive invasion cannot be identified, the diagnosis of "atypical mesothelial proliferation" is appropriate, and further sampling may be indicated. Distinguishing MPM from inflammatory pleural disease requires a full-thickness biopsy sample, with correct orientation of histological sections, perpendicular to the pleural surface [77, 78].

Moreover, some specific morphologic criteria are recognized in order to distinguish MPM from reactive mesothelial hyperplasia and organizing pleuritis and these are depicted in Table 2. However, the application of these criteria might be challenging due to size of the specimens, sampling problems, entrapment of mesothelial cells and superficial or tangential cuts [71, 79, 80].

Table 2: Tissue features of reactive atypical mesothelial hyperplasia versus epithelioid MPM. Adopted from [71]

Histological Features	Atypical Mesothelial Hyperplasia	Malignant Mesothelioma
Major criteria		
Stromal invasion	Absent	Present (the deeper the more definitive)
Cellularity	Confined to the pleural surface	Dense, with stromal reaction
Papillae	Simple, lined by single-cell layer	Complex, with cellular stratification
Growth pattern	Surface growth	Expansile nodules, complex and disorganized pattern
Zonation	Process becomes less cellular towards chest wall	No zonation of process, often more cellular away from effusion
Vascularity	Capillaries are perpendicular to the surface	Irregular and haphazard
Minor criteria		
Cytological atypia	Confined to areas of organizing effusion	Present in any area, but many cells are deceptively bland and relatively monotonous
Necrosis	Rare (necrosis may be within pleural exudates)	Necrosis of tumor area is usually a sign of malignancy
Mitoses	Mitoses may be plentiful	Many mesotheliomas show few mitoses (but atypical mitoses favor malignancy)

2.1.5 Screening, clinical diagnosis, prognosis and staging of MPM

In current international guidelines (ie. European Society of Thoracic Surgeons/European Respiratory Society (ESTS/ERS) guidelines), no general screening methods in order to detect asymptomatic MPM are recommended. This is based on the low sensitivity of even advanced imaging techniques such as low-dose computed tomography (CT) in screening of asbestos workers [81]. Circulating biomarkers such as fibulin-3, osteopontin, mesothelin-related peptides and soluble mesothelin-related peptide (SMRP) have been extensively investigated in MPM and asbestos exposed individuals

[82-84]. However, none of them are yet to be considered as a reliable screening tool [81, 85, 86].

Specific clinical symptoms of MPM are comprised of dyspnea, cough and chest pain on initial examination. Dyspnea is often caused by pleural effusion and later by extensive restriction due to pleural and pulmonary tumor masses in the thoracic cavity. Chest pain might be diffuse, sometimes radiating into the shoulders, arms or abdomen. Tumorous invasion of the brachial plexus and the intercostal or paravertebral nerves can additionally cause neuropathic pain. Weight loss is a symptom of more advanced disease [73]. Since MPM development is associated with previous asbestos exposure (as described above), it is recommended to obtain a detailed occupational history [86].

Typically, MPM occurs as an initially unilateral disease. During disease progression, the tumor can, however, spread to the contralateral pleural cavity or into the peritoneum. Compared with lung cancer, distant metastases in the extrathoracic lymph nodes or in other parenchymal organs are usually rare, although they do occur at more advanced stages [87].

In patients with suspected MPM and recurrent pleural effusions and/or pleural thickening, the recommended initial evaluation includes contrast-enhanced computed tomography (CT) scan of the chest, thoracentesis for cytological assessment of the effusion, pleural biopsy and general laboratory blood tests [73, 81, 85, 86]. Plain chest x-ray lacks sufficient sensitivity for routine diagnosis and staging as significant pleural effusions can mask pleural lesions.

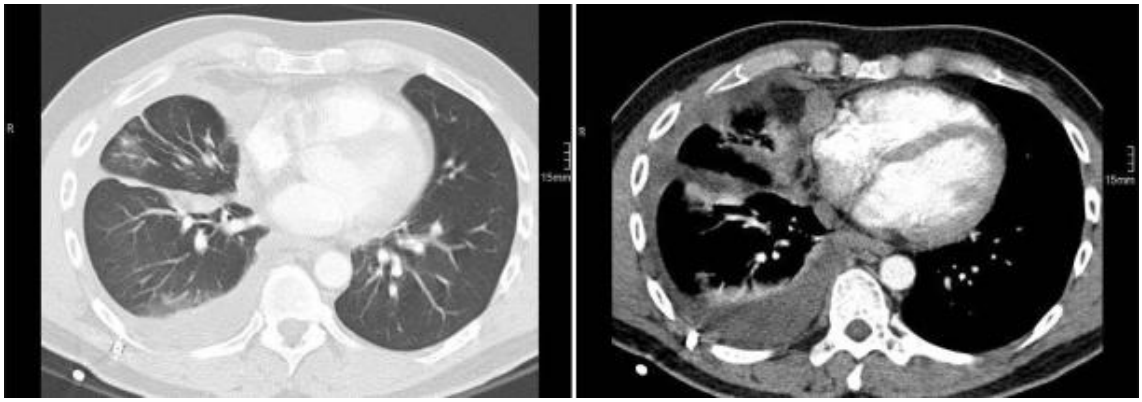

Figure 7: Computer tomography of a patient with MPM showing circular involvement of the visceral and parietal pleura, pericardium and mediastinum. Pulmonary window (left) and mediastinal window (right). Adopted from [73]

By radiological approaches, it can be difficult to distinguish between malignant and benign pleural disease and also to distinguish MPM from other malignant tumors spreading to the pleura such as metastatic carcinomas, sarcomas and thymomas [78].

A thoracoscopy is recommended to obtain adequate biopsies for histological verification, to optimally stage and to allow pleural fluid evacuation (with or without pleurodesis). This can usually be performed as a pleuroscopy or as video-assisted thoracic surgery (VATS). Fine-needle aspiration (FNA) is not routinely recommended for diagnosing MPM as its diagnostic yield is inferior compared to thoracoscopy [88]. MPM can be difficult to pathologically identify and it is therefore recommended to obtain biopsies from tissue of both abnormal and normal appearance. In case a VATS is not feasible or contra-indicated, ultrasound-guided true-cut biopsies are a good alternative [86]. When a biopsy is not possible, appropriate clinical and radiological features may assist in suggesting a diagnosis of MPM. In very rare cases, a surgical open biopsy might be necessary for definitive tissue diagnosis [8]. Micro-anatomical assessment of tissue biopsy samples permits to measure the level of host tissue invasion. Immunohistochemistry (IHC) is pivotal in confirming the mesothelial origin of MPM cells, but cannot confirm their biological potential. A larger tissue biopsy and more targeted sampling approach (radiological or surgical (VATS or open procedure)) might result in a more reliable and definitive diagnosis. Thus, in the vast majority of cases, adequate tissue biopsies and the use of appropriate IHC for definitive, primary diagnosis of MPM are necessary. Consequently, definitive histopathological diagnosis of MPM by using frozen sections is not recommended [86]. Cytological features in effusions may permit a diagnosis of malignancy but reported sensitivities and specificities vary widely [77, 89]. In a high number of cases, MPM lacks significant cytological atypia and it is impossible to distinguish between benign, reactive mesothelial proliferations and MPM. Cytology sample cells may show variable atypia (usually low grade) and exhibit a mesothelial immune phenotype, but malignancy cannot be confirmed in most of the cases.

Because the precise diagnosis of MPM requires histopathological confirmation, thoracoscopy via VATS in operable patients remains the standard procedure for obtaining tissue and performing macroscopic staging at the same time (Figure 8) [73, 81].

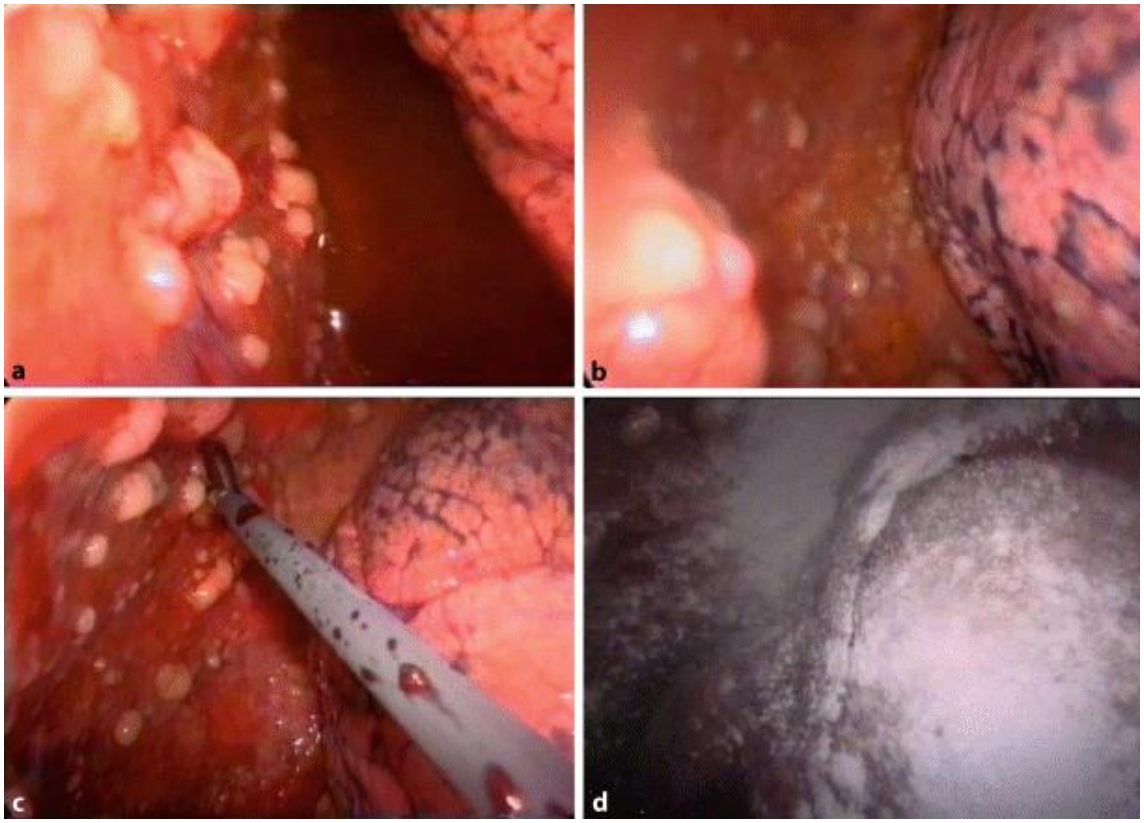

Figure 8: VATS view of forceps biopsy taken from tumor nodules located on the parietal pleura of a patient with MPM. Note the black streaks of anthracotic pigment visible between lobules of the lung beneath the visceral pleura (a). Macroscopic view of the chest cavity after talc pleurodesis (d). Adopted from [73]

Thoracoscopy can be performed under local anesthesia or via surgical approach by VATS in general anesthesia. The VATS procedure allows the combination of a diagnostic procedure with an initial palliative/therapeutic step of talc pleurodesis.

Multiple and deep tissue biopsies obtained by a thoracoscopic procedure are strongly recommended by the Guidelines of the ERS and ESTS, except in the case of pre-operative contraindication or pleural symphysis [81]. Thoracoscopy should be preferred for diagnostic investigation as it allows complete visual examination of the pleura, taking multiple, deep and large biopsies (preferably including fat and/or muscle to assess tumor invasion) and providing a diagnosis in >90% of cases [81].

In cases being considered inoperable, the aforementioned diagnostic procedures should be performed obligatory before starting local or systemic treatment. In order to stage and assess patients diagnosed with MPM whether they are candidates for surgery, the National Comprehensive Cancer Network (NCCN) guidelines as well as the guidelines

of the Austrian Mesothelioma Interest Group (AMIG) suggest to perform contrast enhanced CT of the chest and the abdomen and combined fluorodeoxyglucose positron emission tomography (FDG-PET) CT to rule out distant metastasis and involvement of the abdomen and the mediastinal lymph nodes (Figure 9) [85]. VATS or laparoscopy can be considered if contralateral pleural or peritoneal spread is suspected. If indicated, integrated FDG-PET/CT should ideally be performed before pleurodesis, since talc causes pleural inflammation, which can result in false positive findings by affected FDG avidity [90].

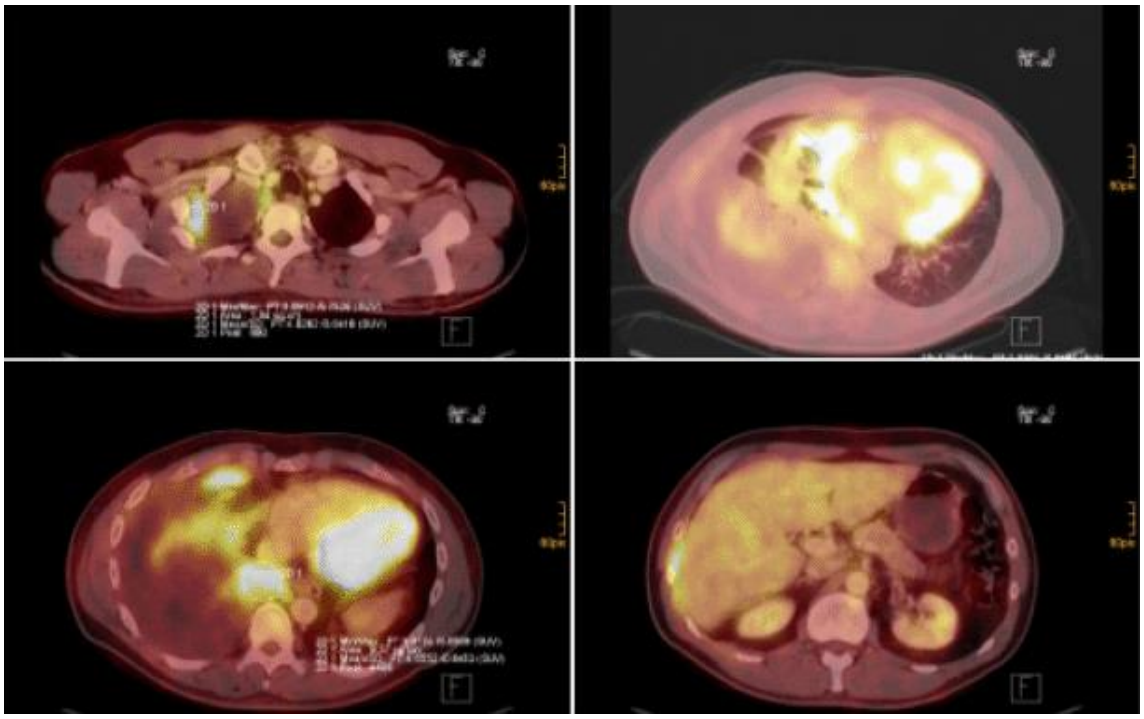

Figure 9: FDG PET-CT images: MPM of the right pleural cavity. Various slides of CT/PET fusion imaging showing pleural tumor apical right (top left), involving the visceral and parietal pleura of the costodiaphragmatic area (bottom left and right) and pericardium (top right). Adopted from [73]

In order to rule out involvement of mediastinal lymph nodes, histological confirmation has to be made either by endobronchial/endo-esophageal ultrasonography and transbronchial needle aspiration (EBUS/EUS-TBNA) or mediastinoscopy or VATS according to the lymph node station involvement and the involved side [91, 92].
A possible algorithm for diagnosis and staging as proposed by the NCCN is depicted in Figure 10.

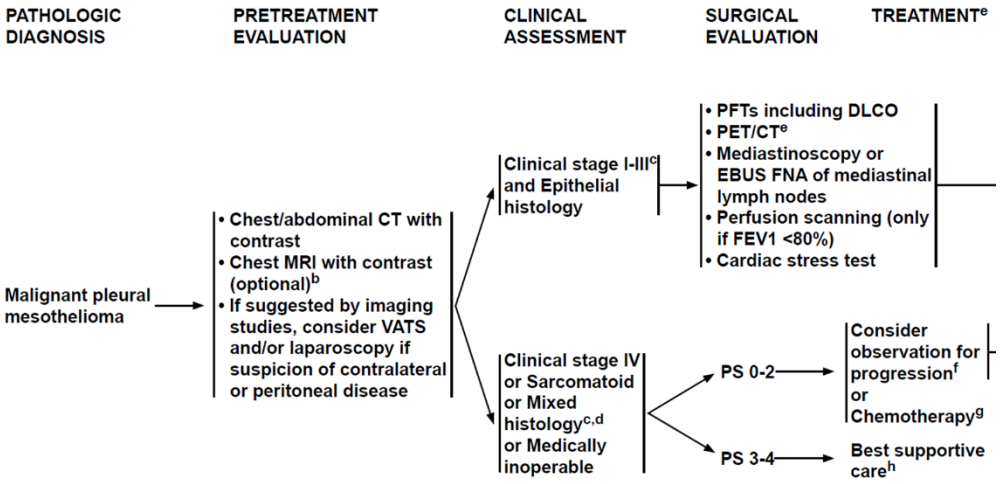

Figure 10: Initial diagnostic and staging procedures for patients with MPM as proposed by the NCCN [85].

Staging procedures are standard in all malignant tumors including MPM. An appropriate staging system describes the anatomical extent of the tumor, correlates with prognosis and facilitates treatment decisions. In case of MPM, different staging systems have evolved during the past 30 years, most initially developed from small single-center experiences and predominantly retrospective surgical cases [93]. The first staging system by Butchart consisted of four stages and was based on observations from 29 patients only [94]. Another staging system as proposed by the International Mesothelioma Interest Group (IMIG) and the International Association for the Study of Lung Cancer (IASLC) was developed in 1995 [95]. Although the IMIG staging system could predict prognosis, it failed to be an independent prognostic factor when analyzed in the clinical setting using multivariate analysis [96]. The most recent staging system is based on a large international database analysis, set up by the IASLC and was recently published in 2016 [47, 97, 98].

	N0		N1/N2	N1
Stage	Seventh Edition	Eighth Edition	Seventh Edition	Eighth Edition
T1	I (A, B)	IA	III	II
T2	II	IB	III	II
T3	II	IB	III	IIIA
T4	IV	IIIB	IV	IIIB
M1	IV	IV	IV	IV

	N3	N2
Stage	Seventh Edition	Eighth Edition
T1	IV	IIIB
T2	IV	IIIB
T3	IV	IIIB
T4	IV	IIIB
M1	IV	IV

Figure 11: TNM stage groupings of the revised TNM-8 versus the previous TNM-7 staging system in MPM. Adopted from [97]

The most important changes compared to the previous IMIG/IASLC staging system include: the incorporation of both clinical and pathological T1a and T1b into a T1 category and both clinical and pN1 and pN2 categories into a single N category (comprising ipsilateral, intrathoracic nodal metastases (N1)). Nodes that have been previously categorized as N3 are reclassified as N2 now (Table 3). Furthermore, measurement of pleural thickness on CT scans has been proposed for further studies, as tumor thickness and nodular or rind-like morphology were significantly associated with survival. These recently proposed revisions for the TNM-8 stage descriptors and groupings should provide a better estimation of outcomes. In the future, additional data collected from both surgically and non-surgically managed patients will also help to refine these stage groupings (Figure 11) [97].

Table 3: Definitions for T, N and M descriptors in the recently revised TNM-8 staging system for MPM. Adopted from [97]

Stage	Definition
Primary tumor (T)	
TX	Primary tumor cannot be assessed
T0	No evidence of primary tumor
T1	Tumor limited to the ipsilateral parietal ± visceral ± mediastinal ± diaphragmatic pleura
T2	Tumor involving each of the ipsilateral pleural surfaces (parietal, mediastinal, diaphragmatic, and visceral pleura) with at least one of the following features: • involvement of diaphragmatic muscle • extension of tumor from visceral pleura into the underlying pulmonary parenchyma
T3	Describes locally advanced but *potentially resectable* tumor. Tumor involving all of the ipsilateral pleural surfaces (parietal, mediastinal, diaphragmatic, and visceral pleura) with at least one of the following features: • involvement of the endothoracic fascia • extension into the mediastinal fat • solitary, completely resectable focus of tumor extending into the soft tissues of the chest wall • nontransmural involvement of the pericardium
T4	Describes locally advanced *technically unresectable* tumor. Tumor involving all of the ipsilateral pleural surfaces (parietal, mediastinal, diaphragmatic, and visceral pleura) with at least one of the following features: • diffuse extension or multifocal masses of tumor in the chest wall, with or without associated rib destruction • direct transdiaphragmatic extension of tumor to the peritoneum • direct extension of tumor to the contralateral pleura • direct extension of tumor to mediastinal organs • direct extension of tumor into the spine • tumor extending through to the internal surface of the pericardium with or without a pericardial effusion, or tumor involving the myocardium
Regional lymph nodes (N)	
NX	Regional lymph nodes cannot be assessed
N0	No regional lymph node metastases
N1	Metastases in the ipsilateral bronchopulmonary, hilar, or mediastinal (including the internal mammary, peridiaphragmatic, pericardial fat pad, or intercostal lymph nodes) lymph nodes
N2	Metastases in the contralateral mediastinal, ipsilateral, or contralateral supraclavicular lymph nodes
Distant metastasis (M)	
M0	No distant metastasis
M1	Distant metastasis present

Several clinical prognostic factors for MPM have been reported. These are similar to those described in other solid tumor types. The prognosis for MPM highly depends on the tumor stage and histological subtype and, moreover, on the patient's age and gender[99]. Patients with epithelioid MPM has a better OS than those with non-epithelioid tumors (median OS: 19 months vs. 13 months (biphasic) and 8 months (sarcomatoid)) [100]. In addition, performance status (PS) has shown to be an independent prognostic factor for OS [8]. Low hemoglobin levels, high platelet levels and high serum lactate dehydrogenase (LDH) are prognostically unfavorable characteristics. Numerous new laboratory markers with the purpose of facilitating decision making are have been evaluated, but no validated data on their prognostic value are available yet [101]. Additionally, our group has previously shown that high circulating c-reactive protein (CRP) and fibrinogen are generally associated with poor prognosis, independently from stage and histological subtype [102, 103].

In general, MPM is a devastating disease and despite many efforts regarding early detection and treatment, outcome remains poor. Even at early stages, minimal tumor burden and lack of distant metastases, median OS ranges from 18 to 23 months, with an expected 5-year survival rate of 15% only [97].

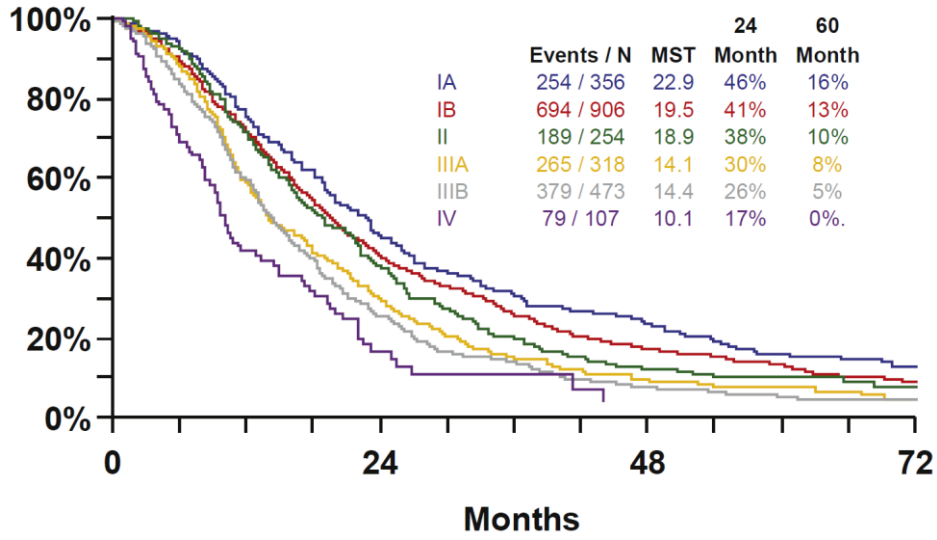

Figure 12: Overall survival according to the TNM-8 staging system for MPM. Adopted from [97]

In more advanced stages, palliative treatment approaches might be necessary and most of the patients die from tumor cachexia, body consumption and respiratory problems caused by secondary pneumonia and respiratory insufficiency with hypoxia and asphyxia.

2.1.6 Therapeutic approaches

Current treatment guidelines recommend that patients with MPM should be managed by a multidisciplinary team with experience in treating MPM. Treatment options in general include chemotherapy (CHT), radiotherapy (RT) and surgery. Selected cases with favorable prognostic parameters (i.e. clinical stage I, medically operable, good PS, epithelioid subtype) might be candidates for combined multimodality therapy [104, 105]. Definitive RT alone is not recommended for unresectable MPM, however, CHT alone is approved in this setting [85, 106]. Appropriate patients should undergo evaluation by medical oncologists, radiation oncologists, thoracic surgeons, diagnostic imaging specialists and pulmonologists in order to assess if they are amenable for multimodality treatment. Best supportive care is recommended for patients with PS 3 to

4. Observation may be considered for patients with PS 0 to 2 who are asymptomatic with minimal disease burden if CHT is planned when progression occurs [85].

2.1.6.1 Systemic therapy

In general, CHT alone is recommended for patients with PS 0 to 2, clinical stage IV disease, sarcomatoid histology (due to the poor prognosis) or who are not candidates for surgery [85].

Prior to the early 2000s a nihilistic attitude persisted among clinical oncologists towards anti-MPM therapy because of the lack of response to standard therapies. Nevertheless, although the role of aggressive surgery remains controversial and no chemo- or targeted therapy has proved fully effective against MPM, robust evidence has emerged to support the use of chemotherapy and angiogenesis inhibition during the past decades [42].

In a meta-analysis including all phase II studies published between 1965 and 2001, cisplatin was found to be the most effective single agent and thus it became the mainstay for combinational therapeutic interventions in MPM [107]. Accordingly, after encouraging data of phase I and II pemetrexed (a multitargeted antifolate that inhibits thymidylate synthase, dihydrofolate reductase and glycinamide ribonucleotide formyl transferase) studies, a single-blinded phase III trial (Evaluation of Mesothelioma in a Phase III Trial of pemetrexed with Cisplatin, EMPHACIS) recruited 448 chemonaive patients with MPM randomly assigned to receive cisplatin at 75 mg/m2 plus either pemetrexed at 500 mg/m2 or placebo every 3 weeks [108]. The median OS in the combination arm was 12.1 months, compared to that of 9.3 months in cisplatin-alone patients. Based on this study, combination chemotherapy with pemetrexed and cisplatin (with folic acid and vitamin B12 supplementation) became the current standard of care for first-line systemic therapy in patients with unresectable MPM and good PS. Although cisplatin monotherapy was never compared to placebo in a randomized trial, these results enforced the recommendation for combination chemotherapy. So far, all current major international guidelines endorse a combination of platinum-based CHT with modern antifolates (pemetrexed or raltitrexed) as the gold standard treatment in MPM [81, 85, 86, 109]. Still, it has to be mentioned that these recommendations are based on two randomized trials published in more than a decade ago [108, 110].

Accordingly, until 2015, no other combination therapy was able to significantly improve OS and progression free survival (PFS) compared to the cisplatin/pemetrexed doublet [86]. However, previous studies have demonstrated that vascular endothelial growth factor (VEGF) signaling plays a crucial part in mesothelioma cell physiopathology [111, 112]. The phase II/III randomized Mesothelioma Avastin Cisplatin Pemetrexed Study (MAPS) was initiated to assess the effect on survival of the VEGF inhibiting monoclonal antibody bevacizumab when added to the present standard of care, cisplatin/pemetrexed, as first-line treatment of advanced MPM[113]. Survival was improved in those who received cisplatin/pemetrexed/bevacizumab (18.8 months, versus 16.1 months in patients receiving CHT alone (hazard ratio 0.77 [95% CI 0.62–0.95]). Although statistically significant, the overall improvement in OS (2.7 months) was still quite modest. Grade 3-4 adverse events were more common in the bevacizumab group (158 [71%] of 222 patients) (139 [62%] of 224 patients), and more patients stopped first-line treatment because of toxic effects in the bevacizumab group (53 [24.3%] of 218 patients) (13 [6.0%] of 217; difference 18.3% [11.7–24.9]). However, these side effects were considered to be tolerable and thus those patients with MPM who are not candidates for clinical trials or who do not have access to such opportunities, but who do not have contraindications to bevacizumab, should be offered three-drug combination treatment as a new standard of care [85].

Some other recent phase II and III trials have assessed the safety and efficacy of different systemic combination treatments. A combination of carboplatin and pemetrexed was investigated in 3 large phase II trials with median OS ranging from 12.7 to 14 months [114-116]. A comparison of 1704 patients with inoperable MPM treated with ether cisplatin/pemetrexed or carboplatin/pemetrexed found that outcomes were similar and concluded that the carboplatin/pemetrexed regimen might be the better choice for patients with poor PS and/or comorbidities [117]. Cisplatin/gemcitabine was assessed in phase II studies and was found to be a valid treatment option for patients not eligible for pemetrexed treatment [118]. Additional acceptable fist-line single agent regimens include pemetrexed or vinorelbine monotherapy (Table 4) [119, 120]. Maintenance therapy after successful first-line systemic treatment might be effective for symptom control and maintaining quality of life [86]. However, the benefit of maintenance treatment in MPM is yet to be evaluated and several studies are ongoing.

For example, a recent phase III randomized trial has failed to show a benefit of adding maintenance thalidomide to pemetrexed-based CHT [121].

Second-line CHT options include pemetrexed (if not administered as first-line), vinorelbine and gemcitabine [122-124]. In patients with good response to first-line pemetrexed, rechallenging pemetrexed might be effective [125]. Limited data are available to guide second-line systemic therapy; however, several agents are investigated in clinical trials [126]. Preliminary data suggest that immune checkpoint inhibitors (nivolumab, pembrolizumab, tremelimumab, ipilimumab) might be effective in the second-line setting and thus currently phase II and III trials are ongoing [127, 128]. In a recent randomized phase IIb trial, the CTLA-4 inhibitor tremelimumab did not significantly prolong OS compared with placebo in patients with previously treated malignant mesothelioma [129]. However, preliminary phase II results from a prospective randomized trial evaluating the safety and efficacy of cisplatin/pemetrexed with the triple angiokinase-inhibitor (TKI) nintedanib revealed a trend for better OS and significantly better PFS for the experimental arm. The confirmatory phase III results are to be awaited [130].

Table 4: Principles of systemic therapy in MPM according to the most recent NCCN guidelines (2.2017). Adopted from [85]

First-line	Later lines
Cisplatin, Pemetrexed	Pemetrexed
Cisplatin, Pemetrexed, Bevacizumab	Vinorelbine
Carboplatin, Pemetrexed	Gemcitabine
Cisplatin, Gemcitabine	Nivolumab, Ipilimumab
Pemetrexed	Pembrolizumab
Vinorelbine	

Interleukins and interferons were tested in studies, as well as the application of targeted therapies with monoclonal antibodies. None of the following substances showed any survival benefit in several studies: gefitinib, erlotinib, or imatinib [131-134].

2.1.6.2 Surgery

2.1.6.2.1 Radical surgical approaches

Macroscopic complete resection (MCR) is goal of every surgical procedure with curative intent [135]. Currently, multimodality treatment including radical surgery as by extrapleural pneumonectomy (EPP) or pleurectomy/decortication (P/D) are the most commonly used surgical techniques for treating MPM with curative intention. However, only a number of mainly retrospective institutional reports using different multimodality regimens and different surgical techniques is available in the current literature, due to the difficulty of prospective randomized trials in this setting. Hence, the question whether P/D or EPP is the more appropriate technique to obtain improved OS in relation to the associated posttreatment quality of life is difficult to address. After the IMIG 2012 meeting and much discussion on the role of surgery and the value of MCR in MPM, the agreement is now that (I) surgical macroscopic complete resection and control of micrometastatic disease play a vital role in the multimodality therapy of MPM, as in case of other solid tumor types. (II) Surgical cytoreduction is indicated when MCR is deemed achievable and (III) the type of surgery (EPP or P/D) depends on clinical factors and on individual surgical judgment and expertise [135].
However, since MPM is a very heterogeneous disease with variability of clinical symptoms, stage, histology, tumor burden and biological behavior, a multidisciplinary discussion of each patient considering age, PS and individual prognosis is mandatory.

2.1.6.2.2 Pleurectomy/Decortication

As the technique of P/D includes a variety of surgical procedures with different clinical indications, the IASLC and IMIG have recently proposed a common nomenclature for these different techniques [136]. Partial pleurectomy was defined as a cytoreductive procedure with partial removal of the visceral and/or parietal tumor gross, without removing the lung or the intention for MCR. P/D was defined as complete resection of the parietal and visceral pleurae, and extended P/D as a technique with additional resection and reconstruction of the pericardium and diaphragm (Figure 13). In a recent systematic review investigating previous results of P/D, mortality and morbidity ranged from 0% to 11% and 13% to 43%, respectively. Median OS ranged from 7.1 to 31.7 months and disease-free survival (DFS) from 6 to 16 months [137]. A detailed analysis

suggested extended P/D to be associated with a higher number of perioperative morbidity whilst favoring the more aggressive approach for improved oncological outcome.

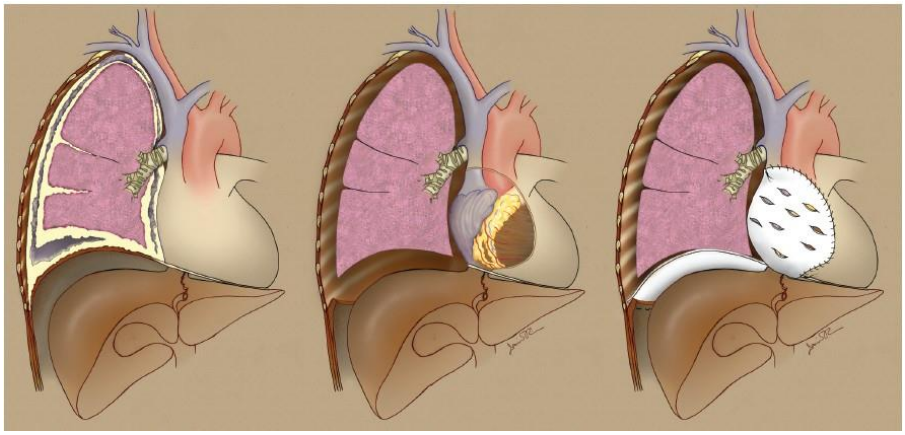

Figure 13: Surgical technique of extended pleurectomy/decortication for MPM. Adopted from [138]

2.1.6.2.3 Extrapleural Pneumonectomy

Extrapleural pneumonectomy (EPP) is a widely standardized surgical technique with en-bloc resection of the lung together with the parietal and visceral pleurae, the pericardium and diaphragm (Figure 15) [139]. The role of EPP has been recently under discussion after the publication of the Mesothelioma and Radical Surgery (MARS I) trial which aimed to assess the outcomes of patients after EPP or no EPP in the context of trimodal therapy within a prospective randomized trial [140]. Median OS was 14.4 months (5.3-18.7) for the EPP group and 19.5 months (13.4 to time not reached) for the no EPP group. EPP was associated with an unacceptably high perioperative mortality of 15.8% and morbidity of 68.8%. However, only 16 of the randomized patients have undergone EPP. The authors of the study concluded that radical surgery by EPP within a multimodality approach offers no benefit in OS and possibly harms patients. However, the MARS I trial was designed to assess the feasibility of randomizing patients with MPM for surgery and not to evaluate morbidity or benefit in OS of EPP. In order to sufficiently address this question an actual number of 670 patients to identify a significant survival benefit would have been needed [135]. A recent systematic review including 58 studies reported a median OS after EPP of 9.4 to 27.5 months, and 1-, 2-, and 5-year survival rates ranging from 36 to 83%, 5 to 59% and 0 to 24%, respectively. Overall perioperative mortality ranged from 0 to 11.8%, and the perioperative morbidity

from 22 to 82% [141]. A recent retrospective evaluation of our own group together with colleagues from Toronto and Zurich including 251 patients completing EPP after platin-based CHT reported a 30-day mortality of 5% and perioperative complication rate of 30% [105]. However, according to the recent ERS/ESTS guidelines, EPP should only be performed at high volume centers within the context of a clinical study and a multimodality regimen [81]. Moreover, the current NCCN guidelines suggest to perform EPP in early stage patients only [85].

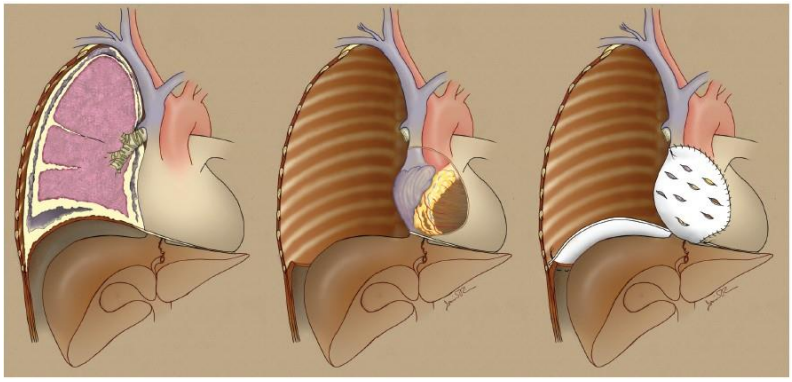

Figure 14: Surgical technique of extrapleural pneumonectomy for MPM. Adopted from [138]

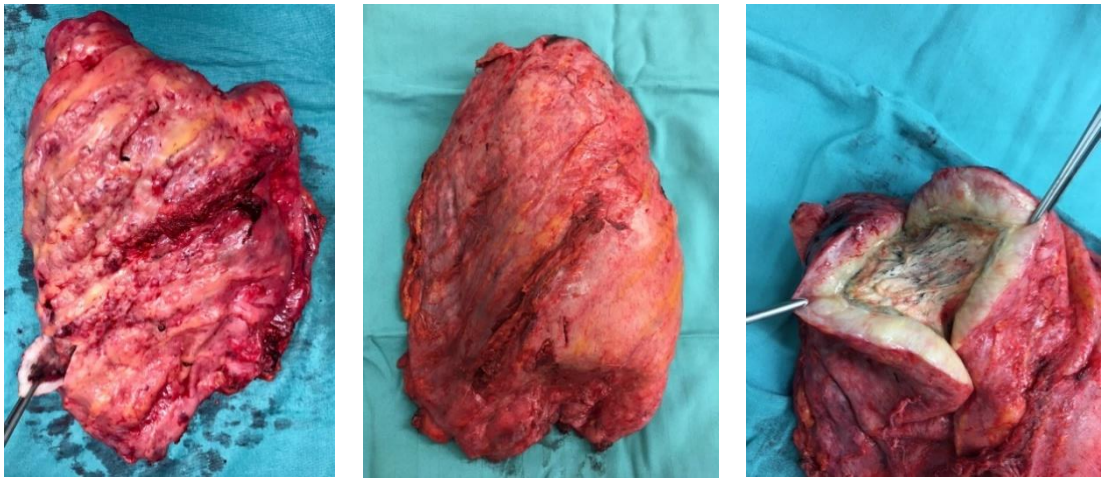

Figure 15: Resection specimens after extrapleural pneumonectomy for MPM at the Division of Thoracic Surgery, Medical University of Vienna

The question whether to perform P/D or EPP in which clinical situation is difficult to address. Initial data from the IASLC Staging Committee Statistical Center including 1494 surgically-treated patients reported that stage I tumors resected by EPP were associated with a median OS of 40 months whereas those managed by P/D had a

median OS of 23 months. No differences in survival between EPP and P/D were identified in patients with higher-stage disease [100]. To date, the largest cohort of patients treated with P/D or EPP was published by Flores et al in 2008. Of 663 consecutive cases, the operative mortality was 7% for EPP (n = 27/385) and 4% for P/D (n = 13/278) [142]. OS was reported to be superior after P/D, however patients selected for EPP had more local advanced disease and P/D was applied in earlier stages. Another recently published meta-analysis compared the perioperative and long-term outcomes of EPP and extended P/D for selected surgical candidates [143]. A comparison between EPP and P/D revealed a significantly lower perioperative mortality and morbidity for patients who underwent extended P/D compared to EPP. Median OS ranged between 13-29 months for extended P/D and 12-22 months for EPP, with a trend favoring extended P/D. The authors concluded that selected patients who underwent extended P/D had lower perioperative morbidity and mortality with similar, if not superior, long-term survival compared to EPP, in the context of multi-modality therapy. However, it must be taken into account that in most of the included studies, P/D was usually chosen for earlier and EPP for more advanced stages.

2.1.6.2.4 Patient selection for surgery

Taken all this information together, patients undergoing surgery for MPM must be carefully selected to outweigh the potential risks with the respective procedures. Several studies have been published assessing clinical prognostic factors and biomarkers for estimating prognosis and proper patient selection. For example, non-epithelioid histology and nodal involvement have consistently been demonstrated to be associated with worse prognosis after EPP [144]. Furthermore, several blood biomarkers and combinations of several clinical factors as prognostic scores have been investigated to identify patients who are more likely to benefit from surgical treatment. Among these, pre-treatment CRP predicted benefit in OS from multimodality therapy including curative intent surgery [102]. Multivariate analysis confirmed that patients with low CRP levels have significantly improved OS compared to those with elevated levels independently from other relevant clinical factors. Moreover, circulating biomarkers such as fibrinogen and albumin were reported to influence prognosis and could be implemented into therapeutic algorithms [103, 145]. Our study group has recently

combined our experience with the colleagues from Zurich, Switzerland in order to assess the prognostic value of a new multimodality score including the following factors: tumor volume, histology, CRP and response to CHT. This prognostic score was found to be useful in allocating patients to surgery after CHT in the two independent cohorts [146]. Moreover, patient data from 10 European Organization for Research and Treatment of Cancer (EORTC) trials of chemotherapy in MPM were pooled and PFS at 18 weeks strongly correlated with OS and discriminated those with improved OS from the patients with poorer prognosis [147].

Finally, not much data is currently available on quality of life (QoL) after surgical treatment of MPM. A recent study has included QoL data in their institutional report and found a superiority of P/D versus EPP in QoL after 6 and 12 months [148]. In line with this, the MARS trial found that QoL scores were higher in the non-EPP group, however, no significant differences between groups were observed in QoL analyses [140].

2.1.6.2.5 Multimodality treatment

Several studies found that surgery alone offers poor prognosis and thus multimodality approaches including CHT and/or RT have been suggested to improve outcome. However, to date, there are no randomized trials available addressing the question if chemo-, radio- or combined chemo/radiotherapy might be the preferred induction treatment before surgery. Before EPP, many high-volume institutions reported on favorable experiences with induction CHT followed by surgery and hemithoracic intensity modulated radiotherapy (IMRT) [142, 149]. The obvious advantage of induction CHT is a possible reduction of tumor volume and the consequent downstaging. The effects of induction CHT and EPP have recently been studied in a prospective multicenter phase II trial including 61 patients. Forty-five patients (74%) underwent EPP and in 37 patients (61%) the resection was complete. Postoperative RT was administered in 36 patients. Median OS of all patients was 19.8 months. In the 45 patients with EPP, median OS was 23 months [150]. A new multimodality protocol was recently suggested by the Toronto group and is currently under investigation in the "Surgery for Mesothelioma After Radiation Therapy (SMART)" trial. A short accelerated course of high-dose hemithoracic IMRT is administered followed by EPP

[151]. A total of 62 patients were included so far. The median OS of all patients was 36 months (51 months in the subgroup of patients with epithelioid MPM). Moreover, complication rates of this protocol were found to be acceptable. These results suggest that this protocol is feasible without elevated perioperative morbidity and mortality and, moreover, that it provides encouraging long-term outcome [152]. However, further prospective trials are needed to investigate the value of different multimodality strategies in the treatment of MPM.

Intracavitary treatment

Because of MPM's locally invasive behavior, it is difficult to achieve R0 resection in MPM patients and thus local recurrence is a frequent problem after MCR (reported in up to 60% of cases [142]). Recently, new treatment strategies have evolved such as intracavitary chemo- or photodynamic therapy to secure resection margins at the end of the operation and consequently lower the risk of local recurrence.

Intracavitary photodynamic therapy (PDT) combines a nontoxic photosensitizing agent with visible light. PDT is used intraoperatively after P/D or EPP [153]. A study combining PDT with radical pleurectomy in patients with stage III/IV disease reported a median OS of 31.7 months for all patients and 41.2 months for patients with epithelial histology [154]. The authors concluded that this combination is effective and safe and thus warrants further prospective investigation.

Hyperthermic intraoperative chemotherapy (HIOC) delivers a high local dose CHT to the resected surface with decreased toxicity compared to systemic therapy [155, 156]. The feasibility and effects of HIOC have recently been investigated in several phase II trials and encouraging PFS and OS have been reported in patients with epithelioid MPM and low risk factors [157-159]. One particular study reported encouraging results after open partial pleurectomy and HIOC with 35.6 months median OS in stage I patients [160].

Other intracavitary treatment concepts of binding cytotoxic (or other agents) to a fibrin carrier are currently evaluated in prospective trials. The concept of localized intracavitary fibrin bound cisplatin-based chemotherapy after MCR is evaluated in a phase IIa trial conducted in Zurich to assess safety and toxicity of this protocol (NCT01644994 Influence Meso) [161].

2.1.6.2.6 Palliative surgery

In a high number of patients, MPM is diagnosed at advanced stage and symptom control plays an important role in achieving palliation [162]. Typical symptoms include dyspnea due to pleural effusion or lung encasement by tumor gross, weight loss, cough and chest pain in case of thoracic wall invasion. In case of effusion, drainage for symptom control is recommended followed by pleurodesis at first relapse [81]. Sterile talc powder is the preferred sclerosing agent and can be installed using a chest tube or after VATS biopsy if the lung expands completely [81, 162]. In case of an entrapped lung, indwelling pleural catheters may be the most practical way to manage recurrent pleural effusions [163, 164]. Recently, the MesoVATS trial has randomized MPM patients to undergo VATS pleurectomy vs. talc pleurodesis via indwelling intercostal chest drain or via thoracoscopy [165]. VATS partial pleurectomy (VATS-PP) did not significantly improve OS and talc pleurodesis was considered to be preferable due to fewer complications and shorter hospital stay. However, VATS-PP was significantly associated with improved control of pleural effusions and improved QoL at 12 months.

2.1.6.3 Radiotherapy

In patients with MPM, RT can be used as part of multimodality treatment protocol. However, RT alone is not recommended due to its poor efficacy. RT as a monotherapy may be used in a palliative attempt in patients not eligible for CHT for relief of chest pain, bronchial or esophageal obstruction or other symptomatic sites associated with metastases such as brain or bone [166, 167]. The dose should be based on the purpose of the treatment (Table 5).

Table 5: Principles of radiotherapy in MPM. Recommended doses and schedules based on the treatment purpose. Adopted from [85]

Recommended Doses for Radiation Therapy

Treatment type	Total dose	Fraction size	Treatment duration
Postoperative after EPP Negative margins Microscopic-macroscopic positive margins	 50–54 Gy 54–60 Gy	 1.8–2 Gy 1.8–2 Gy	 5-6 weeks 6-7 weeks
Palliative Chest wall pain from recurrent nodules Multiple brain or bone metastasis	20–40 Gy or 30 Gy 30 Gy	≥4 Gy 3 Gy 3 Gy	1–2 weeks 2 weeks 2 weeks
Post pleurectomy/decortication Negative margins Microscopic positive margins	 45 Gy–50.4 Gy 50 Gy–54 Gy	 1.8 Gy–2.0 Gy 1.8 Gy–2.0 Gy	 5–6 weeks 5–6 weeks

Neoadjuvant RT can be performed prior to EPP as described in chapter 0. The concept of SMART aims to increase resectability and to decrease local recurrence rate after MCR [151, 152]. In this setting, RT is delivered hypofractionated, applying a higher dose of 5 Gray (Gy) on 5 consecutive days with an integrated boost of 6 Gy to target areas of high tumor volumes. The target volume includes the whole lung including the pleural surface. IMRT is used to reduce high doses at organs at risk such as heart, esophagus, liver or spinal cord. Since a toxic dose is delivered to lung within this protocol, the EPP has to be performed within 1 week after last RT in order to avoid pneumonitis.

Adjuvant IMRT was regarded as an integral part of the classical trimodality treatment including CHT, EPP and adjuvant RT [86]. The dose of 50–54 Gy should be applied with a once daily fraction of 1.8–2.0 Gy (Table 5). The RT is usually administered between 4-12 weeks after surgery, depending on the recovery of the patient. The treatment plans should be reviewed with a thoracic surgeon to ensure coverage of all targets at risk [85]. The safety and efficacy of this approach has been investigated in larger retrospective series and one prospective phase II study [139, 168, 169]. A recent randomized multicenter phase II trial has investigated the role of adjuvant IMRT after CHT and EPP [170]. In total, 151 patients were randomized to receive CHT and EPP followed by adjuvant IMRT or follow-up. Median locoregional relapse-free survival from surgery was 7.6 months in the group without RT and 9.4 months in the RT group (not being statistically significant). Accordingly, the authors concluded that these results do not support the routine use of adjuvant IMRT in this setting.

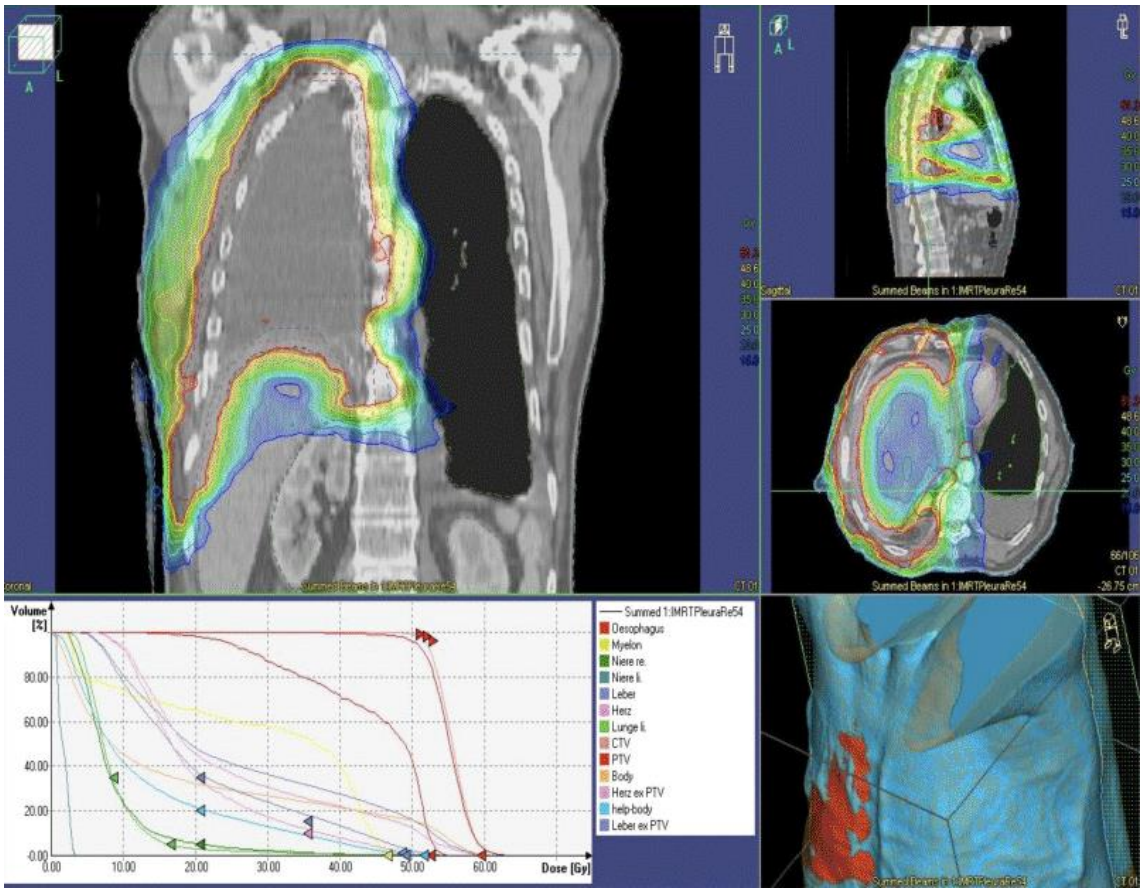

Figure 16: Postoperative situs after EPP of the right lung and pleura. IMRT dose plan. Coronal, sagittal, and axial image of the isodose plan. Steep dose fall to the remaining left lung, liver and kidney. Adopted from [109]

2.1.7 Prognostic tissue biomarkers in MPM

In the past decades, several characteristics of patients with MPM have been reported to be associated with prognosis. These factors include clinicopathological characteristics such as age, gender, performance status, asbestos exposure, stage and histological subtype [171]. In recent years, extensive research aimed to identify new therapeutic targets, predictive biomarkers and prognostic factors in this disease. MPM cells have a number of well investigated chromosomal and genetic aberrations and may express a large array of cancer associated molecules and proteins which are linked to local invasion and disease progression[172]. These molecules include circulating and tissue biomarkers related to multiple cellular pathways affecting cell survival, proliferation, apoptosis, angiogenesis, interaction with immune response and DNA repair mechanisms [172].

Certain cell surface molecules have been shown to mediate proliferation through binding of their circulating ligands and interact with endothelial and immune response cells in many human solid tumor types [173]. Epidermal growth factor receptor (EGFR), a well-studied receptor-tyrosine kinase (RTK), was investigated in 168 MPM samples using IHC. EGFR expression was significantly associated with epithelioid histology and correlated with OS in univariate analysis [174]. C-MET, another RTK known to be frequently overexpressed in human cancers, was associated with OS in IHC studies analyzing its staining intensity and intracellular localization (i.e. membrane or cytoplasm). Also, c-MET localization to the cell membrane was an independent prognostic factor in multivariate analysis [175]. High tissue expression of Axl, a RTK of the TAM subfamily, mediating cell survival and epithelial-to-mesenchymal transition (EMT) by binding to its ligand growth arrest signal-6 (Gas-6), was associated with favorable prognosis and epithelioid histology in 63 MPM patients [176]. Syndecan-1, a member of proteoglycans which mediate adhesion, migration, cytoskeletal organization, angiogenesis, differentiation and proliferation, was overexpressed in epithelioid compared to sarcomatoid MPM, and its presence was associated with longer OS in analysis of 52 cases [177]. Neurotensin (NTS), a regulator of intestinal motility, secretion, smooth muscle activity and epithelial proliferation in physiological conditions, and its receptor NTSR1, have been investigated in 52 MPM samples. NTS expression conferred with inferior survival in univariate and multivariate analysis, with no such tendency for NTSR1 [178]. Expression of CD9, a member of the tetraspanin membrane glycoproteins, exhibiting tumor-promoting or -suppressing effects and involved in cell adhesion, invasion, metastasis and angiogenesis, was associated with younger age, epithelioid subtype and better differentiation as well as longer OS in univariate and multivariate analysis in 112 MPM patients [179]. CD26, involved in immune response modulation primarily via T-cell regulation, has also been shown to be overexpressed in MPM. Similar to CS9, its expression correlates with histology and improved OS in MPM patients after CHT [180]. Aquaporin 1 (AQP1) expression by IHC was analyzed in two independent MPM cohorts. Higher AQP1 expression was associated with significantly longer OS in both cohorts in univariate and multivariate analysis [181]. The pro-inflammatory cytokine migration inhibitory factor (MIF) and its

receptor CD74 was examined in 135 MPM cases and showed significant association with of OS, with no such tendency for MIF [182].

Several studies investigated the prognostic role of different angiogenic molecules and variables in MPM [183]. For example, microvessel density (MVD) as measured by CD34 IHC was reported to be an independent prognostic marker in a study of 93 MPM patients [184].

MPM cells are characterized by sustained proliferation, pro-survival signaling and reduced apoptosis. For example, O'Kane et al. investigated the expressions of p53 and anti- and pro-apoptotic b-cell lymphoma-2 (Bcl-2) family members by IHC in 54 MPM patients. p53 was overexpressed in 81% of the cases with heterogenous expression of pro- and anti-apoptotic Bcl-2 family members and furthermore no influence on survival [185]. Additionally, high cyclooxygenase-2 (COX-2) and low p21 and p27 expressions were associated with significantly shorter OS in a study analyzing 77 MPM patients with IHC [186]. In another recent study, our collaborators from Zurich reported high Ki-67 and p-S6 MPM tissue expressions as measured by IHC. pS6, a downstream molecule in the PI3K pathway, was associated with poor survival only in chemo-naïve MPM, whereas high Ki-67 expression remained associated with shorter PFS also in post-chemotherapy specimens [187]. Furthermore, it has been recently demonstrated that a systemic proinflammatory status is also associated with a more aggressive biological behavior reflected by elevated Ki67 index and VEGF expression in MPM tumor tissues [188].

Biological behavior and response to chemotherapy might be influenced by dysregulated DNA repair mechanisms, frequently detected in cancer cells [189]. One of the most investigated molecules in this regard is the excision repair cross-complementation group 1 (ERCC1). ERCC1 belongs to a group of proteins which remove cisplatin-induced DNA adducts, thereby mediating drug resistance. Lower protein expression of thymidylate synthase (TS), the cellular target of pemetrexed, significantly correlated to longer time to progression and OS in univariate and multivariate analyses investigating 60 MPM patients after pemetrexed treatment. No such association was observed for ERCC1 in 45 patients after cisplatin treatment or for TS in patients without pemetrexed therapy [190]. However, in another more recent study, ERCC1 protein expression was

significantly related to shorter OS. In the same study, both ERCC1 expression and ERCC1 codon 118 polymorphisms were related to PFS [191].

In summary, as shown by the aforementioned examples of prognostic tissue biomarkers, predicting treatment response and identifying reliable prognostic factors remains difficult in MPM. The majority of these reports consists of single-center experiences, some of which may have been certainly of insufficient power. For some specific biomarkers, which were investigated by several groups, results are often conflicting. Differences in case distribution based on gender, histology, stage and treatment, as well as different methodology, have likely contributed to these discrepancies [172].

2.1.7.1 KI-67

The rate of tumor proliferation has long been considered to be related to malignant behavior and thus estimation of tumor cell proliferation has been used as an important adjunct to histopathological diagnosis [192]. A simple and easily applicable method to evaluate tumor cell proliferation is a count of mitotic figures, which showed to be associated with prognosis in several solid tumor types [193, 194]. However, as mitotic counts only reflect one part of the cell cycle (the M-phase) but not the length of this phase, they are not completely reliable and reproducible.

Ki-67 (a nuclear protein detectable in all phases of the cell cycle but absent in G0 cells) was originally defined by the prototype monoclonal antibody Ki-67. This antibody was generated by immunizing mice with nuclei of a Hodgkin lymphoma cell line (L428). The name is derived from the city of origin (Kiel, Germany) and the number of the original clone in the 96-well plate [195]. As the antigen of the Ki-67 antibody was not fully characterized in 1983, it was referred to as the Ki-67 antigen [196]. Soon, the Ki-67 antigen was identified as a nuclear protein, associated with cellular proliferation and ribosomal RNA transcription [197]. To date, however, the exact molecular mechanisms of the Ki-67 protein are still not fully understood.

The characterization of the Ki-67 antibody revealed interesting staining patterns, as it has shown to be reactive only in proliferating cells (Figure 17) [195]. Hence, it soon became evident that it is an excellent marker to determine the fraction of proliferating cells in a given cell population and was increasingly used as a diagnostic tool in different tumor types [198]. Moreover, mitotic rate, as measured by Ki-67 index, was

found to be prognostic in a number tumor types, including thoracic malignancies such as small cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC) [198, 199]. Moreover, it was found to be prognostic in peritoneal mesothelioma [200].

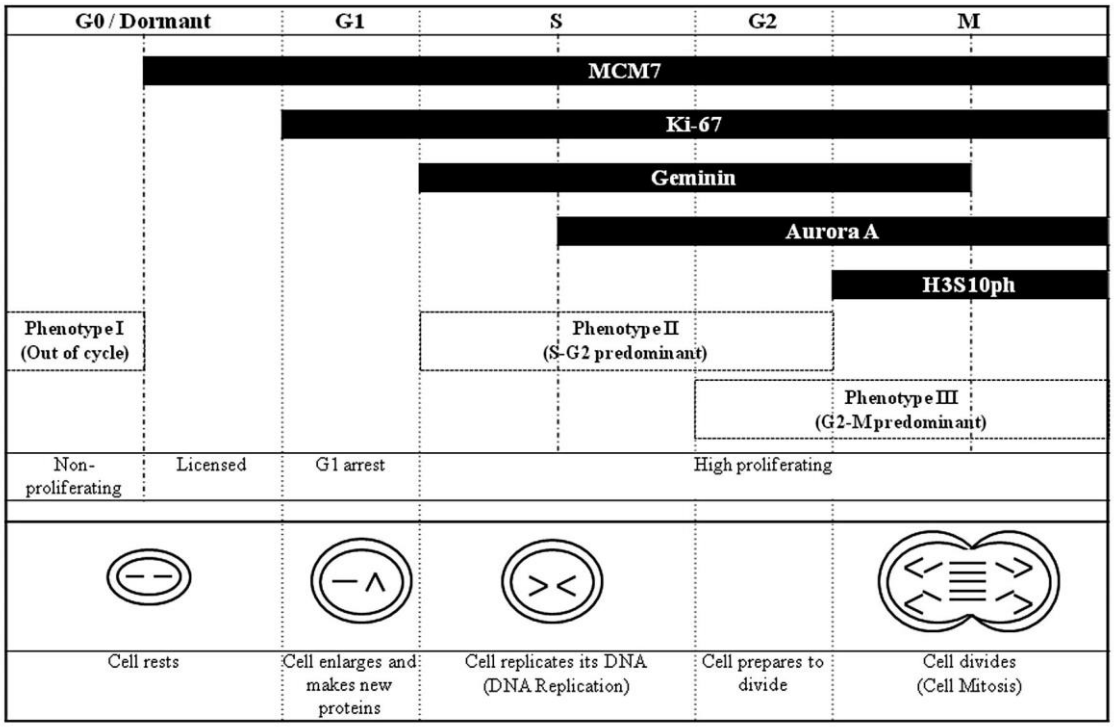

Figure 17: Phase-specific distribution of cell cycle biomarkers in LADC. Ki-67 is expressed during all phases of the cell cycle except the G0 phase. Adopted from [201]

In addition, mitotic index is a well-established parameter for the grading of pre-neoplastic bronchial lesions and used to classify neuroendocrine tumors of the lung [202, 203]. Ki-67 proliferation index was also investigated for diagnostic impact in paraffin-embedded cell blocks of pleural and peritoneal effusions, but failed to show a difference between reactive mesothelial hyperplasia and MPM [204]. The mitotic index, however, was shown to differ significantly between MPM patients with short- and long-term outcome in several studies [205-207]. A recent study proposed a nuclear grading system including nuclear atypia and mitotic count for estimating prognosis in epithelioid MPM [208]. In this study, the combination of nuclear atypia and mitotic count was found to be a stronger discriminator of survival than all currently available clinicopathological factors. Moreover, there was a strong correlation with the time to recurrence after surgical treatment. Another recent study could additionally demonstrate

that high Ki-67 index was related to significantly shorter PFS and OS in chemonaive patients and that it also remained associated with shorter PFS and OS after chemotherapy [187].

However, Ki-67 has still not been validated as a reliable prognostic biomarker for MPM in large international sample collections. Therefore, one of our aims was to examine Ki-67 as a potential independent and reproducible prognostic tissue biomarker in human MPM.

2.1.8 Prognostic circulating biomarkers in MPM

Circulating blood-derived inflammatory or tumor-associated biomarkers were among the first factors predicting poor prognosis in MPM besides stage and histology. Proinflammatory markers include white and red blood cell count, thrombocytosis, and more recently, neutrophil-to-lymphocyte ratio (NLR), platelet-to-lymphocyte ratio (PLR) and the modified Glasgow Prognostic Score (GPS), consisting of CRP and its negative acute phase response counterpart, albumin [209]. Other circulating markers related to dismal prognosis in MPM in different scoring systems include high serum lactate dehydrogenase (LDH) level, anemia and leukocytosis [210]. In addition, our study group identified a prognostic and, furthermore, predictive value for increased CRP as well as elevated fibrinogen in patients with MPM [102, 103]. By analysis of the IASLC database, which to date represents the largest and most comprehensive collection of MPM patient data, others found that high platelet and leucocyte count were significantly related to poor OS, independently from other main clinical prognostic parameters [211]. Moreover, low serum albumin levels were recently shown to be associated with poor OS in two retrospective studies [145].

All the aforementioned markers might somehow reflect the tumor burden and regulatory mechanisms related to the progression of the disease and thus they might act as surrogate parameters reflecting the patients overall condition. Nevertheless, these molecules are not directly associated or secreted by malignant cells. In recent years, research has increasingly focused on tumor-associated circulating biomarkers, which might have therapeutic impact in the future. The prognostic and predictive effect of VEGF serves as a good example of how circulating tumor-associated biomarkers could guide therapy in the future and help to select patients more likely to benefit from anti-

angiogenic treatments. The intrinsic complexity of neo-angiogenesis, and its redundant regulatory mechanisms, suggests that multiple and different biomarkers are needed to predict the efficacy of anti-angiogenic agents and to monitor their biological and therapeutic effects [212]. The predictive role of pre-treatment VEGF levels have been investigated in two parallel phase II studies in patients with advanced MPM treated with thalidomide alone or combined with cisplatin/gemcitabine [213]. This study demonstrated increased survival in patients with low VEGF levels eight weeks post treatment. Change in circulating VEGF levels over the first 8 weeks of treatment was also predictive for survival. When pre-treatment VEGF was >median, decreasing VEGF was associated with increased survival. Another study demonstrated that patients with MPM had significantly higher serum levels of VEGF than those exposed to asbestos but had not developed MPM. In the same study, patients with advanced-stage MPM showed higher levels of VEGF than those with early-stage disease The difference in OS between the groups with high and low VEGF serum levels was significant [214]. Furthermore, it was demonstrated that patients with MPM had significantly higher pleural effusion VEGF levels those with non-malignant pleuritis or lung cancer spreading to the pleural space. Moreover, patients with advanced-stage MPM were reported to have higher circulating VEGF levels than those with early-stage tumors [215]. However, whether circulating VEGF concentrations could serve as prognostic biomarkers or predict bevacizumab efficacy in MPM remains controversial. A randomized phase 3 trial conducted by Zalcman et al. showed that high plasma VEGF concentrations were associated with worse PFS and OS [113]. Most interestingly, patients with baseline plasma VEGF concentrations lower than the median value had a 5.2 month longer OS with cis/pem/beva than with cis/pem, whereas patients with higher baseline VEGF concentrations than the median value treated in the cis/pem/beva group showed a modest benefit in OS only. These results warrant further evaluation of blood VEGF levels as a prognostic and predictive marker for anti-VEGF targeted therapy in combination with other biomarkers.

Mesothelin is a membrane bound protein expressed by normal mesothelial, MPM as well as ovarian and pancreatic cancer cells. Its potential role for early diagnosis and monitoring treatment response has been extensively investigated [216, 217]. Higher levels of serum-soluble mesothelin family proteins (SMRP) were associated with

significantly shorter OS in MPM patients [218]. A study evaluating mesothelin as a predictive factor for disease progression found significantly higher serum SMRP levels in patients with relapse/progression of MPM compared to newly diagnosed patients. Higher SMRP levels also significantly correlated to OS in univariate and multivariate analyses, but lost their significance when analyzed in the subcohort of epithelioid patients [219].

Fibulin-3 is a member of the extracellular glycoprotein fibulin family. Its gene expression is low in healthy tissues with the highest expression in the thyroid gland [220]. Fibulin-3 is related to cell adhesion, communication and growth, and has variable angiogenic effects [221, 222]. It has been shown to be up-regulated in metastases from pancreatic adenocarcinoma [223]. A recent study showed that plasma fibulin-3 levels can distinguish asymptomatic asbestos-exposed individuals from MPM patients and that pleural effusion fibulin-3 levels differentiates MPM effusion from other malignant or benign effusions. Furthermore, plasma fibulin-3 levels were significantly associated with OS [224]. However, another recent study including data from our study group showed a similar association of fibulin-3 levels with OS in two MPM cohorts but no diagnostic power [225].

In summary, similar to tumor tissue-associated biomarkers, a variety of circulating biomarkers in MPM has been investigated in different subcohorts worldwide. However, the prognostic power of these markers varies among studies and only a few ones have shown to be reproducible. Hence, larger international studies are urgently needed in order to identify reliable markers to select patients for individual treatment options from which they might eventually benefit from.

2.1.8.1 Complement component 4d (C4d)

The complement cascade is a major component of the innate immunity and it essentially contributes to immune surveillance, cell homeostasis and tissue regeneration [226, 227]. It promotes inflammation and damages invaders such as microbes or foreign cells by activating a cascade of more than 30 fluid-phase and cell-associated proteins [228]. The development of antibodies against the own host tissue may lead to complement activation-related tissue injury, as it has been described in many auto-immune diseases. Accordingly, specific compounds are currently under investigation in order to interrupt

or hamper complement-related immunresponses [229, 230]. Three main pathways for activation are recognized: the classical, alternative and lectin pathways (Figure 18). The classical pathway is activated by binding of the antigen with a specific antibody. Immunoglobulin M (IgM), IgG3 and IgG1 are the most efficient activators of the classical pathway. Apart from classical antigen-antibody contact, the classical pathway can also be directly activated by other factors such as microorganisms, DNA, CRP, polyanionic molecules or apoptotic cells [228]. Further activation of C3a and C5a leads to the formation of the membrane attack complex (MAC) consisting of the complement factors C5b-C9. MAC is deposited on the target surface with penetration of C9 (the terminal component) into to the lipid layer of the target cell's membrane. This generates pores into the target membrane causing cell death via disruption [231].

The lectin pathway is triggered by binding mannan-binding lectin (MBL - a C-type lectin) to terminal sugars on the surface of microorganisms [232]. MBL is structurally related to C1q, being the first step of compliment activation in the classical pathway. In the circulation, MBL associates with MBL-associated serine protease 1 (MASP1), MASP2 and MASP3. Binding of the MBL-MASP complex to the target cell's membrane leads to C4 and C2 cleavage, resulting in activation of C3, similar to that of the classical pathway.

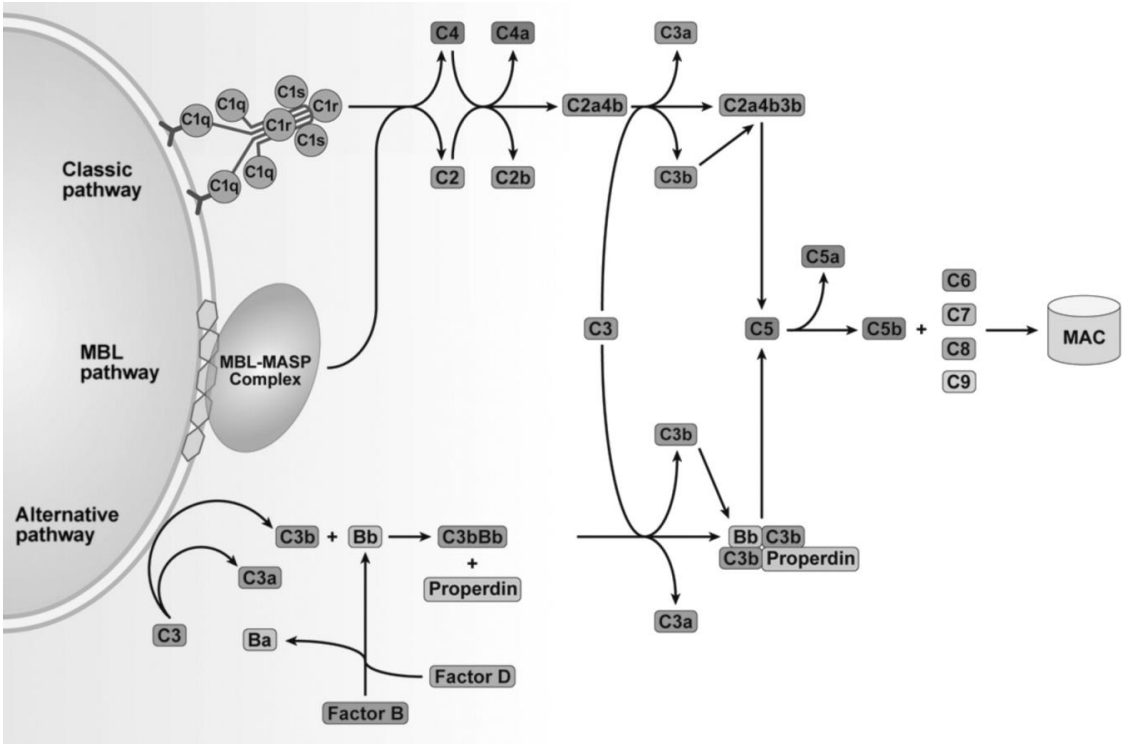

Figure 18: Complement cascade activation pathways: Classical, lectin (MBL) and alternative pathways. C4 is activated by the classical and lectin pathway. Adopted from [226]

Activation of the alternative pathway does not rely on antibodies for antigen recognition [228]. It is triggered by slow hydrolysis of circulating C3, referred as "C3 tickover". Generated C3b is further activated by binding of alternative-pathway-specific proteins factor B, factor D and properdin. Binding of additional C3b to the alternative pathway C3 convertase renders it capable of C5 cleavage, and forms the basis for the amplification loop of the alternative pathway [229]. C3b can then generate the MAC by interacting with polysaccharides or proteins on the surface of microorganisms or endotoxins [233].

As shown in Figure 18, all complement activation pathways merge with C3b deposition on a target. As C3b acts as an additional activating factor of the alternative pathway, complement activation can be initiated by the classical or lectin pathway and amplified by the alternative pathway [229]. Moreover, a number of bypass pathways has been described [234].

Importantly, there is mounting evidence that the complement cascade, apart from auto-immune diseases, is also involved in cancer development and progression by facilitating cellular proliferation and regeneration [226, 235-238].

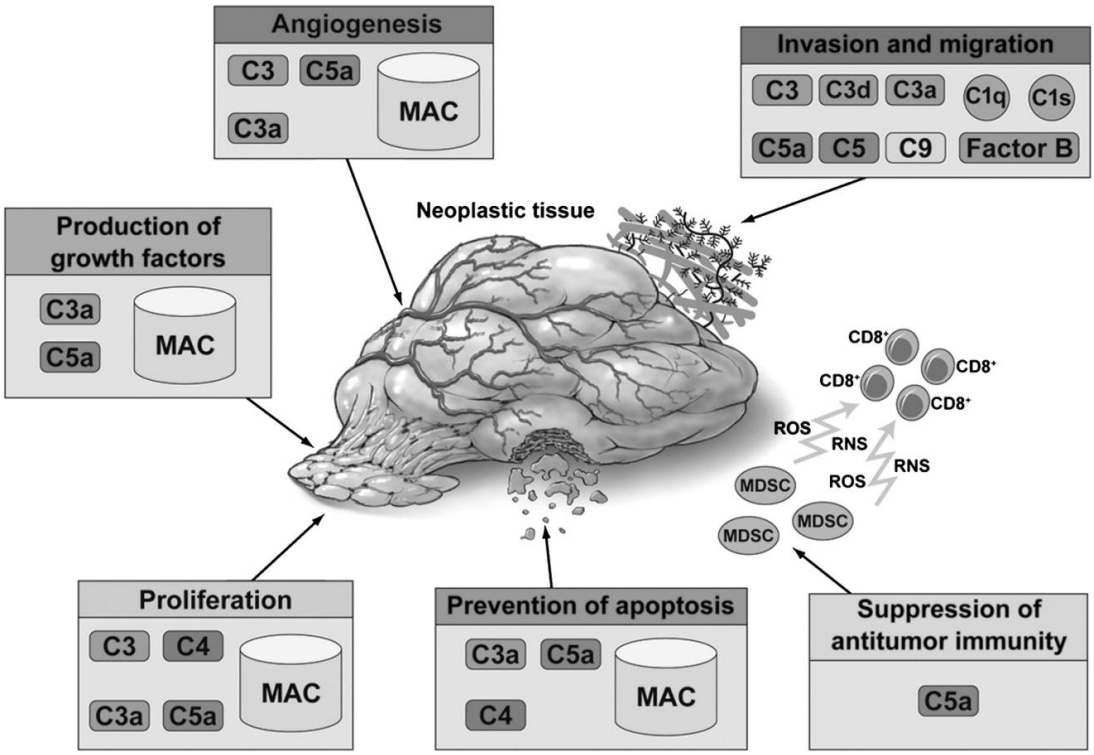

Figure 19: Potential oncogenic roles of complement proteins. Adopted from [226]

Organs chronically exposed to extrinsic toxins such as asbestos or tobacco eventually develop inflammation. Notably, chronic inflammation is a well-established risk factor of MPM and lung cancer [239]. Previous studies have raised the possibility that although acute inflammation interferes with malignant transformation, chronic inflammation promotes carcinogenesis [240]. Complement system activation in acute inflammation is a well-established concept, however, also chronic inflammatory diseases might play a role in triggering and consumption of these proteins [241]. C3a and C5a, for instance, are some of the most powerful proinflammatory molecules, and recent findings have demonstrated that these proteins may aid tumor growth through immunosuppression [242].

Compliment activation promotes cellular proliferation and neoplastic spread by a variety of mechanisms (Figure 19). Activation of complement system proteins promotes oncogenesis by increasing mitogen signaling pathways via C3a and C5a binding and activation of PI3Kinase, Akt and mTOR [243]. Furthermore, MAC has shown to activate the cell cycle and specific oncogenic molecular machineries, such as ERKs, p38 MAPKs, JNKs or PI3Kinase [226] [244]. Additionally, complement related proteins

(especially C3, C6 and MAC) have been shown to prevent cell death by exerting pro-survival, pro-angiogenic and anti-apoptotic effects [245]. Complement proteins also reorganize intra- and extracellular matrix connections and thus facilitate invasion and migration [246]. Finally, the complement system plays an important role in the tumor microenvironment and promote immunosuppression and cancer growth [247].

A specific complement-related protein is the degradation protein C4d, a stable cleavage product of complement protein C4. C4d accumulates following both classical and lectin pathway activation [248-250]. C4d deposition in a tissue is regarded as an indirect proof of an activation of the complement cascade [249]. C4d is usually bound stably to the target structure but may eventually enter the circulation. Of note, C4d is routinely used as a tissue biomarker to investigate allograft tissue rejections in kidney transplants [249-251]. C4d has also been found to have a prognostic role in different types of cancer [248, 249, 252]. Of note, it was recently reported that lung cancer cells produce C5a, a potent proinflammatory mediator creating a favorable microenvironment for tumor progression [253]. Ajona and colleagues found increased C4d levels both in plasma and bronchoalveolar lavage (BAL) samples from LADC patients. In this study, patients with elevated circulating as well as tumor tissue C4d levels had worse prognosis [248, 252]. Furthermore, C4d expression was associated with stage or dismal outcome in different other tumors such as astrocytomas and oropharyngeal squamous cell cancer [249, 254].
To date, there is limited information on the role of the complement activation in asbestos-related diseases and development of MPM. In 2002, a cohort of patients with or without pleural calcifications (PCs) due to prior asbestos exposure was investigated. C4 was only present in BAL samples of patients with PCs but not in those without PCs. Although PCs might trigger MPM development via chronic inflammation and immune response, subjects with PCs were assumed to develop MPM less frequently [255].
Zerva et al. investigated several factors associated with humoral immunity in patients environmentally exposed to asbestos. Asbestos-exposed individuals had significantly increased serum C3 levels independently of the presence of PCs when compared to healthy controls. Although C3 is the target protein of activated C4, serum C4 levels did not differ between these groups [256]. Unfortunately, the authors did not provide any data on the on the activation marker C4d. By analyzing MPM pleural effusions, Nabil et

al. demonstrated that complement factor H, a restrictive cofactor for the cleavage of C3b, potentially recruits monocytes and granulocytes to the malignant site via chemotactic function and thus supports malignant cell phagocytosis [257]. Despite all these efforts in elucidating the role of complement activation in human MPM, no study has yet focused on the prognostic or predictive role of C4d as a marker for complement activation in this disease.

2.1.8.2 Activin A

Activins are cytokines of the TGF-beta super-family of growth and differentiation factors [258] and were named according to their first identification as activators of follicle-stimulating hormone (FSH) release from pituitary cells [259, 260]. Activins are formed via the covalent intracellular dimerization of two subunits [261]. Five different human subunits have been identified (beta A, beta B, beta C and beta E). Multiple biological functions of Activin A (ActA) have been described, including essential roles in mesoderm induction [262], stem cell biology [263], reproductive biology [264], erythroid differentiation [265], systemic inflammation [266], cell death induction [267], wound healing [268] and fibrosis [269]. Because of ActA activation, receptor-regulated Smads (R-Smads) 2 and 3 are recruited to the receptor complex and phosphorylated by the type I receptor. Together with co-factors, Smads are involved in the regulation of gene expression. Important ActA target genes include p15INK4B [270, 271] and the lipid phosphatase SHIP(1) [272]. Moreover, there might be a role of a Smad-independent signaling of ActA via activation of the MAP kinases ERK 1/2 and p38 [273] as well as the PI3Kinase / Akt pathway [274]. Rho and JNK were also found to be stimulated by ActA [275]. Under physiological conditions, activin signaling is kept under stringent control by a number of different mechanisms. Besides regulated expression of different activin beta subunits, the extracellular activin-binding proteins follistatin and follistatin-related gene (FLRG), antagonistic co-receptors like BAMBI and Cripto and inhibitory Smad (I-Smad) 7 are important negative regulators of activin signaling (Figure 20) [276, 277].

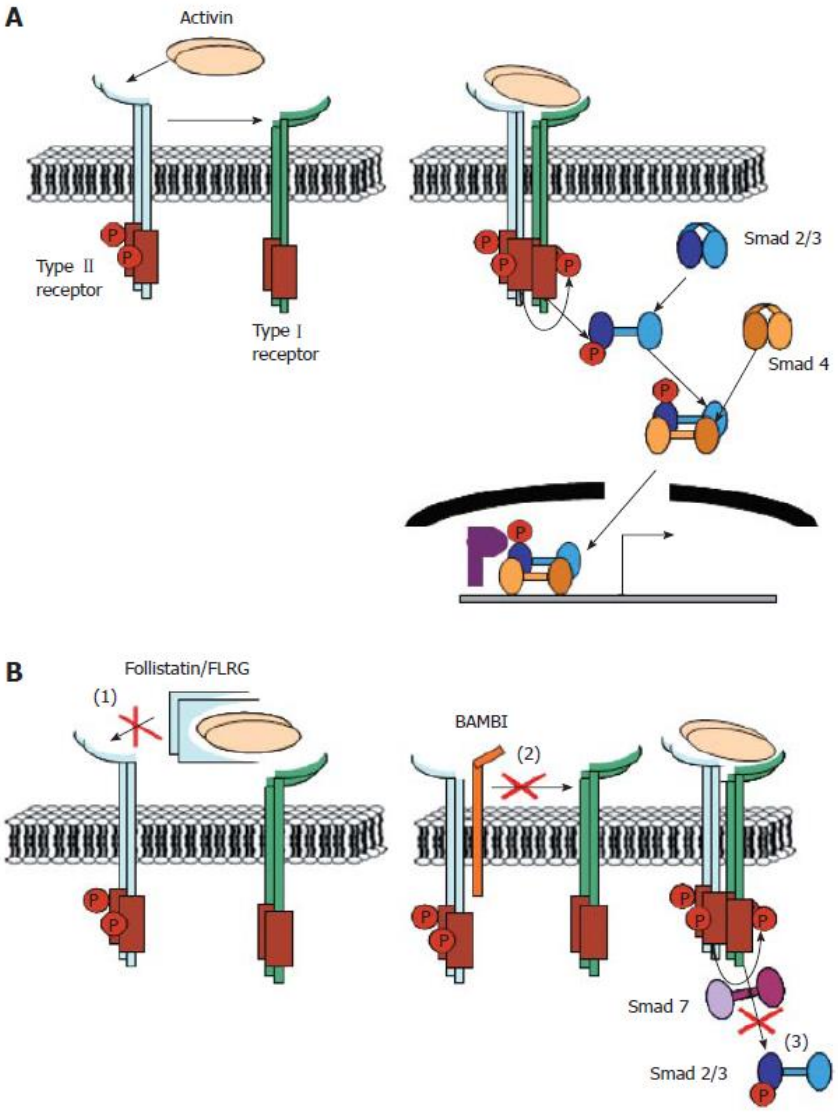

Figure 20: Graphic model of ActA signaling. (A) ActA transduces signals to the cell nucleus via type II and type I activin receptors and Smad proteins. (B) Under physiological conditions ActA signaling is strictly controlled by several mechanisms: (i) extracellular binding proteins for ActA like follistatin or FLRG can block interaction of ActA with activin receptors; (ii) expression of inhibitory co-receptors like BAMBI can block receptor activation; (iii) intracellular proteins interacting with the Smad pathway can modulate ActA signals. All these levels of control can become deregulated during carcinogenesis. Adopted from [276]

ActA is critically involved in cellular differentiation and homeostasis in multiple organs. ActA induces growth arrest and apoptosis in several non-transformed cell types. For example, ActA is essential for maintaining a physiological balance between cell proliferation and cell death by limiting replication in hepatocytes [258]. Deregulated activin signaling has been found in a broad range of malignancies [278-281]. A recent

study demonstrated that ActA is overexpressed in both LADC and squamous cell carcinoma tissue and, furthermore, that NFkB-dependent upregulation of ActA is associated with cancer progression and metastasis [282]. Also, in esophageal carcinoma, a recent report demonstrated that elevated tumor tissue ActA expression is associated with lymph node metastasis and decreased survival [280]. Importantly, activin signaling has also been investigated as a potential new therapeutic target in different cancer types [281].

ActA is secreted to the blood and can be detected in the serum or plasma by ELISA (ELISA). Its circulating levels have been investigated in a variety of human malignancies including hepatocellular, breast, prostate, ovarian, oral, esophageal, pancreatic and lung cancers and multiple myeloma [283-291].

With respect to thoracic malignancies, our group has recently demonstrated that also MPM cells overexpress ActA [292]. ActA increased MPM cells' proliferation and migration whereas inhibition of activin receptors by pharmacological inhibitors or siRNA-mediated silencing of ActA expression decreased their growth and survival. Our data on ActA in MPM were recently confirmed and extended to the closely related growth factor activin B by another group [293]. Thus, in MPM, similar to reports in NSCLC [294] and esophageal carcinoma [295, 296], ActA is associated with malignant behavior. Moreover, our group has recently shown that circulating ActA levels are elevated compared to healthy controls and correlated with disease stage and survival in LADC [297]. Therefore, we decided to study circulating ActA as a biomarker in MPM.

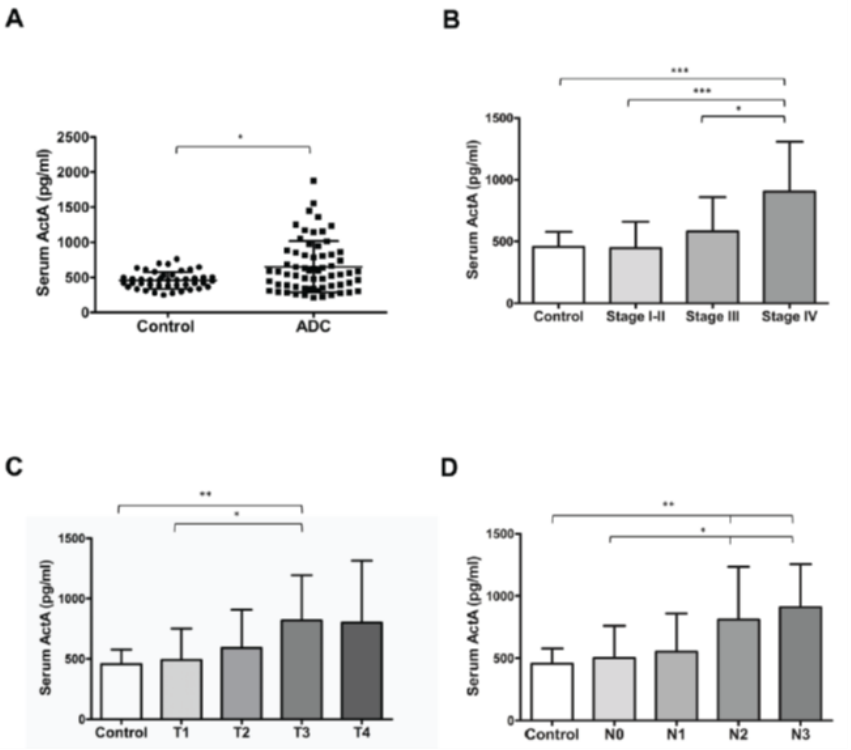

Figure 21: A previous study of our group on ActA and LADC. Serum ActA levels are elevated in patients with LADC and correlate with tumor progression. A.) ActA concentration is significantly higher in blood samples of patients with LADC ($p = 0.015$, vs. controls). B., C., D.) T and N status- and stage-dependent increase of serum ActA in LADC (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$) [297]

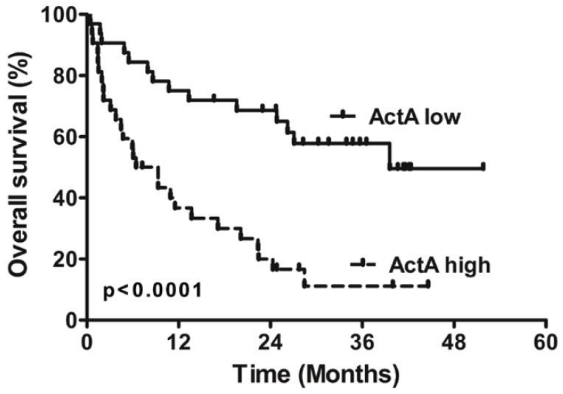

Figure 22: Our previous study on ActA and LADC. Kaplan-Meier curves for OS of LADC patients according to serum ActA level (cut-off value is the median). LADC patients with high serum ActA levels had significantly shorter OS than those with low serum ActA levels (median OS was 7.9 vs. 39.6 months, HR: 0.2768, 95% CI 0.1450 to 0.5286; $p < 0.0001$) [297]

3 Objectives

Based on the current literature and on the previous findings of our MPM study group, we intended to investigate the prognostic and predictive value of specific tissue and circulating biomarkers using large cohorts of blood and tissue samples collected in our clinic at the Medical University of Vienna and through our well-established international cooperation with centers specialized in the treatment of this devastating disease.

3.1 Ki-67 index as a prognostic parameter in MPM

Ki-67 has still not been validated as a reliable prognostic biomarker for MPM in large international sample collections and thus validation studies are needed to prove the independent and reproducible prognostic power of this tissue biomarker. Accordingly, we aimed to investigate whether Ki-67 index as determined by immunolabeling of MPM tissue sections is an independent and, furthermore, reproducible prognostic factor in a large international cohort of MPM samples. We further aimed to study the prognostic power of Ki-67 index compared to other well-established prognostic factors in an independent cohort of MPM patients.

3.2 Circulating C4d as a prognostic biomarker in MPM

Despite efforts in elucidating the role of complement activation in human MPM, no study has yet focused on the prognostic or predictive role of C4d as a marker for complement activation in this disease.

Since the complement degradation product C4d has been shown to have a strong prognostic relevance in LADC, we aimed to investigate the tissue and circulating levels of C4d in MPM patients and thus compared these data with tumor load, chemotherapy response and clinicopathological parameters. We additionally assessed the impact of chemotherapy on circulating C4d levels and also evaluated the potential prognostic relevance of C4d levels on OS.

3.3 Circulating ActA as a prognostic parameter in MPM

Circulating ActA has been shown to correlate with OS in LADC, but no data were available on the prognostic impact of circulating ActA levels in MPM. Thus, we aimed to analyze ActA plasma levels in MPM patients and compared them to clinical, radiological and pathological parameters and assessed the prognostic value of ActA in a large cohort of MPM patients.

4 Methods

4.1 General ethical considerations

Tumor tissue, blood samples and patient data described in chapters 3.1 - 0 (objectives) were collected in the following institutions:

- Division of Thoracic Surgery, Medical University of Vienna, Austria (AUT)
- National Koranyi Institute of Pulmonology, Budapest, Hungary (HUN)
- Department for Respiratory Diseases Jordanovac, School of Medicine, University of Zagreb, Croatia (CRO)
- Department for Pulmonology, University Clinic Golnik, Slovenia (SLO)
- Concord Repatriation General Hospital and Strathfield Private Hospital in Sydney, Australia (AUS)

All of the included patients provided written informed consent to the collection of the biological material and the collection of the clinical data. Analyses of these data and materials were approved by all local ethical committees. No individual patient data is identifiable in the respective publications or in this thesis. All methods included in this thesis were performed in accordance with the relevant guidelines and regulations. All experiments that were reported in this thesis were done complying with all mandatory laboratory health and safety procedures.

4.2 Evaluation of Ki-67 index as a prognostic parameter in MPM

4.2.1 Study population

In order to evaluate Ki-67 IHC in MPM, we investigated 285 patients. The test cohort consisted of 187 patients from three institutions. 91 patients were included in CRO. The HUN cohort consisted of 42 cases. 54 cases were included in AUT. In addition, 98 patients from SLO were analyzed as an independent validation cohort. All patients were referred to one of the four institutions between 1994 and 2012. Inclusion criteria were complete clinical data and reliable follow-up and available representative paraffin-embedded tumor tissue. MPM diagnosis was histologically proven during clinical routine work-up in all cases. The IMIG staging system was routinely used for clinical and pathological tumor staging [95]. All tissue samples were collected during surgery (n=71) or as part of diagnostic work-up.

The CRO cohort consisted of 85 epithelioid and 6 non-epithelioid cases. Forty-six patients were treated by chemo and/or radiotherapy and 29 by best supportive care. Twelve patients received surgery in curative intent. In 4 patients, information about the applied treatment was unavailable in the medical records.

In the 42 HUN cases, only three patients received surgery within a multimodality treatment protocol and two additional patients were treated by surgery alone. Epithelioid morphology was most frequently identified (n=33).

In the AUT cohort, all 54 patients received MCR. EPP was performed in the majority of cases (n=52), as previously described [169]. One patient underwent P/D, and 1 received local MCR followed by adjuvant radiotherapy. All tumor samples were collected during surgery. Thirty-six patients received a combination of surgery with adjuvant and/or neoadjuvant treatment (multimodality treatment group). 29 patients (53.7%) received induction chemotherapy before surgery and 23 patients (42.6%) were treated with adjuvant therapy after resection (radiation: n=10, chemotherapy: n=8, chemo-radiation: n=5). Eighteen patients were treated with surgery alone (surgery-alone group). The most frequent histological subtype was the epithelioid (n=38). 57% had pathologically classified late stage disease according to the postoperative report (stage III and IV; n=31).

As an independent validation cohort, 98 SLO patients were included to re-evaluate Ki67 as a reproducible prognostic marker. For the SLO cohort, we used the median Ki67 expression as a cutoff as later described because the tumor samples were scored by an independent pathologist different than the one in the test cohort. Epithelioid subtype was the most frequent (n=77) followed by biphasic tumors (n=15).

4.2.2 Tumor samples, staining, scoring and blood biomarkers

All tumor samples were fixed in formalin and embedded in paraffin (FFPE). One 4-µm section from a representative, tumor-rich FFPE block was stained by hematoxylin/eosin (HE) to confirm and locate areas of definitive tumor content and consecutive sections were used for Ki67 IHC. Sections were deparaffinised with xylene and rehydrated with decreasing alcohol concentrations. After heat-induced epitope retrieval in citrate buffer (10 mmol l−1, pH 6.0), slides were incubated at room temperature with the Ki67 antibody (monoclonal mouse antibody, Dako Cytomation (Dako, Glostrup, Denmark),

clone MIB-1, dilution 1 : 100, incubation time: 30 min). Antibody binding was detected by means of the UltraVision LP detection system (Lab Vision Corporation, Fremont, CA, USA) according to the manufacturer's recommendations. Color development was achieved by 3-3-diaminobenzidine. Finally, all analyzed slides were counterstained with Mayer's hematoxylin.

Ki-67 scoring

Evaluation of IHC staining was independently performed by one pathologist in the test cohort (AUT/CRO/HUN) and by another independent pathologist in the validation cohort (SLO). They had no information about the clinical history of the cases as they only received anonymized slides for evaluation and scoring. Both pathologists scored the HUN cohort to test the inter-observer reproducibility. Counting was limited to proven tumor tissue and only nuclear staining was scored as positive. First, the whole tissue section was reviewed at low magnification to identify the regions with the highest viable tumor cell content. The hot-spot approach was used to measure areas with the highest proliferative activity. Whenever possible, up to 10 high-power fields were analyzed and at least 500 tumor cells were counted to calculate the mean percentage of Ki67-positive tumor cells per analyzed sample.

4.2.3 Statistical analyses

Metric data is always given as median and corresponding range, or, in case of survival, as median and corresponding 95% confidence interval (CI) if not otherwise indicated. OS was defined as the time between MPM diagnosis and death or between diagnosis and last follow-up date. Survival was analyzed by the Kaplan–Meier method and log rank test or by the Cox-regression model to calculate hazard ratios (HRs) and corresponding CIs. In the test cohort, patients were divided into high and low Ki67 index groups by the median (15%). Since the validation cohort was scored by a different independent pathologist, we again used median Ki67 expression of the validation group (22%) for dichotomizing into the low (n=52) and high Ki67 (n=46) expressing groups. An unpaired t-test was used to compare the mean of parametric distributed metric data between two groups and one-way ANOVA to compare the mean within more than two groups, respectively. Mann–Whitney U-test was used for non-parametric distributed

metric data. $\chi 2$-test was performed for analyzing the association between categorical factors. The correlation of metric data was analyzed by Pearson's correlation coefficient. Receiver operating characteristic (ROC) analysis was performed in the epithelioid subtype of the full cohort with a follow-up of >12 months (n=221) to investigate the sensitivity and specificity of Ki67 in predicting short-term survival using the median OS of 12 months as cutoff. 118 patients were analyzed for the NLR, 97 patients for CRP and 127 patients for fibrinogen using cutoffs from the respective studies [102, 103, 298]. In addition, in 65 patients, all three aforementioned biomarkers were available and could therefore be directly compared with each other and to Ki67 in ROC analyses. All statistical analyses were calculated with the SPSS Statistics 18.0 package (Predictive Analytics Software, SPSS Inc., Chicago, IL, USA). P-values are given as two-sided and were considered statistically significant below 0.05.

4.3 Evaluation of circulating C4d as a prognostic parameter in MPM

4.3.1 Study population

Clinical data and plasma samples of 55 consecutive, histologically verified MPM patients were collected at the time of diagnosis (n=30) or before curative intent surgery after induction chemotherapy (n=25) at the center in AUT between May 2011 and December 2014. All patient blood and tissue samples were retrieved before any diagnostic or curative intervention or in case of post-chemotherapy samples before surgical resection. Patients with acute clinical infections were excluded from the study. In 12 patients, plasma samples at the time of diagnosis as well as before surgery were available, representing pre- and post-chemotherapy samples. Of 32 of these 55 patients, FFPE tissue specimens were collected for IHC analysis. Furthermore, plasma samples from an age-matched cohort of 21 healthy volunteers (HV) as well as from 14 patients diagnosed with non-malignant pleural diseases (NMPD) were included.

4.3.2 Tumor and blood samples, staining and blood biomarkers

32 FFPE MPM tissue specimens were collected and analyzed by C4d IHC. Additionally, we performed C1q IHC in 14 FFPE tissue specimens deriving from patients with high (n = 7) and low (n = 7) circulating C4d levels. After the sections were deparaffinized and rehydrated, endogenous peroxidase activity was blocked with 0.3%

H2O2 in phosphate buffered saline. Heat-induced epitope retrieval was performed by using TRIS/EDTA buffer (pH=9). Primary antibodies (anti-human C4d; Biomedica, Vienna, Austria and anti-human C1q; ab71089, Abcam, Cambridge, UK) were diluted 1:500 and 1:1000, respectively, and incubated for 60 min at RT. Antibody binding was detected with the UltraVision LP Detection System (Lab Vision Corporation). Color development was achieved by DAB followed by hematoxylin counterstaining. All stainings were evaluated by a pathologist.

Blood was collected via peripheral venous puncture from MPM patients and controls into 10 ml EDTA Vacutainers (BD Biosciences). Within 30 min after blood collection, centrifugation was performed. Plasma supernatant was stored in aliquots at -80°C until use. C4d and C3a fragments ELISA kits were purchased from Quidel (San Diego, CA, USA). For the analyses of C4d and C3a, samples were diluted 1:70 and 1:200, respectively, according to the manufacturers' instructions. Standard curve generations and measurements in duplicates were performed according to the guidelines of the company.

4.3.3 Tumor volumetry

In 20 of 55 patients, tumor volumetry measurement was achievable. Volumetric analyses were performed in patients with digitally available CT imaging data before and/or following chemotherapy. Chest CT images were analyzed by using dedicated software featuring semi-automatic segmentation with linear interpolation, allowing manual adjustments if necessary (Myrian; Intrasense, Paris, France) - as previously described [299]. In summary, the segmentation and tumor volume quantification contained the following steps: the normal lung tissue, including bronchi and vessels, was marked semi-automatically by thresholding and region growing. Then pleural effusion and atelectatic lung areas were marked with a magnetic lasso function. After marking normal lung tissue and pleural effusion, atelectatic lung and the outer part of the pleura was segmented semi-automatically. By using a lineal algorithm, interpolation between the marked slices was applied automatically. Finally, tumor volume was calculated by multiplying the sum of the voxels. Data was independently analyzed by two radiologists.

4.3.4 Statistical analyses

To investigate the role of circulating plasma levels of C4d, first we stratified our cohort by the cut-off of 3 µg/mL which was previously used in LADC [248]. Nevertheless, because only one patient had C4d plasma level above 3 µg/mL at time of diagnosis, this cut-off value proved to be not suitable for MPM. However, when we analyzed circulating C4d values of patients with early- and late-stage disease, we found a distinct separation when using 1.5 µg/mL concentration as a potential cut-off. We also found that 1.5 µg/mL level was the best cut-off in our cohort by performing ROC curve analysis and Youden's Index calculation for twelve months' survival (data not shown). Hence, calculations described below were performed by using 1.5 µg/mL as cut-off.
Categorical data was compared by performing Fishers' exact or chi-square tests. Statistical differences between two groups were tested by Mann-Whitney U test. The significance of potential correlations of continuous parameters was investigated by using Pearson correlation. OS was defined as time between initial MPM diagnosis and date of death or last follow-up. OS was estimated by the Kaplan-Meier method and a log rank test was used to calculate survival differences between two groups. A multivariate cox regression model was used to calculate hazard ratios and 95% confidence intervals for factors independently influencing OS. To investigate the accuracy of C4d as a predictor for survival and to distinguish between MPM and non-malignant pleural disease, ROC curve analysis was used. For each cut-off point, the Youden's Index was calculated (sensitivity + specificity). All results were considered statistically significant when $p<0.05$ two-sided. Analyses were performed using the SPSS Statistics 23.0 package (SPSS Inc., Chicago, IL, USA) and GraphPad Prism 5.0.

4.4 Evaluation of circulating ActA as a prognostic parameter in MPM

4.4.1 Study population

Thirty consecutive patients with blood withdrawn at the time of diagnosis were included at the institution in CRO between 2013 and 2014. Between 2011 and 2014, 62 consecutive patients were included at the center in AUT with blood collected either at the time of diagnosis or before surgical resection or both. The center in AUS contributed 37 non-consecutive samples either from the time of diagnosis or following therapy (collected between 2009 and 2013). 16 patients who were diagnosed with

pleuritis or pleural fibrosis and demonstrated no sign of MPM were also included. Additionally, plasma samples were collected from an age- and gender-matched cohort of 45 healthy individuals.

4.4.2 Blood samples

Blood was collected by venous puncture from suspected MPM patients and healthy subjects into 10 ml EDTA Vacutainers (BD Biosciences). Centrifugation was performed within 30 min after blood collection. Plasma supernatant was stored in aliquots at −80 °C. Quantikine ActA ELISA kits were purchased from R&D Systems (Minneapolis, MN, United States of America [USA]). Sample preparation, standard curve generation and measurement of samples in duplicates were performed according to the guidelines of the manufacturer.

4.4.3 Tumor volumetry

Tumor volumetry in this project was performed applying the same methods as described in chapter 4.3.3.

4.4.4 Statistical analyses

Statistical differences between two groups were tested by Mann-Whitney test unless otherwise stated. Kruskal–Wallis test was used with the post hoc Dunn-test for the comparison of more than two groups. The Bonferroni correction was calculated for the adjustment of the alpha type I error. Accordingly, in multiple comparisons the P-value was divided by the number of comparisons being made. In order to analyze the correlation of two parameters, Spearman-test was applied. OS was defined as the time between date of diagnosis and death, otherwise patients were censored at the time of last contact. Kaplan–Meier curves were used to demonstrate patients' OS. Differences in OS were compared by the log-rank test. Prior to the performance of the multivariate analysis of the clinical parameters using the Cox regression model, the proportional hazards (PH) assumption was evaluated graphically by plotting the logarithm of the cumulative hazards functions for each covariate. The PH assumption was considered satisfied when the lines were approximately parallel and did not cross each other. Furthermore, interaction terms between plasma ActA levels and covariates were

introduced to the multivariate model. Multiple imputation by chain equation (MICE) was employed to handle the missing data, in order to avoid the omission of valuable information. ROC curve analysis was used to assess the accuracy of fibrinogen and ActA levels to predict survival. The Youden index was calculated as (sensitivity + specificity)−1 to determine the optimal cut-off point. Differences were considered statistically significant when $P < 0.05$. All statistical analyses were performed using the PASW Statistics 18.0 package (Predictive Analytics Software, SPSS Inc., Chicago, IL, USA) and GraphPad Prism 5.0.

5 Results

5.1 Ki-67 index as a prognostic parameter in MPM

5.1.1 OS is influenced by treatment modality and histological subtype

The test cohort consisted of 40 female (21.4%) and 147 male (78.6%) histologically verified MPM patients. 156 patients suffered from epithelioid MPM (83.4%) compared with 31 non-epithelioid MPM patients (16.6%) including 22 biphasic (11.8%) and 9 sarcomatoid (4.8%) cases.

Median OS in the test cohort was 12.0 months (CI 9.3–14.7). In univariate survival analyses, histology (Figure 23A) and treatment (Figure 23C) were found to have prognostic value. Gender, age and disease stage (Figure 23B) had no significant impact on OS in univariate survival analyses of the test cohort (Table 6).

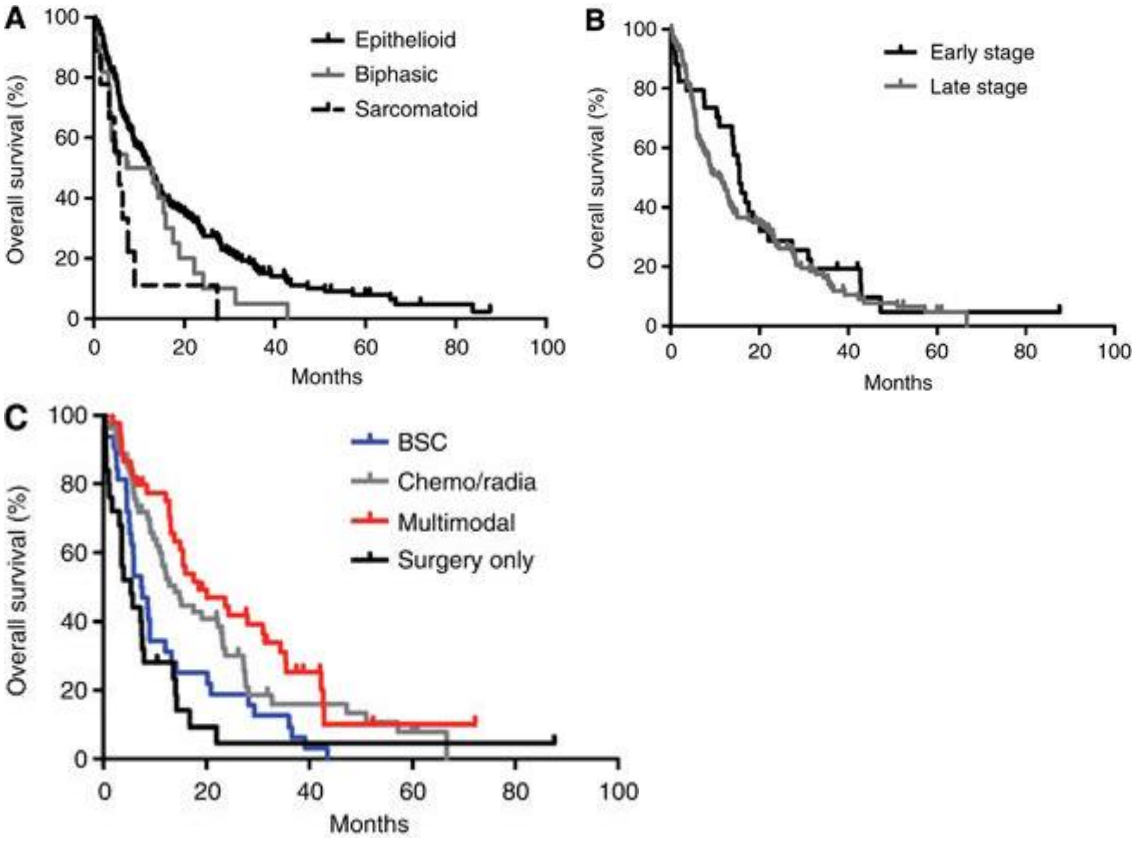

Figure 23: OS prognosticators in MPM. (A) The three histological subtypes are characterised by different outcomes (epithelioid 12.8 (CI 10.5–15.0) months vs biphasic 7.2 (CI 0–20.7) vs sarcomatoid 5.6 (CI 2.5–8.7) months, P=0.005). (B) Disease stage had no significant impact on OS (late stage 10.8 (CI 7.3–14.3) months vs early stage 15.4 (CI 13.1–17.8) months, P=0.305). (C) Treatment has robust prognostic

impact on OS (multimodality therapy 18.5 (CI 8.2–28.8) months vs chemo and/or radiotherapy 13.9 (CI 9.6–18.3) months vs best supportive care (BSC) 7.6 (CI 4.4–10.8) months vs surgery alone 5.3 (CI 0.8–9.7) months, P<0.001).

Table 6: Univariate survival analyses in the test cohort (n=187). CI=confidence interval; HR=hazard ratio; OS=overall survival. a Two-sided log rank test. b Missing cases: n=30 (16.0%).

Characteristics	OS (CI)	P[a]	HR (CI)
Age, years			
≤61.5	12.2 (10.0–14.5)	0.84	0.97 (0.72–1.31)
>61.5	10.3 (5.2–15.4)	—	—
Sex			
Female	11.3 (9.6–13.0)	0.17	0.76 (0.51–1.13)
Male	12.1 (8.6–15.6)	—	—
Histology			
Epithelioid	12.8 (10.5–15.0)	<0.01	1
Biphasic	7.2 (0–20.7)	—	—
Sarcomatoid	5.6 (2.5–8.7)	—	—
Stage[b]			
Early stage	15.4 (13.1–17.8)	0.31	1.24 (0.82–1.87)
Late stage	10.8 (7.3–14.3)	—	—
Treatment[b]			
Surgery alone	5.3 (0.8–9.7)	<0.01	1.30 (0.76–2.21)
Best supportive care	7.6 (4.4–10.8)	—	1
Chemo and/or radiotherapy	13.9 (9.6–18.3)	—	0.61 (0.39–0.95)
Multimodality treatment including radical surgery	18.5 (8.2–28.8)	—	0.46 (0.28–0.74)

5.1.2 Ki67 index is not associated with histology and stage but significantly decreased in post-chemotherapy cases

Next, Ki67 index was determined on tumor sections following IHC staining (Figure 24). In the HUN cohort, both pathologists scored the tissue slides to test the inter-observer reproducibility. The scoring by the two pathologists showed a strong and significant correlation (Pearson's correlation coefficient: 0.668, P<0.001, n=41) and thus was considered to be reliable.

The median percentage of Ki67-positive tumor cells was 15.0% (range: 0–60%) for the entire test cohort. Table 7 shows the main patient characteristics by Ki-67 index. There was no significant difference in the distribution of Ki67 index with regard to age, sex, histology or stage. Furthermore, there was no association with histology (Figure 24B).

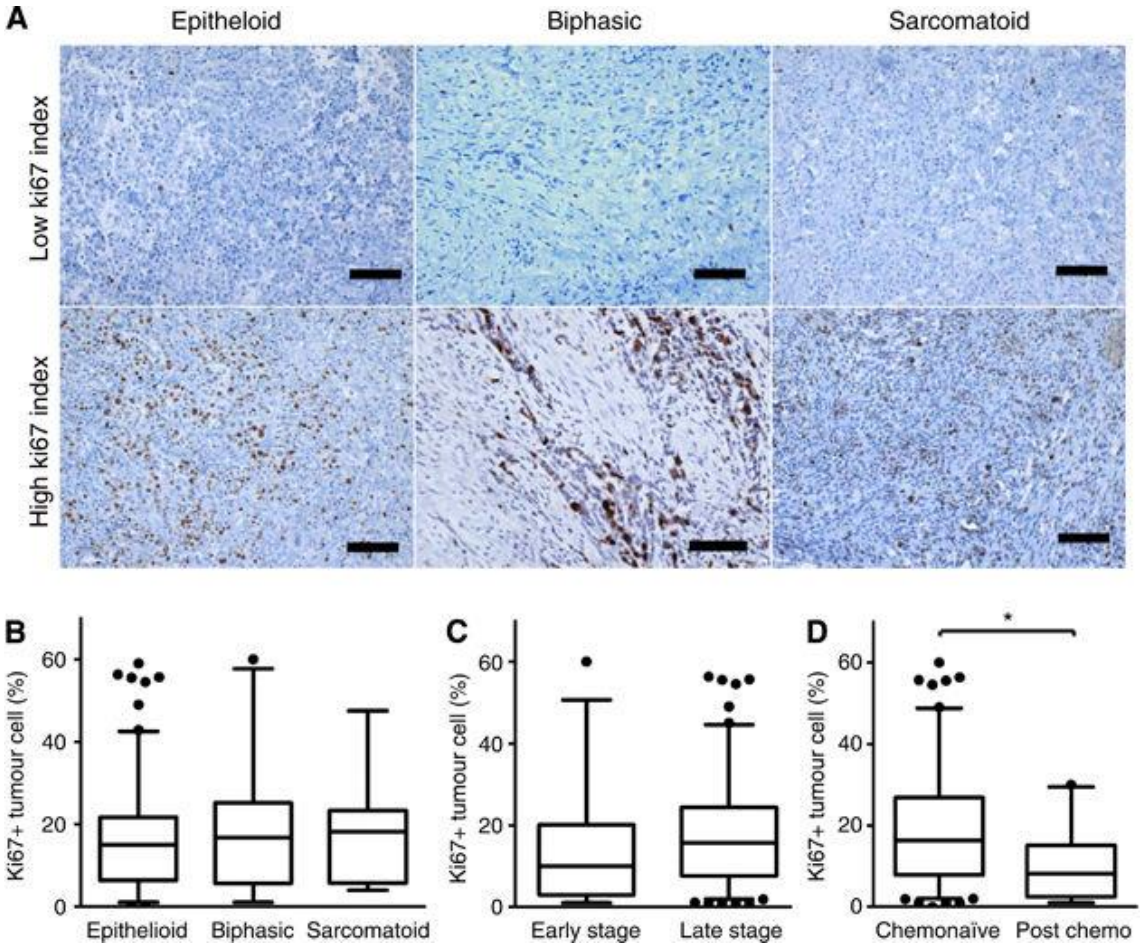

Figure 24: Ki67 index in MPM tissue samples. (A) Ki67 nuclear staining in all three major histological subtypes of MPM. Scale bar is 100 µm. (B) There was no significant difference between the three histological subtypes with regard to Ki67 index (mean±s.d.: epithelioid 16.3±12.9 vs biphasic 18.3±15.3 vs sarcomatoid 18.2±13.6, P=0.766). (C) Modest increase of Ki67 index in late stage of disease (mean±s.d.: 17.6±13.2) vs early stage (13.9±13.6, P=0.144). (D) Patients after induction chemotherapy had significantly lower amount of Ki67-positive tumor cells when compared with the treatment naïve patients in the test cohort (mean±s.d.: induction chemotherapy 10.5±8.5 vs chemo-naïve 18.3±13.9, asterisk denotes significance of P<0.001).

Accordingly, the distribution of the histological subtypes within the high and low Ki67 index groups did not differ significantly (Table 7). No association of Ki67 index and disease stage was found in the test cohort (Figure 24C). However, patients that were treated in a multimodality setting had lower Ki67 index when compared with those in other treatment groups. Accordingly, the Ki67 index was significantly lower in patients treated by induction therapy than others in the test cohort who had not received chemotherapy tissue retrieval (Figure 24D).

Table 7: Patient characteristics and distribution according to Ki67 expression in the test cohort (n=187). *=two-sided χ2-test; #=missing cases: n=30 (16.0%)

	Low Ki67		High Ki67		All patients		
Characteristics	Numbers	%	Numbers	%	Numbers	%	P
Age, Years							
61.5	50	53.2	44	46.8	94	50.3	NS*
>61.5	45	48.4	48	51.6	93	49.7	—
Sex							
Female	25	62.5	15	37.5	40	21.4	NS*
Male	70	47.6	77	52.4	147	78.6	—
Histology							
Epithelioid	81	51.9	75	48.1	156	83.4	NS*
Non- Epithelioid	14	45.2	17	54.8	31	16.6	—
Stage[#]							
early stage	22	64.7	12	35.3	34	21.7	NS*
late stage	60	48.8	63	51.2	123	78.3	—
Treatment[#]							
Surgery alone	12	46.2	14	53.8	26	16.6	0.02*
Best supportive care	13	39.4	20	60.6	33	21	—
Chemo and/or Radiotherapy	24	45.3	29	54.7	53	33.8	—
Multimodal treatment	32	71.1	13	28.9	45	28.7	—
Surgery[#]							
Surgery	44	62	27	38	71	45.2	0.02*
No Surgery	37	43	49	57	86	54.8	—
Induction Therapy[#]							
Induction	25	75.8	8	24.2	33	21	0.01*
Chemo-naïve at sampling time	56	45.2	68	54.8	124	79	—

5.1.3 Ki67 is an independent prognostic marker only in epithelioid MPM

Outcome of MPM patients with high Ki67 index was significantly worse compared to the low Ki67 index group (median OS 7.5 vs. 19.1 months, HR 2.3, CI 1.7–3.2, P<0.001, Figure 25A) in univariate survival analyses. In multivariate survival analyses, Ki67 index remained to be a significant prognostic parameter (HR: 2.1, CI: 1.4–3.1, P<0.001) independently from histology (P=0.013) and treatment modality (P=0.001), whereas age, gender and stage had no significant independent prognostic impact on OS (Table 8). In subgroup analyses, Ki67 showed prognostic power only in the epithelioid subgroup (Figure 25B) whereas it had no prognostic impact in the non-epithelioid subtype (Figure 25C). In all other subgroup analyses (for age, gender, stage and treatment), Ki67 proved to be a prognostic marker in all except the surgery-alone subgroup. Moreover, Ki-67 proved to be a prognostic parameter in the validation cohort as well (Figure 25D).

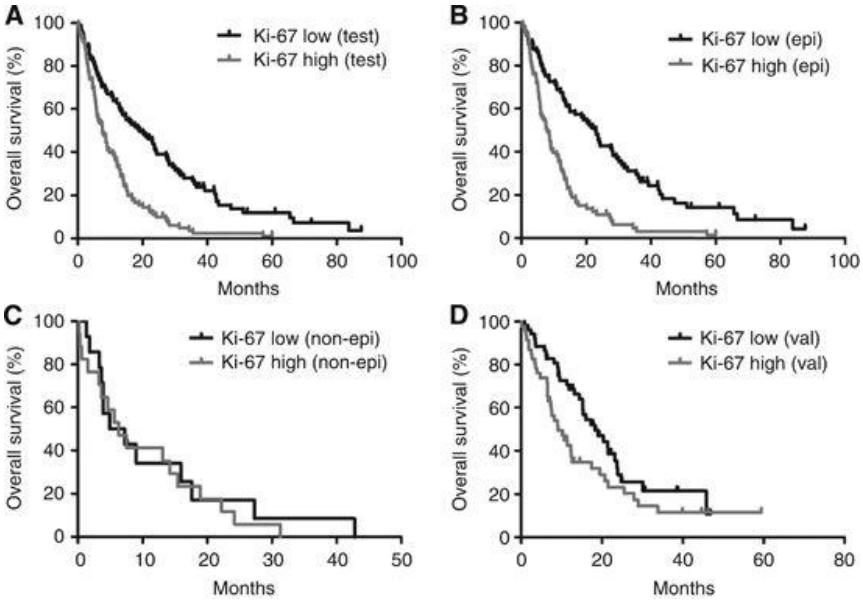

Figure 25: Prognostic power of Ki67 index in MPM. (A) Kaplan–Meier survival analysis of the test cohort (n=187). There was a significant difference in the OS between patients with high (n=92) vs low (n=95) Ki67 index (HR 2.3, CI 1.7–3.2, P<0.001). Median OS was higher in low Ki67 index (<15%) group (19.1 (CI 11.7–26.5) months) than in patients with high Ki67 index (7.5 (CI 5.2–9.8) months, P<0.001)). (B) Kaplan–Meier survival curve shows that Ki67 is prognostic in epithelioid MPM. (C) In contrast, there is no impact of Ki67 index on OS in patients with non-epithelioid MPM (n=31). (D). Kaplan–Meier survival analysis in the validation cohort (n=98). Ki67 had a significant impact on OS (P=0.048) when using median Ki67 expression of the validation cohort as cutoff (22%).

Table 8: Cox-regression model adjusted for patient characteristics (n=149)

Characteristics	HR for death	95% CI	P
Age, years			
61.5	0.98	0.69–1.40	0.92
>61.5	1	—	—
Sex			
Female	0.94	0.53–1.67	0.84
Male	1	—	—
Histology			
Epithelioid	1	—	0.01
Non-epithelioid	1.90	1.14–3.15	—
Stage			
Early stage	0.64	0.36–1.12	0.12
Late stage	1	—	—
Treatment			
Surgery alone	1.44	0.75–2.80	0.28
Best supportive care	1	—	—
Chemo and/or Radiotherapy	0.58	0.36–0.93	0.02
Multimodal treatment	0.52	0.30–0.91	0.02
Ki67 Expression			
Low Ki67	1	—	<0.01
High Ki67	2.11	1.44–3.10	

5.1.4 Ki67 is an accurate marker predicting short-term survival in epithelioid MPM

Next, we performed a ROC curve analysis to test the sensitivity and specificity for detecting early death within one year after diagnosis. For this analysis, all epithelioid MPM patients with clinical follow-up for at least 12 months (n=221) were included. Ki67 showed an area under the curve of 0.68 for predicting 1-year survival (Figure 26A). The sensitivity and specificity at 15% Ki-67 positivity were 0.69 and 0.57, respectively.

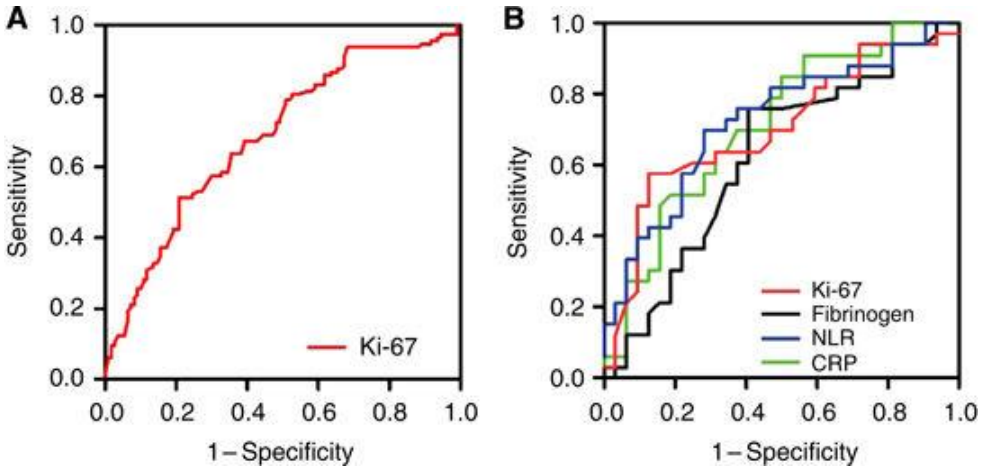

Figure 26: Sensitivity and specificity analyses of Ki67 by ROC analysis. (A) in all epithelioid MPM patients with follow-up of at least 12 months (n=221) showed an area under the curve of 0.68. The sensitivity and specificity at 15% (cutoff used in the test cohort) were 0.69 and 0.57, respectively. (B) Ki67 index, NLR, fibrinogen and CRP showed areas under the curve of 0.70, 0.72, 0.62 and 0.71, respectively (n=65).

We further compared the ability of fibrinogen, NLR and CRP to predict 1-year OS in MPM patients with Ki67 and performed an additional ROC analysis. Ki67, NLR, fibrinogen and CRP showed similar areas under the curve of 0.70, 0.72, 0.62 and 0.71. (Figure 26B).

5.2 C4d as a circulating prognostic biomarker in MPM

The clinicopathological parameters with regard to high and low plasma C4d levels of the entire patient cohort are presented in Table 9. A total of 34 patients (61.8%) underwent multimodality treatment, consisting of induction chemotherapy (platinum-based) followed by MCR with or without IMRT. In 31 (91.2%) of these patients, EPP was performed. Neoadjuvant CHT was applied in 35 patients (63.6%) and consisted of three to four cycles of a platinum-based regimen. Fifteen patients (38.2%) received CHT and/or RT only due to advanced disease at the time of diagnosis.

Table 9: Clinicopathological characteristics of MPM patients grouped by circulating C4d levels with a cut-off of 1.5 µg/mL. MMT, multimodal treatment; EPP, extrapleural pneumonectomy; CHT, chemotherapy; RT, radiotherapy; NA, not available

		Total (n= 55)	**C4d low (n= 37)**	**C4d high (n= 18)**	**p**
Gender	Male Female	40 15	28 9	12 6	0.529
Age	Mean ± SD	62.0 ± 12.3	64.6 ± 11.4	56.8 ± 12.6	**0.02**
Smoker (NA = 9)	Never Former Current	15 24 7	9 17 4	6 7 3	0.700
Histology	Epithelioid Non-epi	41 14	29 8	6 12	**0.002**
IMIG Stage (NA = 11)	Early (I/II) Late (III/IV)	12 32	11 18	1 14	**0.035**
Treatment (NA = 6)	CHT/RT MMT	15 34	9 24	10 6	**0.028**
EPP	Yes No	31 24	23 14	8 10	0.255
CHT (NA = 1)	CHT No induction	35 19	25 11	10 8	0.372

5.2.1 Lack of tumor cell-specific expression of C4d

We performed IHC of 32 MPM FFPE tissue specimens to assess C4d expression within tumor tissue. Interestingly, we found no tumor cell-specific C4d expression in these samples (Figure 27A). Therefore, correlations between circulating C4d plasma levels and tumor cell expression could not be performed. However, several germinal centers of ectopic lymphoid structures within the tumor strongly stained positive for C4d (Figure 27B).

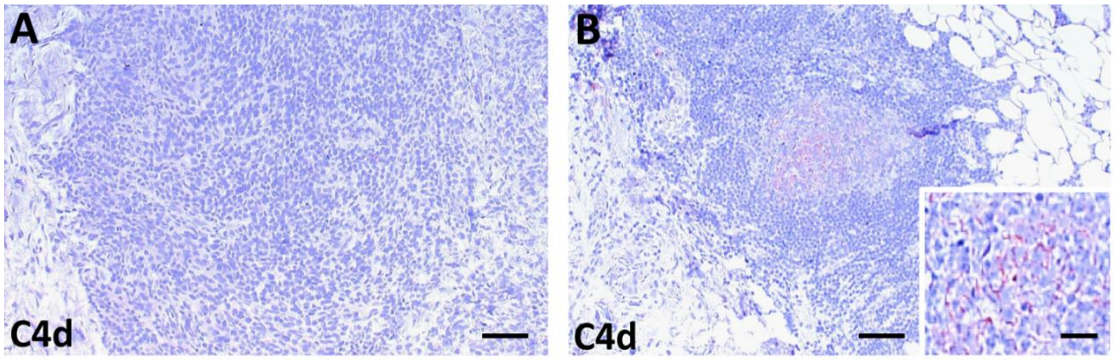

Figure 27: Tissue expression of C4d in MPM. (A) There was no C4d labeling found in MPM cells. Scale bar is 100µm (B) However, strong C4d expression was observed in the germinal center of ectopic lymphoid structures (scale bar 100µm). The plasma membrane specific labeling is demonstrated in the high magnification inset (scale bar 40µm).

5.2.2 C4d plasma levels are not elevated in MPM patients compared to HV or NMPD

To investigate the relevance of circulating C4d as a potential diagnostic biomarker, we compared C4d plasma levels of MPM patients (n=55), HVs (n=21) and with NMPD (n=14) (Figure 28A). Interestingly, we found no significant differences between these three cohorts.

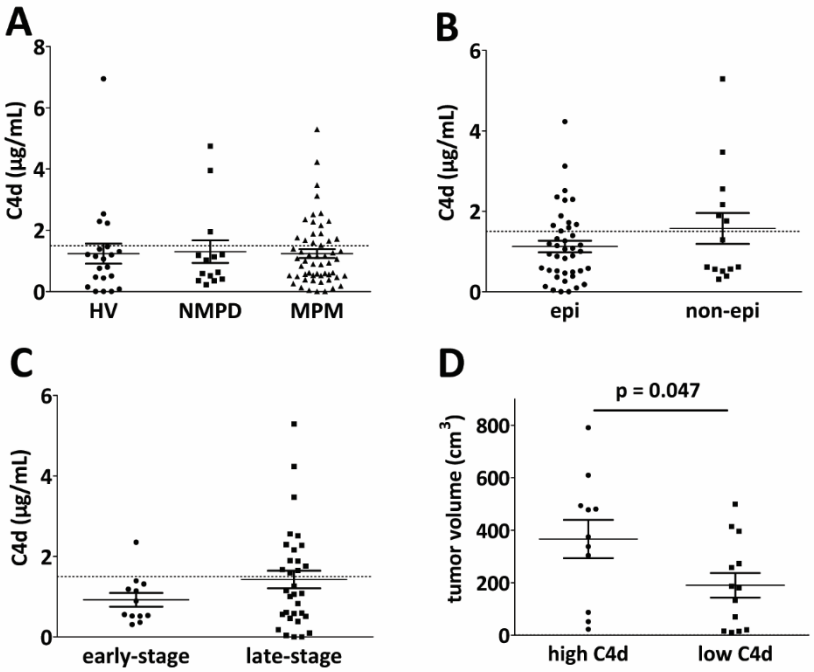

Figure 28: Plasma levels of C4d in MPM patients. (A) There were no significant differences between MPM patients and healthy volunteers (HV) or patients with non-malignant pleural disease (NMPD) in

their circulating C4d levels (MPM versus HVs: Mann Whitney U test, p=0.833; MPM versus NMPDs: Mann Whitney U test, p=0.851). (B) Within the MPM cohort, we found a non-significant tendency for higher C4d levels in non-epithelioid compared to epithelioid subtype (Mann Whitney U test, p=0.182). (C) Also, patients with advanced disease (stage III or IV) had the tendency to have higher circulating C4d plasma levels (Mann Whitney U test, p=0.079). (D) Comparison of radiologically assessed tumor volumetric data and circulating C4d levels. After dividing the cohort (cut-off 1.5 µg/mL), high C4d levels were significantly associated with a higher tumor load (366.7±72.7 versus 190.3±46.7 cm3, p=0.047).

C4d levels were much more heterogeneous in MPM patients than in HV (18 (33%) MPM patients showed a C4d level above 1.5µg/mL - only 4 (20%) HVs had levels above 1.5µg/mL). MPM patients presenting with epithelioid subtype tended to have lower circulating C4d levels (Figure 28B). Moreover, patients presenting with advanced disease showed a tendency to have higher circulating C4d plasma levels when compared to patients with early-stage disease (Figure 28C). Interestingly, nearly all HV, NMPD as well as the majority of MPM patients had circulating C4d levels equal to or lower to our established cut-off of 1.5 µg/mL. Only a distinct subgroup of MPM patients had clearly increased circulating C4d plasma levels (Figure 28A).

We also evaluated the association of C4d with main clinicopathological parameters including gender, age, smoking status and treatment regimen. Patients with epithelioid subtype, IMIG stage I/II, advanced age or those who underwent multimodality treatment were significantly more likely to have C4d levels below 1.5 µg/mL (Table 9). Notably, circulating C4d levels decreased significantly with advanced age, though with a weak correlation (Pearson r: -0.34; p=0.003).

5.2.3 Correlation of C4d with CT-based tumor volumetry

Circulating C4d correlated with disease stage and thus we evaluated the correlation of tumor load assessed by CT-based tumor volumetry with plasma C4d levels. Pre- and post-chemotherapeutic volumetric data were available for 20 patients. The average period between CT and blood collection was 21.5 days. After dichotomizing the sub-cohort according to our established C4d cut-off (1.5 µg/mL), high circulating C4d levels were significantly associated with a higher tumor load (Figure 28D). When we directly correlated tumor volumetry with circulating C4d plasma levels, we found the non-significant tendency that C4d increased with increasing tumor volume (Pearson r: 0.34; p=0.099).

5.2.4 Correlation of C4d with circulating inflammatory-based markers

Based on the observation that C4d is expressed intratumorally within ectopic germinal lymphoid structures, we correlated C4d with well-known inflammation-related biomarkers. Higher circulating C4d levels were associated with higher plasma levels of fibrinogen (Figure 29A) and CRP (Figure 29B). We detected no correlation between C4d and white blood count (WBC) (Figure 29C).

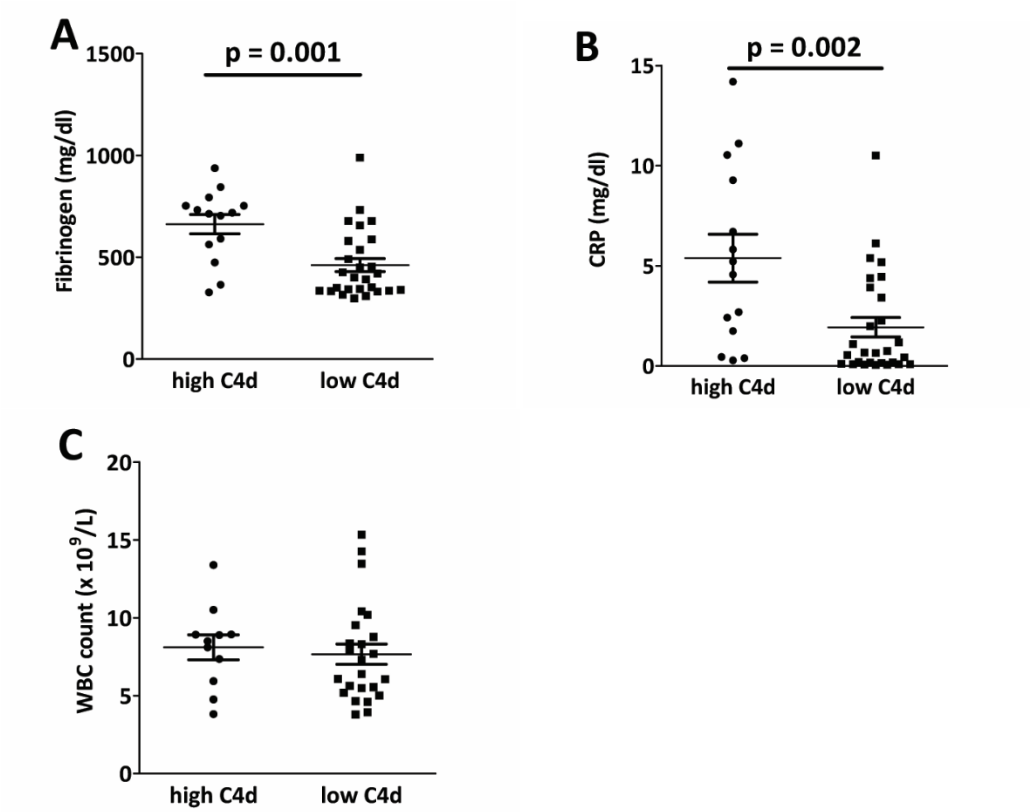

Figure 29: Correlations of circulating C4d levels with other inflammatory-related biomarkers. Strong correlations were found between circulating fibrinogen (A) as well as CRP (B) and circulating C4d levels in MPM patients (Fibrinogen: Pearson r: 0.48; p=0.001; CRP: Pearson r: 0.44; p=0.002;). (C) In contrast, WBC did not correlate with circulating C4d plasma level (Pearson r: -0.14; p=0.697).

5.2.5 Plasma levels of C4d predict chemotherapeutic response after induction treatment

Next, we investigated the effect of CHT on circulating C4d levels and evaluated C4d values of patients following platinum-based neo-adjuvant chemotherapy. Interestingly, patients with stable disease or progressive disease (SD/PD) after CHT had significantly

higher C4d levels compared to those with a partial or major response (Figure 30A). In contrary, inflammatory-based markers including fibrinogen and CRP showed no significant correlation with chemotherapeutic response.

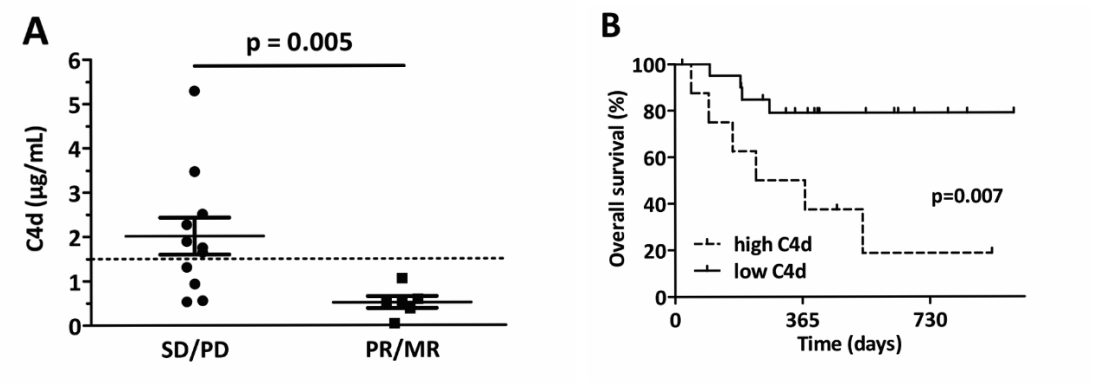

Figure 30: Circulating plasma level of C4d correlates with chemotherapy response and prognosis in MPM patients. (A) Following induction chemotherapy, patients with partial or major response (PR/MR) had significantly lower circulating C4d values when compared to patients with SD/PD (0.52±0.13 versus 2.02±0.42 µg/mL, p=0.005). (B) Kaplan-Meier survival curve of all MPM patients with plasma samples collected at time of diagnosis (n=30). After dividing the cohort (cut-off 1.5 µg/mL) into low (n=21) and high (n=9) C4d plasma levels, those with low plasma C4d had significantly shorter OS (HR 7.33, CI 1.71 – 31.44, p=0.007).

In a series of 12 patients we also measured C4d levels before and after CHT. Interestingly, patients with SD/PD showed a tendency for an increase in circulating C4d levels following CHT whereas patients with PR/MR showed a weak tendency for a decrease (relative differences between pre- and post-chemotherapy C4d levels: SD/PD +0.39±0.37 versus PR/MR -0.10±0.21 µg/mL, p=0.300).

5.2.6 Circulating C4d has a prognostic impact in MPM

As a next step, we investigated the prognostic impact of circulating plasma C4d level and dichotomized our cohort into patients with high (≥1.5 µg/mL; n=9) and low median C4d levels (<1.5 µg/mL; n=21) at time of diagnosis. Patients with high C4d had significantly worse prognosis compared to patients with low plasma levels (Figure 30B). Importantly, a multivariate cox regression analysis revealed that plasma C4d levels at diagnosis influenced OS independently from histological subtype, IMIG stage and type of treatment (Table 10).

Table 10: Multivariate Cox-regression analyses adjusted for clinical factors influencing OS of MPM patients. MMT, multimodal treatment; CHT, chemotherapy; RT, radiotherapy; NA, not available; HR, hazard ratio; CI, confidence interval.

		HR	95% CI	p
Histology	Epithelioid Non-epithelioid	0.695 1	0.250-1.935	0.486
IMIG Stage	Early (I/II) Late (III/IV)	0.711 1	0.176-2.875	0.633
Treatment	MMT CHT and/or RT	0.277 1	0.101-0.761	**0.013**
Plasma C4d levels	Low High	0.263 1	0.096-0.725	**0.010**

5.2.7 Correlation of circulating C4d and C3a levels

C4d is the degradation product of C4 in the classical and lectin complement pathways. Together with C2a it forms a proteolytic convertase which cleaves C3 releasing C3a into the surrounding. Here, we investigated the specificity of circulating C4d as a representative marker for complement activation and measured plasma C3a levels in patients with high (n = 14) and low (n = 13) circulating C4d levels as well as in HVs (n = 12). In this cohort, plasma C3a levels were significantly higher in MPM patients compared to HVs (Figure 31A). Within the cohort of MPM patients, C3a levels did not correlate with histological subtype (epithelioid versus non-epithelioid: 122.70±13.01 versus 151.50 ±22.24 µg/mL, p=0.244), stage (IMIG stage I or II versus III or IV: 119.90±21.82 versus 144.10 ±15.69 µg/mL, p=0.406,) or age (Pearson r: -0.05; p=0.807). However, we found a modest but significant positive correlation between circulating C3a and C4d plasma levels (Figure 31B).

Finally, to investigate whether C3a shows a similar reliability as a prognostic marker in MPM as C4d, we divided the selected C3a sub-cohort into low (n=14) and high (n=13) C3a categories by using the median plasma C3a level as a cut-off (119 ng/mL). There was a tendency for decreased OS in patients with increased circulating C3a (Figure 31C). Of note, a similar tendency for worse OS in patients with high circulating C4d levels was found in the same subcohort (HR 2.542, CI 0.86 – 7.56, p=0.106). These data strongly suggest that the elevated C4d levels found in late stage MPM is the result of the activation of either the classical of the lectin complement activation pathway.

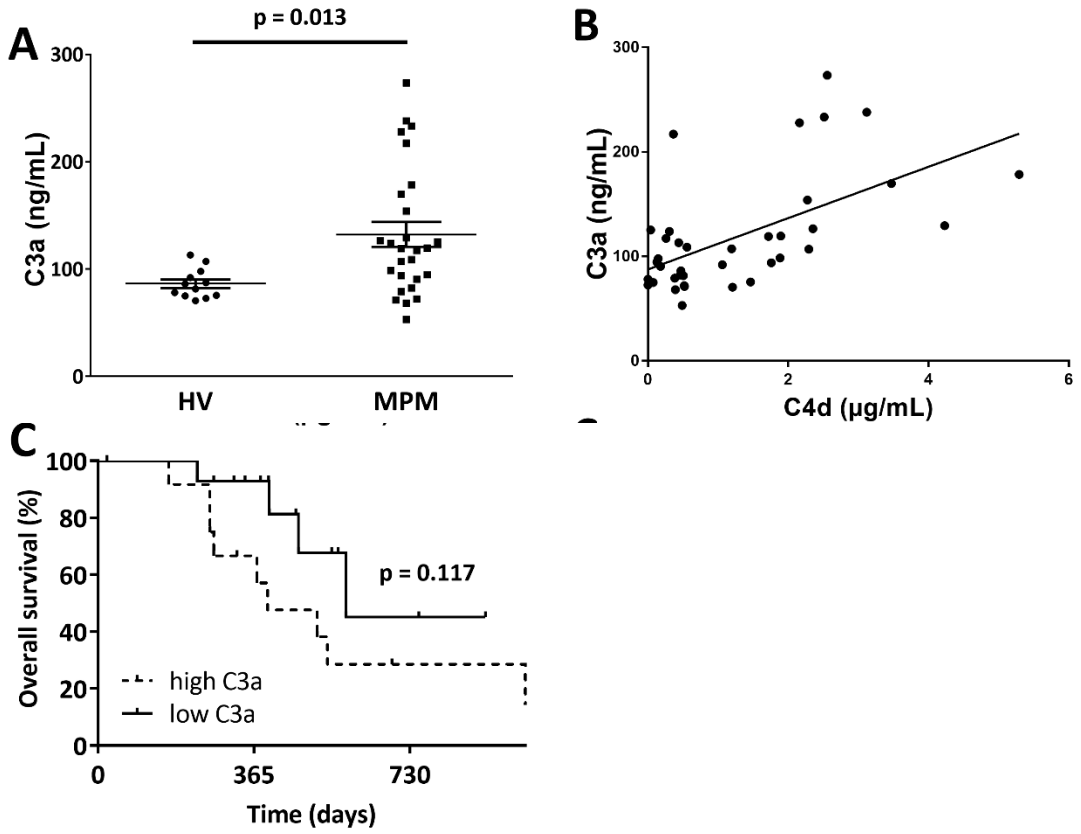

Figure 31: Plasma levels of C3a in MPM patients. (A) MPM patients had significantly higher circulating C3a levels compared to HVs (MPM versus HVs: Mann Whitney U test, p=0.013). (B) There was a modest but significantly positive correlation between circulating C3a and C4d levels (Pearson r = 0.57, p<0.001). (C) Kaplan-Meier survival curve of selected MPM patients with C3a plasma samples. After dividing the cohort (median plasma C3a as cut-off: 119 ng/mL) into sub-cohorts of patients with low (n=14) versus high (n=13) C3a plasma levels, there was a tendency for difference in OS between the two groups (C3a plasma levels >119ng/mL: HR 2.52, CI 0.75 – 8.38, p=0.117).

5.2.8 Limited tumor cell-specific expression of C1q in MPM

The classical complement activation pathway is initiated by the binding of C1q to the target molecule. To investigate whether circulating C4d derives from this pathway, we performed C1q IHC in 14 FFPE tissue specimens deriving from selected patients with high (n = 7) and low (n = 7) circulating C4d levels. Similar to C4d, we found no significant C1q expression by tumor cells. Only two MPM samples, both deriving from patients with low circulating C4d levels, showed weak tumor cell-specific C1q staining

(Figure 32A). However, inflammatory cells stained positive for C1q regardless of circulating C4d values (Figure 32B). Based on this observation, we next performed semiquantitative evaluation for tumor-associated inflammatory cells in HE-stained sections from the aforementioned 14 specimens and found no correlation between circulating C4d levels and inflammatory cell infiltration.

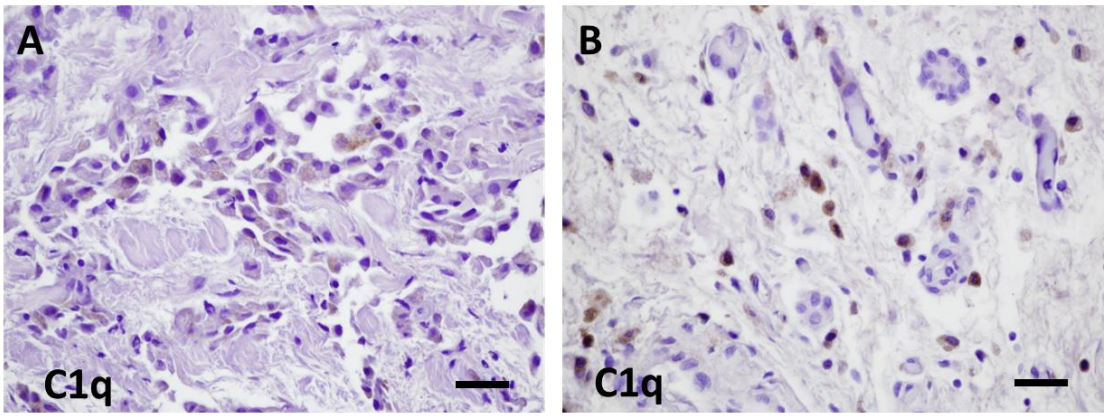

Figure 32: Tissue expression of C1q in MPM. Scale bar is 50 μm. (A) Slightly positive tumor cells were only found in two patients (both with low circulating C4d levels). (B) Scattered positive staining (i.e. inflammatory cells) for C1q was found in all cases.

5.3 Circulating ActA as a prognostic biomarker in MPM

5.3.1 Impact of ActA in a in distinguishing MPM from non-malignant pleural disease

First, we evaluated circulating ActA regarding its potential to act as a diagnostic biomarker for distinguishing MPM from NMPD or HVs. Circulating ActA levels were significantly elevated compared to HVs or patients with NMPD. Patients with NMPD also showed a trend for elevation of ActA levels (Figure 33A).

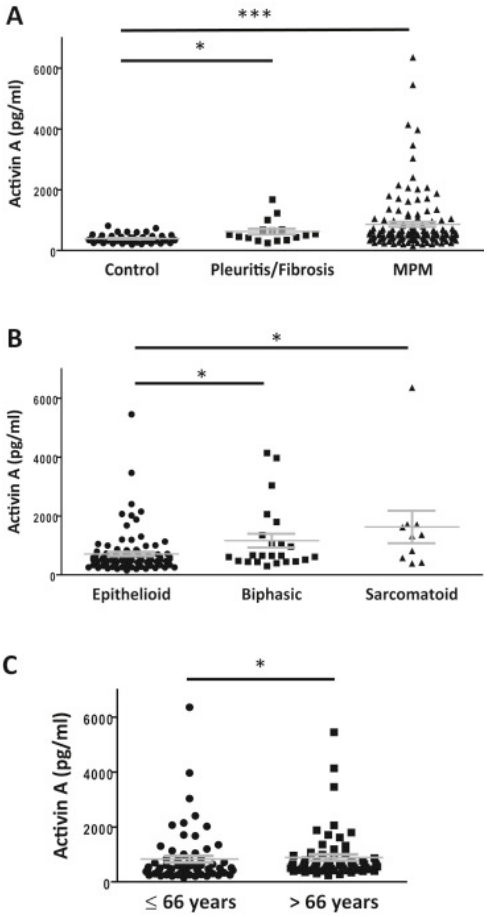

Figure 33: Circulating activin A levels are increased in MPM patients. A, There was a highly significant difference between healthy controls and patients with MPM ($P < 0.0001$, Bonferroni correction $P = 0.0029$). Interestingly, there was also a small but significant increase in patients with pleuritis or fibrosis (NMPD) compared to the control group ($P = 0.0067$). B, A significant difference was revealed within the three MPM histological subtypes ($P = 0.0022$, Bonferroni correction $P = 0.0032$). Sarcomatoid patients had a significantly higher circulating ActA level than those with epithelioid tumours ($P = 0.0019$). Additionally, biphasic cases showed a modest but significant elevation in plasma ActA levels ($P = 0.0188$). C, A significantly lower circulating activin A level was observed in patients below the median age (66 years) when compared to older patients ($P = 0.0362$). * $P \leq 0.05$; *** $P \leq 0.001$; MPM, malignant pleural mesothelioma.

Next, basic clinicopathological characteristics were compared using median ActA levels as a cut-off (Table 11 and Table 12). Patients with ActA levels below the median were significantly younger, of epithelioid subtype, and had lower stage and more often MMT including MCR (Table 11). There was a significant difference in ActA levels between the 3 main histological subtypes of MPM (Figure 33B). Moreover, ActA levels were significantly decreased in patients below the median age of 66 years (Figure 33C).

Table 11: Clinicopathological characteristics of MPM patients grouped by circulating ActA level. BSC, best supportive care; MMT, multimodal treatment; EPP, extrapleural pneumonectomy; NA, not available; SD, standard deviation

		Total	ActA low	ActA high	p
Gender	Male	102	49	53	0.387
	Female	27	16	11	
Age	Mean ± SD	65 ± 11.7	63 ± 11.6	67 ± 11.6	0.072
Smoker (NA = 12)	Never	48	26	22	0.602
	Former	45	22	23	
	Current	24	10	14	
Histology (NA = 1)	Epithelioid	94	54	40	**0.016**
	Non-epithelioid	34	11	23	
IMIG stage (NA = 37)	I	7	5	2	**0.049**
	II	17	9	8	
	III	38	23	15	
	IV	30	9	21	
Treatment (NA = 12)	BSC	21	9	12	**0.007**
	Chemo or radio	46	17	29	
	MMT	50	34	16	
Surgery	EPP	39	28	11	**0.002**
	No	90	37	53	

5.3.2 ActA level has prognostic impact in epithelioid MPM only

The prognostic impact of circulating ActA was further examined by dichotomizing the patient cohort with reliable OS data (n = 119, median follow-up time 1028 d) by its median plasma ActA level (Figure 34A and Table 12). The Youden index analysis was performed in order to determine the best cut-off level for OS, with the highest index being 0.3377 at 574 pg/ml (similar to our chosen median cut-off). Patients with high plasma ActA levels had significantly worse outcome compared to patients with low levels. With regards to age, plasma ActA proved to be a prognostic marker only in patients under the age of 66 years (Figure 34B).

Table 12: Univariate survival analyses (n = 119). BSC, best supportive care; CHT, chemotherapy; RT, radiotherapy; MMT, multimodal treatment; OS, overall survival; CI, confidence interval; HR, hazard ratio; NA, not available.

		N	OS	Ratio (CI)	p	HR (CI)
Gender	Female	25	537	0.808 (0.213–1.404)	0.353	1.264 (0.771–2.074)
	Male	94	434			
Age	>66	59	408	1.316 (0.658–1.974)	**0.024**	0.603 (0.389–0.935)
	≤66	60	537			
Histology (NA = 1)	Non-epi	32	271	2.092 (1.457–2.727)	**0.001**	0.399 (0.230–0.695)
	Epi	86	567			
ActA	High	59	365	2.014 (1.358–2.670)	**0.001**	0.387 (0.247–0.606)
	Low	60	735			
Stage (NA = 33)	Late	67	438	1.676 (1.132–2.220)	0.183	0.685 (0.393–1.196)
	Early	19	734			
Treatment (N = 12)	BSC	20	182	0.236 (0.309-0.780)	**0.001**	6.756 (3.024–15.09)
	CHT/RT	44	395	0.512 (0.083-1.106)		2.901 (1.703–4.941)
	MMT	43	772	1 (0.439–1.561)		1 (0.557–1.796)

Importantly, plasma ActA had a prognostic impact only in the subgroup of patients with epithelioid MPM (Figure 34C). In patients with non-epithelioid histology, there was a non-significant trend for different OS regarding ActA levels only (Figure 34D).

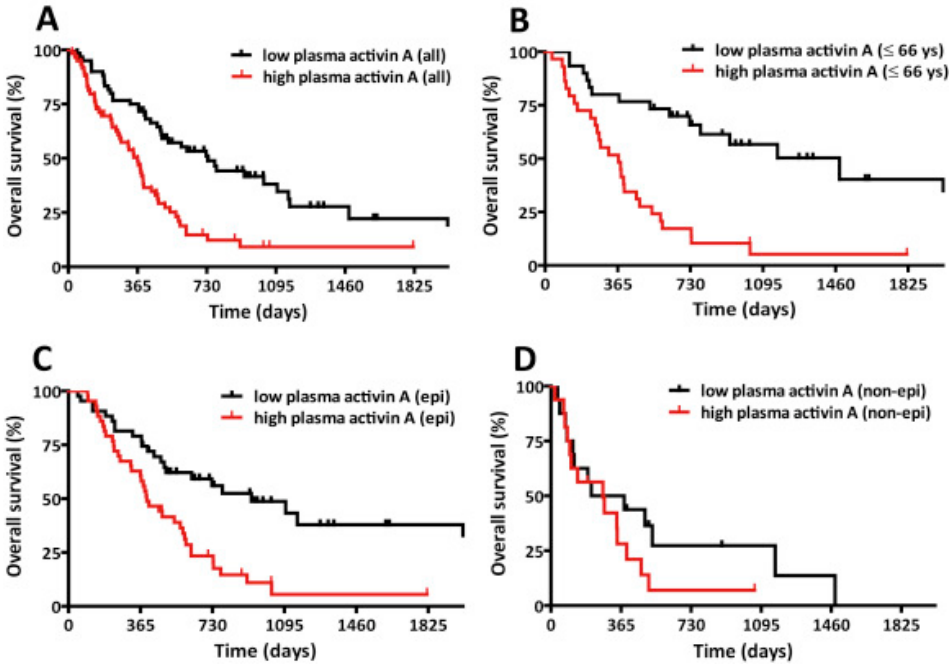

Figure 34: Prognostic power of circulating plasma ActA in MPM. A, Kaplan–Meier survival analysis of all MPM patients (n = 119). There was a significant difference in the OS between MPM patients with low (n = 60, 735 d) versus high (n = 59, 365 d) circulating plasma ActA level when median plasma level was

used as a cutoff (574.0 pg/ml, P < 0.0001). B, The plasma level of ActA is prognostic only in patients younger than 66 years of age (P < 0.0001). C, Plasma level of ActA has a prognostic value in epithelioid MPM patients (n = 86, P = 0.0003). D, In contrast, plasma level of ActA has no impact on the OS in patients with non-epithelioid MPM (n = 32, P = 0.1998). MPM, malignant pleural mesothelioma; OS, overall survival.

Moreover, in patients with ActA level measured at the time of diagnosis only (n = 65) ActA remained as a significant prognostic parameter (HR 0.57, CI 0.31–1.02, P = 0.058).

To assess the prognostic value of circulating ActA independently from other clinical parameters, we performed a multivariate analysis including age, gender, histological subtype and stage (Table 13). To ensure the applicability of the respective subgroup analysis, interaction terms for covariates were calculated for ActA and introduced to the multivariate cox regression model. A significant interaction term was found between age and plasma ActA levels (P = 0.014), whereas the interaction between histology and plasma ActA levels failed to reach statistical significance (P = 0.943).

Table 13: Multivariate analysis by Cox regression model adjusted for patient characteristics (n = 85). CI, confidence interval; HR, hazard ratio.

		N	HR	P	95% CI	
					Lower	Upper
Gender	Female	18	1			
	Male	67	1.142	0.673	0.617	2.114
Age	>66	33	1			
	≤66	52	0.750	0.284	0.443	1.270
Histology	Non-epi	19	1			
	Epi	66	0.621	0.112	0.345	1.117
ActA	High	42	1			
	Low	43	0.420	**0.002**	0.245	0.721
Stage	Late	66	1			
	Early	19	0.818	0.540	0.431	1.553

In this multivariate cox regression model, ActA was the only significant cofactor, independently influencing OS apart from gender, age, MPM subtype and stage of disease.

5.3.3 Association of circulating ActA and fibrinogen

In a previous study, our group has demonstrated that circulating fibrinogen can act as a strong prognostic parameter in MPM [103]. Accordingly, we correlated ActA with fibrinogen levels and found a modest but significant association between these two parameters (Figure 35A). Furthermore, we performed a ROC curve analysis to estimate the predictive value of these parameters with regard to 1-year OS and found a similar sensitivity and specificity of both biomarkers (Figure 35B).

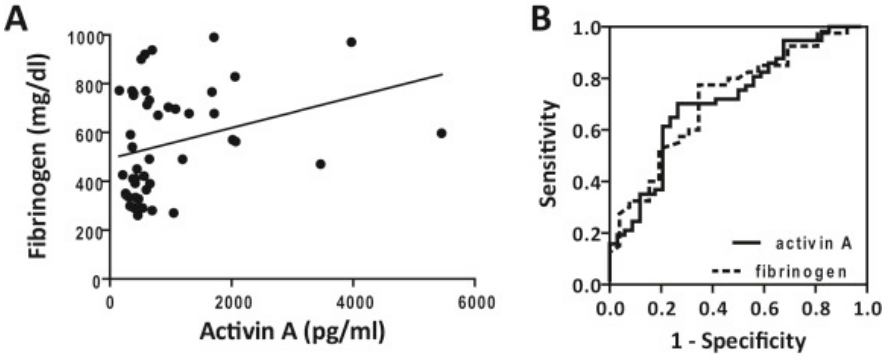

Figure 35: Comparison of sensitivity and specificity for prognostic power of circulating ActA and fibrinogen by ROC curve analysis. A, A weak but significant correlation was found between fibrinogen and plasma ActA levels in 67 MPM patients (Spearman r = 0.37, P = 0.002). B, Circulating ActA levels proved to have a similar sensitivity and specificity as fibrinogen levels with an area under the curve of 0.657 and 0.687, respectively. MPM, malignant pleural mesothelioma; ROC, receiver operating characteristics.

5.3.4 Correlation of ActA with tumor volume and chemotherapy treatment

Tumor volumetry analysis was performed in 19 patients and correlated with circulating ActA levels (Figure 36 A+B). Tumor volume showed a significant positive correlation with the corresponding plasma ActA levels (Figure 36C).

In an exploratory analysis, we compared matched chemo-naïve and post-chemotherapy (CHT) samples (n = 14). Increased levels were found in ten patients whereas plasma ActA level decreased after treatment in four patients (Figure 36D). Of note, the patient with the dramatic decrease in plasma ActA level after CHT experienced a major response.

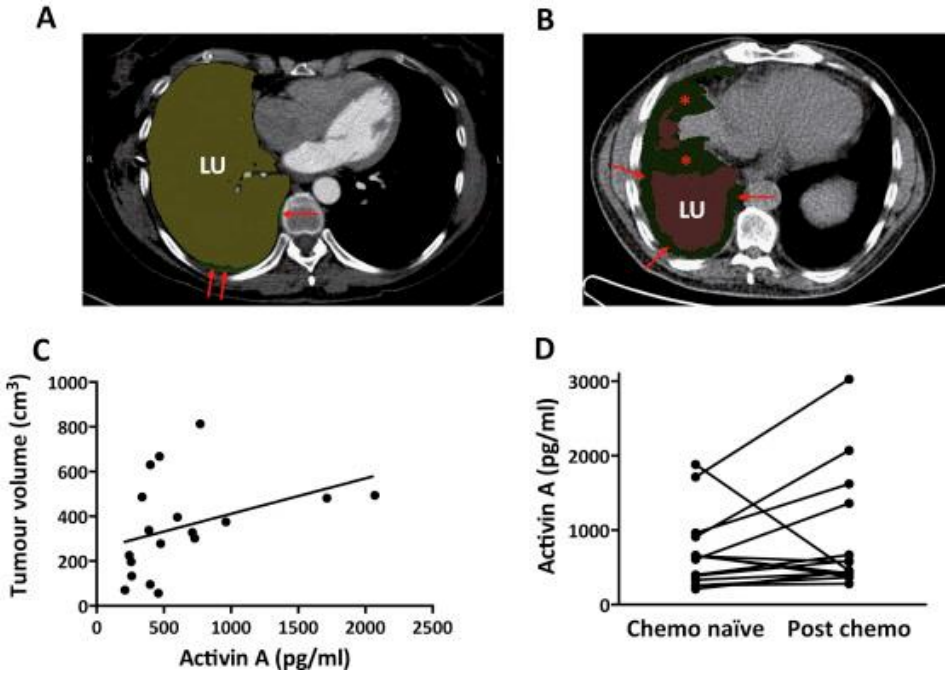

Figure 36: Exploratory subgroup analysis for the correlation of circulating ActA levels with tumor volume and cytotoxic chemotherapy. A and B, Representative CT-images from patients with a low (A) and high (B) tumor load. Red stars and arrows indicate the localization of the tumor lesions; 'LU' depicts the healthy lung tissue. C, Tumor volumes measured at the time of diagnosis showed a significant correlation with the corresponding plasma ActA values. (Spearman r = 0.548, P = 0.019). D, Matched chemo-naive and post-chemotherapy samples were available in 14 MPM patients. The paired t-test revealed a non-significant relationship between the chemo-naïve and post-chemotherapy plasma ActA levels (P = 0.287). CT, computed tomography; MPM, malignant pleural mesothelioma.

6 Discussion

MPM is a rare but devastating disease. Despite many efforts in prevention, early detection, identification of prognostic parameters and new therapeutic approaches, its outcome is extremely dismal. Accordingly, new markers are urgently needed to guide selection for new therapeutic compounds and to identify patients for more aggressive treatment approaches. The research conducted in part within this thesis deals with the so far unmet need for molecular prognosticators, which might be easily available, reproducible and detectable and could lead to a better understanding of the underlying mechanisms of resistance to more aggressive multimodal treatment approaches and eventually to improved patient selection to avoid dismal outcome.

6.1 Diagnostic biomarkers in MPM

As has been pointed out in the introduction of this thesis, mesothelioma occurs unfrequently, but is still gradually on rise and previous asbestos exposure is a main risk factor for MPM development. In Europe, the average incidence is 0.02 per 1000 inhabitants and the frequency is highly dependent on the amount of asbestos removal, asbestos import and industrialization. The peak incidence of MPM is to be expected between around 2020 due to the long latency period [13]. Exposure to asbestos is highly labor-dependent and occupations associated with exposure are traditionally carried out by men. This association leads to a 2 to 8-fold higher risk for MPM development in men, depending on the geographic region. However, asbestos exposure not only triggers chronic pleural inflammation to contribute to the formation of malignant mesothelial cells, but can also cause NMPDs such as pleuritis or chronic pleural plaques [32]. This leads to the frequent clinical problem of determining appropriate diagnostic strategies for asbestos-exposed individuals with recurrent pleural effusions or pleural thickening. Blood- or pleural effusion-derived diagnostic biomarkers could substantially improve early diagnosis by distinguishing MPM from NMPD and thus increase the potential chances for curative treatment attempts. Several new circulating diagnostic markers have recently been investigated to rule out or confirm the suspicion for MPM and to individualize treatment strategies. Until recently, fibulin-3, a member of the extracellular glycoprotein fibulin family, was the most promising biomarker for early

diagnosis so far. A recent study including 92 MPM patients, 136 asbestos-exposed individuals without cancer, 93 patients with pleural effusions due to NMPD and 43 healthy controls from 2 different centers, investigated whether plasma and pleural effusion fibulin-3 could meet the criteria for a robust diagnostic biomarker [224]. In this study, plasma and pleural effusion fibulin-3 levels were significantly elevated compared to controls. ROC curve analysis for plasma fibulin-3 levels revealed a sensitivity of 96.7% and a specificity of 95.5% at a cutoff value of 52.8 ng/ml. In early-stage MPM, the sensitivity was 100% and the specificity was 94.1% at a cutoff value of 46.0 ng/ml for distinguishing from asbestos-exposed individuals. The authors of this study consequently considered fibulin-3 as an excellent robust blood and effusion derived biomarker to early diagnose MPM in an asbestos exposed cohort and to distinguish between benign and malignant, MPM-associated pleural effusion. After publication of this study, many health care specialists, especially those dealing with occupational diseases, were excited about the high diagnostic power of this recently discovered biomarker. Unfortunately, the results could not be reproduced when applied to different study populations and it remains still unclear, if fibulin-3 should be routinely examined in patients with suspicion for MPM [225, 300]. In addition to fibulin-3, osteopontin has been suggested to serve as an additional diagnostic biomarker for early MPM detection. Osteopontin (OPN) is an extracellular matrix glycoprotein which is overexpressed in several human neoplasms such as lung, breast, prostate and colon cancer [301]. Seven studies have so far assessed the diagnostic value of OPN in MPM [302]. A recent meta-analysis of these studies on circulating osteopontin revealed a pooled sensitivity of 0.57 and specificity of 0.81, with an AUC of 0.8. This is clearly a lower diagnostic accuracy compared to fibulin-3.

In addition to its prognostic value in MPM, plasma ActA was also investigated as a diagnostic marker to distinguish between HV, NMPD and MPM in the project described in this thesis [303]. There was a significant difference between circulating ActA levels when HVs (median 361.3 pg/ml, lower quartile 302.3 pg/ml, upper quartile 473.1 pg/ml) and MPM patients (n=129, median 562.0 pg/ml, lower quartile 395.2 pg/ml, upper quartile 859.8 pg/ml) were compared, but no significant difference was found between MPM and NMPD. We observed the same finding in our project with regards to C4d, as it was not able to distinguish between MPM and NMPD. Both proteins (i.e.

ActA and C4d) are expressed in normal tissues and released into circulation but might also promote tumor development and progression [252, 292]. However, the precise molecular mechanisms of ActA and C4d synthesis related to MPM formation and thereby the use of these proteins as potential circulating diagnostic markers for early MPM detection are yet to be further investigated. Furthermore, the results derived from the so far most promising diagnostic biomarkers, fibulin-3 and osteopontin, warrant further studies in larger cohorts before these markers can be recommended for early MPM detection in occupationally asbestos exposed individuals in the routine practice.

6.2 Prognostic factors and biomarkers in MPM

Apart from early MPM detection, tremendous efforts have been undertaken to optimize patient selection for different treatment modalities in MPM patients. Routinely assessed clinical factors were among the first to estimate prognosis. It has been clearly shown in large database analyses that the expected survival for MPM patients highly depends on the age, gender, performance status, tumor stage and histological subtype [99, 100]. Epithelioid MPM has a better overall prognosis than non-epithelioid histological subtypes (median OS 19 months vs. 13 months (biphasic) and 8 months (sarcomatoid)) and thus it became clinical practice to exclude non-epithelioid cases from aggressive treatment approaches due to the expected poor outcome [85]. A recent phase II study evaluating a new multimodality approach with neoadjuvant accelerated IMRT followed by EPP (SMART protocol) reported benefit from treatment only in epithelioid cases, whereas patients with biphasic subtype had significantly poorer OS [151]. Moreover, analysis of the IASLC MPM database revealed a trend for improved OS after surgical treatment in the epithelioid subgroup only. Based on these observations, surgery is not routinely recommended for non-epithelioid cases according to the latest NCCN, ESMO or ESTS/ERS guidelines for management of MPM [81, 85, 86]. The same holds truth for patients in advanced disease and poor performance status.

In recent years, a number of tissue and circulating biomarkers has been proposed in order to determine prognosis of different patient subgroups or biomarker-based treatment guidance. Usually, a protein of interest at tissue level can be easily detected and quantified via IHC and its expression pattern can be correlated with clinical data and survival. These frequently investigated markers include EGFR, c-MET, Axl,

Syndecan-1, NTS, CD9, CD26, AQP1, CD72, CD43, p53, COX-2, ERCC1 or TS (reviewed in chapter 2.1.7.) However, the majority of these studies consists of single-center experiences, some of which may have been certainly underpowered. For some specific biomarkers, which were investigated by several groups, results have been conflicting. Differences in case distribution based on gender, histology, stage and treatment, as well as different methodology, have likely contributed to these discrepancies and larger international cooperation and tissue biobanks are needed to overcome these obstacles in the future.

Ki-67 is a frequently used IHC marker to determine the fraction of proliferating cells in a given cell population and was increasingly used as a diagnostic tool in different tumor types [198]. In our above described study including MPM tissue samples from 4 different international institutions, we found that Ki-67 index is a significant prognostic parameter independently from sex, age, stage, histology and treatment modality. This finding was observed in a test cohort including patients from 3 centers and validated in another cohort from a forth center. Furthermore, we found that Ki-67 assessed in MPM patients warrants prognostic value similar to circulating inflammatory based parameters including CRP, NLR and fibrinogen.

Ki-67 proliferative index was previously investigated for diagnostic yield in FFPE cell blocks of pleural and peritoneal effusion, but failed to show a difference between reactive mesothelial hyperplasia and MPM [204]. Another recent study could additionally demonstrate that high Ki-67 tissue expression was related to significantly shorter PFS and OS in chemonaive patients and remained associated with shorter PFS and OS after chemotherapy [187]. Importantly, our study revealed for the first time that Ki-67 index is only prognostic in the epithelioid but not in the non-epithelioid subgroup of MPM. Non-epithelioid tumors are generally associated with worse prognosis due to early disease progression. The underlying mechanisms of reduced prognostic influence of tumor proliferation in biphasic and sarcomatoid MPM remain uncovered yet. In line with a previous study, however, we did not observe a significant difference in the proportion of Ki-67 positive tumor cells between the epithelioid and non-epithelioid histology, suggesting that the poor prognosis in biphasic/sarcomatoid tumors is not directly associated with high proliferation rates [304]. In our study, Ki-67 showed a non-significant association with more advanced disease stage, but not with other clinical

characteristics, such as gender or age. However, in subgroup analyses, Ki-67 revealed significant prognostic power in early and late stage patients, suggesting that Ki-67 expression does not simply reflect clinically undetected late stage of disease.

Our study additionally demonstrated that the prognostic impact of Ki-67 index is similar to other recently investigated inflammation-based circulating biomarkers [102, 103, 298]. According to our observation, the AUC of Ki-67 in predicting 1-year OS was 0.70. Importantly, there was a strong correlation between fibrinogen and CRP, suggesting that these two acute phase proteins reflect the same biological characteristics of MPM patients. Additionally, CRP, fibrinogen and NLR showed similar predictive values compared to Ki-67 index in the ROC curve analysis, suggesting similar biological importance during disease progression.

Here, we also report that Ki-67 expression significantly differs between MPM patients undergoing different treatment regimens. Expression was significantly lower in the multimodality group. However, in this subset of patients, a high number of cases received platinum-based induction CHT, which induces cell death in tumors with highly proliferating cells. We have additionally shown that Ki-67 index is significantly higher in pre- compared to post-induction cases, suggesting reduced cell proliferation after CHT. This observation was also reported in a previous study [187]. Thus, we believe that Ki-67 index might also serve as a clinically useful marker to assess the biological efficacy of (induction) CHT. Of note, induction CHT was recently shown to be an additional important prognostic factor for MPM in a multicenter study [146]. In another subgroup analysis, we demonstrate here that Ki-67 had prognostic impact in patients undergoing surgery and in those with conservative treatment (i.e. systemic treatment or BSC), suggesting that this marker might be useful for selecting patients both in surgical and conservative treatment arms. In the multivariate cox regression model, Ki-67 remained as a significant prognostic variable for OS, independently from other clinical parameters. In addition, histological subtype (i.e. epithelioid vs. non-epithelioid) and treatment modality had an independent influence on survival, which is well in line with other previous reports on prognostic factors in larger MPM cohorts [8, 100, 142]. Moreover, the rather historical subgroup of patients undergoing a surgery-only approach tended to have significantly shorter OS compared with patients who receive multimodality treatment. This observation has been previously reported by a number of

other high-volume centers and thus the strategy to perform surgery exclusively within a multimodality approach is well perceived among MPM specialists worldwide [8, 100, 139]. Our multicenter study strongly supports this approach.

The feasibility, importance and application of certain tissue-derived biomarkers as prognostic factors in MPM have been well established. The tissue needed for analysis, however, obviously has to be retrieved at the time of diagnosis. Yet, tissue biopsy can be quite challenging. VATS biopsy usually obtains large tissue samples for diagnosis and investigation of additional markers, but this applies for patients only that are candidates for surgery. In unresectable cases due to poor PS or advanced tumor stage, CT-guided biopsy is the diagnostic method of choice and hence tumor tissue might be consumed for establishing definite diagnosis but might not be available for additional investigations (i.e. for further molecular profiling).

Taken this into account, blood-derived prognostic biomarkers represent an important tool to overcome the common lack of tissue availability. Furthermore, they can be easily retrieved and simply reassessed at multiple time points. Some of the reported blood biomarkers are actually commonly assessed during routine investigations. These include white and red blood cell and thrombocyte counts, and more recently, NLR, PLR and the modified Glasgow Prognostic Score (GPS), consisting of CRP and its negative acute phase response counterpart albumin [209]. Our study group has additionally shown the prognostic significance of acute phase proteins such as CRP and fibrinogen [102, 103]. Some of these markers have even shown to be able to predict benefit from certain treatment modalities. Accordingly, these molecules can be useful in allocating patients to multimodality approaches. The majority of these markers might reflect the burden of the disease and are clearly associated with stage and PS, however, are not directly associated with malignant cells. In recent years, research has increasingly focused on tumor-associated circulating biomarkers, which might have therapeutic impact in the future. Among these, drugs against circulating VEGF, mesothelin or fibulin-3 have been investigated for their therapeutic value [84, 213, 218, 224, 305, 306].

Today, there is increasing evidence that the complement system is not only an important player within the inert immune system but is also involved in cancer development and progression by facilitating cellular proliferation and regeneration by a variety of mechanisms [226]. However, molecular mechanisms of complement protein-associated

MPM formation and progression had not been investigated before. Nevertheless, it has been shown that C4d, the stable cleavage product of complement protein C4, accumulating following classical and lectin pathway activation, might play a role in various tumor types including MPM [254, 255]. Although MPM development is strongly associated with chronic asbestos-related inflammation, complement activation in human MPM was barely understood and thus no study had focused on the prognostic or predictive role of C4d as a marker for complement activation in this disease. Thus, the aim of the study described in this thesis was to investigate the tissue and circulating levels of C4d in MPM patients and to compare these data with tumor load, chemotherapy response and clinicopathological parameters. Importantly, we found that late-stage MPM patients had higher plasma C4d levels and high circulating C4d was associated with a higher tumor volume. Plasma C4d levels following induction chemotherapy were significantly higher in patients with SD/PD and patients with low C4d levels at diagnosis had a significantly better OS as confirmed in a multivariate cox regression model. To the best of our knowledge, our study was the first to indicate the prognostic relevance of plasma C4d in MPM.

Of note, in our cohort, C4d plasma levels were lower compared to LADC patients from previous studies and thus we used 1.5 µg/mL as a cut-off in our cohort in contrary to the 3.0 µg/mL used by others. Lung cancer cells were shown to be able to directly activate the classical complement pathway via C1q binding, suggesting higher circulating levels of complement activation proteins [248]. Circulating C4d levels in MPM patients showed similar prognostic power for OS, comparable to other well-established biomarkers in thoracic tumors [102, 103, 294, 303, 307, 308]. However, we did not observe a significant difference in C4d levels between MPM patients and HVs or patients with NMPD and thus C4d may not be used as a diagnostic marker in this disease.

In contrast to LADC, MPM cells did not positively stain for C4d by IHC. Of note, C4d tumor expression was found to correlate with prognosis, disease-stage and/or nodal invasion in a variety of other tumor types [248, 249, 252, 254]. However, IHC staining for C4d did not reveal tumor specific expression in lymphoma cells [309]. Hence, this lack of C4d expression in MPM cells might suggest that the complement cascade does not get directly activated by malignant cells. Importantly, we found C4d expression in

the germinal centers of intratumoral ectopic lymphoid structures. Accordingly, as has been described within this thesis, circulating C4d was also associated with other inflammatory-based markers including CRP and fibrinogen.

In the classical complement pathway, C1q activates C4. Accordingly, we analyzed the staining pattern of C1q and found a modest tumor cell specific C1q expression only. This is in line with a previous study on transcriptome analyses of MPM cell lines, demonstrating the up-regulation of C1q-binding protein (a negative regulator of C1q) [310]. However, C1q was clearly expressed in tumor-infiltrating immune cells. Whether these infiltrating immune cells themselves express C1q or the expression is related to tumor associated complement activation remains unclear and could not be uncovered by our study.

As has been reported in LADC [248], we observed a correlation of C4d and disease stage in our cohort. Of note, tumor tissue-specific expression of C4d was shown to additionally correlate with stage and tumor size in oral squamous cell carcinoma and astrocytomas [249, 254]. However, the correlation between tumor load and circulating C4d has not been studied. Accordingly, our study is the first to show a correlation between circulating C4d levels and tumor volume assessed by CT-scans. Our results suggest that a more advanced disease as well as a higher local tumor load might lead to amplification of complement activation resulting in opsonization of malignant cells. We further hypothesize that not MPM tumor cells themselves but the local immune infiltrative cells might trigger the activation of the complement cascade. The increase in tumor load during disease progression might be related to a higher amount of immune cell infiltration resulting ultimately in higher circulating complement activation proteins.

In our study, we further investigated the specificity of circulating C4d as a marker for complement activation in MPM and consequently measured plasma C3a levels in corresponding patients. Similar to C4d, C3a is a complement-specific cleavage product and can be induced by all three complement activation pathways. Accordingly, it is a very unspecific marker for complement activation. In contrast to C4d, C3a levels were increased in MPM patients compared to HVs and there was a modest correlation between C3a and C4d only. C3a showed a prognostic significance, similar to C4d. These findings further support the presence of complement activation in certain MPM

patients and underline the clinical role of complement activation in MPM. It is important to note that while the investigation of the additional markers C3a and C1q contributes to establishing the specificity of C4d as a biomarker, these correlative studies cannot dissect the upstream events and thus the specific mechanisms of complement activation in MPM. Further studies, including preclinical in vitro and in vivo experiments, are needed to identify the specific complement activation pathways that contribute to the progression of MPM.

To date, no blood derived biomarker exists in order to differentiate between histological MPM subtypes [311]. In line with other studies on blood biomarkers, in our study, C4d levels tended to be modestly higher in non-epithelioid patients, with no significant difference [217, 303, 311, 312].

Here, importantly, we had the opportunity to correlate C4d with chemotherapeutic response to CHT. Remarkably, patients with progression after CHT treatment had significantly higher C4d levels when compared to those with treatment response. Thus, we concluded that investigating C4d levels may help re-staging and re-evaluation of patients following induction CHT before surgical intervention. This is of importance, since response evaluation and examining tumor expansion by RECIST criteria remains challenging in MPM [255, 313].

So far, our study is the first to report that circulating C4d levels directly correlate with primary tumor load, chemotherapeutic response and OS in MPM. Therefore, additionally to conventional response assessment, measuring C4d levels might aid re-staging in order to estimate individual treatment efficacy more comprehensively.

In the last part of this thesis, we demonstrate that circulating ActA can serve as an additional prognostic biomarker in MPM. Our group has recently shown that MPM cells overexpress ActA [292] and, moreover, that ActA exposure causes increased proliferation and migration whereas inhibition of activin receptors decreases MPM cell growth and survival. Thus, in MPM, similar to reports in NSCLC [294] and esophageal carcinoma [295, 296], ActA is associated with malignant behavior. Moreover, our group has recently shown that circulating ActA levels are elevated compared to healthy controls and correlated with disease stage and survival in LADC [297]. However, no data were available on the impact of circulating ActA levels in MPM.

As described above, in our study, circulating ActA levels were significantly increased in MPM patients when compared to HVs. Non-epithelioid cases were associated with high ActA levels when compared to epithelioid histology. As has been described for C4d, also tumor volume showed a positive correlation with increased circulating ActA levels. MPM patients with low ActA levels exhibited significantly improved OS, being exclusively prognostic in epithelioid cases.

As mentioned, no circulating biomarker has been reported to reliably distinguish between histological subtypes of MPM. Importantly, in our study, we found an association between ActA levels and non-epithelioid subtypes. Previously, as has been described above, also blood derived fibulin-3 showed no correlation with histological MPM subtypes [224]. However, effusion derived fibulin-3 was significantly increased in non-epithelioid MPM. Due to the relatively small number of non-epithelioid cases in this study, the potential diagnostic impact of ActA should be further evaluated in larger cohorts with increased numbers of biphasic and sarcomatoid cases.

In our cohort, we demonstrated a correlation of circulating ActA levels with increased age. Of note, accordingly, ActA was prognostic only in patients aged 66 years or younger. However, in an additional multivariate analysis, we found the interaction term between ActA and age to be significant.

Nevertheless, plasma ActA levels were highly prognostic in this large multi-center cohort analysis. By using the cox regression model, we also confirmed that low circulating ActA is an independent prognostic factor associated with better OS in MPM patients.

A previous study from our group identified fibrinogen as a novel prognostic biomarker in MPM [103]. Our correlation analysis revealed a significant relationship between ActA and fibrinogen. Moreover, fibrinogen and ActA levels demonstrated a similar prognostic power in predicting 1-year OS when ROC curve analysis was performed. Furthermore, in contrast to ActA, fibrinogen did not tend to be elevated in non-epithelioid cases in our previous study.

In contrast to several other investigated biomarkers (including C4d), the biological function of ActA has been well described and ActA was shown to exert protumorigenic effects in several cancer types including MPM. Currently, novel inhibitors of the activin

signaling cascade are being investigated for use in a variety of diseases [281]. Accordingly, it may represent a novel therapeutic target in MPM.
Hence, our findings suggest that the measurement of circulating ActA may support the histological classification of MPM and at the same time help to identify epithelioid MPM patients with poor prognosis.

In summary, this thesis deals with the emerging but yet unmet need for diagnostic, prognostic and predictive biomarkers in MPM. We could additionally demonstrate that an international cooperation is needed to establish larger tissue and data collections to study this rare disease in depth. Only a tight integration of translational research from bed to bench and vice versa will allow researchers to effectively investigate new compounds and guide therapy individually using reliable and reproducible biomarkers.

7 Conclusion

Considering the results of this thesis, the following main conclusions can be drawn in order to answer the questions as formulated in the aims of the thesis.

1. Ki-67 index is a reproducible and easily available prognostic tissue derived biomarker in MPM. We could demonstrate that high Ki-67 expression is associated with significantly worse prognosis in a large international tissue collection and this result was additionally reproduced in an independent validation cohort. Importantly, Ki-67 was exclusively prognostic for OS in epithelioid MPM. Moreover, we showed that chemotherapy significantly decreased tumor proliferation (as measured by Ki-67 expression) in MPM and thus Ki-67 might be used as marker to monitor response to chemotherapy.

2. Complement activation might play a role in the formation and progression of MPM. Here we report for the first time that C4d, a marker for complement cascade activation, is significantly elevated in late stage MPM and patients with high tumor volume. Furthermore, high circulating C4d levels were significantly associated with lack of clinical response to chemotherapy and with decreased OS. Considering these results, we suggest that compliment activation plays an important role in MPM, partly influencing prognosis. We also conclude that assessing circulating C4d levels might help to select patients for surgery following induction chemotherapy.

3. The biological function and protumorigenic effect of ActA in MPM has been well described. In our study we demonstrate that circulating ActA is significantly elevated in MPM, especially in cases with non-epithelioid subtype. As in the case of C4d, high circulating ActA was associated with tumor volume and worse prognosis. Similar to Ki-67 index of MPM tissue, circulating ActA was exclusively prognostic in epithelioid cases. In summary, circulating ActA may support the histological classification of MPM and at the same time help to identify epithelioid MPM patients with poor prognosis.

8 Summary

Objective: Malignant pleural mesothelioma (MPM) is a rare but devastating disease. Despite many efforts in prevention, early detection, identification of prognostic parameters and new therapeutic approaches, outcome is extremely dismal. Accordingly, new markers are urgently needed to guide selection for new therapeutic compounds and to identify patients for more aggressive treatment approaches. The aim of these works was to study selected tissue and blood derived biomarkers with regard to their diagnostic and prognostic value in a large international cohort of MPM cases.

Methods: Due to our international cooperation, we could collect tissue, blood and clinical data from 5 specialized MPM centers (4 in Europe, 1 in Australia). The collected biological material also included tissue and blood samples from healthy volunteers and patients with non-malignant pleural diseases. We investigated the diagnostic and prognostic role of the following proteins: Ki-67, complement degradation product 4 (C4d) and Activin A (ActA).

Results: We found that circulating ActA is significantly elevated in MPM cases compared to healthy controls and in non-epithelioid versus epithelioid tumors. Tissue expression of Ki-67 and circulating C4d did not show such associations. Importantly, expression of Ki67 was significantly lower in patients after chemotherapy. We found no C4d expression by tumor cells, but within intratumoral ectopic lymphoid structures. However, circulating C4d was significantly elevated in late stage MPM and in patients with high tumor volume. Furthermore, C4d was significantly associated with lack of clinical response to chemotherapy. Elevated ActA was additionally associated with high tumor volume. All three investigated biomarkers significantly influenced overall survival, independently from clinical variables such as age, gender, stage and histological subtype. However, Ki-67 and ActA revealed to be prognostic exclusively in patients with epithelioid subtype.

Conclusion: These analyses of large international MPM patient cohorts demonstrate that 1.\ Ki-67 index is an independent and reproducible prognostic factor in epithelioid MPM 2.\ induction chemotherapy decreases the proliferative capacity of MPM. 3.\ circulating plasma C4d is a promising new prognostic biomarker in MPM and it may help to select patients for surgery following induction chemotherapy and 4.\ ActA may support the histological classification of MPM and at the same time can help to identify epithelioid MPM patients with poor prognosis.

9 Összefoglalás

Célkitűzés: A malignus pluerális mesothelioma (MPM) egy ritka, de rendkívül rossz prognózisú betegség. Annak ellenére, hogy igen nagy erőfeszítéseket tesznek a prevenció, a korai felismerés, a prognosztikai faktorok felfedezése és új kezelési módok kifejlesztésének területén, a túlélési adatok lesújtóak. Éppen ezért sürgős szükség van új markerek felfedezésére, amelyek segíthetnek célzott hatóanyagok előállításában és azon betegek kiválasztásában, akiknél agresszívabb kezelési mód szükséges. Kutatásaink célja az volt, hogy megvizsgáljuk bizonyos szöveti és keringő biomarkerek diagnosztikus és prognosztikus értékét egy nagy, nemzetközi MPM kohortban.

Módszerek: Nemzetközi együttműködésünknek köszönhetően 5 specializált MPM centrumból (4 európai, 1 ausztrál) tudtunk szövet- és vérmintákat, valamint klinikai adatokat gyűjteni. Ezek részét képezték egészséges önkéntesekből és nem-malignus pleurális betegségben szenvedő páciensekből gyűjtött szövet- és vérminták is. A következő fehérjék diagnosztikus és prognosztikus szerepét vizsgáltuk: Ki-67, complement degradációs termék 4 (C4d) és Activin A (ActA).

Eredmények: Vizsgálatainkban megfigyeltük, hogy a keringő ActA szignifikánsan emelkedettebb MPM esetekben, mint egészséges kontrol személyekben, valamint, hogy az ActA szint magasabb nem-epitheloid tumoros esetekben (vs. epitheloid MPM). A Ki-67 szöveti expressziója és a keringő C4d nem mutatott hasonló összefüggést. Fontos megfigyelésünk volt azonban, hogy a Ki67 index szignifikánsan alacsonyabbnak bizonyult olyan betegekben, akik már kemoterápiában részesültek. Szemben a tumorsejtekkel, C4d expresszió intratumorálisan csak ectopiás lymphaticus struktúrákban volt megfigyelhető. Ennek ellenére a keringő C4d szintje szignifikánsan magasabb volt előrehaladott MPM esetekben, akikben nagy tumor-térfogat volt jelen. Továbbá szignifikánsan összefüggés volt kimutatható a C4d szintje és a kemoterápiára mutatott rossz klinikai válasz között. A növekedett ActA szintén összefüggést mutatott a nagy tumormérettel. Mind a három megvizsgált biomarker befolyásolta a túlélést, a klinikai változóktól (életkor, nem, a betegség stádiuma és pontos szövettana) függetlenül. Azonban a Ki-67 és ActA kizárólag epitheoild szövettani szubtípusban bizonyult prognosztikus értékűnek.

Konklúziók: Ezek a nagy és nemzetközi kohorton végzett vizsgálatok bizonyítják, hogy 1. \ A Ki-67 index egy független és reprodukálható prognosztikus faktor epitheloid MPM-ben, 2. \ Az indukciós kemoterápia csökkenti a MPM proliferációs képességét, 3. \ a keringő plazma C4d egy ígéretes új biomarker MPM-ben mely segíthet azon betegek kiválasztásában, akiknél érdemes operációt végezni indukciós kemoterápiát követően, és 4. \ ActA támogathatja az MPM szövettani klasszifikációját és egyben segíthet a rossz prognózisú epitheloid MPM esetek identifikálásában.

10 Bibliography

[1] Torre LA, Bray F, Siegel RL, Ferlay J, Lortet-Tieulent J, Jemal A. (2015) Global cancer statistics, 2012. CA Cancer J Clin, 65: 87-108.

[2] Bray F, Møller B. (2006) Predicting the future burden of cancer. Nat Rev Cancer, 6: 63-74.

[3] de Martel C, Ferlay J, Franceschi S, Vignat J, Bray F, Forman D, Plummer M. (2012) Global burden of cancers attributable to infections in 2008: a review and synthetic analysis. Lancet Oncol, 13: 607-615.

[4] Robinson BW, Lake RA. (2005) Advances in malignant mesothelioma. N Engl J Med, 353: 1591-1603.

[5] Bianchi C, Bianchi T. (2014) Global mesothelioma epidemic: Trend and features. Indian J Occup Environ Med, 18: 82-88.

[6] Novello S, Pinto C, Torri V, Porcu L, Di Maio M, Tiseo M, Ceresoli G, Magnani C, Silvestri S, Veltri A, Papotti M, Rossi G, Ricardi U, Trodella L, Rea F, Facciolo F, Granieri A, Zagonel V, Scagliotti G. (2016) The Third Italian Consensus Conference for Malignant Pleural Mesothelioma: State of the art and recommendations. Crit Rev Oncol Hematol, 104: 9-20.

[7] Henley SJ, Larson TC, Wu M, Antao VC, Lewis M, Pinheiro GA, Eheman C. (2013) Mesothelioma incidence in 50 states and the District of Columbia, United States, 2003-2008. Int J Occup Environ Health, 19: 1-10.

[8] Klikovits T, Hoda MA, Dong Y, Arns M, Baumgartner B, Errhalt P, Geltner C, Machan B, Pohl W, Hutter J, Eckmayr J, Studnicka M, Flicker M, Cerkl P, Kirchbacher K, Klepetko W. (2016) Management of malignant pleural mesothelioma - part 3 : Data from the Austrian Mesothelioma Interest Group (AMIG) database. Wien Klin Wochenschr, 128: 627-634.

[9] Lehnert M, Kraywinkel K, Heinze E, Wiethege T, Johnen G, Fiebig J, Brüning T, Taeger D. (2017) Incidence of malignant mesothelioma in Germany 2009-2013. Cancer Causes Control, 28: 97-105.

[10] Jennings CJ, Walsh PM, Deady S, Harvey BJ, Thomas W. (2014) Malignant pleural mesothelioma incidence and survival in the Republic of Ireland 1994-2009. Cancer Epidemiol, 38: 35-41.

[11] López-Abente G, García-Gómez M, Menéndez-Navarro A, Fernández-Navarro P, Ramis R, García-Pérez J, Cervantes M, Ferreras E, Jiménez-Muñoz M, Pastor-Barriuso R. (2013) Pleural cancer mortality in Spain: time-trends and updating of predictions up to 2020. BMC Cancer, 13: 528.

[12] Courtice MN, Lin S, Wang X. (2012) An updated review on asbestos and related diseases in China. Int J Occup Environ Health, 18: 247-253.

[13] Montanaro F, Bray F, Gennaro V, Merler E, Tyczynski JE, Parkin DM, Strnad M, Jechov'a M, Storm HH, Aareleid T, Hakulinen T, Velten M, Lef'evre H, Danzon A, Buemi A, Daur'es JP, Ménégoz F, Raverdy N, Sauvage M, Ziegler H, Comber H, Paci E, Vercelli M, De Lisi V, Tumino R, Zanetti R, Berrino F, Stanta G, Langmark F, Rachtan J, Mezyk R, Blaszczyk J, Ivan P, Primic-Zakelj M, Martínez AC, Izarzugaza I,

Borràs J, Garcia CM, Garau I, Sánchez NC, Aicua A, Barlow L, Torhorst J, Bouchardy C, Levi F, Fisch T, Probst N, Visser O, Quinn M, Gavin A, Brewster D, Mikov M, Group EW. (2003) Pleural mesothelioma incidence in Europe: evidence of some deceleration in the increasing trends. Cancer Causes Control, 14: 791-803.

[14] Taioli E, Wolf AS, Camacho-Rivera M, Flores RM. (2014) Women with malignant pleural mesothelioma have a threefold better survival rate than men. Ann Thorac Surg, 98: 1020-1024.

[15] Liu B, van Gerwen M, Bonassi S, Taioli E, Force IAftSoLCMT. (2017) Epidemiology of Environmental Exposure and Malignant Mesothelioma. J Thorac Oncol, 12: 1031-1045.

[16] Luo S, Liu X, Mu S, Tsai SP, Wen CP. (2003) Asbestos related diseases from environmental exposure to crocidolite in Da-yao, China. I. Review of exposure and epidemiological data. Occup Environ Med, 60: 35-41.

[17] Joshi TK, Bhuva UB, Katoch P. (2006) Asbestos ban in India: challenges ahead. Ann N Y Acad Sci, 1076: 292-308.

[18] Joshi TK, Gupta RK. (2003) Asbestos-related morbidity in India. Int J Occup Environ Health, 9: 249-253.

[19] McDonald JC. (2010) Epidemiology of malignant mesothelioma--an outline. Ann Occup Hyg, 54: 851-857.

[20] McDonald JC, McDonald AD. (1996) The epidemiology of mesothelioma in historical context. Eur Respir J, 9: 1932-1942.

[21] Baumann F, Ambrosi JP, Carbone M. (2013) Asbestos is not just asbestos: an unrecognised health hazard. Lancet Oncol, 14: 576-578.

[22] Røe OD, Stella GM. (2015) Malignant pleural mesothelioma: history, controversy and future of a manmade epidemic. Eur Respir Rev, 24: 115-131.

[23] Stanton MF, Wrench C. (1972) Mechanisms of mesothelioma induction with asbestos and fibrous glass. J Natl Cancer Inst, 48: 797-821.

[24] Lippmann M. (1994) Deposition and retention of inhaled fibres: effects on incidence of lung cancer and mesothelioma. Occup Environ Med, 51: 793-798.

[25] Wagner JC, Berry G, Skidmore JW, Pooley FD. (1980) The comparative effects of three chrysotiles by injection and inhalation in rats. IARC Sci Publ: 363-372.

[26] Lanphear BP, Buncher CR. (1992) Latent period for malignant mesothelioma of occupational origin. J Occup Med, 34: 718-721.

[27] Wagner JC, Berry G, Skidmore JW, Timbrell V. (1974) The effects of the inhalation of asbestos in rats. Br J Cancer, 29: 252-269.

[28] Bourdès V, Boffetta P, Pisani P. (2000) Environmental exposure to asbestos and risk of pleural mesothelioma: review and meta-analysis. Eur J Epidemiol, 16: 411-417.

[29] Maule MM, Magnani C, Dalmasso P, Mirabelli D, Merletti F, Biggeri A. (2007) Modeling mesothelioma risk associated with environmental asbestos exposure. Environ Health Perspect, 115: 1066-1071.

[30] Ferrante D, Bertolotti M, Todesco A, Mirabelli D, Terracini B, Magnani C. (2007) Cancer mortality and incidence of mesothelioma in a cohort of wives of asbestos workers in Casale Monferrato, Italy. Environ Health Perspect, 115: 1401-1405.

[31] Tarrés J, Albertí C, Martínez-Artés X, Abós-Herràndiz R, Rosell-Murphy M, García-Allas I, Krier I, Cantarell G, Gallego M, Canela-Soler J, Orriols R. (2013) Pleural mesothelioma in relation to meteorological conditions and residential distance from an industrial source of asbestos. Occup Environ Med, 70: 588-590.

[32] McDonald JC. (1985) Health implications of environmental exposure to asbestos. Environ Health Perspect, 62: 319-328.

[33] Roushdy-Hammady I, Siegel J, Emri S, Testa JR, Carbone M. (2001) Genetic-susceptibility factor and malignant mesothelioma in the Cappadocian region of Turkey. Lancet, 357: 444-445.

[34] Metintas M, Hillerdal G, Metintas S, Dumortier P. (2010) Endemic malignant mesothelioma: exposure to erionite is more important than genetic factors. Arch Environ Occup Health, 65: 86-93.

[35] Metintas S, Metintas M, Ucgun I, Oner U. (2002) Malignant mesothelioma due to environmental exposure to asbestos: follow-up of a Turkish cohort living in a rural area. Chest, 122: 2224-2229.

[36] Sakellariou K, Malamou-Mitsi V, Haritou A, Koumpaniou C, Stachouli C, Dimoliatis ID, Constantopoulos SH. (1996) Malignant pleural mesothelioma from nonoccupational asbestos exposure in Metsovo (north-west Greece): slow end of an epidemic? Eur Respir J, 9: 1206-1210.

[37] Taskinen E, Ahlamn K, Wükeri M. (1973) A current hypothesis of the lymphatic transport of inspired dust to the parietal pleura. Chest, 64: 193-196.

[38] Jean D, Daubriac J, Le Pimpec-Barthes F, Galateau-Salle F, Jaurand MC. (2012) Molecular changes in mesothelioma with an impact on prognosis and treatment. Arch Pathol Lab Med, 136: 277-293.

[39] Christensen BC, Houseman EA, Godleski JJ, Marsit CJ, Longacker JL, Roelofs CR, Karagas MR, Wrensch MR, Yeh RF, Nelson HH, Wiemels JL, Zheng S, Wiencke JK, Bueno R, Sugarbaker DJ, Kelsey KT. (2009) Epigenetic profiles distinguish pleural mesothelioma from normal pleura and predict lung asbestos burden and clinical outcome. Cancer Res, 69: 227-234.

[40] Røe OD, Anderssen E, Sandeck H, Christensen T, Larsson E, Lundgren S. (2010) Malignant pleural mesothelioma: genome-wide expression patterns reflecting general resistance mechanisms and a proposal of novel targets. Lung Cancer, 67: 57-68.

[41] Hamilton RF, Thakur SA, Holian A. (2008) Silica binding and toxicity in alveolar macrophages. Free Radic Biol Med, 44: 1246-1258.

[42] Mossman BT, Shukla A, Heintz NH, Verschraegen CF, Thomas A, Hassan R. (2013) New insights into understanding the mechanisms, pathogenesis, and management of malignant mesotheliomas. Am J Pathol, 182: 1065-1077.

[43] Liu G, Beri R, Mueller A, Kamp DW. (2010) Molecular mechanisms of asbestos-induced lung epithelial cell apoptosis. Chem Biol Interact, 188: 309-318.

[44] Herrick SE, Mutsaers SE. (2004) Mesothelial progenitor cells and their potential in tissue engineering. Int J Biochem Cell Biol, 36: 621-642.

[45] Kothmaier H, Quehenberger F, Halbwedl I, Morbini P, Demirag F, Zeren H, Comin CE, Murer B, Cagle PT, Attanoos R, Gibbs AR, Galateau-Salle F, Popper HH. (2008) EGFR and PDGFR differentially promote growth in malignant epithelioid mesothelioma of short and long term survivors. Thorax, 63: 345-351.

[46] Boutin C, Schlesser M, Frenay C, Astoul P. (1998) Malignant pleural mesothelioma. Eur Respir J, 12: 972-981.

[47] Nowak AK, Chansky K, Rice DC, Pass HI, Kindler HL, Shemanski L, Billé A, Rintoul RC, Batirel HF, Thomas CF, Friedberg J, Cedres S, de Perrot M, Rusch VW, Staging and Prognostic Factors Committee AvBaPI. (2016) The IASLC Mesothelioma Staging Project: Proposals for Revisions of the T Descriptors in the Forthcoming Eighth Edition of the TNM Classification for Pleural Mesothelioma. J Thorac Oncol, 11: 2089-2099.

[48] Kanbay A, Ozer Simsek Z, Tutar N, Yılmaz I, Buyukoglan H, Canoz O, Demir R. (2014) Non-asbestos-related malignant pleural mesothelioma. Intern Med, 53: 1977-1979.

[49] Kiss B, Bíró T, Czifra G, Tóth BI, Kertész Z, Szikszai Z, Kiss AZ, Juhász I, Zouboulis CC, Hunyadi J. (2008) Investigation of micronized titanium dioxide penetration in human skin xenografts and its effect on cellular functions of human skin-derived cells. Exp Dermatol, 17: 659-667.

[50] Schinwald A, Murphy FA, Prina-Mello A, Poland CA, Byrne F, Movia D, Glass JR, Dickerson JC, Schultz DA, Jeffree CE, Macnee W, Donaldson K. (2012) The threshold length for fiber-induced acute pleural inflammation: shedding light on the early events in asbestos-induced mesothelioma. Toxicol Sci, 128: 461-470.

[51] Donaldson K, Murphy FA, Duffin R, Poland CA. (2010) Asbestos, carbon nanotubes and the pleural mesothelium: a review of the hypothesis regarding the role of long fibre retention in the parietal pleura, inflammation and mesothelioma. Part Fibre Toxicol, 7: 5.

[52] Gurr JR, Wang AS, Chen CH, Jan KY. (2005) Ultrafine titanium dioxide particles in the absence of photoactivation can induce oxidative damage to human bronchial epithelial cells. Toxicology, 213: 66-73.

[53] Hattori K, Nakadate K, Morii A, Noguchi T, Ogasawara Y, Ishii K. (2017) Exposure to nano-size titanium dioxide causes oxidative damages in human mesothelial cells: The crystal form rather than size of particle contributes to cytotoxicity. Biochem Biophys Res Commun, 492: 218-223.

[54] Goodman JE, Nascarella MA, Valberg PA. (2009) Ionizing radiation: a risk factor for mesothelioma. Cancer Causes Control, 20: 1237-1254.

[55] Travis LB, Fosså SD, Schonfeld SJ, McMaster ML, Lynch CF, Storm H, Hall P, Holowaty E, Andersen A, Pukkala E, Andersson M, Kaijser M, Gospodarowicz M, Joensuu T, Cohen RJ, Boice JD, Dores GM, Gilbert ES. (2005) Second cancers among 40,576 testicular cancer patients: focus on long-term survivors. J Natl Cancer Inst, 97: 1354-1365.

[56] Hodgson DC, Gilbert ES, Dores GM, Schonfeld SJ, Lynch CF, Storm H, Hall P, Langmark F, Pukkala E, Andersson M, Kaijser M, Joensuu H, Fosså SD, Travis LB. (2007) Long-term solid cancer risk among 5-year survivors of Hodgkin's lymphoma. J Clin Oncol, 25: 1489-1497.

[57] Carbone M, Stach R, Di Resta I, Pass HI, Rizzo P. (1998) Simian virus 40 oncogenesis in hamsters. Dev Biol Stand, 94: 273-279.

[58] Poulin DL, DeCaprio JA. (2006) Is there a role for SV40 in human cancer? J Clin Oncol, 24: 4356-4365.

[59] Vilchez RA, Kozinetz CA, Arrington AS, Madden CR, Butel JS. (2003) Simian virus 40 in human cancers. Am J Med, 114: 675-684.

[60] Kroczynska B, Cutrone R, Bocchetta M, Yang H, Elmishad AG, Vacek P, Ramos-Nino M, Mossman BT, Pass HI, Carbone M. (2006) Crocidolite asbestos and SV40 are cocarcinogens in human mesothelial cells and in causing mesothelioma in hamsters. Proc Natl Acad Sci U S A, 103: 14128-14133.

[61] Bott M, Brevet M, Taylor BS, Shimizu S, Ito T, Wang L, Creaney J, Lake RA, Zakowski MF, Reva B, Sander C, Delsite R, Powell S, Zhou Q, Shen R, Olshen A, Rusch V, Ladanyi M. (2011) The nuclear deubiquitinase BAP1 is commonly inactivated by somatic mutations and 3p21.1 losses in malignant pleural mesothelioma. Nat Genet, 43: 668-672.

[62] Murthy SS, Testa JR. (1999) Asbestos, chromosomal deletions, and tumor suppressor gene alterations in human malignant mesothelioma. J Cell Physiol, 180: 150-157.

[63] Kato S, Tomson BN, Buys TP, Elkin SK, Carter JL, Kurzrock R. (2016) Genomic Landscape of Malignant Mesotheliomas. Mol Cancer Ther, 15: 2498-2507.

[64] Bueno R, De Rienzo A, Dong L, Gordon GJ, Hercus CF, Richards WG, Jensen RV, Anwar A, Maulik G, Chirieac LR, Ho KF, Taillon BE, Turcotte CL, Hercus RG, Gullans SR, Sugarbaker DJ. (2010) Second generation sequencing of the mesothelioma tumor genome. PLoS One, 5: e10612.

[65] van Meerbeeck JP, Scherpereel A, Surmont VF, Baas P. (2011) Malignant pleural mesothelioma: the standard of care and challenges for future management. Crit Rev Oncol Hematol, 78: 92-111.

[66] Bueno R, Stawiski EW, Goldstein LD, Durinck S, De Rienzo A, Modrusan Z, Gnad F, Nguyen TT, Jaiswal BS, Chirieac LR, Sciaranghella D, Dao N, Gustafson CE, Munir KJ, Hackney JA, Chaudhuri A, Gupta R, Guillory J, Toy K, Ha C, Chen YJ, Stinson J, Chaudhuri S, Zhang N, Wu TD, Sugarbaker DJ, de Sauvage FJ, Richards WG, Seshagiri S. (2016) Comprehensive genomic analysis of malignant pleural mesothelioma identifies recurrent mutations, gene fusions and splicing alterations. Nat Genet, 48: 407-416.

[67] Hylebos M, Van Camp G, van Meerbeeck JP, Op de Beeck K. (2016) The Genetic Landscape of Malignant Pleural Mesothelioma: Results from Massively Parallel Sequencing. J Thorac Oncol, 11: 1615-1626.

[68] Carbone M, Yang H, Pass HI, Krausz T, Testa JR, Gaudino G. (2013) BAP1 and cancer. Nat Rev Cancer, 13: 153-159.

[69] Testa JR, Cheung M, Pei J, Below JE, Tan Y, Sementino E, Cox NJ, Dogan AU, Pass HI, Trusa S, Hesdorffer M, Nasu M, Powers A, Rivera Z, Comertpay S, Tanji M, Gaudino G, Yang H, Carbone M. (2011) Germline BAP1 mutations predispose to malignant mesothelioma. Nat Genet, 43: 1022-1025.

[70] Travis WD, Brambilla E, Muller-Hermelink HK, Harris CC. World Health Organization Classification of Tumours. Pathology and Genetics of Tumours of the Lung, Pleura, Thymus and Heart. IARC Press: Lyon 2004.

[71] Galateau-Salle F, Churg A, Roggli V, Travis WD, Pleura WHOCfTot. (2016) The 2015 World Health Organization Classification of Tumors of the Pleura: Advances since the 2004 Classification. J Thorac Oncol, 11: 142-154.

[72] Travis WD, Brambilla E, Burke AP, Marx A, Nicholson AG. (2015) Introduction to The 2015 World Health Organization Classification of Tumors of the Lung, Pleura, Thymus, and Heart. J Thorac Oncol, 10: 1240-1242.

[73] Geltner C, Errhalt P, Baumgartner B, Ambrosch G, Machan B, Eckmayr J, Klikovits T, Hoda MA, Popper H, Klepetko W, (AMIG) AMIG. (2016) Management of malignant pleural mesothelioma - part 1: epidemiology, diagnosis, and staging : Consensus of the Austrian Mesothelioma Interest Group (AMIG). Wien Klin Wochenschr, 128: 611-617.

[74] Brčić L, Jakopović M, Brčić I, Klarić V, Milošević M, Sepac A, Samaržija M, Seiwerth S. (2014) Reproducibility of histological subtyping of malignant pleural mesothelioma. Virchows Arch, 465: 679-685.

[75] Kadota K, Suzuki K, Sima CS, Rusch VW, Adusumilli PS, Travis WD. (2011) Pleomorphic epithelioid diffuse malignant pleural mesothelioma: a clinicopathological review and conceptual proposal to reclassify as biphasic or sarcomatoid mesothelioma. J Thorac Oncol, 6: 896-904.

[76] Goldberg M, Imbernon E, Rolland P, Gilg Soit Ilg A, Savès M, de Quillacq A, Frenay C, Chamming's S, Arveux P, Boutin C, Launoy G, Pairon JC, Astoul P, Galateau-Sallé F, Brochard P. (2006) The French National Mesothelioma Surveillance Program. Occup Environ Med, 63: 390-395.

[77] Henderson DW, Reid G, Kao SC, van Zandwijk N, Klebe S. (2013) Challenges and controversies in the diagnosis of mesothelioma: Part 1. Cytology-only diagnosis, biopsies, immunohistochemistry, discrimination between mesothelioma and reactive mesothelial hyperplasia, and biomarkers. J Clin Pathol, 66: 847-853.

[78] Husain AN, Colby T, Ordonez N, Krausz T, Attanoos R, Beasley MB, Borczuk AC, Butnor K, Cagle PT, Chirieac LR, Churg A, Dacic S, Fraire A, Galateau-Salle F, Gibbs A, Gown A, Hammar S, Litzky L, Marchevsky AM, Nicholson AG, Roggli V, Travis WD, Wick M, Group IMI. (2013) Guidelines for pathologic diagnosis of malignant mesothelioma: 2012 update of the consensus statement from the International Mesothelioma Interest Group. Arch Pathol Lab Med, 137: 647-667.

[79] Churg A, Sheffield BS, Galateau-Salle F. (2016) New Markers for Separating Benign From Malignant Mesothelial Proliferations: Are We There Yet? Arch Pathol Lab Med, 140: 318-321.

[80] Churg A, Galateau-Salle F. (2012) The separation of benign and malignant mesothelial proliferations. Arch Pathol Lab Med, 136: 1217-1226.

[81] Scherpereel A, Astoul P, Baas P, Berghmans T, Clayson H, de Vuyst P, Dienemann H, Galateau-Salle F, Hennequin C, Hillerdal G, Le Péchoux C, Mutti L, Pairon JC, Stahel R, van Houtte P, van Meerbeeck J, Waller D, Weder W, Force ERSESoTST. (2010) Guidelines of the European Respiratory Society and the European Society of Thoracic Surgeons for the management of malignant pleural mesothelioma. Eur Respir J, 35: 479-495.

[82] Park EK, Yates DH, Creaney J, Thomas PS, Robinson BW, Johnson AR. (2012) Association of biomarker levels with severity of asbestos-related diseases. Saf Health Work, 3: 17-21.

[83] Park EK, Thomas PS, Creaney J, Johnson AR, Robinson BW, Yates DH. (2009) Blood-based early detection of malignant mesothelioma. J Clin Oncol, 27: 160.

[84] Pei D, Li Y, Liu X, Yan S, Guo X, Xu X. (2017) Diagnostic and prognostic utilities of humoral fibulin-3 in malignant pleural mesothelioma: Evidence from a meta-analysis. Oncotarget, 8: 13030-13038.

[85] Ettinger DS, Wood DE, Akerley W, Bazhenova LA, Borghaei H, Camidge DR, Cheney RT, Chirieac LR, D'Amico TA, Dilling T, Dobelbower M, Govindan R, Hennon M, Horn L, Jahan TM, Komaki R, Lackner RP, Lanuti M, Lilenbaum R, Lin J, Loo BW, Martins R, Otterson GA, Patel JD, Pisters KM, Reckamp K, Riely GJ, Schild SE, Shapiro TA, Sharma N, Swanson SJ, Stevenson J, Tauer K, Yang SC, Gregory K, Hughes M. (2017) NCCN Guidelines: Malignant Pleural Mesothelioma, Version 1.2017. J Natl Compr Canc Netw.

[86] Baas P, Fennell D, Kerr KM, Van Schil PE, Haas RL, Peters S, Committee EG. (2015) Malignant pleural mesothelioma: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. Ann Oncol, 26 Suppl 5: v31-39.

[87] Finn RS, Brims FJH, Gandhi A, Olsen N, Musk AW, Maskell NA, Lee YCG. (2012) Postmortem findings of malignant pleural mesothelioma: a two-center study of 318 patients. Chest, 142: 1267-1273.

[88] Greillier L, Cavailles A, Fraticelli A, Scherpereel A, Barlesi F, Tassi G, Thomas P, Astoul P. (2007) Accuracy of pleural biopsy using thoracoscopy for the diagnosis of histologic subtype in patients with malignant pleural mesothelioma. Cancer, 110: 2248-2252.

[89] Paintal A, Raparia K, Zakowski MF, Nayar R. (2013) The diagnosis of malignant mesothelioma in effusion cytology: a reappraisal and results of a multi-institution survey. Cancer Cytopathol, 121: 703-707.

[90] Ahmadzadehfar H, Palmedo H, Strunk H, Biersack HJ, Habibi E, Ezziddin S. (2007) False positive 18F-FDG-PET/CT in a patient after talc pleurodesis. Lung Cancer, 58: 418-421.

[91] Rice DC, Steliga MA, Stewart J, Eapen G, Jimenez CA, Lee JH, Hofstetter WL, Marom EM, Mehran RJ, Vaporciyan AA, Walsh GL, Swisher SG. (2009) Endoscopic ultrasound-guided fine needle aspiration for staging of malignant pleural mesothelioma. Ann Thorac Surg, 88: 862-868; discussion 868-869.

[92] Pilling JE, Stewart DJ, Martin-Ucar AE, Muller S, O'Byrne KJ, Waller DA. (2004) The case for routine cervical mediastinoscopy prior to radical surgery for malignant pleural mesothelioma. Eur J Cardiothorac Surg, 25: 497-501.

[93] Sugarbaker DJ, Strauss GM, Lynch TJ, Richards W, Mentzer SJ, Lee TH, Corson JM, Antman KH. (1993) Node status has prognostic significance in the multimodality therapy of diffuse, malignant mesothelioma. J Clin Oncol, 11: 1172-1178.

[94] Butchart EG, Gibbs AR. (1990) Pleural mesothelioma. Curr Opin Oncol, 2: 352-358.

[95] Rusch VW. (1995) A proposed new international TNM staging system for malignant pleural mesothelioma. From the International Mesothelioma Interest Group. Chest, 108: 1122-1128.

[96] Tammilehto L, Kivisaari L, Salminen US, Maasilta P, Mattson K. (1995) Evaluation of the clinical TNM staging system for malignant pleural mesothelioma: an assessment in 88 patients. Lung Cancer, 12: 25-34.

[97] Rusch VW, Chansky K, Kindler HL, Nowak AK, Pass HI, Rice DC, Shemanski L, Galateau-Sallé F, McCaughan BC, Nakano T, Ruffini E, van Meerbeeck JP, Yoshimura M, IASLC Staging and Prognostic Factors Committee ab, and participating institutions. (2016) The IASLC Mesothelioma Staging Project: Proposals for the M Descriptors and for Revision of the TNM Stage Groupings in the Forthcoming (Eighth) Edition of the TNM Classification for Mesothelioma. J Thorac Oncol, 11: 2112-2119.

[98] Rice D, Chansky K, Nowak A, Pass H, Kindler H, Shemanski L, Opitz I, Call S, Hasegawa S, Kernstine K, Atinkaya C, Rea F, Nafteux P, Rusch VW, Mesothelioma Domain of the IASLC Staging and Prognostic Factors Committee abapi. (2016) The IASLC Mesothelioma Staging Project: Proposals for Revisions of the N Descriptors in the Forthcoming Eighth Edition of the TNM Classification for Pleural Mesothelioma. J Thorac Oncol, 11: 2100-2111.

[99] Spirtas R, Connelly RR, Tucker MA. (1988) Survival patterns for malignant mesothelioma: the SEER experience. Int J Cancer, 41: 525-530.

[100] Rusch VW, Giroux D, Kennedy C, Ruffini E, Cangir AK, Rice D, Pass H, Asamura H, Waller D, Edwards J, Weder W, Hoffmann H, van Meerbeeck JP, Committee IS. (2012) Initial analysis of the international association for the study of lung cancer mesothelioma database. J Thorac Oncol, 7: 1631-1639.

[101] Herndon JE, Green MR, Chahinian AP, Corson JM, Suzuki Y, Vogelzang NJ. (1998) Factors predictive of survival among 337 patients with mesothelioma treated between 1984 and 1994 by the Cancer and Leukemia Group B. Chest, 113: 723-731.

[102] Ghanim B, Hoda MA, Winter MP, Klikovits T, Alimohammadi A, Hegedus B, Dome B, Grusch M, Arns M, Schenk P, Pohl W, Zielinski C, Filipits M, Klepetko W, Berger W. (2012) Pretreatment serum C-reactive protein levels predict benefit from multimodality treatment including radical surgery in malignant pleural mesothelioma: a retrospective multicenter analysis. Ann Surg, 256: 357-362.

[103] Ghanim B, Hoda MA, Klikovits T, Winter MP, Alimohammadi A, Grusch M, Dome B, Arns M, Schenk P, Jakopovic M, Samarzija M, Brcic L, Filipits M, Laszlo V,

Klepetko W, Berger W, Hegedus B. (2014) Circulating fibrinogen is a prognostic and predictive biomarker in malignant pleural mesothelioma. Br J Cancer, 110: 984-990.

[104] Krug LM, Pass HI, Rusch VW, Kindler HL, Sugarbaker DJ, Rosenzweig KE, Flores R, Friedberg JS, Pisters K, Monberg M, Obasaju CK, Vogelzang NJ. (2009) Multicenter phase II trial of neoadjuvant pemetrexed plus cisplatin followed by extrapleural pneumonectomy and radiation for malignant pleural mesothelioma. J Clin Oncol, 27: 3007-3013.

[105] Lauk O, Hoda MA, de Perrot M, Friess M, Klikovits T, Klepetko W, Keshavjee S, Weder W, Opitz I. (2014) Extrapleural Pneumonectomy After Induction Chemotherapy: Perioperative Outcome in 251 Mesothelioma Patients From Three High-Volume Institutions. Ann Thorac Surg, 98: 1748-1754

[106] Baldini EH. (2009) Radiation therapy options for malignant pleural mesothelioma. Semin Thorac Cardiovasc Surg, 21: 159-163.

[107] Berghmans T, Paesmans M, Lalami Y, Louviaux I, Luce S, Mascaux C, Meert AP, Sculier JP. (2002) Activity of chemotherapy and immunotherapy on malignant mesothelioma: a systematic review of the literature with meta-analysis. Lung Cancer, 38: 111-121.

[108] Vogelzang NJ, Rusthoven JJ, Symanowski J, Denham C, Kaukel E, Ruffie P, Gatzemeier U, Boyer M, Emri S, Manegold C, Niyikiza C, Paoletti P. (2003) Phase III study of pemetrexed in combination with cisplatin versus cisplatin alone in patients with malignant pleural mesothelioma. J Clin Oncol, 21: 2636-2644.

[109] Hoda MA, Klikovits T, Arns M, Dieckmann K, Zöchbauer-Müller S, Geltner C, Baumgartner B, Errhalt P, Machan B, Pohl W, Hutter J, Eckmayr J, Studnicka M, Flicker M, Cerkl P, Klepetko W. (2016) Management of malignant pleural mesothelioma-part 2: therapeutic approaches : Consensus of the Austrian Mesothelioma Interest Group (AMIG). Wien Klin Wochenschr, 128: 618-626.

[110] van Meerbeeck JP, Gaafar R, Manegold C, Van Klaveren RJ, Van Marck EA, Vincent M, Legrand C, Bottomley A, Debruyne C, Giaccone G, Group EOfRaToCLC, Canada NCIo. (2005) Randomized phase III study of cisplatin with or without raltitrexed in patients with malignant pleural mesothelioma: an intergroup study of the European Organisation for Research and Treatment of Cancer Lung Cancer Group and the National Cancer Institute of Canada. J Clin Oncol, 23: 6881-6889.

[111] Ohta Y, Shridhar V, Bright RK, Kalemkerian GP, Du W, Carbone M, Watanabe Y, Pass HI. (1999) VEGF and VEGF type C play an important role in angiogenesis and lymphangiogenesis in human malignant mesothelioma tumours. Br J Cancer, 81: 54-61.

[112] Strizzi L, Catalano A, Vianale G, Orecchia S, Casalini A, Tassi G, Puntoni R, Mutti L, Procopio A. (2001) Vascular endothelial growth factor is an autocrine growth factor in human malignant mesothelioma. J Pathol, 193: 468-475.

[113] Zalcman G, Mazieres J, Margery J, Greillier L, Audigier-Valette C, Moro-Sibilot D, Molinier O, Corre R, Monnet I, Gounant V, Rivière F, Janicot H, Gervais R, Locher C, Milleron B, Tran Q, Lebitasy MP, Morin F, Creveuil C, Parienti JJ, Scherpereel A, (IFCT) FCTI. (2016) Bevacizumab for newly diagnosed pleural mesothelioma in the Mesothelioma Avastin Cisplatin Pemetrexed Study (MAPS): a randomised, controlled, open-label, phase 3 trial. Lancet, 387: 1405-1414.

[114] Katirtzoglou N, Gkiozos I, Makrilia N, Tsaroucha E, Rapti A, Stratakos G, Fountzilas G, Syrigos KN. (2010) Carboplatin plus pemetrexed as first-line treatment of patients with malignant pleural mesothelioma: a phase II study. Clin Lung Cancer, 11: 30-35.

[115] Castagneto B, Botta M, Aitini E, Spigno F, Degiovanni D, Alabiso O, Serra M, Muzio A, Carbone R, Buosi R, Galbusera V, Piccolini E, Giaretto L, Rebella L, Mencoboni M. (2008) Phase II study of pemetrexed in combination with carboplatin in patients with malignant pleural mesothelioma (MPM). Ann Oncol, 19: 370-373.

[116] Ceresoli GL, Castagneto B, Zucali PA, Favaretto A, Mencoboni M, Grossi F, Cortinovis D, Del Conte G, Ceribelli A, Bearz A, Salamina S, De Vincenzo F, Cappuzzo F, Marangolo M, Torri V, Santoro A. (2008) Pemetrexed plus carboplatin in elderly patients with malignant pleural mesothelioma: combined analysis of two phase II trials. Br J Cancer, 99: 51-56.

[117] Santoro A, O'Brien ME, Stahel RA, Nackaerts K, Baas P, Karthaus M, Eberhardt W, Paz-Ares L, Sundstrom S, Liu Y, Ripoche V, Blatter J, Visseren-Grul CM, Manegold C. (2008) Pemetrexed plus cisplatin or pemetrexed plus carboplatin for chemonaïve patients with malignant pleural mesothelioma: results of the International Expanded Access Program. J Thorac Oncol, 3: 756-763.

[118] Arrieta O, López-Macías D, Mendoza-García VO, Bacon-Fonseca L, Muñoz-Montaño W, Macedo-Pérez EO, Muñiz-Hernández S, Blake-Cerda M, Corona-Cruz JF. (2014) A phase II trial of prolonged, continuous infusion of low-dose gemcitabine plus cisplatin in patients with advanced malignant pleural mesothelioma. Cancer Chemother Pharmacol, 73: 975-982.

[119] Scagliotti GV, Shin DM, Kindler HL, Vasconcelles MJ, Keppler U, Manegold C, Burris H, Gatzemeier U, Blatter J, Symanowski JT, Rusthoven JJ. (2003) Phase II study of pemetrexed with and without folic acid and vitamin B12 as front-line therapy in malignant pleural mesothelioma. J Clin Oncol, 21: 1556-1561.

[120] Muers MF, Stephens RJ, Fisher P, Darlison L, Higgs CM, Lowry E, Nicholson AG, O'Brien M, Peake M, Rudd R, Snee M, Steele J, Girling DJ, Nankivell M, Pugh C, Parmar MK, Group MTM. (2008) Active symptom control with or without chemotherapy in the treatment of patients with malignant pleural mesothelioma (MS01): a multicentre randomised trial. Lancet, 371: 1685-1694.

[121] Buikhuisen WA, Burgers JA, Vincent AD, Korse CM, van Klaveren RJ, Schramel FM, Pavlakis N, Nowak AK, Custers FL, Schouwink JH, Gans SJ, Groen HJ, Strankinga WF, Baas P. (2013) Thalidomide versus active supportive care for maintenance in patients with malignant mesothelioma after first-line chemotherapy (NVALT 5): an open-label, multicentre, randomised phase 3 study. Lancet Oncol, 14: 543-551.

[122] Zauderer MG, Kass SL, Woo K, Sima CS, Ginsberg MS, Krug LM. (2014) Vinorelbine and gemcitabine as second- or third-line therapy for malignant pleural mesothelioma. Lung Cancer, 84: 271-274.

[123] Jänne PA, Wozniak AJ, Belani CP, Keohan ML, Ross HJ, Polikoff JA, Mintzer DM, Ye Z, Monberg MJ, Obasaju CK, investigators Peap. (2006) Pemetrexed alone or

in combination with cisplatin in previously treated malignant pleural mesothelioma: outcomes from a phase IIIB expanded access program. J Thorac Oncol, 1: 506-512.

[124] Jassem J, Ramlau R, Santoro A, Schuette W, Chemaissani A, Hong S, Blatter J, Adachi S, Hanauske A, Manegold C. (2008) Phase III trial of pemetrexed plus best supportive care compared with best supportive care in previously treated patients with advanced malignant pleural mesothelioma. J Clin Oncol, 26: 1698-1704.

[125] Zucali PA, Simonelli M, Michetti G, Tiseo M, Ceresoli GL, Collovà E, Follador A, Lo Dico M, Moretti A, De Vincenzo F, Lorenzi E, Perrino M, Giordano L, Farina G, Santoro A, Garassino M. (2012) Second-line chemotherapy in malignant pleural mesothelioma: results of a retrospective multicenter survey. Lung Cancer, 75: 360-367.

[126] Kotova S, Wong RM, Cameron RB. (2015) New and emerging therapeutic options for malignant pleural mesothelioma: review of early clinical trials. Cancer Manag Res, 7: 51-63.

[127] Alley EW, Lopez J, Santoro A, Morosky A, Saraf S, Piperdi B, van Brummelen E. (2017) Clinical safety and activity of pembrolizumab in patients with malignant pleural mesothelioma (KEYNOTE-028): preliminary results from a non-randomised, open-label, phase 1b trial. Lancet Oncol, 18: 623-630.

[128] (2017) Dual Checkpoint Blockade Takes Aim at Relapsed Mesothelioma. Cancer Discov, 7: OF7.

[129] Maio M, Scherpereel A, Calabrò L, Aerts J, Perez SC, Bearz A, Nackaerts K, Fennell DA, Kowalski D, Tsao AS, Taylor P, Grosso F, Antonia SJ, Nowak AK, Taboada M, Puglisi M, Stockman PK, Kindler HL. (2017) Tremelimumab as second-line or third-line treatment in relapsed malignant mesothelioma (DETERMINE): a multicentre, international, randomised, double-blind, placebo-controlled phase 2b trial. Lancet Oncol, 18: 1261-1273.

[130] Grosso F, Steele N, Novello S, Nowak AK, Popat S, Greillier L, John T, Leighl NB, Reck M, Taylor P, Planchard D, Sørensen JB, Socinski MA, von Wangenheim U, Loembé AB, Barrueco J, Morsli N, Scagliotti G. (2017) Nintedanib Plus Pemetrexed/Cisplatin in Patients With Malignant Pleural Mesothelioma: Phase II Results From the Randomized, Placebo-Controlled LUME-Meso Trial. J Clin Oncol, 35: 3591-3600.

[131] Govindan R, Kratzke RA, Herndon JE, Niehans GA, Vollmer R, Watson D, Green MR, Kindler HL, 30101) CaLGBC. (2005) Gefitinib in patients with malignant mesothelioma: a phase II study by the Cancer and Leukemia Group B. Clin Cancer Res, 11: 2300-2304.

[132] Garland LL, Rankin C, Gandara DR, Rivkin SE, Scott KM, Nagle RB, Klein-Szanto AJ, Testa JR, Altomare DA, Borden EC. (2007) Phase II study of erlotinib in patients with malignant pleural mesothelioma: a Southwest Oncology Group Study. J Clin Oncol, 25: 2406-2413.

[133] Jackman DM, Kindler HL, Yeap BY, Fidias P, Salgia R, Lucca J, Morse LK, Ostler PA, Johnson BE, Jänne PA. (2008) Erlotinib plus bevacizumab in previously treated patients with malignant pleural mesothelioma. Cancer, 113: 808-814.

[134] Mathy A, Baas P, Dalesio O, van Zandwijk N. (2005) Limited efficacy of imatinib mesylate in malignant mesothelioma: a phase II trial. Lung Cancer, 50: 83-86.

[135] Rusch V, Baldini EH, Bueno R, De Perrot M, Flores R, Hasegawa S, Klepetko W, Krug L, Lang-Lazdunski L, Pass H, Weder W, Sugarbaker DJ, Congress piIMIG. (2013) The role of surgical cytoreduction in the treatment of malignant pleural mesothelioma: meeting summary of the International Mesothelioma Interest Group Congress, September 11-14, 2012, Boston, Mass. J Thorac Cardiovasc Surg, 145: 909-910.

[136] Rice D, Rusch V, Pass H, Asamura H, Nakano T, Edwards J, Giroux DJ, Hasegawa S, Kernstine KH, Waller D, Rami-Porta R, Group IAftSoLCISCatIMI. (2011) Recommendations for uniform definitions of surgical techniques for malignant pleural mesothelioma: a consensus report of the international association for the study of lung cancer international staging committee and the international mesothelioma interest group. J Thorac Oncol, 6: 1304-1312.

[137] Cao C, Tian DH, Pataky KA, Yan TD. (2013) Systematic review of pleurectomy in the treatment of malignant pleural mesothelioma. Lung Cancer, 81: 319-327.

[138] Rice D. (2012) Standardizing surgical treatment in malignant pleural mesothelioma. Ann Cardiothorac Surg, 1: 497-501.

[139] Sugarbaker DJ, Jaklitsch MT, Bueno R, Richards W, Lukanich J, Mentzer SJ, Colson Y, Linden P, Chang M, Capalbo L, Oldread E, Neragi-Miandoab S, Swanson SJ, Zellos LS. (2004) Prevention, early detection, and management of complications after 328 consecutive extrapleural pneumonectomies. J Thorac Cardiovasc Surg, 128: 138-146.

[140] Treasure T, Lang-Lazdunski L, Waller D, Bliss JM, Tan C, Entwisle J, Snee M, O'Brien M, Thomas G, Senan S, O'Byrne K, Kilburn LS, Spicer J, Landau D, Edwards J, Coombes G, Darlison L, Peto J, trialists M. (2011) Extra-pleural pneumonectomy versus no extra-pleural pneumonectomy for patients with malignant pleural mesothelioma: clinical outcomes of the Mesothelioma and Radical Surgery (MARS) randomised feasibility study. Lancet Oncol, 12: 763-772.

[141] Cao CQ, Yan TD, Bannon PG, McCaughan BC. (2010) A systematic review of extrapleural pneumonectomy for malignant pleural mesothelioma. J Thorac Oncol, 5: 1692-1703.

[142] Flores RM, Pass HI, Seshan VE, Dycoco J, Zakowski M, Carbone M, Bains MS, Rusch VW. (2008) Extrapleural pneumonectomy versus pleurectomy/decortication in the surgical management of malignant pleural mesothelioma: results in 663 patients. J Thorac Cardiovasc Surg, 135: 620-626.

[143] Cao C, Tian D, Park J, Allan J, Pataky KA, Yan TD. (2014) A systematic review and meta-analysis of surgical treatments for malignant pleural mesothelioma. Lung Cancer, 83: 240-245.

[144] Cao C, Yan TD, Bannon PG, McCaughan BC. (2011) Summary of prognostic factors and patient selection for extrapleural pneumonectomy in the treatment of malignant pleural mesothelioma. Ann Surg Oncol, 18: 2973-2979.

[145] Yao ZH, Tian GY, Yang SX, Wan YY, Kang YM, Liu QH, Yao F, Lin DJ. (2014) Serum albumin as a significant prognostic factor in patients with malignant pleural mesothelioma. Tumour Biol, 35: 6839-6845.

[146] Opitz I, Friess M, Kestenholz P, Schneiter D, Frauenfelder T, Nguyen-Kim TD, Seifert B, Hoda MA, Klepetko W, Stahel RA, Weder W. (2015) A New Prognostic Score Supporting Treatment Allocation for Multimodality Therapy for Malignant Pleural Mesothelioma: A Review of 12 Years' Experience. J Thorac Oncol, 10: 1634-1641.

[147] Hasan B, Greillier L, Pallis A, Menis J, Gaafar R, Sylvester R, Fennell DA, Baas P, Surmont V, Van Meerbeeck JP, O'brien ME. (2014) Progression free survival rate at 9 and 18 weeks predict overall survival in patients with malignant pleural mesothelioma: An individual patient pooled analysis of 10 European Organisation for Research and Treatment of Cancer Lung Cancer Group studies and an independent study validation. Eur J Cancer, 50: 2771-2781.

[148] Rena O, Casadio C. (2012) Extrapleural pneumonectomy for early stage malignant pleural mesothelioma: a harmful procedure. Lung Cancer, 77: 151-155.

[149] Sugarbaker DJ, Garcia JP. (1997) Multimodality therapy for malignant pleural mesothelioma. Chest, 112: 272-275.

[150] Weder W, Stahel RA, Bernhard J, Bodis S, Vogt P, Ballabeni P, Lardinois D, Betticher D, Schmid R, Stupp R, Ris HB, Jermann M, Mingrone W, Roth AD, Spiliopoulos A, Research SGfCC. (2007) Multicenter trial of neo-adjuvant chemotherapy followed by extrapleural pneumonectomy in malignant pleural mesothelioma. Ann Oncol, 18: 1196-1202.

[151] Cho BC, Feld R, Leighl N, Opitz I, Anraku M, Tsao MS, Hwang DM, Hope A, de Perrot M. (2014) A feasibility study evaluating Surgery for Mesothelioma After Radiation Therapy: the "SMART" approach for resectable malignant pleural mesothelioma. J Thorac Oncol, 9: 397-402.

[152] de Perrot M, Feld R, Leighl NB, Hope A, Waddell TK, Keshavjee S, Cho BC. (2016) Accelerated hemithoracic radiation followed by extrapleural pneumonectomy for malignant pleural mesothelioma. J Thorac Cardiovasc Surg, 151: 468-473.

[153] Friedberg JS. (2009) Photodynamic therapy as an innovative treatment for malignant pleural mesothelioma. Semin Thorac Cardiovasc Surg, 21: 177-187.

[154] Friedberg JS, Culligan MJ, Mick R, Stevenson J, Hahn SM, Sterman D, Punekar S, Glatstein E, Cengel K. (2012) Radical pleurectomy and intraoperative photodynamic therapy for malignant pleural mesothelioma. Ann Thorac Surg, 93: 1658-1665; discussion 1665-1657.

[155] Ried M, Potzger T, Braune N, Diez C, Neu R, Sziklavari Z, Schalke B, Hofmann HS. (2013) Local and systemic exposure of cisplatin during hyperthermic intrathoracic chemotherapy perfusion after pleurectomy and decortication for treatment of pleural malignancies. J Surg Oncol, 107: 735-740.

[156] Sugarbaker PH, Stuart OA, Eger C. (2012) Pharmacokinetics of Hyperthermic Intrathoracic Chemotherapy following Pleurectomy and Decortication. Gastroenterol Res Pract, 2012: 471205.

[157] Tilleman TR, Richards WG, Zellos L, Johnson BE, Jaklitsch MT, Mueller J, Yeap BY, Mujoomdar AA, Ducko CT, Bueno R, Sugarbaker DJ. (2009) Extrapleural pneumonectomy followed by intracavitary intraoperative hyperthermic cisplatin with pharmacologic cytoprotection for treatment of malignant pleural mesothelioma: a phase II prospective study. J Thorac Cardiovasc Surg, 138: 405-411.

[158] Richards WG, Zellos L, Bueno R, Jaklitsch MT, Jänne PA, Chirieac LR, Yeap BY, Dekkers RJ, Hartigan PM, Capalbo L, Sugarbaker DJ. (2006) Phase I to II study of pleurectomy/decortication and intraoperative intracavitary hyperthermic cisplatin lavage for mesothelioma. J Clin Oncol, 24: 1561-1567.

[159] Sugarbaker DJ, Gill RR, Yeap BY, Wolf AS, DaSilva MC, Baldini EH, Bueno R, Richards WG. (2013) Hyperthermic intraoperative pleural cisplatin chemotherapy extends interval to recurrence and survival among low-risk patients with malignant pleural mesothelioma undergoing surgical macroscopic complete resection. J Thorac Cardiovasc Surg, 145: 955-963.

[160] Bertoglio P, Ambrogi MC, Chella A, Aprile V, Dini P, Korasidis S, Fanucchi O, Mussi A. (2017) Is less also better? A single-institution experience on treatment of early stage Malignant Pleural Mesothelioma. Eur J Surg Oncol, 43: 1365-1371.

[161] Opitz I. (2014) Management of malignant pleural mesothelioma-The European experience. J Thorac Dis, 6 Suppl 2: 238-252.

[162] Committee BTSSoC. (2007) BTS statement on malignant mesothelioma in the UK, 2007. Thorax, 62 Suppl 2: ii1-ii19.

[163] Lee YC, Fysh ET. (2011) Indwelling pleural catheter: changing the paradigm of malignant effusion management. J Thorac Oncol, 6: 655-657.

[164] Suzuki K, Servais EL, Rizk NP, Solomon SB, Sima CS, Park BJ, Kachala SS, Zlobinsky M, Rusch VW, Adusumilli PS. (2011) Palliation and pleurodesis in malignant pleural effusion: the role for tunneled pleural catheters. J Thorac Oncol, 6: 762-767.

[165] Rintoul RC, Ritchie AJ, Edwards JG, Waller DA, Coonar AS, Bennett M, Lovato E, Hughes V, Fox-Rushby JA, Sharples LD, Collaborators obotM. (2014) Efficacy and cost of video-assisted thoracoscopic partial pleurectomy versus talc pleurodesis in patients with malignant pleural mesothelioma (MesoVATS): an open-label, randomised, controlled trial. Lancet, 384: 1118-1127.

[166] Husain AN, Colby TV, Ordóñez NG, Allen TC, Attanoos RL, Beasley MB, Butnor KJ, Chirieac LR, Churg AM, Dacic S, Galateau-Sallé F, Gibbs A, Gown AM, Krausz T, Litzky LA, Marchevsky A, Nicholson AG, Roggli VL, Sharma AK, Travis WD, Walts AE, Wick MR. (2017) Guidelines for Pathologic Diagnosis of Malignant Mesothelioma: 2017 Update of the Consensus Statement From the International Mesothelioma Interest Group. Arch Pathol Lab Med, 142: 89-108.

[167] Price A. (2011) What is the role of radiotherapy in malignant pleural mesothelioma? Oncologist, 16: 359-365.

[168] Van Schil PE, Baas P, Gaafar R, Maat AP, Van de Pol M, Hasan B, Klomp HM, Abdelrahman AM, Welch J, van Meerbeeck JP. (2010) Trimodality therapy for

malignant pleural mesothelioma: results from an EORTC phase II multicentre trial. Eur Respir J, 36: 1362-1369.

[169] Aigner C, Hoda MA, Lang G, Taghavi S, Marta G, Klepetko W. (2008) Outcome after extrapleural pneumonectomy for malignant pleural mesothelioma. Eur J Cardiothorac Surg, 34: 204-207.

[170] Stahel RA, Riesterer O, Xyrafas A, Opitz I, Beyeler M, Ochsenbein A, Früh M, Cathomas R, Nackaerts K, Peters S, Mamot C, Zippelius A, Mordasini C, Caspar CB, Eckhardt K, Schmid RA, Aebersold DM, Gautschi O, Nagel W, Töpfer M, Krayenbuehl J, Ribi K, Ciernik LF, Weder W. (2015) Neoadjuvant chemotherapy and extrapleural pneumonectomy of malignant pleural mesothelioma with or without hemithoracic radiotherapy (SAKK 17/04): a randomised, international, multicentre phase 2 trial. Lancet Oncol, 16: 1651-1658.

[171] Baas P. (2003) Predictive and prognostic factors in malignant pleural mesothelioma. Curr Opin Oncol, 15: 127-130.

[172] Davidson B. (2015) Prognostic factors in malignant pleural mesothelioma. Hum Pathol, 46: 789-804.

[173] Marcq E, Siozopoulou V, De Waele J, van Audenaerde J, Zwaenepoel K, Santermans E, Hens N, Pauwels P, van Meerbeeck JP, Smits EL. (2017) Prognostic and predictive aspects of the tumor immune microenvironment and immune checkpoints in malignant pleural mesothelioma. Oncoimmunology, 6: e1261241.

[174] Edwards JG, Swinson DE, Jones JL, Waller DA, O'Byrne KJ. (2006) EGFR expression: associations with outcome and clinicopathological variables in malignant pleural mesothelioma. Lung Cancer, 54: 399-407.

[175] Levallet G, Vaisse-Lesteven M, Le Stang N, Ilg AG, Brochard P, Astoul P, Pairon JC, Bergot E, Zalcman G, Galateau-Sallé F. (2012) Plasma cell membrane localization of c-MET predicts longer survival in patients with malignant mesothelioma: a series of 157 cases from the MESOPATH Group. J Thorac Oncol, 7: 599-606.

[176] Pinato DJ, Mauri FA, Lloyd T, Vaira V, Casadio C, Boldorini RL, Sharma R. (2013) The expression of Axl receptor tyrosine kinase influences the tumour phenotype and clinical outcome of patients with malignant pleural mesothelioma. Br J Cancer, 108: 621-628.

[177] Kumar-Singh S, Jacobs W, Dhaene K, Weyn B, Bogers J, Weyler J, Van Marck E. (1998) Syndecan-1 expression in malignant mesothelioma: correlation with cell differentiation, WT1 expression, and clinical outcome. J Pathol, 186: 300-305.

[178] Alifano M, Loi M, Camilleri-Broet S, Dupouy S, Régnard JF, Forgez P. (2010) Neurotensin expression and outcome of malignant pleural mesothelioma. Biochimie, 92: 164-170.

[179] Amatya VJ, Takeshima Y, Aoe K, Fujimoto N, Okamoto T, Yamada T, Kishimoto T, Morimoto C, Inai K. (2013) CD9 expression as a favorable prognostic marker for patients with malignant mesothelioma. Oncol Rep, 29: 21-28.

[180] Aoe K, Amatya VJ, Fujimoto N, Ohnuma K, Hosono O, Hiraki A, Fujii M, Yamada T, Dang NH, Takeshima Y, Inai K, Kishimoto T, Morimoto C. (2012) CD26

overexpression is associated with prolonged survival and enhanced chemosensitivity in malignant pleural mesothelioma. Clin Cancer Res, 18: 1447-1456.

[181] Kao SC, Armstrong N, Condon B, Griggs K, McCaughan B, Maltby S, Wilson A, Henderson DW, Klebe S. (2012) Aquaporin 1 is an independent prognostic factor in pleural malignant mesothelioma. Cancer, 118: 2952-2961.

[182] Otterstrom C, Soltermann A, Opitz I, Felley-Bosco E, Weder W, Stahel RA, Triponez F, Robert JH, Serre-Beinier V. (2014) CD74: a new prognostic factor for patients with malignant pleural mesothelioma. Br J Cancer, 110: 2040-2046.

[183] Chia PL, Russell PA, Scott AM, John T. (2016) Targeting the vasculature: anti-angiogenic agents for malignant mesothelioma. Expert Rev Anticancer Ther, 16: 1235-1245.

[184] Edwards JG, Cox G, Andi A, Jones JL, Walker RA, Waller DA, O'Byrne KJ. (2001) Angiogenesis is an independent prognostic factor in malignant mesothelioma. Br J Cancer, 85: 863-868.

[185] O'Kane SL, Pound RJ, Campbell A, Chaudhuri N, Lind MJ, Cawkwell L. (2006) Expression of bcl-2 family members in malignant pleural mesothelioma. Acta Oncol, 45: 449-453.

[186] Mineo TC, Ambrogi V, Cufari ME, Pompeo E. (2010) May cyclooxygenase-2 (COX-2), p21 and p27 expression affect prognosis and therapeutic strategy of patients with malignant pleural mesothelioma? Eur J Cardiothorac Surg, 38: 245-252.

[187] Bitanihirwe BK, Meerang M, Friess M, Soltermann A, Frischknecht L, Thies S, Felley-Bosco E, Tsao MS, Allo G, de Perrot M, Seifert B, Moch H, Stahel R, Weder W, Opitz I. (2014) PI3K/mTOR signaling in mesothelioma patients treated with induction chemotherapy followed by extrapleural pneumonectomy. J Thorac Oncol, 9: 239-247.

[188] Pinato DJ, Mauri FA, Ramakrishnan R, Wahab L, Lloyd T, Sharma R. (2012) Inflammation-based prognostic indices in malignant pleural mesothelioma. J Thorac Oncol, 7: 587-594.

[189] Curtin NJ. (2012) DNA repair dysregulation from cancer driver to therapeutic target. Nat Rev Cancer, 12: 801-817.

[190] Righi L, Papotti MG, Ceppi P, Billè A, Bacillo E, Molinaro L, Ruffini E, Scagliotti GV, Selvaggi G. (2010) Thymidylate synthase but not excision repair cross-complementation group 1 tumor expression predicts outcome in patients with malignant pleural mesothelioma treated with pemetrexed-based chemotherapy. J Clin Oncol, 28: 1534-1539.

[191] Ting S, Mairinger FD, Hager T, Welter S, Eberhardt WE, Wohlschlaeger J, Schmid KW, Christoph DC. (2013) ERCC1, MLH1, MSH2, MSH6, and βIII-tubulin: resistance proteins associated with response and outcome to platinum-based chemotherapy in malignant pleural mesothelioma. Clin Lung Cancer, 14: 558-567.

[192] Brown DC, Gatter KC. (1990) Monoclonal antibody Ki-67: its use in histopathology. Histopathology, 17: 489-503.

[193] Graziano F, Cascinu S. (2003) Prognostic molecular markers for planning adjuvant chemotherapy trials in Dukes' B colorectal cancer patients: how much evidence is enough? Ann Oncol, 14: 1026-1038.

[194] Ranchod M, Kempson RL. (1977) Smooth muscle tumors of the gastrointestinal tract and retroperitoneum: a pathologic analysis of 100 cases. Cancer, 39: 255-262.

[195] Scholzen T, Gerdes J. (2000) The Ki-67 protein: from the known and the unknown. J Cell Physiol, 182: 311-322.

[196] Gerdes J, Schwab U, Lemke H, Stein H. (1983) Production of a mouse monoclonal antibody reactive with a human nuclear antigen associated with cell proliferation. Int J Cancer, 31: 13-20.

[197] Bullwinkel J, Baron-Lühr B, Lüdemann A, Wohlenberg C, Gerdes J, Scholzen T. (2006) Ki-67 protein is associated with ribosomal RNA transcription in quiescent and proliferating cells. J Cell Physiol, 206: 624-635.

[198] Yerushalmi R, Woods R, Ravdin PM, Hayes MM, Gelmon KA. (2010) Ki67 in breast cancer: prognostic and predictive potential. Lancet Oncol, 11: 174-183.

[199] Scagliotti GV, Micela M, Gubetta L, Leonardo E, Cappia S, Borasio P, Pozzi E. (1993) Prognostic significance of Ki67 labelling in resected non small cell lung cancer. Eur J Cancer, 29A: 363-365.

[200] Baratti D, Kusamura S, Cabras AD, Bertulli R, Hutanu I, Deraco M. (2013) Diffuse malignant peritoneal mesothelioma: long-term survival with complete cytoreductive surgery followed by hyperthermic intraperitoneal chemotherapy (HIPEC). Eur J Cancer, 49: 3140-3148.

[201] Haruki T, Shomori K, Shiomi T, Taniguchi Y, Nakamura H, Ito H. (2012) Multiparameter analysis using cell cycle biomarkers for small-size lung adenocarcinoma: prognostic implications. Oncol Rep, 28: 915-922.

[202] Meert AP, Feoli F, Martin B, Verdebout JM, Mascaux C, Verhest A, Ninane V, Sculier JP. (2004) Ki67 expression in bronchial preneoplastic lesions and carcinoma in situ defined according to the new 1999 WHO/IASLC criteria: a preliminary study. Histopathology, 44: 47-53.

[203] Skov BG, Holm B, Erreboe A, Skov T, Mellemgaard A. (2010) ERCC1 and Ki67 in small cell lung carcinoma and other neuroendocrine tumors of the lung: distribution and impact on survival. J Thorac Oncol, 5: 453-459.

[204] Hasteh F, Lin GY, Weidner N, Michael CW. (2010) The use of immunohistochemistry to distinguish reactive mesothelial cells from malignant mesothelioma in cytologic effusions. Cancer Cytopathol, 118: 90-96.

[205] Comin CE, Anichini C, Boddi V, Novelli L, Dini S. (2000) MIB-1 proliferation index correlates with survival in pleural malignant mesothelioma. Histopathology, 36: 26-31.

[206] Bongiovanni M, Cassoni P, De Giuli P, Viberti L, Cappia S, Ivaldi C, Chiusa L, Bussolati G. (2001) p27(kip1) immunoreactivity correlates with long-term survival in pleural malignant mesothelioma. Cancer, 92: 1245-1250.

[207] Leonardo E, Zanconati F, Bonifacio D, Bonito LD. (2001) Immunohistochemical MIB-1 and p27kip1 as prognostic factors in pleural mesothelioma. Pathol Res Pract, 197: 253-256.

[208] Kadota K, Suzuki K, Colovos C, Sima CS, Rusch VW, Travis WD, Adusumilli PS. (2012) A nuclear grading system is a strong predictor of survival in epitheloid diffuse malignant pleural mesothelioma. Mod Pathol, 25: 260-271.

[209] Linton A, van Zandwijk N, Reid G, Clarke S, Cao C, Kao S. (2012) Inflammation in malignant mesothelioma - friend or foe? Ann Cardiothorac Surg, 1: 516-522.

[210] Campbell NP, Kindler HL. (2011) Update on malignant pleural mesothelioma. Semin Respir Crit Care Med, 32: 102-110.

[211] Pass HI, Giroux D, Kennedy C, Ruffini E, Cangir AK, Rice D, Asamura H, Waller D, Edwards J, Weder W, Hoffmann H, van Meerbeeck JP, Rusch VW, Institutions ISCaP. (2014) Supplementary prognostic variables for pleural mesothelioma: a report from the IASLC staging committee. J Thorac Oncol, 9: 856-864.

[212] Ceresoli GL, Zucali PA. (2012) Anti-angiogenic therapies for malignant pleural mesothelioma. Expert Opin Investig Drugs, 21: 833-844.

[213] Kao SC, Harvie R, Paturi F, Taylor R, Davey R, Abraham R, Clarke S, Marx G, Cullen M, Kerestes Z, Pavlakis N. (2012) The predictive role of serum VEGF in an advanced malignant mesothelioma patient cohort treated with thalidomide alone or combined with cisplatin/gemcitabine. Lung Cancer, 75: 248-254.

[214] Yasumitsu A, Tabata C, Tabata R, Hirayama N, Murakami A, Yamada S, Terada T, Iida S, Tamura K, Fukuoka K, Kuribayashi K, Nakano T. (2010) Clinical significance of serum vascular endothelial growth factor in malignant pleural mesothelioma. J Thorac Oncol, 5: 479-483.

[215] Hirayama N, Tabata C, Tabata R, Maeda R, Yasumitsu A, Yamada S, Kuribayashi K, Fukuoka K, Nakano T. (2011) Pleural effusion VEGF levels as a prognostic factor of malignant pleural mesothelioma. Respir Med, 105: 137-142.

[216] Robinson BW, Creaney J, Lake R, Nowak A, Musk AW, de Klerk N, Winzell P, Hellstrom KE, Hellstrom I. (2005) Soluble mesothelin-related protein--a blood test for mesothelioma. Lung Cancer, 49 Suppl 1: 109-111.

[217] Robinson BW, Creaney J, Lake R, Nowak A, Musk AW, de Klerk N, Winzell P, Hellstrom KE, Hellstrom I. (2003) Mesothelin-family proteins and diagnosis of mesothelioma. Lancet, 362: 1612-1616.

[218] Cristaudo A, Foddis R, Vivaldi A, Guglielmi G, Dipalma N, Filiberti R, Neri M, Ceppi M, Paganuzzi M, Ivaldi GP, Mencoboni M, Canessa PA, Ambrosino N, Chella A, Mutti L, Puntoni R. (2007) Clinical significance of serum mesothelin in patients with mesothelioma and lung cancer. Clin Cancer Res, 13: 5076-5081.

[219] Schneider J, Hoffmann H, Dienemann H, Herth FJ, Meister M, Muley T. (2008) Diagnostic and prognostic value of soluble mesothelin-related proteins in patients with malignant pleural mesothelioma in comparison with benign asbestosis and lung cancer. J Thorac Oncol, 3: 1317-1324.

[220] Kobayashi N, Kostka G, Garbe JH, Keene DR, Bächinger HP, Hanisch FG, Markova D, Tsuda T, Timpl R, Chu ML, Sasaki T. (2007) A comparative analysis of the fibulin protein family. Biochemical characterization, binding interactions, and tissue localization. J Biol Chem, 282: 11805-11816.

[221] Segade F. (2010) Molecular evolution of the fibulins: implications on the functionality of the elastic fibulins. Gene, 464: 17-31.

[222] Albig AR, Neil JR, Schiemann WP. (2006) Fibulins 3 and 5 antagonize tumor angiogenesis in vivo. Cancer Res, 66: 2621-2629.

[223] Seeliger H, Camaj P, Ischenko I, Kleespies A, De Toni EN, Thieme SE, Blum H, Assmann G, Jauch KW, Bruns CJ. (2009) EFEMP1 expression promotes in vivo tumor growth in human pancreatic adenocarcinoma. Mol Cancer Res, 7: 189-198.

[224] Pass HI, Levin SM, Harbut MR, Melamed J, Chiriboga L, Donington J, Huflejt M, Carbone M, Chia D, Goodglick L, Goodman GE, Thornquist MD, Liu G, de Perrot M, Tsao MS, Goparaju C. (2012) Fibulin-3 as a blood and effusion biomarker for pleural mesothelioma. N Engl J Med, 367: 1417-1427.

[225] Kirschner MB, Pulford E, Hoda MA, Rozsas A, Griggs K, Cheng YY, Edelman JJ, Kao SC, Hyland R, Dong Y, László V, Klikovits T, Vallely MP, Grusch M, Hegedus B, Dome B, Klepetko W, van Zandwijk N, Klebe S, Reid G. (2015) Fibulin-3 levels in malignant pleural mesothelioma are associated with prognosis but not diagnosis. Br J Cancer, 113: 963-969.

[226] Rutkowski MJ, Sughrue ME, Kane AJ, Mills SA, Parsa AT. (2010) Cancer and the complement cascade. Mol Cancer Res, 8: 1453-1465.

[227] Ricklin D, Hajishengallis G, Yang K, Lambris JD. (2010) Complement: a key system for immune surveillance and homeostasis. Nat Immunol, 11: 785-797.

[228] Walport MJ. (2001) Complement. First of two parts. N Engl J Med, 344: 1058-1066.

[229] Wagner E, Frank MM. (2010) Therapeutic potential of complement modulation. Nat Rev Drug Discov, 9: 43-56.

[230] Mollnes TE, Kirschfink M. (2006) Strategies of therapeutic complement inhibition. Mol Immunol, 43: 107-121.

[231] Cole DS, Morgan BP. (2003) Beyond lysis: how complement influences cell fate. Clin Sci (Lond), 104: 455-466.

[232] Thiel S. (2007) Complement activating soluble pattern recognition molecules with collagen-like regions, mannan-binding lectin, ficolins and associated proteins. Mol Immunol, 44: 3875-3888.

[233] Holers VM. (2008) The spectrum of complement alternative pathway-mediated diseases. Immunol Rev, 223: 300-316.

[234] Atkinson JP, Frank MM. (2006) Bypassing complement: evolutionary lessons and future implications. J Clin Invest, 116: 1215-1218.

[235] Coussens LM, Werb Z. (2002) Inflammation and cancer. Nature, 420: 860-867.

[236] Dunn GP, Bruce AT, Ikeda H, Old LJ, Schreiber RD. (2002) Cancer immunoediting: from immunosurveillance to tumor escape. Nat Immunol, 3: 991-998.

[237] Mantovani A, Allavena P, Sica A, Balkwill F. (2008) Cancer-related inflammation. Nature, 454: 436-444.

[238] Markiewski MM, Lambris JD. (2009) Is complement good or bad for cancer patients? A new perspective on an old dilemma. Trends Immunol, 30: 286-292.

[239] Lin WW, Karin M. (2007) A cytokine-mediated link between innate immunity, inflammation, and cancer. J Clin Invest, 117: 1175-1183.

[240] Loveland BE, Cebon J. (2008) Cancer exploiting complement: a clue or an exception? Nat Immunol, 9: 1205-1206.

[241] Köhl J. (2001) Anaphylatoxins and infectious and non-infectious inflammatory diseases. Mol Immunol, 38: 175-187.

[242] Markiewski MM, DeAngelis RA, Benencia F, Ricklin-Lichtsteiner SK, Koutoulaki A, Gerard C, Coukos G, Lambris JD. (2008) Modulation of the antitumor immune response by complement. Nat Immunol, 9: 1225-1235.

[243] Venkatesha RT, Berla Thangam E, Zaidi AK, Ali H. (2005) Distinct regulation of C3a-induced MCP-1/CCL2 and RANTES/CCL5 production in human mast cells by extracellular signal regulated kinase and PI3 kinase. Mol Immunol, 42: 581-587.

[244] Fosbrink M, Niculescu F, Rus H. (2005) The role of c5b-9 terminal complement complex in activation of the cell cycle and transcription. Immunol Res, 31: 37-46.

[245] Bora PS, Sohn JH, Cruz JM, Jha P, Nishihori H, Wang Y, Kaliappan S, Kaplan HJ, Bora NS. (2005) Role of complement and complement membrane attack complex in laser-induced choroidal neovascularization. J Immunol, 174: 491-497.

[246] Shinjyo N, Ståhlberg A, Dragunow M, Pekny M, Pekna M. (2009) Complement-derived anaphylatoxin C3a regulates in vitro differentiation and migration of neural progenitor cells. Stem Cells, 27: 2824-2832.

[247] Ostrand-Rosenberg S. (2008) Cancer and complement. Nat Biotechnol, 26: 1348-1349.

[248] Ajona D, Pajares MJ, Corrales L, Perez-Gracia JL, Agorreta J, Lozano MD, Torre W, Massion PP, de-Torres JP, Jantus-Lewintre E, Camps C, Zulueta JJ, Montuenga LM, Pio R. (2013) Investigation of complement activation product c4d as a diagnostic and prognostic biomarker for lung cancer. J Natl Cancer Inst, 105: 1385-1393.

[249] Makela K, Helen P, Haapasalo H, Paavonen T. (2012) Complement activation in astrocytomas: deposition of C4d and patient outcome. BMC Cancer, 12: 565.

[250] Feucht HE. (2003) Complement C4d in graft capillaries -- the missing link in the recognition of humoral alloreactivity. Am J Transplant, 3: 646-652.

[251] Murata K, Baldwin WM, 3rd. (2009) Mechanisms of complement activation, C4d deposition, and their contribution to the pathogenesis of antibody-mediated rejection. Transplant Rev (Orlando), 23: 139-150.

[252] Ajona D, Razquin C, Pastor MD, Pajares MJ, Garcia J, Cardenal F, Fleischhacker M, Lozano MD, Zulueta JJ, Schmidt B, Nadal E, Paz-Ares L, Montuenga LM, Pio R. (2015) Elevated levels of the complement activation product C4d in bronchial fluids for the diagnosis of lung cancer. PLoS One, 10: e0119878.

[253] Corrales L, Ajona D, Rafail S, Lasarte JJ, Riezu-Boj JI, Lambris JD, Rouzaut A, Pajares MJ, Montuenga LM, Pio R. (2012) Anaphylatoxin C5a creates a favorable microenvironment for lung cancer progression. J Immunol, 189: 4674-4683.

[254] Ajona D, Pajares MJ, Chiara MD, Rodrigo JP, Jantus-Lewintre E, Camps C, Suarez C, Bagan JV, Montuenga LM, Pio R. (2015) Complement activation product C4d in oral and oropharyngeal squamous cell carcinoma. Oral Dis, 21: 899-904.

[255] Galani V, Constantopoulos S, Manda-Stachouli C, Frangou-Lazaridis M, Mavridis A, Vassiliou M, Dalavanga Y. (2002) Additional proteins in BAL fluid of Metsovites environmentally exposed to asbestos: more evidence of "protection" against neoplasia? Chest, 121: 273-278.

[256] Zerva LV, Constantopoulos SH, Moutsopoulos HM. (1989) Humoral immunity alterations after environmental asbestos exposure. Respiration, 55: 237-241.

[257] Nabil K, Rihn B, Jaurand MC, Vignaud JM, Ripoche J, Martinet Y, Martinet N. (1997) Identification of human complement factor H as a chemotactic protein for monocytes. Biochem J, 326 (Pt 2): 377-383.

[258] Rodgarkia-Dara C, Vejda S, Erlach N, Losert A, Bursch W, Berger W, Schulte-Hermann R, Grusch M. (2006) The activin axis in liver biology and disease. Mutat Res, 613: 123-137.

[259] Ling N, Ying SY, Ueno N, Shimasaki S, Esch F, Hotta M, Guillemin R. (1986) A homodimer of the beta-subunits of inhibin A stimulates the secretion of pituitary follicle stimulating hormone. Biochem Biophys Res Commun, 138: 1129-1137.

[260] Ling N, Ying SY, Ueno N, Shimasaki S, Esch F, Hotta M, Guillemin R. (1986) Pituitary FSH is released by a heterodimer of the beta-subunits from the two forms of inhibin. Nature, 321: 779-782.

[261] Schmierer B, Hill CS. (2007) TGFbeta-SMAD signal transduction: molecular specificity and functional flexibility. Nat Rev Mol Cell Biol, 8: 970-982.

[262] McDowell N, Gurdon JB. (1999) Activin as a morphogen in Xenopus mesoderm induction. Semin Cell Dev Biol, 10: 311-317.

[263] Beattie GM, Lopez AD, Bucay N, Hinton A, Firpo MT, King CC, Hayek A. (2005) Activin A maintains pluripotency of human embryonic stem cells in the absence of feeder layers. Stem Cells, 23: 489-495.

[264] de Kretser DM, Hedger MP, Loveland KL, Phillips DJ. (2002) Inhibins, activins and follistatin in reproduction. Hum Reprod Update, 8: 529-541.

[265] Maguer-Satta V, Bartholin L, Jeanpierre S, Ffrench M, Martel S, Magaud JP, Rimokh R. (2003) Regulation of human erythropoiesis by activin A, BMP2, and BMP4, members of the TGFbeta family. Exp Cell Res, 282: 110-120.

[266] Jones KL, de Kretser DM, Patella S, Phillips DJ. (2004) Activin A and follistatin in systemic inflammation. Mol Cell Endocrinol, 225: 119-125.

[267] Chen JG, Zhu J, Parkin DM, Zhang YH, Lu JH, Zhu YR, Chen TY. (2006) Trends in the incidence of cancer in Qidong, China, 1978-2002. Int J Cancer, 119: 1447-1454.

[268] Munz B, Smola H, Engelhardt F, Bleuel K, Brauchle M, Lein I, Evans LW, Huylebroeck D, Balling R, Werner S. (1999) Overexpression of activin A in the skin of transgenic mice reveals new activities of activin in epidermal morphogenesis, dermal fibrosis and wound repair. Embo J, 18: 5205-5215.

[269] Werner S, Alzheimer C. (2006) Roles of activin in tissue repair, fibrosis, and inflammatory disease. Cytokine Growth Factor Rev, 17: 157-171.

[270] Burdette JE, Jeruss JS, Kurley SJ, Lee EJ, Woodruff TK. (2005) Activin A mediates growth inhibition and cell cycle arrest through Smads in human breast cancer cells. Cancer Res, 65: 7968-7975.

[271] Ho J, de Guise C, Kim C, Lemay S, Wang XF, Lebrun JJ. (2004) Activin induces hepatocyte cell growth arrest through induction of the cyclin-dependent kinase inhibitor p15INK4B and Sp1. Cellular signalling, 16: 693-701.

[272] Valderrama-Carvajal H, Cocolakis E, Lacerte A, Lee EH, Krystal G, Ali S, Lebrun JJ. (2002) Activin/TGF-beta induce apoptosis through Smad-dependent expression of the lipid phosphatase SHIP. Nature cell biology, 4: 963-969.

[273] Murase Y, Okahashi N, Koseki T, Itoh K, Udagawa N, Hashimoto O, Sugino H, Noguchi T, Nishihara T. (2001) Possible involvement of protein kinases and Smad2 signaling pathways on osteoclast differentiation enhanced by activin A. J Cell Physiol, 188: 236-242.

[274] Dupont J, McNeilly J, Vaiman A, Canepa S, Combarnous Y, Taragnat C. (2003) Activin signaling pathways in ovine pituitary and LbetaT2 gonadotrope cells. Biol Reprod, 68: 1877-1887.

[275] Zhang L, Deng M, Parthasarathy R, Wang L, Mongan M, Molkentin JD, Zheng Y, Xia Y. (2005) MEKK1 transduces activin signals in keratinocytes to induce actin stress fiber formation and migration. Mol Cell Biol, 25: 60-65.

[276] Deli A, Kreidl E, Santifaller S, Trotter B, Seir K, Berger W, Schulte-Hermann R, Rodgarkia-Dara C, Grusch M. (2008) Activins and activin antagonists in hepatocellular carcinoma. World J Gastroenterol, 14: 1699-1709.

[277] Grusch M, Rodgarkia-Dara C, Bursch W, Schulte-Hermann R. Activins and the liver. In: Jakowlew S, editor. Transforming Growth Factor-Beta in Cancer Therapy, Volume I. Totowa, NJ: Humana press; 2008. p. 483-508.

[278] Jeruss JS, Sturgis CD, Rademaker AW, Woodruff TK. (2003) Down-regulation of activin, activin receptors, and Smads in high-grade breast cancer. Cancer Res, 63: 3783-3790.

[279] Razanajaona D, Joguet S, Ay AS, Treilleux I, Goddard-Leon S, Bartholin L, Rimokh R. (2007) Silencing of FLRG, an antagonist of activin, inhibits human breast tumor cell growth. Cancer Res, 67: 7223-7229.

[280] Wang Z, Zhang N, Song R, Fan R, Yang L, Wu L. (2015) Activin A expression in esophageal carcinoma and its association with tumor aggressiveness and differentiation. Oncol Lett, 10: 143-148.

[281] Tsuchida K, Nakatani M, Hitachi K, Uezumi A, Sunada Y, Ageta H, Inokuchi K. (2009) Activin signaling as an emerging target for therapeutic interventions. Cell Commun Signal, 7: 15.

[282] Wamsley JJ, Kumar M, Allison DF, Clift SH, Holzknecht CM, Szymura SJ, Hoang SA, Xu X, Moskaluk CA, Jones DR, Bekiranov S, Mayo MW. (2015) Activin Upregulation by NF-kappaB Is Required to Maintain Mesenchymal Features of Cancer Stem-like Cells in Non-Small Cell Lung Cancer. Cancer Res, 75: 426-435.

[283] Voumvouraki A, Notas G, Koulentaki M, Georgiadou M, Klironomos S, Kouroumalis E. (2012) Increased serum activin-A differentiates alcoholic from cirrhosis of other aetiologies. Eur J Clin Invest, 42: 815-822.

[284] Pirisi M, Fabris C, Luisi S, Santuz M, Toniutto P, Vitulli D, Federico E, Del Forno M, Mattiuzzo M, Branca B, Petraglia F. (2000) Evaluation of circulating activin-A as a serum marker of hepatocellular carcinoma. Cancer Detect Prev, 24: 150-155.

[285] Leto G, Incorvaia L, Badalamenti G, Tumminello FM, Gebbia N, Flandina C, Crescimanno M, Rini G. (2006) Activin A circulating levels in patients with bone metastasis from breast or prostate cancer. Clin Exp Metastasis, 23: 117-122.

[286] Lambert-Messerlian GM, DePasquale SE, Maybruck WM, Steinhoff MM, Gajewski WH. (1999) Secretion of activin A in recurrent epithelial ovarian carcinoma. Gynecol Oncol, 74: 93-97.

[287] Chang KP, Kao HK, Liang Y, Cheng MH, Chang YL, Liu SC, Lin YC, Ko TY, Lee YS, Tsai CL, Wang TH, Hao SP, Tsai CN. (2010) Overexpression of activin A in oral squamous cell carcinoma: association with poor prognosis and tumor progression. Ann Surg Oncol, 17: 1945-1956.

[288] Liu SG, Li HC, Zhao BS, Cao F. (2013) [Expression of activin A in tissue and serum of patients with esophageal squamous cell carcinoma and its clinical significance]. Zhonghua Zhong Liu Za Zhi, 35: 843-847.

[289] Togashi Y, Kogita A, Sakamoto H, Hayashi H, Terashima M, de Velasco MA, Sakai K, Fujita Y, Tomida S, Kitano M, Okuno K, Kudo M, Nishio K. (2015) Activin signal promotes cancer progression and is involved in cachexia in a subset of pancreatic cancer. Cancer Lett, 356: 819-827.

[290] Hoda MA, Rozsas A, Lang E, Klikovits T, Lohinai Z, Torok S, Berta J, Bendek M, Berger W, Hegedus B, Klepetko W, Renyi-Vamos F, Grusch M, Dome B, Laszlo V. (2016) High circulating activin A level is associated with tumor progression and predicts poor prognosis in lung adenocarcinoma. Oncotarget, 7: 13388-13399.

[291] Terpos E, Kastritis E, Christoulas D, Gkotzamanidou M, Eleutherakis-Papaiakovou E, Kanellias N, Papatheodorou A, Dimopoulos MA. (2012) Circulating activin-A is elevated in patients with advanced multiple myeloma and correlates with extensive bone involvement and inferior survival; no alterations post-lenalidomide and dexamethasone therapy. Ann Oncol, 23: 2681-2686.

[292] Hoda MA, Munzker J, Ghanim B, Schelch K, Klikovits T, Laszlo V, Sahin E, Bedeir A, Lackner A, Dome B, Setinek U, Filipits M, Eisenbauer M, Kenessey I, Torok S, Garay T, Hegedus B, Catania A, Taghavi S, Klepetko W, Berger W, Grusch M. (2012) Suppression of activin A signals inhibits growth of malignant pleural mesothelioma cells. Br J Cancer, 107: 1978-1986.

[293] Tamminen JA, Yin M, Ronty M, Sutinen E, Pasternack A, Ritvos O, Myllarniemi M, Koli K. (2015) Overexpression of activin-A and -B in malignant mesothelioma - Attenuated Smad3 signaling responses and ERK activation promote cell migration and invasive growth. Exp Cell Res, 332: 102-115.

[294] Seder CW, Hartojo W, Lin L, Silvers AL, Wang Z, Thomas DG, Giordano TJ, Chen G, Chang AC, Orringer MB, Beer DG. (2009) Upregulated INHBA expression

may promote cell proliferation and is associated with poor survival in lung adenocarcinoma. Neoplasia, 11: 388-396.

[295] Seder CW, Hartojo W, Lin L, Silvers AL, Wang Z, Thomas DG, Giordano TJ, Chen G, Chang AC, Orringer MB, Beer DG. (2009) INHBA overexpression promotes cell proliferation and may be epigenetically regulated in esophageal adenocarcinoma. J Thorac Oncol, 4: 455-462.

[296] Yoshinaga K, Yamashita K, Mimori K, Tanaka F, Inoue H, Mori M. (2008) Activin a causes cancer cell aggressiveness in esophageal squamous cell carcinoma cells. Ann Surg Oncol, 15: 96-103.

[297] Hoda MA, Rozsas A, Lang E, Klikovits T, Lohinai Z, Torok S, Berta J, Bendek M, Berger W, Hegedus B, Klepetko W, Renyi-Vamos F, Grusch M, Dome B, Laszlo V. (2016) High circulating activin A level is associated with tumor progression and predicts poor prognosis in lung adenocarcinoma. Oncotarget, 7: 13388-13399

[298] Kao SC, Pavlakis N, Harvie R, Vardy JL, Boyer MJ, van Zandwijk N, Clarke SJ. (2010) High blood neutrophil-to-lymphocyte ratio is an indicator of poor prognosis in malignant mesothelioma patients undergoing systemic therapy. Clin Cancer Res, 16: 5805-5813.

[299] Frauenfelder T, Tutic M, Weder W, Gotti RP, Stahel RA, Seifert B, Opitz I. (2011) Volumetry: an alternative to assess therapy response for malignant pleural mesothelioma? Eur Respir J, 38: 162-168.

[300] Kaya H, Demir M, Taylan M, Sezgi C, Tanrikulu AC, Yilmaz S, Bayram M, Kaplan I, Senyigit A. (2015) Fibulin-3 as a diagnostic biomarker in patients with malignant mesothelioma. Asian Pac J Cancer Prev, 16: 1403-1407.

[301] Fedarko NS, Jain A, Karadag A, Van Eman MR, Fisher LW. (2001) Elevated serum bone sialoprotein and osteopontin in colon, breast, prostate, and lung cancer. Clin Cancer Res, 7: 4060-4066.

[302] Lin H, Shen YC, Long HY, Wang H, Luo ZY, Wei ZX, Hu SQ, Wen FQ. (2014) Performance of osteopontin in the diagnosis of malignant pleural mesothelioma: a meta-analysis. Int J Clin Exp Med, 7: 1289-1296.

[303] Hoda MA, Dong Y, Rozsas A, Klikovits T, Laszlo V, Ghanim B, Stockhammer P, Ozsvar J, Jakopovic M, Samarzija M, Brcic L, Bendek M, Szirtes I, Reid G, Kirschner MB, Kao SC, Opitz I, Weder W, Frauenfelder T, Nguyen-Kim TD, Aigner C, Klepetko W, van Zandwijk N, Berger W, Dome B, Grusch M, Hegedus B. (2016) Circulating activin A is a novel prognostic biomarker in malignant pleural mesothelioma - A multi-institutional study. Eur J Cancer, 63: 64-73.

[304] Hirano H, Tsuji M, Kizaki T, Sashikata T, Yoshi Y, Okada Y, Mori H. (2002) Expression of matrix metalloproteinases, tissue inhibitors of metalloproteinase, collagens, and Ki67 antigen in pleural malignant mesothelioma: an immunohistochemical and electron microscopic study. Med Electron Microsc, 35: 16-23.

[305] Brosseau S, Assoun S, Naltet C, Steinmetz C, Gounant V, Zalcman G. (2017) A review of bevacizumab in the treatment of malignant pleural mesothelioma. Future Oncol, 13: 2537-2546

[306] Tian L, Zeng R, Wang X, Shen C, Lai Y, Wang M, Che G. (2017) Prognostic significance of soluble mesothelin in malignant pleural mesothelioma: a meta-analysis. Oncotarget, 8: 46425-46435.

[307] Jing X, Huang C, Zhou H, Li C, Fan L, Chen J, Zhang G, Liu Y, Cui Z, Qi D, Ma J. (2015) Association between serum C-reactive protein value and prognosis of patients with non-small cell lung cancer: a meta-analysis. Int J Clin Exp Med, 8: 10633-10639.

[308] Kim KH, Park TY, Lee JY, Lee SM, Yim JJ, Yoo CG, Kim YW, Han SK, Yang SC. (2014) Prognostic significance of initial platelet counts and fibrinogen level in advanced non-small cell lung cancer. J Korean Med Sci, 29: 507-511.

[309] Bu X, Zheng Z, Wang C, Yu Y. (2007) Significance of C4d deposition in the follicular lymphoma and MALT lymphoma and their relationship with follicular dendritic cells. Pathol Res Pract, 203: 163-167.

[310] Mohr S. NA, Bottin M.C., Micillino J.C. , Keith G., Rihn B.H. (2005) Immune Signature of Malignant Pleural Mesothelioma as Assessed by Transcriptome Analysis. Cancer genomics proteomics, 2: 12.

[311] Kirschner MB, Pulford E, Hoda MA, Rozsas A, Griggs K, Cheng YY, Edelman JJ, Kao SC, Hyland R, Dong Y, Laszlo V, Klikovits T, Vallely MP, Grusch M, Hegedus B, Dome B, Klepetko W, van Zandwijk N, Klebe S, Reid G. (2015) Fibulin-3 levels in malignant pleural mesothelioma are associated with prognosis but not diagnosis. Br J Cancer, 113: 963-969.

[312] Hollevoet K, Reitsma JB, Creaney J, Grigoriu BD, Robinson BW, Scherpereel A, Cristaudo A, Pass HI, Nackaerts K, Rodriguez Portal JA, Schneider J, Muley T, Di Serio F, Baas P, Tomasetti M, Rai AJ, van Meerbeeck JP. (2012) Serum mesothelin for diagnosing malignant pleural mesothelioma: an individual patient data meta-analysis. J Clin Oncol, 30: 1541-1549.

[313] Byrne MJ, Nowak AK. (2004) Modified RECIST criteria for assessment of response in malignant pleural mesothelioma. Ann Oncol, 15: 257-260.

11 Bibliography of the candidate's publications

11.1 Publications related to the thesis

Klikovits T*, Stockhammer P*, Laszlo V, Dong Y, Hoda M, Ghanim B, Opitz I, Frauenfellner T, Nguyen-Kim T, Weder W, Berger W, Grusch M, Aigner C, Klepetko W, Dome B, Renyi-Vamos F, Oehler R, Hegedus B
Circulating level of the complement component 4d (C4d) correlates with tumor volume, chemotherapeutic response and survival in patients with malignant pleural mesothelioma
Scientific Reports 2017; 7:16456, *shared first authorship KT & SP

Klikovits T, Hoda M, Arns M, Baumgartner B, Errhalt P, Geltner C, Machan B, Pohl W, Hutter J, Eckmayr J, Studnicka M, Flicker M, Cerkl P, Klepetko W
Management of malignant pleural mesothelioma - part 3: data from the Austrian Mesothelioma Interest Group (AMIG) database
Wien Klin Wochenschr 2016; 128:627-34

Hoda M, Dong Y, Rozsas A, Klikovits T, Laszlo V, Ghanim V, Stockhammer P, Jakopovic M, Samarzija M, Brcic L, Bendek M, Szirtes I, Reid G, Kirschner M, Kao S, Opitz-Schmitz I, Weder W, Frauenfelder T, Nguyen-Kim T, Aigner C, Klepetko W, van Zandwijk N, Berger W, Dome B, Grusch M, Hegedus B
Circulating activin A is a novel prognostic biomarker in malignant pleural mesothelioma – a multi-institutional study
Eur J Cancer 2016; 63:64-73

Ghanim B*, Klikovits T*, Hoda M, Lang G, Szirtes I, Setinek U, Rozsas A, Renyi-Vamos F, Laszlo V, Grusch M, Filipits M, Scheed A, Jakopovic M, Samarzija M, Brcic L, Stancic–Rokotov D, Kern I, Rozman A, Dekan G, Klepetko W, Berger W, Glasz T, Dome B, Hegedus B
Ki67 index is an independent prognostic factor in epithelioid but not in non-epithelioid malignant pleural mesothelioma: a multicenter study
Br J Cancer 2015; 112:783-92, *shared first authorship GB & KT

11.2 Publications not related to the thesis

Decaluwé H, Dooms C, D'Journo X, Call S, Sanchez D, Haager B, Beelen R, Kara V, Klikovits T, Aigner C, Tournoy K, Moons J, Brioude G, Trujillo J, Bethe B, Klepetko W, Turna A, Passlick B, Molins L, Rami-Porta R, Thomas R, De Leyn P
Mediastinal staging by videomediastinoscopy in clinical N1 non-small cell lung cancer: a prospective multicentre study
Eur Resp J 2017; 50:1701493

Marulli G, Rendina EA, Klepetko W, Perkmann R, Zampieri D, Maurizi G, Klikovits T, Zaraca F, Venuta F, Perissinotto E, Rea F
Surgery for T4 lung cancer invading the thoracic aorta: Do we push the limits?
J Surg Oncol 2017; 116:1141-1149

Waseda R*, Klikovits T*, Hoda MA, Hoetzenecker K, Dieckmann K, Zöchbauer-Müller S, Pirker R, Prosch H, Dome B, Klepetko W
Trimodality therapy for Pancoast tumors: T4 is not a contraindication to radical surgery
J Surg Oncol 2017; 116:227-235, *shared first authorship WR & KT

Lohinai Z*, Klikovits T*, Moldvay J, Ostoros G, Raso E, Timar J, Fabian K, Kovalszky I, Kenessey I, Aigner C, Renyi-Vamos F, Klepetko W, Dome B, Hegedus B
KRAS mutation incidence and prognostic value are metastatic site-specific in lung adenocarcinoma: poor prognosis in patients with KRAS mutation and bone metastasis
Sci Rep 2017; 7:39721, *shared first authorship LZ & KT

Torok S, Rezeli M, Vegvari A, Watanabe K, Sugihara Y, Tisza A, Marton T, Kelemen O, Hegedus B, Tovari J, Helbich T, Laszlo V, Klikovits T, Paku S, Marko-Varga G, Dome B
Limited tumor tissue drug penetration contributes to primary resistance against angiogenesis inhibitors
Theranostics 2017; 7:400-412

Hoda M, Pirker C, Dong Y, Schelch K, Heffeter P, Kryeziu K, van Schoonhoven S, Klikovits T, Laszlo V, Rozsas A, Ozsvar J, Klepetko W, Doeme B, Grusch M, Hegedues B, Berger W
Trabectedin is active against malignant pleural mesothelioma cell and xenograft models and synergizes with chemotherapy and bcl-2 inhibition in vitro
Mol Cancer Ther 2016; 15:2357-2369

Klikovits T, Lambers C, Ghanim B, Dome B, Muraközy G, Zöchbauer-Müller S, Waseda R, Aigner C, Lang G, Taghavi S, Klepetko W, Jaksch P, Hoda M
Lung transplantation in patients with incidental early stage lung cancer – institutional experience of a high volume center
Clinical Transplantation 2016; 30:912-7

Hoda M, Rozsas A, Lang E, Klikovits T, Lohinai Z, Torok S, Berta J, Bendek M, Berger W, Hegedus B, Klepetko W, Renyi-Vamos F, Grusch M, Dome B, Laszlo V
High circulating activin A level is associated with tumor progression and predicts poor prognosis in lung adenocarcinoma
Oncotarget 2016; 7:13388-99

Klikovits T, Slama A, Hoetzenecker K, Waseda R, Lambers C, Murakoezy G, Jaksch P, Aigner C, Taghavi S, Klepetko W, Lang G, Hoda M
A rare indication for lung transplantation – pulmonary alveolar microlithiasis: institutional experience of 5 consecutive cases
Clinical Transplantation 2016; 30:429-34

Kirschner M, Pulford E, Hoda M, Rozsas A, Griggs K, Cheng Y, Edelman J, Kao S, Hyland R, Dong Y, László V, Klikovits T, Vallely M, Grusch M, Hegedus B, Dome B, Klepetko W, van Zandwijk N, Klebe S, Reid G
Fibulin-3 has a potential prognostic, but not diagnostic role in malignant pleural mesothelioma
Br J Cancer 2015; 113:963-9

Laszlo V, Hoda M, Garay T, Pirker C, Ghanim B, Klikovits T, Dong Y, Rozsas A, Kenessey I, Szirtes I, Grusch M, Jakopovic M, Samarzija M, Brcic L, Kern I, Rozman A, Popper H, Zoechbauer-Müller S, Heller G, Altenberger C, Ziegler B, Klepetko W, Berger W, Dome B, Hegedus B
Epigenetic downregulation of integrinα7 increases migratory potential and confers poor prognosis in malignant pleural mesothelioma
J Pathol 2015; 237:203-14

Lang G, Ghanim B, Hoetzenecker K, Klikovits T, Matilla J, Aigner C, Taghavi S, Klepetko W
Extracorporeal membrane oxygenation support for complex tracheo-bronchial procedures
Eur J Cardiothorac Surg 2015; 47:250-6

Slama A, Ghanim B, Klikovits T, Scheed A, Hoda MA, Hoetzenecker K, Jaksch P, Matilla J, Taghavi S, Klepetko W, Aigner C
Lobar lung transplantation - is it comparable with standard lung transplantation?
Transpl Int 2014; 27:909-16

Schelch K, Hoda M, Klikovits T, Münzker J, Ghanim B, Wagner C, Garay T, Laszlo V, Setinek U, Dome B, Filipits M, Pirker C, Heffeter P, Selzer E, Tovari J, Torok S, Kenessey I, Holzmann K, Grasl-Kraupp B, Marian B, Klepetko W, Berger W, Hegedus B, Grusch M
FGF Receptor Inhibition is Active against Mesothelioma and Synergizes with Radio- and Chemotherapy
Am J Respir Crit Care Med 2014; 190:763-72

Lauk O, Hoda M, de Perrot M, Friess M, Klikovits T, Klepetko W, Keshavjee S, Weder W, Opitz I
Extrapleural pneumonectomy after induction chemotherapy: Perioperative outcome in 251 mesothelioma patients from 3 high volume institutions
Ann Thorac Surg 2014; 98: 1748-54

Cserepes M, Ostoros G, Lohinai Z, Raso E, Barbai T, Timar J, Rozsas A, Moldvay J, Kovalszky I, Fabian K, Gyulai M, Ghanim B, Laszlo V, Klikovits T, Hoda M, Grusch M, Berger W, Klepetko W, Hegedus B, Dome B
Subtype-specific KRAS mutations in advanced lung adenocarcinoma: a retrospective study of patients treated with platinum-based chemotherapy
Eur J Cancer 2014; 50:1819-28

Ghanim B, Hoda M, Klikovits T, Winter M, Alimohammadi A, Grusch M, Dome B, Arns M, Schenk P, Jakopovic M, Samarzija M, Brcic L, Filipits M, Laszlo V, Klepetko W, Berger W, Hegedus B
Circulating fibrinogen is a prognostic and predictive biomarker in malignant pleural mesothelioma patients
Br J Cancer 2014; 110:984-90

Ghanim B, Ankersmit H, Prosch H, Klikovits T, Marta G, Klepetko W, Lang G
A Rare Indication for Video-Assisted Thoracoscopic Surgery: Headscarf Needle Aspiration
Clin Respir J 2013; 7: 15-17

Hoda M, Muenzker J, Ghanim B, Schelch K, Klikovits T, Laszlo V, Sahin E, Bedeir A, Lackner A, Dome B, Setinek U, Filipits M, Eisenbauer M, Kenessey I, Török S, Garay T, Hegedus B, Taghavi S, Klepetko W, Berger W, Grusch M
Suppression of Activin A Signals Inhibits Growth of Malignant Pleural Mesothelioma Cells
Br J Cancer 2012; 107:1978-86

Ghanim B, Hoda M, Winter M, Klikovits T, Alimohammadi A, Hegedus B, Dome B, Grusch M, Arns M, Schenk P, Pohl W, Zielinski C, Filipits M, Klepetko W, Berger W
Pretreatment serum C-reactive protein levels predict benefit from multimodality treatment including radical surgery in malignant pleural mesothelioma: a retrospective multicenter analysis
Ann Surg 2012; 256: 357-62

Klikovits T, End A, Dekan G, Riedl G, Stiebler M, Klepetko W
Effects of reclassification from the TNM-6 into the TNM-7 staging system in bronchoplastic resection for non-small cell lung cancer
Interact Cardiovasc Thorac Surg 2011; 13: 29-34

12 List of figures

13 List of tables

14 Acknowledgements

Cancer is an epidemic and dreadful disease. During my training so far, I had the great opportunity to be educated in both, clinical and scientific medicine and thus learn how to prevent, detect, treat and follow-up malignant diseases. Moreover, I was taught the basic aspects translational cancer research. As a surgical oncologist focusing on thoracic malignancies, I am dealing with patients suffering from lung cancer or malignant pleural mesothelioma every day. Many of these patients lost their life to cancer and I am thankful to work within a highly professional and dedicated team aiming to fight these devastating diseases.

For this very reason, I am thanking number of people who supported me during the last years. Without their patience and encouragement, my achievements so far would not have been possible.

First, I want to thank my main PhD supervisors Ferenc Renyi-Vamos and Balazs Döme for guiding me in my clinical and translational research activity.

Furthermore, I am thankful for the help and support from my colleagues at the Translational Thoracic Oncology Lab at the Medical University of Vienna, especially Viktoria Laszlo, Mir Alireza Hoda and Balazs Hegedüs. Particularly Alireza Hoda always supported and motivated me during many hours of clinical work and cancer research, together as a team.

I want to thank my colleagues from the Division of Thoracic Surgery at the Medical University of Vienna, especially my boss Walter Klepetko and also Shahrokh Taghavi. Moreover, I am thankful for the help and support from our highly motivated clinical thoracic oncology team, namely Alireza Hoda, Katharina Sinn and Theresa Stork.

I also want to thank my colleagues Bahil Ghanim and Paul Stockhammer, with whom together I conducted parts of the research included in this thesis.

I am grateful for my family and friends. Most importantly, I want to thank my wife Sabine Klikovits for her great patience, understanding and support. She constantly motivates and encourages me. Without her I would have never come so far. This thesis is dedicated to her.

I am also grateful for the support of my parents, Gerda Heissenberger and Helmut Klikovits, who were always there when I needed advice and motivation. They gave me the chance to attend medical school and thus to become a surgical oncologist. I also thank my brother, Bernhard Klikovits.