DOI:10.14753/SE.2020.2421

MOLECULAR AND HISTOLPATHOLOGIC PROGNOSTIC FACTORS IN MALIGNANT PLEURAL MESOTHELIOMA

PhD Thesis

Ágnes Bilecz, MD

Pathological Studies Doctoral School

Semmelweis University

Supervisor: Balázs Hegedűs, Ph.D

Official reviewers: Hajnalka Rajnai, MD, Ph.D

János Szőke, MD, Ph.D

Head of the Final Examination Committee: Attila Tordai, MD, D.Sc

Members of the Final Examination Committee: Erika Tóth, MD, Ph.D
Attila Patócs, MD, Ph.D

Budapest
2020

DOI:10.14753/SE.2020.2421

TABLE OF CONTENTS

DOI:10.14753/SE.2020.2421

DOI:10.14753/SE.2020.2421

DOI:10.14753/SE.2020.2421

LIST OF ABBREVIATIONS

3D CRT	three-dimensional conformal radiation therapy
A	adenine
AACR	American Association of Cancer Research
ACS	active symptom control
AE1/AE3	pan cytokeratin antybody AE1/AE3
AJCC	American Joint Committee on Cancer
AKT	Protein kinase B
ALT	alternative lengthening of telomeres
AP1	Activator Protein 1
ARID2	AT-Rich Interaction Domain 2
ASSI	Argininosuccinate synthase 1
ATP	Adenosine triphosphate
ATRX	Alpha Thalassemia/Mental Retardation Syndrome X-Linked
ATS	American Thoracic Society
AUT	Austia
BAP-1	BRCA1-associated protein-1
BAP1-TPDS	BRCA1-associated protein-1 tumor predisposition syndrome
BARD1	BRCA1-associated RING domain protein
Ber-EP4	anty-Epithelial cell adhesion molecule-antibody Ber-EP4
BG8	Blood group 8
BMM	biphasic malignant mesothelioma
BRIP1	BRCA1 Interacting Protein C-Terminal Helicase 1
BSC	best supportive care
BTS	British Thoracic Society
C	cytosine
c.	codone
CANT1	Calcium Activated Nucleotidase 1
CAR	Chimeric antigen receptor
CD15	cluster of differentiation 15
CD79B	B-Cell Antigen Receptor Complex-Associated Protein Beta Chain

CD80	cluster of differentiation 80
CDKN2A	cyclin-dependent kinase Inhibitor 2A
cDNA	complementary DNA
CEA	Carcinoembryonic antigen
CHT	chemotherapy
CI	confidence intervall
CK	cytokeratin
CK 5/6	cytokeratin 5/6
cKIT	KIT proto-oncogene, receptor tyrosine kinase
CRO	Croatia
CRP	C Reactive Protein
CT	computed tomography
CTLA4	cytotoxic T-lymphocyte-associated protein 4
D2-40	monoclonal anti-podoplanin-antibody D2-40
DAXX	Death Domain Associated Protein
DNA	deoxyribonucleic acid
dsDNA	double-strand DNA
E2F1	E2F Transcription Factor 1
ECOG PS	Eastern Cooperative Oncology Group Scale of Performance Status
EGFR	epidermal growth factor receptor
EMM	epithelioid malignant mesothelioma
EORTC	European Organisation for Research and Treatment of Cancer
ePD	extended pleurectomy/decortication
EPP	extrapleural pulmonectomy
ERS	European Respiratory Society
ETS	E-twenty-six
EURACAN	European Network for Rare Adult Solid Cancers
EZH2	Enhancer of zeste homolog 2
FAK	Focal adhesion kinase
FAT4	FAT Atypical Cadherin 4
FDG	fluorodeoxyglucose
FFPE	Formalin fixed paraffin embedded

FISH	fluorescent in situ hybridization
G	guanine
GABPA/B	GA Binding Protein Transcription Factor Subunit Alpha/Beta
GATA3	GATA Binding Protein 3
GCDFP15	Gross cystic disease fluid protein 15
Gly	glycine
Gy	Grey
HE	hematoxylin eosin
HIF1	Hypoxia Inducible Factor
HMGB-1	High mobility group box 1 protein
HPF	high power field
HR	hazard ratio
HSV1716	Herpes simplex virus 1716
IASLC	International Association for the Study of Lung Cancer
IC50	half maximal inhibitory concentration
IFN-β	interferon beta
IMIG	International Mesothelioma Interest Group
IMRT	Intensity-modulated radiotherapy
IQR	interquartile range
KIBRA	Kidney and brain protein
KPS	Karnofsky performance status scale
LADC	lung adenocarcinoma
LATS2	Large Tumor Suppressor Kinase 2
Leu-M1	monoclonal anti-CD15-antibody Leu-M1
M/N score	mitosis-necrosis score
MARS	Mesothelioma and Radical Surgery
MCR	macroscopic complete resection
MDM2	Mouse Double Minute 2,
MLLT3	MLLT3 Super Elongation Complex Subunit
MM	malignant mesothelioma
MMT	Multimodality treatment
MOB1	Mps One Binder Kinase Activator-Like 1B

MOC31	anty-Epithelial cell adhesion molecule-antibody MOC31
MPM	malignant pleural mesothelioma
mRNA	messenger RNA
MST1/2	Mammalian STE20-Like Protein Kinase 1/2
MTAP	Methylthioadenosine Phosphorylase
mTOR	mammalian target of rapamycin
mut	mutant
MYC	V-Myc Avian Myelocytomatosis Viral Oncogene Homolog
NA	not available
NF2	Neurofibromatosis type 2
OD	optical density
OR	overall risk
OS	overall survival
p.	protein amino acid
p16	cyclin-dependent kinase inhibitor 2A
p40	anty-deltaNp63-antibody
PARP	Poly (ADP-ribose) polymerase
PAX2	Paired box gene 2
PAX8	Paired box gene 8
PBRM1	Polybromo 1
PBS	Phosphate-buffered saline
PCR	polymerase chain reaction
PD	pleurectomy/decortication
PD(L)1	programmed death (ligand) 1
PET-CT	Positron emission tomography–computed tomography
PFS	progressio-free survival
PI3K	Phosphoinositide 3-kinase (
POT1	Protection Of Telomeres 1
pRb	retinoblastoma protein
PSEN1	presenilin-1
PTCH1	Patched 1
PTPRD	Protein Tyrosine Phosphatase Receptor Type D

qRT-PCR	quantitative reverse transcription polymerase chain reaction
RAD51	RAD51 Recombinase
RAP1	Repressor/Activator Protein 1 Homolog
RNA	ribonucleic acid
RPL41	Ribosomal Protein L41
RT	raditherapy
SAV1	Salvador Family WW Domain Containing Protein 1
SE	standard error
SETD2	SET Domain Containing 2, Histone Lysine Methyltransferase
SETDB1	SET Domain Bifurcated Histone Lysine Methyltransferase 1
SLO	Slovenia
SMM	sarcomatoid malignant mesothelioma
SMYD3	SET And MYND Domain Containing 3
SNP	single nucleotid polymorphism
SP1	Specificity Protein 1
SQCC	squamous cell carcinoma
SRB	Sulforhodamine B
T	thymine
TAZ	Tafazzin
TCGA	The Cancer Genome Atlas
TEAD	transcription-enhancer activator domain transcripition factor
TEK	TEK Receptor Tyrosine Kinase
TERC	Telomerase RNA Component
TERT	Telomerase Reverse Transcriptase
TERTp	TERT promoter
THOR	TERT hypermethylation oncological region
TIN2	TRF interacting protein 2
TNM	tumor, nodes and metastases
TP53	Tumor Protein P53
TPP1	TINT1/PTOP/PIP1
TRF	Telomeric Repeat Binding Factor
Tris	tris(hydroxymethyl)aminomethane

DOI:10.14753/SE.2020.2421

Trp	tryptophan
TTF-1	thyroid transcription factor 1
UICC	Union for International Cancer Control
VATS	video-assisted thoracoscopy
VGFR	Vascular endothelial growth factor
WBC	white blood cell count
WHO	World Health Organisation
wt	wild-type
WT-1	Wilms' tumour 1
WWTR1	WW Domain Containing Transcription Regulator 1
YAP	Yes Associated Protein

DOI:10.14753/SE.2020.2421

1. INTRODUCTION

1.1 Epidemiology of malignant pleural mesothelioma

Malignant mesothelioma (MM) is a rare malignancy arising from the mesothelial cells of serous membranes such as the pleura, peritoneum[1], pericardium[2], tunica vaginalis of the testis[3] and ovarial surface epithelium[4]. Malignant pleural mesothelioma (MPM) is the most common form of malignant mesothelioma accounting for 80-85% of the cases [5].

According to the World Health Organisation (WHO) mortality database 92,253 malignant mesothelioma deaths were reported in the period between 1994 and 2008 from 83 countries across the world. Worldwide, crude and age-adjusted mortality rates were 6.2 and 4.9 deaths per million population, respectively, the latter showing a yearly increase of 5.37%. During the studied time period malignant mesothelioma associated deaths occurred more frequently in the high-income countries of the Americas and Europe [6].

MPM's incidence varies substantially across the world. MPM's age standardized-rate incidence was 1.93 per 100,000 among men, and 0.41 among women in the United States. Standardized-rate incidences were 3.5 among men and 1.25 per 100,000 among men and women in Italy [7], while among males in Great Britain it was 3.4/100,000, 2.3/100,000 in France, and 3.2/100,000 in the Netherlands [8]. Lower MPM incidence rates are reported n Central and Eastern Europe, 1.84 in Croatia and 1 per 100,000 men in Austria [9].

MPM incidence and mortality not only shows spatial variations, but also changes over time. Number of MPM associated deaths has been rising during the 20th century due to the rising production and consumption of asbestos. In North America and Western Europe the rise in incidence is expected to level out in the near future and then decrease. During the first decade of the 2000s Sweden already experienced a decrease in the number of MPM cases thanks to early adaptation of strict regulation of asbestos handling [10, 11]. In other countries like Italy, Netherlands and France show stagnant MPM mortality rates, which are expected to decrease in the near future [12, 13]. In contrast, Eastern European countries are still witnessing an increase in the burden of asbestos related carcinogenesis [14]. 80% of the world's population still lives in

countries where there is no ban on asbestos (Figure 1) [15, 16] which causes a continued increase of MPM incidence worldwide [8].

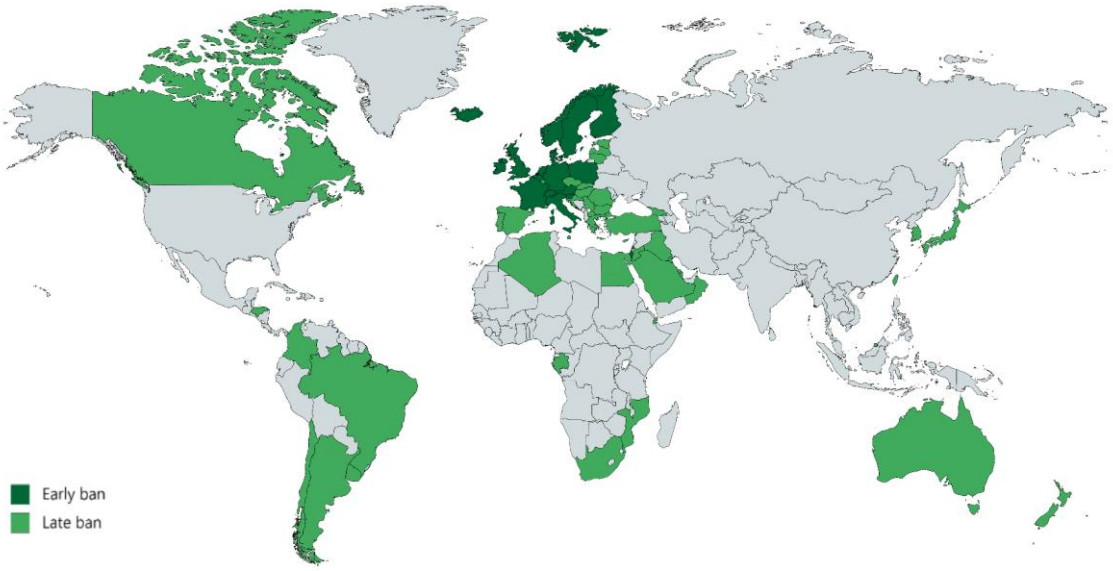

Figure 1. The use and production of asbestos is currently banned in 67 countries. Early bans were introduced in Western Europe before 2000 (dark green). Several countries have implemented such measures only after 2000 (light green). The author's drawing based on data from [17, 18].

Due to the long latency period after exposure MPM is most commonly diagnosed in patients older than 65 years [14]. 2% of all MPM patients are younger than 40 years, and they have a significantly better overall survival among all three main histological subtype, than those older than 40 (11 months vs. 8 months) [19].

MPM is approximately four times more common among males than females, which might be explained by males traditionally working in positions with higher risk of occupational asbestos exposure [19]. The French National Mesothelioma Surveillance Program calculated the MPM risk fraction attributable to occupational exposure for both genders, and estimated it to be 83.2% (95% CI 76.8-89.6) for men, while only 38.4% (95% CI 26.8-50.0) for women [20]. Enviromental exposure, however is a higher burden for women, the male-to-female ratio being approximately 1, and MPM risk associated with environmental exposure in women being 38.7% and 20% in men [21]. Women with mesothelioma have been reported to have a significantly longer survival

compared to men, a phenomenon also in part attributable to differences in the doses of asbestos exposure [22].

1.1.1 Asbestos exposure and malignant pleural mesothelioma

Of all MPM cases, approximately 80% are estimated to be linked to inhalation of asbestos fibers (Figure 2) [23], while only 10% of those substantially exposed to asbestos develop MPM [24]. Asbestos carcinogenesis is linked to DNA damage caused by direct mechanical interference of asbestos fibers whit chromosomes, as well as by reactive oxygen and nitrogen species secreted by mesothelial cells and macrophages [25]. The HMGB-1 mediated necrosis and chronic inflammation induced by the depositions of asbestos fibers also plays a role in the development of MPM [26].

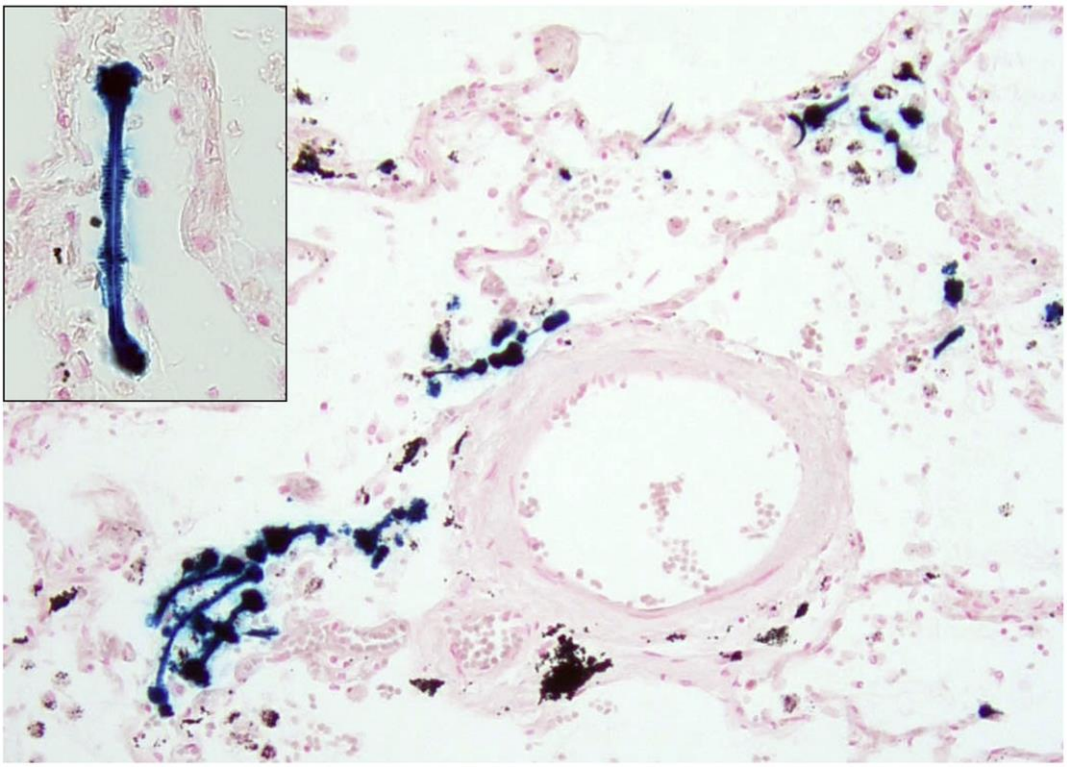

Figure 2. Asbestos bodies, also known as ferruginous bodies are dumbbell-shaped, thin structures covered by a coat of proteins and iron-containing mucopolysacchirdes, that stain blue with Prussian blue staining [27]. Reprinted with the permission of John Wiley and Sons from [28].

Ecological correlations have been shown to be robust between a country's historical asbestos consumption given in kg per person per year and its age-adjusted annual MM and MPM mortality rates [29].

Crocidolite, amosite and chrysotile are the three types of asbestos associated with the induction of MPM, the ratio of exposure specific risk of MPM from the three principal types of asbestos is estimated to be 500:100:1 [30]. Eternit workers and wives were typically exposed to a mixture of crocidolite and chrysotile, while railway stock workers were predominantly exposed to crocidolite, and amosite factory workers to amosite [31].

Regional clustering of MPM cases was observed within several Western European countries [32]. The hotspots were identified most commonly in the vicinity of harbors with oil refieneries or shipyards due to historical asbestos use in shipbuilding and repair (eg. South-East England [33], Genoa and Trieste, Italy [34]), asbestos mines and asbestos-cement industries (eg. Casale Monferrato, Italy) or near railway carriage construction and repair sites (eg.Veneto, Italy) [35]. Men between the ages of 40 and 74 years in Scotland and England had an age standardized MPM incidence rate of 8.8 and 8.0 per 100,000, in the Trieste and Genova region of Italy 17.2 and 14.4 per 100,000 persons, respectively, while for the remaining European countries an incidence of 0.6-4.2 per 100,000 was observed in the time period beween 1991 and 1995 [24].

A study carried out by the French National Mesothelioma Program identified industries associated with the highest risk for MPM. French men working in shipbuilding and repair had more than 9 times higher risk (OR=9.3, 95% CI: 5.20-16.06) for developing MPM compared to those never having worked in asbestos related occupations. Among others, the men working in the manufacturing of astbestos products, of metal constructions, plumbers, construction workers, electrical wiremen and those working in railroad equipment production were also at substantially higher risk for MPM [36]. Patients in household contact with workers exposed to asbestos also have an elevated risk for pleural disease [37].

A large pooled analysis of cohort studies including workers with occupational exposure and individuals with environmental asbestos exposure found the median age at the time of first exposure to be in the early- to mid-20s, and the median length of exposure to be 3.75 years (IQR 0.7-18.2). The median time between exposure and the diagnosis of

MPM was 38.4 years (IQR 31.3-45.3). The risk of developing MPM increased for 45 years after exposure, after that the increase in risk appeared to level out [31].

1.1.2 Non-asbestos related MPM

Approximately 20% of all MPM cases occur without asbestos exposure. The role of potential alternative risk factors remains unclear. Non-asbestos minerals that have a similar fibrous form and high biopersistence to that of commercial asbestos varieties also have carcinogenic potential, especially erionite [38]. An in vivo experiment showed that carbon nanotubules beyond the threshold length of 4 μm caused acute pleural inflammation, that is considered an early event in MPM carcinogenesis [39]. Exposure to ionizing radiation [40], and Simian virus 40-like virus infection [41] have been proposed as risk factors in a subset of MPM patients, however, their role needs further verification [42].

1.1.3 Genetic predisposition to MPM

The germline mutations in the gene encoding BRCA1-associated protein-1 (BAP-1) have recently been described as a predisposing genetic factor of MPM [43]. This high penetrance germline mutation causes a newly recognized cancer syndrome, namely the BAP-1 tumor predisposition syndrome (BAP1-TPDS), which is characterized by the development of distinct tumor types by the age of 55 years [44]. Carriers show an increased risk to develop peritoneal or pleural mesothelioma, but are also predisposed to other tumor types, such as atypical Spitz tumor [45, 46], cutaneous or uveal melanoma [47], renal cell carcinoma [48], breast cancer, basal cell carcinoma [49] and less frequently to further malignancies [50, 51]. The MM patients carrying these germline mutations are typically younger than those with sporadic MM, more than 60% of them are female, and they have a significantly longer overall survival compared to all MM patients [52, 53].

A recent study suggests that germline mutations of the CDKN2A gene predispose not only to malignant melanoma, but also to MPM. However, the associated potential cancer syndrome needs further investigation, since there is only one identified carrier of

the germline CDKN2A mutation (c.301G > T, p.Gly101Trp) who developed malignant cutaneous melanoma and has a history of both melanoma and MPM in her family [54].

1.2 Clinical diagnosis and staging of MPM

The diagnosis of MPM is often challenging, as symptoms present at a late stage of the disease progression, and are non-specific [55]. The most common symptoms are dyspnoe and chest pain. Dyspnoe is caused by a typically unilateral pleural effusion. Chest pain might be diffuse and dull, or less often of pleuritic nature [56]. Other patients present with weight loss, fatigue, or sweats. Local spread of the tumor into mediastinal structures can cause dysphagia, superior vena cava syndrome or recurrent laryngeal nerve palsy [57].

The diagnostic pathway for MPM proposed by the British Thoracic Society (BTS) includes chest radiography as first line imaging modality for patients with symptoms suspicious for MPM [58]. On radiographs, unilateral pleural effusions are present in 94% of the cases. Further findings typical for MPM include a diffuse thickening of the pleura, which might cause a loss in the lung volume, or show a spread along the interlobar fissures [59]. For patients with radiographic features of MPM the recommended second-line imaging method is venous-phase, contrast-enhanced CT of the thorax and the upper part of the abdomen (Figure 3).

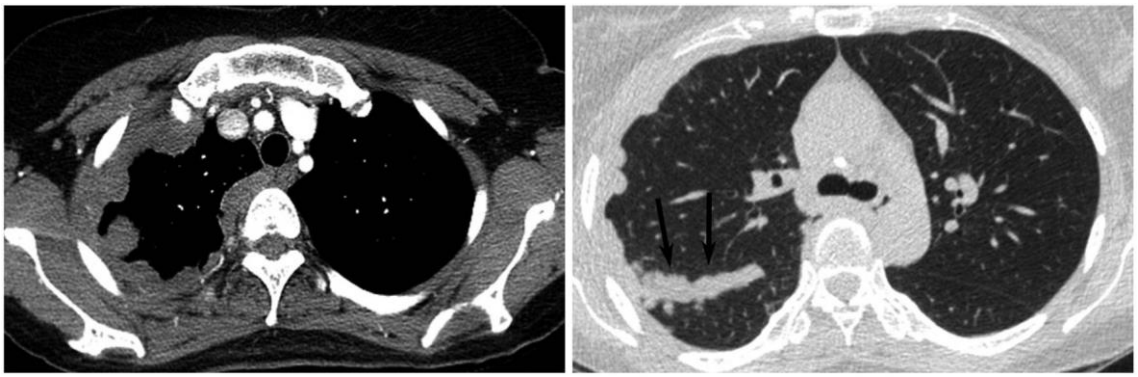

Figure 3. Chest CT scan showing concentric and fissural (arrows) pleural thickening in the right thoracic cavity corresponding to MPM. Reprinted from: [60]

For a high rate of false positivity -especially after talc pleurodesis-, PET-CT is only recommended for assessment of patients' eligibility for surgical resection, such as for evaluation of depth of chest wall invasion or for exclusion of distant metastases [58]. In each case, the diagnosis of MPM has to rely on pathologic evaluation, for there are no specific radiological or clinical features of the disease. There are several methods available for diagnostic sample acquisitions which differ widely in invasiveness and sensitivity. For patients presenting with unilateral pleural effusion, an ultrasound-guided pleurocentesis followed by the cytological evaluation of the pleural fluid is recommended [8, 61]. The sensitivity of cytology in the diagnosis of MPM varies substantially, ranging from 30% to 75%, mainly depending on the experience of the laboratories and the availability of ancillary testing [62-66].

The observed high false-negative rate might be explained by the fact that MPM cells lack specific features of malignancy, malignant epithelioid cells and reactive mesothelial cells share many cytological features, such as low nuclear to cytolplasmic ratios, cell clumps with scalloped borders. Another important factor is that the sarcomatoid component is usually not shed into the malignant effusion, that, as a consequence, results paucicellular [55]. In summary, the first diagnosis of MPM is often based on cytology, but in most cases a tissue biopsy is needed to assess invasion and to confirm the primary MPM diagnosis [61, 67]. However, in patients to frail for further invasive interventions, a diagnosis based on cytology alone is accepted [8, 68].

In patients who are candidates for chemotherapy or multimodal therapy, a tissue sample should be obtained. This might be carried out through video-assisted thoracoscopy (VATS) providing an opportunity to directly visualize any suspicious lesions throughout the pleural surface and to gain sufficiently large and deep tissue samples (Figure 4). Thoracoscopy allows a histologic diagnosis in more than 90% of the cases [69].

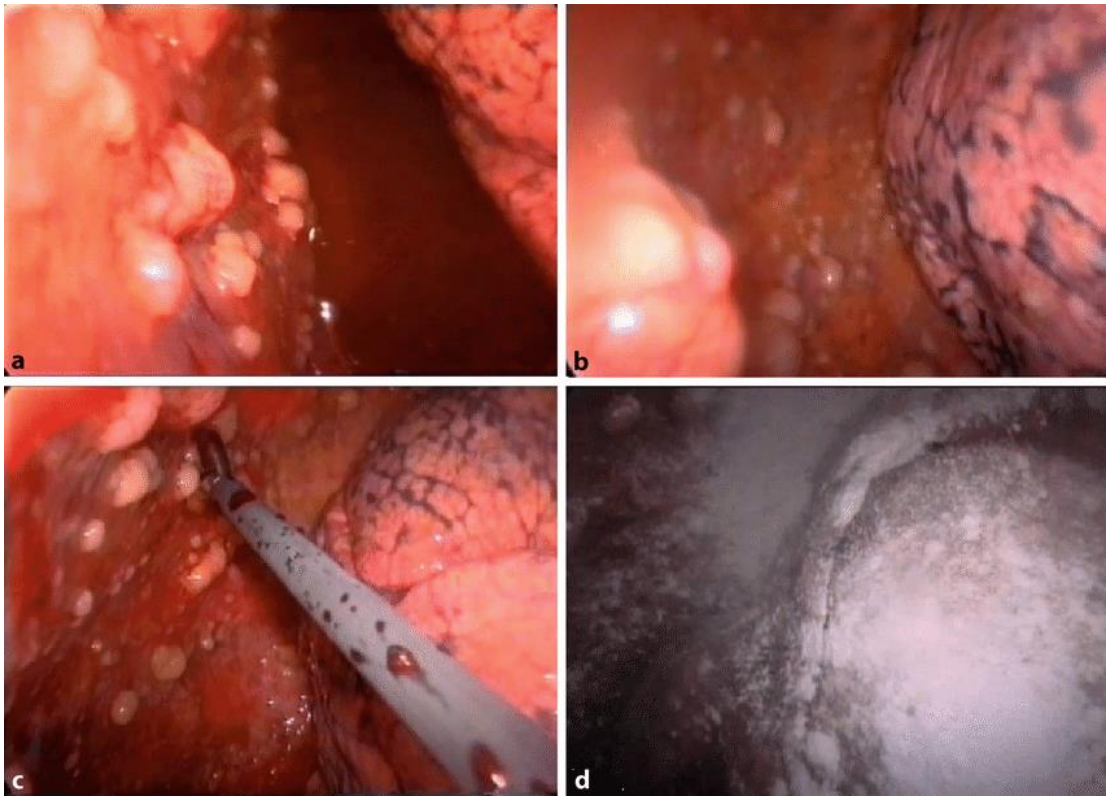

Figure 4. (A) and (B) Visualization of the pleural surface and MPM through VATS. (C) Forceps obtaining a tissue biopsy. (D) VATS view of talc pleurodesis [70].

If the extent of the disease does not allow a thoracoscopic approach, an open surgical biopsy might be carried out. In patients who are not fit for VATS or surgical biopsy and do not have a cytologic diagnosis, an imaging guided percutan core needle biopsy should be carried out [61]. Blind biopsies have a lower sensitivity due to sampling error [71] and higher complication rate including pneumothorax in 9.4% of the cases [72].

Initial staging of MPM is based on contrast enhanced chest and upper abdominal CT scan and usually an FDG PET-CT scan. If any of these suggest lesions suspicious for mediastinal lymph node metastases, these should be confirmed through an endobronchial ultrasound-guided fine needle aspiration biopsy or mediastinoscopy in cases where a radical surgical intervention is considered [61, 73-75]. Also, if suspicious lesions on the contralateral pleura or in the abdominal cavity are the only contraindication for radical surgery, a contralateral thoracoscopy or laparoscopy needs to be performed [61].

Individual patients' functional status is commonly described using the Karnofsky performance status scale (KPS). It ranges from 0% (dead) to 100% (no sign of disease)

and measures the patient's ability to carry out ordinary tasks [76]. The Eastern Cooperative Oncology Group (ECOG) Scale of Performance Status (PS) ranging between grade 0 and 5 is a similar measure of disease related changes in the amount of daytime spent in bed and the patient's need for care [77].

The prognostic score system of the European Organisation for Research and Treatment of Cancer (EORTC) is a composit score developed to assess the prognosis of MPM patients. It includes patient's gender, ECOG PS, the tumor's histological subtype, certainty of the MPM diagnosis and white blood cell count (WBC) [78]. Male gender, non-epithelioid histology, an uncertain/possible diagnosis of MPM, WBC over 8.3x 10^9/L and an ECOG PS other than 0 are associated with poor prognosis, and are summarized in the final score after multiplication with a constant specified for each [79]. The prognosis is considered poor if the EORTC score is below 1.27 [80].

The TNM staging system proposed by the IASLC is also used to predict patient outcomes and to help guide treatment decisions (Table 1) [81, 82].

Table 1. Definitions of T, N and M categories according to the IASLC proposal for the 8th edition of TNM, reprinted from [83].

T1	Tumor involving the ipsilateral parietal or visceral pleura only
T2	Tumor involving ipsilateral pleura (parietal or visceral pleura) with invasion involving at least one of the following: – diaphragmatic muscle – pulmonary parenchyma
T3	Tumor involving ipsilateral pleura (parietal or visceral pleura) with invasion involving at least one of the following: – endothoracic fascia – mediastinal fat – chest wall, with or without associated rib destruction (solitary, resectable) – pericardium (nontransmural invasion)
T4	Tumor involving ipsilateral pleura (parietal or visceral pleura) with invasion involving at least one of the following: – chest wall, with or without associated rib destruction (diffuse or multifocal, unresectable) – peritoneum (via direct transdiaphragmatic extension) – contralateral pleura – mediastinal organs (esophagus, trachea, heart, great vessels) – vertebrae, neuroforamen, spinal cord, or brachial plexus – pericardium (transmural invasion with or without pericardial effusion)
NX	Regional lymph nodes cannot be assessed
N0	No regional lymph-node metastases
N1	Metastases to ipsilateral intrathoracic lymph nodes (including ipsilateral bronchopulmonary, hilar, subcarinal, paratracheal, aortopulmonary, paraesophageal, peridiaphragmatic, pericardial, intercostals, and internal mammary nodes)
N2	Metastases to contralateral intrathoracic lymph nodes, metastases to ipsilateral or contralateral supraclavicular lymph nodes
M0	No distant metastasis
M1	Distant metastases present

Stage	Tumor	Lymph nodes	Metastases
IA	T1	N0	M0
IB	T2, T3	N0	M0
II	T1, T2	N1	M0
IIIA	T3	N1	M0
IIIB	T1–T3	N2	M0
	T4	N0–N2	M0
IV	Any T4	Any N	M1

Despite all efforts to achieve early detection, sometimes heroic surgical and oncological treatment, the prognosis of MPM remains dismal. Even in patients with disease limited to the pleura without lymph node or distant metastases (stage IA), the 5-year overall survival is only 16% (Figure 5) [82].

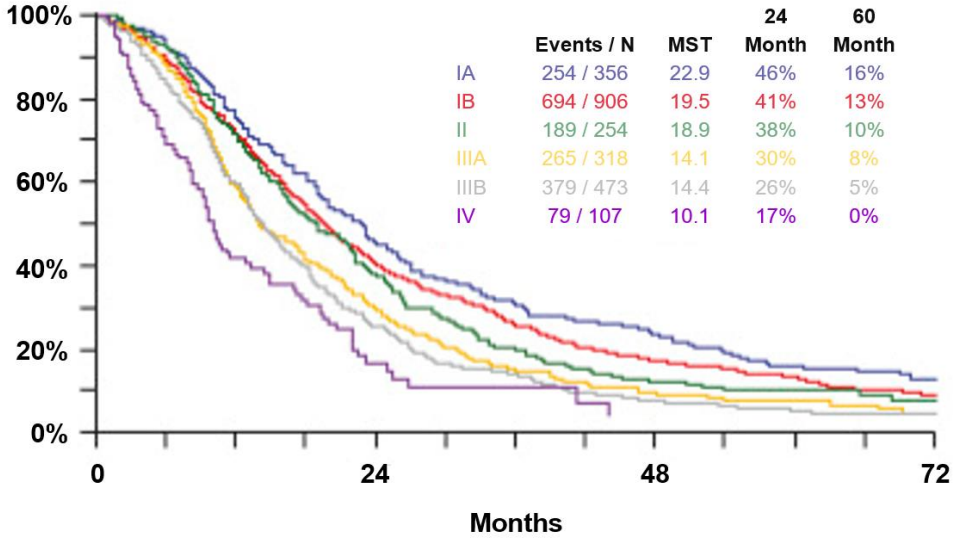

Figure 5. Overall survival of MPM patients based on the IASLC staging system from the 8th edition of TNM Classification of Malignant Tumors by UICC. Reprinted with the permission of Elsevier from [82]

The need for early detection of MPM in patients with known asbestos-exposure has emerged, and multiple screening methods, such as breath tests and circulating tumor markers were tested [84-88]. Nonetheless, screening remains not advised due to MPM's low incidence even in a high-risk population, its subtle radiologic presentation and the lack of curative therapeutic options [8, 58, 61]. Although there are currently no biomarkers recommended for screening or as a single diagnostic test, biomarker testing is, however, used in the diagnosis of patients with suspicious cytology who are not fit enough for further invasive diagnostic procedures [89].

1.3 Histopathologic features of MPM

Mesothelioma remains a challenging histopathological diagnosis requiring expertise and extensive use of additional immunohistochemical markers. The French National Mesothelioma Surveillance Program reviewed the initial histological diagnosis in over

600 MPM cases with the involvement of at least three expert mesothelioma pathologists and supplemental immunohistochemical analysis. The study was able to confirm the diagnosis of MPM in only 67%. The study found false positive diagnoses in 13% of the initial MPM cases and an uncertain diagnosis was made in 17% of the reviewed cases [20]. The 2015 edition of the WHO Classification of Tumours of the Lung, Pleura, Thymus and Heart recognizes three main histological subtypes of diffuse malignant mesothelioma, namely the epithelioid (EMM), biphasic (BMM) and sarcomatoid (SMM) types [90] (Figure 6).

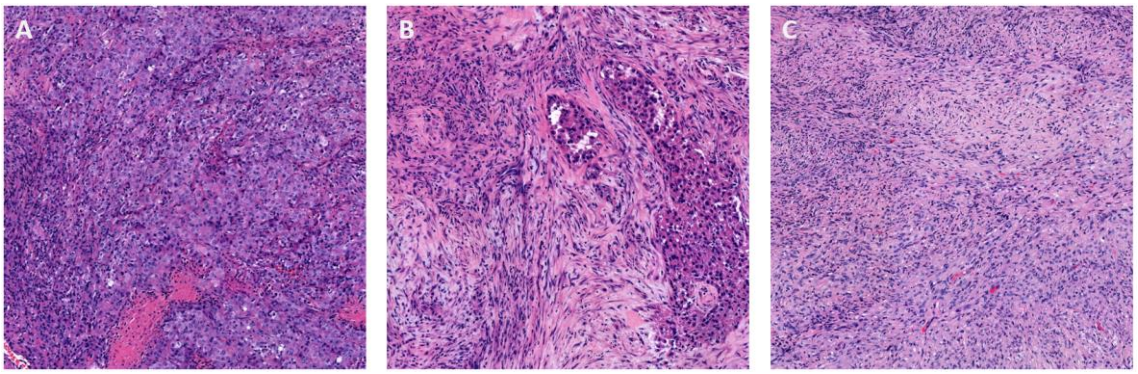

Figure 6. The major histological types of mesothelioma are (A) epithelioid, (B) biphasic and (C) sarcomatoid. (HE, 100x, author's photomicrographs).

According to the recent IMIG recommendations, the distinction between these three subtypes is a mandatory part of the pathological reporting of all MPM cases, because the histological subtype is one of the most robust prognostic factor in MPM known to this date, and also influences crucial treatment decisions [63].

EMM is associated with the longes overall survival (12–27 months), BMM confers intermediate prognosis (8–21 months OS), while SMM is associated with dismal prognosis (7–18 months OS) [42]. Patients with EMM and BMM are more often treated by radical surgery, than those with SMM. EMM shows a survival benefit associated with multimodal therapy, however, the data in relation to BMM is unclear, and patients with SMM do not appear to benefit from macroscopic complete resection [91-93].

1.3.1 Epithelioid type

Epithelioid type MPMs are usually composed of bland, mostly cuboidal tumor cells with eosinophilic cytoplasm and inconspicuous nuclei, however marked atypia can also be present in a fraction of the cases. Mitoses are tipically infrequent [90].

EMM has a wide range of morphological subtypes, and often shows various growth patterns within the same tumor. The most common growth patterns of EMM include the solid pattern that consists of sheets of monomorphic, mostly cuboidal cells without specific architectural arrangement. Tubulopapillary EMM is composed of a mixture of tumor cells arranged around fibrovascular cores and tubular structures. The microcystic variant is composed of structures similar to adenomatoid tumors, forming a lace-like architecture of cysts of variable size. Microcystic morphological variants are sometimes associated with a myxoid stroma. The trabecular subtype is characterized by tumor cells arranged in thin rows embedded in desmoplastic stroma. Micropapillary subtype tumors are composed of small papillary structures lacking a fibrovascular core [90]. The pleomorphic variant is characterized by prominent giant cells and anaplastic tumor cells, often with multiple nuclei, nuclear enlargement and hyperchromasia [94]. The rare histological variant composed of plump, elongated epithelioid cells with marked cellular borders and a sheet-like growth has been termed transitional pattern [95]. EMM not only shows a wide variability in growth patterns, but also exhibits unconventional cytologic features in a minority of the cases. Variant cytologic features of EMM include deciduoid [96], lymphohistiocytoid [97] small cell [98], rhabdoid, signet ring and clear cell features [99].

The prognostic role of histomorphology, with an emphasis on growth patterns has been studied extensively in a variety of solid malignancies [100-102]. The 2011 IASLC/ATS/ERS proposal recommended the use of an architecture based classification for invasive lung adenocarcinoma (LADC), introducing the lepidic, acinar, papillary, solid and micropapillary predominant histological subtypes [103]. Growth patterns of lung adenocarcinomas have since been established as independent prognostic factors by several studies [104, 105]. A predominant lepidic growth pattern of LADC shows an indolent clinical behavior and excellent 5-year survival after surgical resection [106, 107]. Predominant solid and micropapillary patterns, however, were associated with

significantly shorter OS [108, 109]. The presence of non-predominant solid or micropapillary patterns in resection specimens was associated with intermediate patient outcomes: significantly worse than those without such areas, however, significantly better than those with predominant solid or micropapillary growth [110]. Solid and micropapillary patterns were associated with an elevated risk for lymph node metastases [106], and in case of solid predominant tumors with multiplex, early, extrathoracic recurrences [111]. Growth pattern based classification of LADC is not only of prognostic relevance, but might be associated with distinct driver gene alterations [112-114], as well as a predictor of patients benefiting from adjuvant chemotherapy after surgical resection [115, 116]. Patients with early stage disease and a solid or micropapillary predominant component are found to benefit from adjuvant chemotherapy, while no significant benefit from adjuvant chemotherapy was found in the patients subgroup with acinar or papillary patterns [117]. Similarly, on eximanation of small biopsies of advanced stage patients receiving adjuvant platinum based therapy high-grade (micropapillary and solid predominant) patterns were significantly associated with an increased progression-free and overall survival in comparison to intermediate grade tumors [118].

In contrast to lung cancer, limited data is available in the literature on the potential prognostic role of the different predominant patterns in malignant pleural mesothelioma. A study analyzing 114 EMM samples found 16 myxoid-microcystic variants and identified it as positive prognostic factors in EMM, being associated with significantly longer OS than solid, micropapillary and pleomorphic subtypes [119]. Another study found predominant solid pattern tumors associated with worse patient outcomes in comparison to non-solid variants among 708 EMM samples [120]. The tumors showing a transitional pattern are associated with exceptionally short OS [95]. Similarly, the pleomorphic subtype also shows an association with dismal clinical outcomes comparable to that of BMM [94, 95, 119, 121, 122]. Although the 2015 WHO classification of MPM included the pleomorphic and transitional patterns among the variants of EMM, the 2019 proposal of European Network for Rare Adult Solid Cancers/International Association for the Study of Lung Cancer (EURACAN/IASLC) on histologic classification of MPM includes these two patterns not only in the EMM,

but also among the subtypes of SMM based on their dismal prognosis, a finding, however, that still needs confirmation [99].

Several studies investigated cellular features of mesothelioma tumor cells in correlation with patient outcomes. The presence of necrosis was found to be associated with worse prognosis in multiple studies, as well as the degree of nuclear atypia and elevated mitotic counts [120, 123, 124]. Additionally, more delicate nuclear and cytological features were also evaluated in EMM. While the presence of atypical mitoses and prominent nucleoli showed significant prognostic power, intranuclear inclusions and a low cytoplasmic/nuclear ratio did not exhibit such properties, and the impact of chromatin architecture and density is still ambigous [123, 124].

The resently established nuclear grading system predicts patient outcomes. It is based on a three-tier assessment of nuclear atypia, and a three-tier scoring of mitotic counts. These scores are combined into nuclear grades I to III [120, 123].

A further grading system, the recently proposed mitosis-necrosis score is computed based on the presence of necrosis and a two-tier scoring of mitotic figures, the cut-off value being 5 per 10 high power fields [120]. In a recent validation study, both the three-tier nuclear grading and the mitosis-necrosis score was confirmed to be useful in predicting patient outcomes in a cohort where 87% of the tissue samples were small biopsies [125].

1.3.2. Differential diagnostics of EMM regarding other carcinomas in the lung

The distinction between EMM and lung carcinomas involving the pleura or pleural metastases is often challenging and requires the use of immunohistochemistry. Due to the variable specificity and sensitivity of the commonly used antibodies, the IMIG guideline for the diagnosis of MPM recommends the use of a minimum of two positive markers for confirmation of mesothelial origin, and two negative markers to exclude carcinomas [63].

Lung malignancies and metastases involving the pleura are far more common than MPM, thus, it is important to use a panel of organ-specific immunhistochemical markers selected based on the patient’s clinical history and the differential diagnosis. The most commonly applied negative markers are listed in Table 2.

Table 2. List of immunohistochemical markers commonly negative in MPM and positive in carcinomas that frequently involve the pleura either through direct infiltration or through metastases [63, 90].

Markers negative in MPM and positive in carcinomas		
	Sensitivity	Specificity vs. MPM
Lung squamous cell carcinoma		
P40	100%	97.5%
Claudin 4	95%	0%
MOC31	97-100%	85-98%
BG8	80%	93-97%
Adenocarcinoma markers		
MOC31	95-100%	85-98%
Ber-EP4	95-100%	74-87%
BG8	90-100%	93-97%
CEA (monoclonal)	80-100%	> 95%
Markers of lung origin		
TTF-1	80%	High
Napsin-A	80%	High
Markers of breast origin		
GCDFP15	30-40%	High
Mammaglobin	50-85%	High
Markers of renal origin		
PAX8	70-100%	Unknown
PAX2	80%	Unknown
Claudin 4	90%	0%
CD15 (Leu-M1)	60%	High

The most commonly used mesothelial markers include WT-1, calretinin, podoplanin (D2-40 and CK 5/6 [90]. The sensitivity and specificity of these markers are described in detail in Table 3.

Table 3. The most common and sensitive mesothelial markers used in the immunohistochemical diagnosis of epithelioid MPM, and their reactivity in lung squamous cell carcinoma and adenocarcinoma [63].

Positive markers for epithelioid mesothelioma				
Marker	**Staining in MPM**	**Positivity in MPM**	**Positivity in lung squamous cell carcinomas**	**Positivity in lung adenocarcinomas**
WT-1	Nuclear, diffuse, strong	70-95%	˜0%	˜0%
Calretinin	Nuclear and cytoplasmic, often diffuse, strong	˜100%	40%	5-10% (usually focal)
Podoplanin (D2-40)	Membrane positivity, diffuse	90-100%	50%	<15%
Cytokeratin 5/6	Citoplasmic, diffuse	75-100%	100%	2-20% (focal)

Discrimination between reactive mesothelial proliferations and EMM is yet another diagnostic challenge. In addition to morphological characteristics, immunohistochemical detection of the loss of nuclear BAP1 staining is useful. The loss of nuclear BAP1 staining was detected by immunohistochemistry in 40-77% of epithelioid MPMs [126-128] and was found to be significantly associated with nonsynonimous genetic alterations of the BAP1 gene [129]. BAP1 negativity was exclusively observed in MPM but not in benign, reactive lesions of the pleura [130]. In a further study, the sensitivity, specificity, positive predictive value, and negative predictive value of the loss of nuclear BAP1 staining was estimated to be 61%, 100%, 100%, and 32%, respectively [127]. Another study proposes that combined use of MTAP – a highly sensitive surrogate marker of 9p21 deletions, a common event in

MPM – and BAP1 immunohistochemistry improves sensitivity of the distinction between MPM and benign proliferations [131].

1.3.3. Biphasic type

BMM contains an epithelioid component intermixed with a sarcomatoid or spindle cell component, both constituting at least 10% of the tumor area.

The diagnosis of biphasic MPM often represents a diagnostic challenge. In an international interobserver agreement study 42 patients' MPM samples originally classified as biphasic MPM were reviewed by fourteen pathologists with special interest in mesothelioma. The 544 expert opinions on the diagnosis for 42 cases showed moderate interobserver correlation (weighted κ-value=0.45). The original diagnosis of BMM was agreed in 71% of the cases, in 17% the case was reclassified as EMM, and in 12% as pure SMM [95].

The identification of a sarcomatoid component is of outmost importance, since it is a negative prognostic factor and is associated with worse patient outcomes in a radical surgery setting [95]. Both the WHO and the EURACAN/IASLC recommend that the amount of spindle cell component be reported because of its possible prognostic role [90, 99]. Patients with BMM containing less than 20% sarcomatoid elements were found to have significantly longer median OS [95], while another study reported a similar association between the amount of sarcomatoid elements and OS using a cutoff or 50% [132].However, a frank sarcomatoid component of BMM is hard to be distinguished from reactive fibrosis accompanying an epithelioid MPM. The malignant spindle cell population almost invariably shows an at least focal positivity with pancytokeratin antibodies [133] and broad spectrum anti-keratin cocktails such as AE1/AE3 [90]. Reactive fibroblastic proliferations might also be positive with pancytokeratins, but are arranged in regular fascicles that respect mesothelial boundaries, in contrast to the haphazard appearance of a malignant proliferation [63]. Other ancillary techniques are helpful in this setting, such as the BAP-1 immunhistochemical staining [134] and the detection of homozygous p16/CDKN2A deletion by fluorescent in situ hybridization (FISH). The homozygous deletion of p16/CDKN2A was detected in 94.7% of BMM, and the concordance between the

p16/CDKN2A status of the epithelioid and sarcomatous component was 100%. The non-neoplastic fibrous stroma showed intact p16/CDKN2A status in 100% of the cases. The loss of the nuclear BAP-1 staining was reported in 38.5% of the BMM cases, but no such loss was observed in the atypical fibrous stroma of EMM cases [135].

1.3.4 Sarcomatoid type

SMM is composed fascicles of spindle cells arranged in a haphazard pattern. The sarcomatoid tumor cells show remarkable morphological variability, and might have plump or thin cytoplasm, nuclei with various degree of atypia and exhibit a wide range of mitotic counts [90]. Heterologous elements such as rhabdomyo-, osteo- or chondrosarcomatous components might be present [136]. Desmoplastic mesothelioma is a distinct subtype of SMM, and is characterized by dense, eosinophilic, hyalinized stroma, and bland, atypical spindle cells forming no remarkable structure (also known as patternless pattern) [90]. The pleomorphic and transitional patterns -currently regarded by the WHO classification as variants of EMM - might be reclassified as SMM subcategories in the future [94, 95].The main differential diagnoses for SMM are various metastatic or primary soft tissue sarcomas, which are mostly CK negative, while virtually all SMM show at least focal CK positivity [133]. The diagnostic role of broad spectrum keratins as positive markers is especially important, since mesothelial markers, such as WT1 and calretinin, only stain SMM cells in about 50% of the cases, and the D2-40 immunostaining, while highly sensitive, lacks specificity [137, 138]. GATA3 recently emerged as a positive marker for SMM that might play a role in distinguishing between SMM and the sarcomatoid carcinoma of the lung [139]. The homozygous deletion of p16/CDKN2A was detected through FISH in 100% of SMM samples by a recent study [140]. The diagnostic challenge of discriminating organizing pleuritis from low grade sarcomatous or desmoplastic MPM is similar to BMM.

1.4 Treatment modalities in MPM

1.4.1 Systemic therapy

Since the early 2000s the first line treatment of MPM patients not eligible for surgery has been a combination chemotherapy based on antifolate and platinum agents [141]. Cisplatin in combination with either raltitrexed or pemetrexed improves overall survival in comparison to cisplatin alone [8, 142, 143], and the combination of cisplatin and pemetrexed is the most commonly used frontline treatment to date [144]. Carboplatin is also an acceptable alternative to cisplatin in combination with antifolates, and might be better tolerated for patients of elder age or comorbidities [145]. In unresectable cases the median overall survival achievable through combination chemotherapeutic treatment was found to be approximately 12 months in a randomized trial [142], while on a population-based level median overall survival of patients treated with chemotherapy increased from 10.1 months observed before the introduction of combined chemotherapy to 13.1 months after that [146]. The combined use of bevacizumab and cisplatin plus pemetrexed provided significantly longer OS for patients newly diagnosed with MPM, and improved 20-month survival rates to 90%, and 40-month survival to 20% in contrast to 77% and 16% achived through cisplatin-pemetrexed only [147]. Based on these findings the combination containing bevacizumab is now included among the first line treatment regimens in the National Comprehensive Cancer Network's Guidline [148].

The single randomized trial comparing the patient outcome between active symptom control (ASC) alone and ASC in combination vinorelbine as first-line chemotherapy treatment found a 2-month survival benefit for the latter group (7.6 months vs. 9.5 months) [149]. However, there is no established biomarker recommended for standard use for the prediction of patient's response to first-line chemotherapy [141].

Among patients receiving first-line chemotherapy the median time to progression is 5 months, and 25% of the patients are refractory to first-line agents, thus, a large number of patients receive second-line treatment [13]. In spite of all efforts in developing efficient options for patients after progression of disease, no validated second line treatment of MPM has been established so far [150]. Vinorelbine [151] and gemcitabine alone [152] are both commonly used in this clinical setting, and have shown efficacy in

retrospective studies, but due to the study designs interpretation of these data remains difficult. Premetexed has been found to be effective as a single agent [153], however, its common inclusion in first-line regimens limits its use in second-line in a variety of cases, although rechallenge therapy remains an option still to be evaluated [154].

1.4.2 Radiotherapy

The application of RT alone is not recommended because of its poor efficacy and is only used either as part of palliative care in an attempt to control chest pain and other tumor mass related obstructive symptoms, or in multimodality treatment protocols [8, 155]. The results of RT in terms of local control are complicated by the complex growth of tumors along interlobar fissures and into diaphragmal recesses. The associated toxicity is high due to the vicinity of vital organs including the remaining lungs after pleural decortication [8, 156].

Recent retrospective studies analysed patient outcomes after receiving either intensity modulated radiation therapy or 3D conformal radiation as part of multimodality therapy. One study found that of 2846 patients undergone surgical treatment, 213 (7%) received adjuvant RT. The study found a survival benefit after adjuvant RT only in stage I-II patients ($p=0.024$) in contrast to stage III ($p=0.890$) and IV patients ($p=0.183$) [157]. Another study analysed data of 24914 patients, 23.8% received surgical therapy only, and 3.1% surgery plus at least 40 Gy radiaton therapy. The two subgroups had 16.59 months and 21.4 months OS, respectively ($p<0.001$). In multivariable analysis, receiving chemotherapy, surgery plus radiotherapy and a higher socioeconomic status were found to be independent predictor of improved survival [158]. Analysis of retrospective data of The National Cancer Data Base in the United States identified IMRT as the most commonly used technique for adjuvant RT, and did not find a significant difference among patients receiving 3D CRT or IMRT [159].

1.4.3 Surgical therapy

Only few cases are eligible for radical intent surgery, mostly young patients with localized disease, good performance status and epithelioid histology [150, 160]. The

aim of radical procedures is to remove all visible tumor tissue, however, due to the highly complicated location of these tumors it is virtually impossible to achieve microscopically confirmed complete tumor-free resection margins [8]. The surgical procedures currently applied with the intent of achiving macroscopic complete resection include extrapleural pulmonectomy (EPP, also known as pleuropneumectomy), which involves the en bloc resection of both the parietal and visceral pleura, as well as the ipsilateral lung, or lung sparing options pleurectomy/decortication (PD) or extended pleurectomy/decortication (ePD), also meaning the removal of both pleural plates, but - if required- with the removal of the diaphragm and/or pericardium [161].

In patients who underwent extrapulmonal pneumonectomy a median overall survival of 12 months was observed, while those having received pleurectomy/decortication treatment had 16 months median overall survival, with operative mortality rates of 7% and 4%, respectively [162]. Outcomes after EPP have been assessed in the MARS feasibility study, in which 50 patients all eligible for surgical resection were randomly assigned to either EPP plus hemithoracic irradiation of the affected side or to no EPP, both arms in combination with three cycles of platinum-based neoadjuvant chemotherapy and further adjuvant chemothrapy. In the no-EPP arm of the study an OS of 19.5 months (13.4-time not reached at the time of publication), while for patients receiving EPP as part of trimodality treatment OS was 14.4 months (5.3–18.7) [163]. Further systematic review of data on the efficacy and safety of EPP reported, that patients receiving EPP as part of trimodality treatment also involving adjuvant chemoradiotherapy had a median OS of 13-23.9 months, as well as perioperative mortality ranging between 0-11.8%, perioperative morbidity of 22-82% and major morbidity rates between 12.5 and 48% [164].

Due to a possibly more favorable patient outcomes, lower perioperative mortality rate and its feasibility for patients over 65 years [165, 166], as well as its superiority in QoL analyses [167] pleurectomy/decortication is becoming the preferred surgical intervention for MPM patients.

1.4.4 Multimodality treatment

Most guidelines on MPM management recommend the application of radical surgery only in a selected set of patients, in specialized centers, and favorably in combination with chemo- and/or radiotherapy [168].

In a study mainly including patients with epithelioid histology tumors (87.3%), a median OS of 35.6 months (15.4–42.6) and good locoregional disease control was observed among patients who were able to complete MMT. However, due to serious complications only 45% of the patients concluded induction chemotherapy, surgery and postoperative irradiation. Postoperative mortality was 11.1%, and 44.4% experienced major complications including rethoracotomy for haemothorax, acute respiratory distress syndrome, pulmonary embolism, cardiac or gastric herniation and bronchopleural fistula among others [169]. Further studies also suggest that surgery alone provides dissatisfactory results, and it be used in combination with other treatment modalities. However, questions regarding the preferred type of induction chemotherapy and radiation therapy are yet to be settled [170, 171].

1.4.5 Emerging therapeutic approaches

Given the dismal prognosis of MPM even in cases suitable for radical multimodal treatments, there are several novel therapeutic approaches currently tested in clinical trials, including antiangiogenic agents bevacizumab and nintedanib [147, 172], anti-mesothelin targeted therapy[173-175], anti-WT1 vaccination [176], arginin deprivation [177], dendritic cell vaccination [178], anti-CTLA4 antibodies [179], anti-PD(L)1 inhibitors [180, 181], FAK inhibitors [182], intrapleural viral therapy [183] (Figure 7). So far, neither of these approaches provided the anticipated substantial improvement in survival.

DOI:10.14753/SE.2020.2421

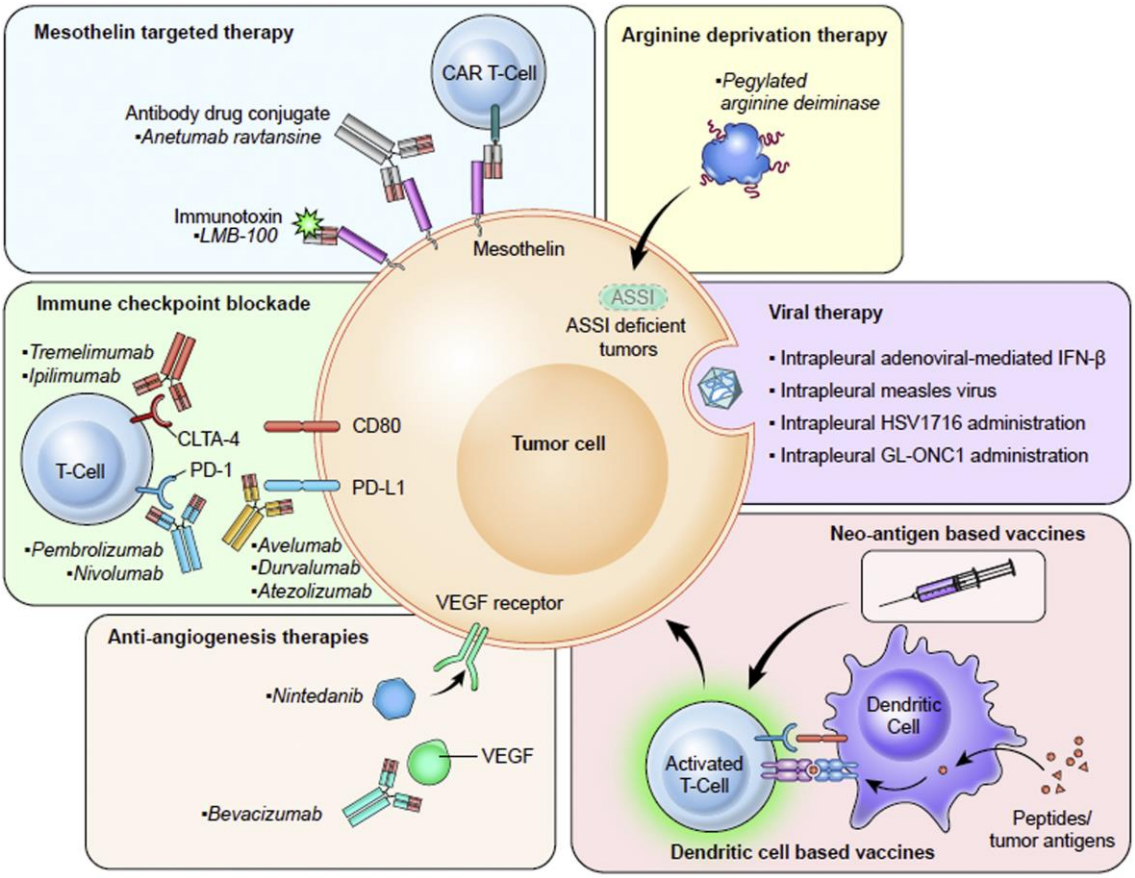

Figure 7. Summary of novel therapeutic approaches for MPM management. Reprinted with the permission of Elsevier from [184].

Bevacizumab is used in combination with cistplatin and pemetrexed as first-line treatment, thus, the potential role of other angiogenesis inhibitors was also investigated. In the phase 3 trial the addition of nintedanib to cisplatin and pemetrexed was compared to placebo plus cisplatin and pemetrexed in MPM patients not receiving surgical resection. The trial failed to confirm any of the positive effects of nintedanib on outcomes previously observed in a phase 2 trial [172].

There are various genetic alterations in MPM that might be tested in biopsy samples and predict which molecularly targeted therapeutic approach is most likely to be beneficial for the patient (Figure 8).

DOI:10.14753/SE.2020.2421

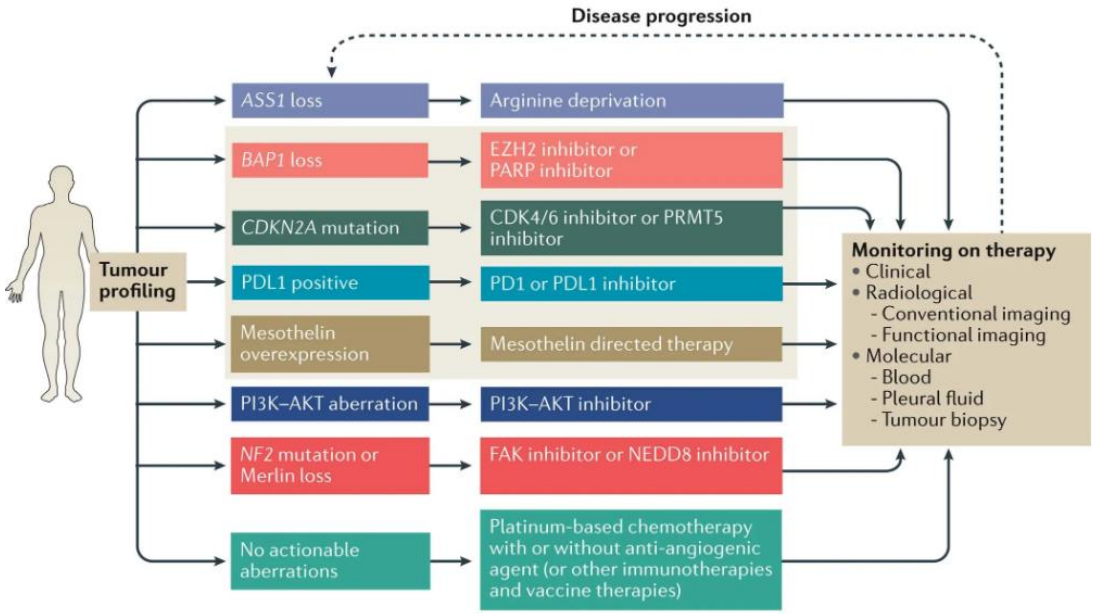

Figure 8. There are several genetic alterations wich are proposed to have a predictive implication in targeted therapy of MPM. Reprinted with the permission of Springer Nature from [42].

Tumors harboring NF2 mutations might be targeted by FAK inhibitors, such as defactinib. However, a phase 2 clinical trial failed to prove any statistical difference in PFS, OS or quality of life between patients who after first line chemotherapy received defactinib maintenance treatment versus those who received placebo, and the result was found to be independent of NF2 mutation status [182].

Another potential target is the subgroup of ASS1 (argininosuccinate synthetase 1) deficient MPMs. The use of arginin-lowering agent ADI-PEGO20 in a phase 2 trial involving 68 patients, has provided a statistically significant improvement in median PFS (3.2 months versus 2.0 months) [177].

The anti-CTLA4 antibody tremalimumab failed to increase OS in patients pretreated with first and second line chemotherapy [179]. PD1 inhibition is studied in several clinical trials, of which the most promising so far has achieved 12-week disease control in 44% and 52% of the patients using either nivolumab or nivolumab plus ipilimumab, respectively [180]. The combination of durvalumamb and platinum plus pemetrexed chemotherapy has resulted of sufficient activity, a median PFS of 6.9 months and objective tumor response in approximately 50% of the cases [181].

To overcome the relatively immunosuppressing microenviroment typical of MPM, various immune-activating therapies have emerged and are currently tested in pilot studies involving a limited number of patients. Chimeric antigen receptor (CAR) T-cells extracted from the patients and then genetically engineered to be activated by MPM specific cell surface protein mesothelin and readministered the modified T-cells into the patients represent another novel direction that is currently being investigated in various solid malignancies [173]. Another approach is the presentation of allogenic tumor lysate to monocytes extracted from the patient and the re-injection of allogenic activated dendritic cells into the patient [178].

1.5 The molecular landscape of MPM

Molecular alterations in MPM include mutations and copy number alterations, as well as epigenetic changes. Strikingly, the most frequently involved genes are tumor suppressors and regulators of gene expression (Figure 9).

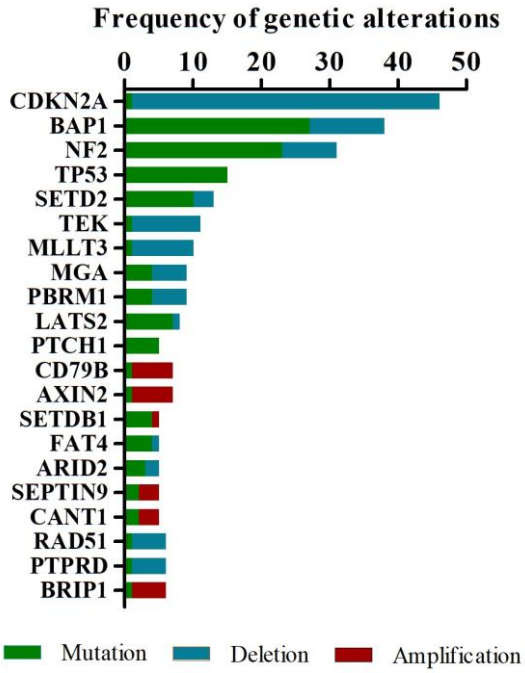

Figure 9. Frequency (%) of genetic alterations detected in MPM based on DNA-sequencing and copy number analysis of 87 samples from TCGA-MESO cohort [185]. and 22 samples published by Guo et al. [186]. Based on data downloaded from cBioPortal [187].

Despite the growing number of high throughput genomic analyses and the increasing data on the molecular characteristics of MPM, no frequent oncogenic driver has been discovered to this date [188-190].

1.5.1 Cell cycle regulation pathways

The CDKN2A locus encodes the p16^{INK4a} and p14^{ARF} tumor suppressor proteins that are inhibitors of the cell cycle as depicted in Figure 10. The protein p16^{INK4a} binds to cyclin dependent kinases (CDK4/6) and inhibits their kinase activity. Uninhibited CDK4/6 binds cyclin D1 and their complex phosphorylates the retinoblastoma protein (pRb) which releases the transcription factor E2F1. The latter protein promotes the transcripition of genes involved in the transition from G1 to S phase. The alternate reading frame product of CDKN2A, p14^{ARF} inhibits MDM2, thus, activates p53 and prevents its MDM2-mediated degradation [191, 192]. The activation of transcription factor p53 results in the transcription of numerous genes involved in cell cycle arrest, senescence, apoptosis and differentiation [193].

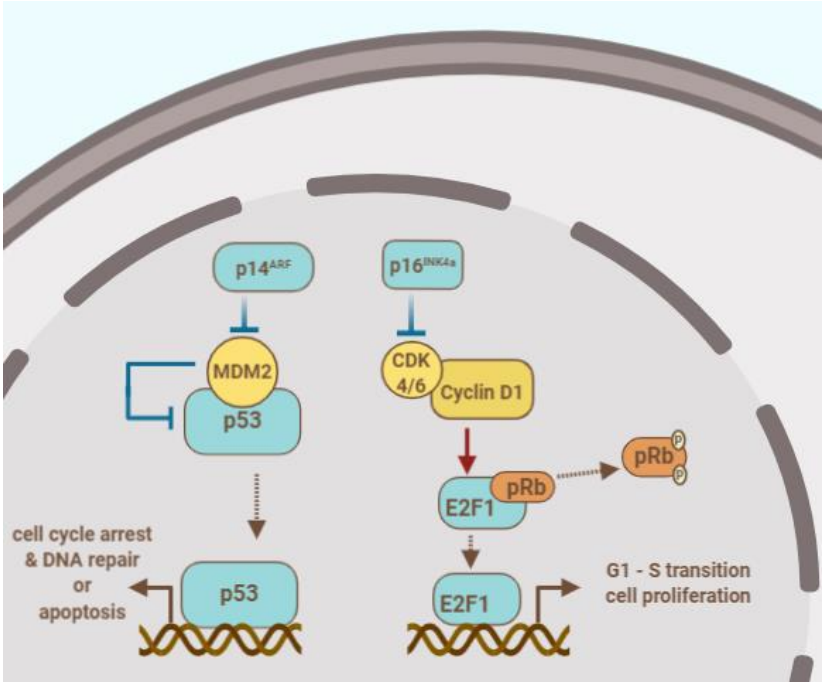

Figure 10: The products of the CDKN2A gene p14^{ARF} and p16 ^{INK4a} play a role in the regulation of cell cycle and apoptosis. The author's drawing based on: [191, 193].

The loss of CDKN2A locus through homozygous deletion of 9p21 occurs in 67-83% of all MPM cases, while its frequency is up to 100% in SMM [140, 186, 191, 194, 195]. Less frequent causes for p16 inactivation are hypermethylation and point mutations of the gene CDKN2A [196, 197]. Several studies reported a strong association between the loss of CDKN2A and significantly shorter OS in MPM patients [197-199].

Recurrent mutations in the gene TP53 are relatively infrequent in MPM [186, 194], however, its reported frequency varies widely and was found to be 57% in one retrospective study [129], while only 16% by another recent study [200]. The germline mutation of the TP53 gene is associated with the Li-Fraumeni cancer syndrome. The patients carrying this type of TP53 mutations frequently develop breast cancer, carcinomas of the adrenal cortex or sarcomas, however, are only occasionally diagnosed with MPM [28].

1.5.2 BAP1 and DNA damage repair

BRCA1-Associated Protein 1 (BAP1) is a deubiqitinating enzyme consisting of three main domains, namely the N-terminal ubiquitin carboxyl hydrolase domain, a middle portion containing binding sites for complex forming and a C-terminal domain also important in interactions with other proteins [44]. When located in the nucleus BAP1 acts as a tumor suppressor through regulation of the cell cycle and differentiation [201] and plays an essential role in the repair of double strand DNA break repair through an interaction with a variety of recombination proteins, such as Breast cancer type 1 susceptibility protein (BRCA1) and BRCA1-associated RING domain protein (BARD1) [202] as shown in Figure 11. When located in the endoplasmic reticulum (ER) BAP1 modulates intracellular calcium levels and promotes apoptosis [203]. Cells with an impaired BAP1 function show reduced mitochondrial Ca^{2+} levels, and as a consequence are more likely to show a metabolic shift towards aerobic glycolysis [204], and are not able to initiate the apoptotic process through a Ca^{2+} release from the ER [28].

DOI:10.14753/SE.2020.2421

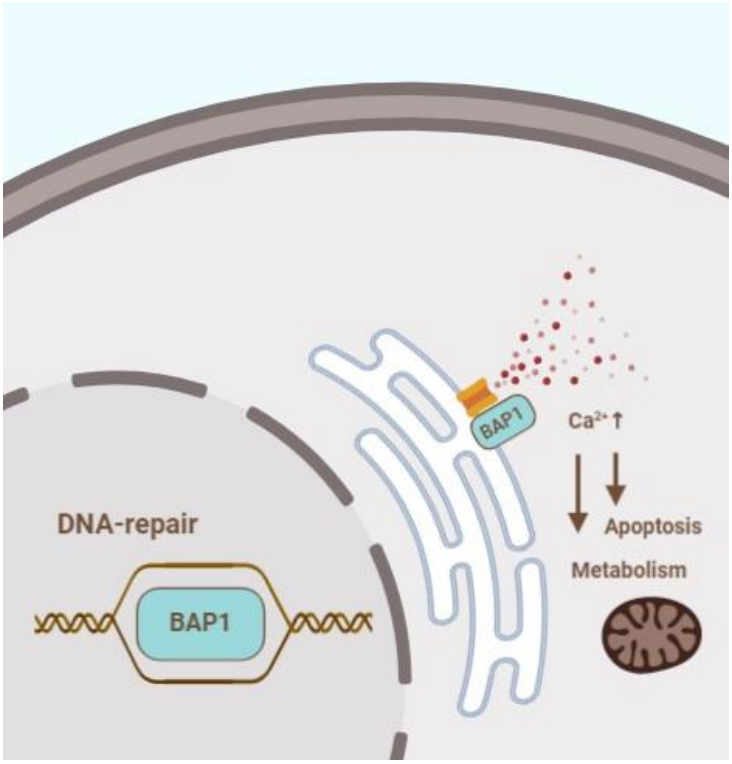

Figure 11. BAP1 has different functions in a nuclear localisation and in the endoplasmic reticulum. The author's drawing based on [28].

The gene encoding BRCA1-Associated Protein 1 (BAP1) is located on chromosome 3's short arm (3p21.1) and is one of the most frequently affected by genetic alterations in MPM. The frequency of alterations leading to the loss of BAP1 function is within a wide range between different studies, it is reported to occur in 23-63% of MPM cases [194, 200, 205-207]. The mechanisms of the inactivation of BAP1 include loss-of-function mutations, copy number loss of chromosome 3p21 and gene fusions [206]. Hotspot regions of the BAP1 gene with genetic alterations are exon 13 and 17, where a study identified variations in 38% and 25% of the patients, respectively [129]. The loss of nuclear localization of the BAP1 protein detected by immunohistochemistry correlates with the nonsynonimous variations of the BAP1 gene identified by next generation sequencing [129].

DOI:10.14753/SE.2020.2421

1.5.3 Hippo pathway

The Hippo pathway is a highly conserved signaling pathway that plays a regulatory role in organ growth, tissue regeneration and preventing tumorigenesis through restraining the cell cycle, controlling cellular differentiation and promoting apoptosis.

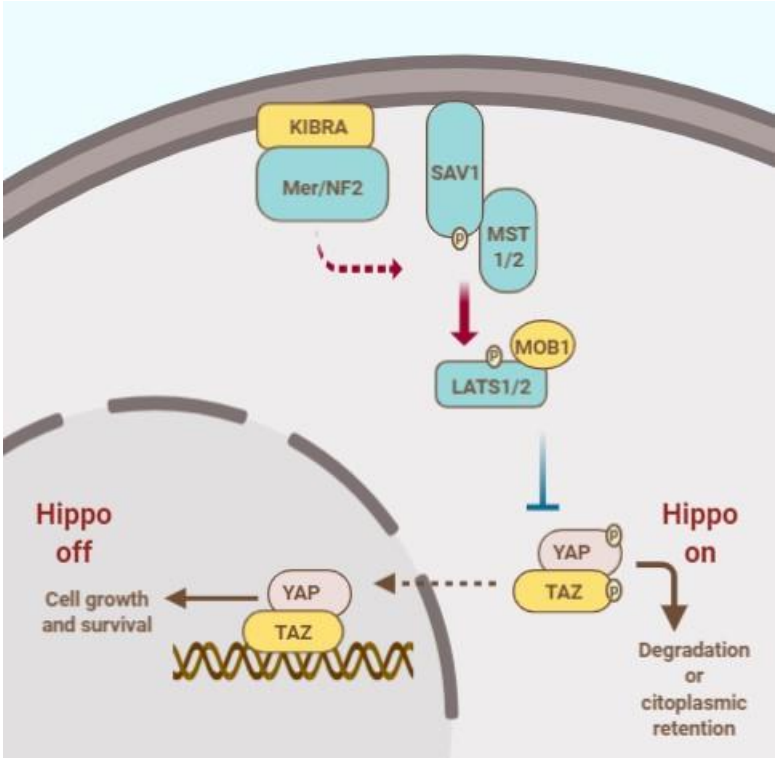

Figure 12. Main actors of the Hippo pathway. The author's drawing based on [208]

The kinase cascade of the Hippo pathway (Figure 12) include the MST1/2-SAV1 complex that activates the LATS1/2-MOB1A/B complex through phosphorylation, which then phosphorylates the YAP/TAZ complex. When phosphorylated, the nuclear effector YAP/TAZ transcriptional coactivators are excluded from the nucleus and thus inactivated. When the activity of the Hippo pathway is low, YAP/TAZ is able to enter the nucleus, where it interacts with transcription-enhancer activator domain transcripition factor (TEAD) and activates the transcription of several target genes involved in cell proliferation and the evasion of apoptosis. Upstream regulators of the pathway are reported to mediate extra- and intracellular signals, such as polarity, cellular interactions through adherens junctions, mechanical and other stress signals.

Among these regulators, NF2/Merlin and KIBRA are cooperating proteins located at the apical membrane of cells that interact with LATS1/2 and through the activation of the Hippo pathway mediate contact inhibition in cell cultures [208].

In MPM the loss of function alterations of genes NF2 and LATS1/2 occur relatively frequently, while alterations of MST1 and SAV1 are also reported [206]. The inactivation of the negative regulators of YAP/TAZ complex leads to the constitutive activation of the complex [209]. Although oncogenic alterations of the YAP1 and WWTR1 gene (encoding TAZ) are relatively frequent in triple-negative breast cancers, non-small cell lung cancer, it is a rare occurrence in MPM [210, 211].

The frequency of genetic abnormalities affecting the NF2 gene is reported to be 14-50%, mostly being missense, nonsense or splice site mutations, and less commonly losses of chromosome region 22q12 encoding NF2 [186, 194, 200, 205, 206, 212]. Alterations of the NF2 locus is reported to occure significantly more often in patients not exposed to asbestos [190].

Neurofibromatosis type 2 is associated with germ-line mutations in the NF2 gene, but this autosomal dominant disease is not associated with increased risk to develop MPM, even though there is an overlap between somatic mutations detected in MPM and those in hereditary neurofibromatosis [213].

The loss of function alterations of tumor suppressor LATS2 occur through the homozygous deletion of 13q12 encoding the LATS2 gene, which was reported to occur in 10 out of 45 MPM samples [214], or through somatic mutations of the LATS2 gene [206, 215]. The loss of LATS1 function occurs most frequently through a chromosomal translocation which leads to the fusion of the LATS1 and PSEN1 (presenilin-1) genes, and the fusion protein product lacks the kinase activity which is essential in the inhibition of YAP [215].

Other altered pathways include the mTOR, histone methylation and RNA helicases signaling pathways, which, however, occur in a small fraction of MPM cases [206].

DOI:10.14753/SE.2020.2421

1.6 Telomere and telomerase

1.6.1 Structure and function

The telomere region of eukaryotic chromosomes is located at the extremes of the chromosomes and is “capped” by a large nucleoprotein complex that prevents breakdowns and fusions between chromosome ends during mitosis [216]. Its DNA component contains several kb of the repetitive sentence d(TTAGGG) in humans [217]. Telomeric DNA is characterized by the protruding extreme of the G-rich strand, which is approximately 200 nucleotides long and is a consequence of the mechanism of terminal replication [218]. The overhang produced at the end of the lagging strand might form G-quadruplexes [219] or a T-loop which is a circle of curled-up single strand DNA forming a triple-strand structure at the very end, called displacement loop [220] (Figure 13A).

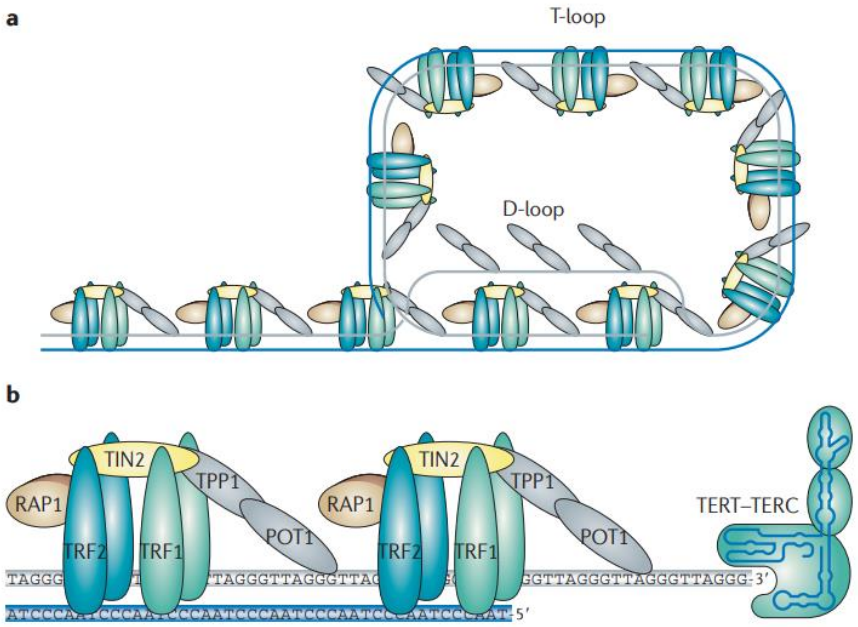

Figure 13. Telomeric region of human chromosomes. (A) G overhang forms a protective T-loop and through invading the dsDNA to form a D-loop. (B) Shelterin complex of telomere binding proteins protecting the chromosomes’ ends from triggering a DNA damage response. The telomerase complex consisting of the protein TERT and template RNA TERC components recognizes the 3’-end of the single strand G overhang and elongates it. Reprinted with the permission of Springer Nature from [221].

The telomeric region is bound by the shelterin complex in human cells that consists of six proteins, namely TRF1 and TRF2, RAP1, TIN2, TPP1 and POT1. TRF1 and TRF2 both need to build dimers to be able to bind to 5'-YTAGGGTTR-3' sequences of double strand telomeric DNA. POT1 interacts with single strand G overhang at 5'-TAGGGTTAG-3' sequences and interacts with the TRF1 and TRF2 homodimers through proteins RAP1, TIN2 and TPP1 (Figure 13B).

During cell division the length of the telomere decreases at each passage of the replication fork due to the inability of conventional DNA polymerases to fully duplicate the 3' end of linear DNA molecules [222]. Telomerase plays an essential role in maintaining chromosomal integrity by preventing the loss of genetic material caused by incomplete terminal replication and compensating for the shortening of the telomere region through *de novo* addition of TTAGGG repeats. The telomerase enzyme complex is a reverse transcriptase containing the catalytic subunit TERT encoded by the gene hTERT in humans and the RNA template component TERC [221] (Figure 13B).

Telomerase is physiologically expressed in a strictly regulated manner in germ cells and stem cells, but its activity is restrained in somatic cells [216]. Telomere repression is a mechanism for the prevention of uncontrolled cellular proliferation. In cells lacking telomerase expression the erosion of the telomere leads to the activation of DNA damage response pathway and cells enter senescence [223]. TERT also plays telomere-independent roles both in cooperation with the TERC RNA template and independently of that. Such functions of TERT include the regulation of targets of the Wnt-pathway, the genesis of double strand precursors of silencing RNAs, and the maintenance of mitochondrial fitness [224].

1.6.2 Telomere lengthening and telomerase in disease

Telomere shortening can lead to impaired tissue regeneration and accelerated aging. On the other hand, constitutive expression of the telomerase permits uninhibited cell division and immortalization but is also associated with increased chromosomal instability [225].

The constitutive expression of the TERT gene and telomerase activity is detected in 85-90% of all malignant tumors [223, 226], and is considered a hallmark of cancer

[227].There are several mechanisms underlying the telomerase reactivation and telomere lengthening in malignant cells.

Abnormal expression of positive regulators of the TERT gene such as the oncogene MYC induce TERT expression and leads to an increased telomerase activity [228, 229]. Epigenetic factors might also lead to an increase in TERT activity. The TERT promoter is generally not methylated in normal cells, however, hypermethylation of the promoter at the TERT hypermethylation oncological region (THOR) occurs in malignant cells and accounts for upregulation of TERT expression [230]. SMYD3 regulated histone H3-K4 trimethylation are factors leading to constitutive activation of the telomerase [231], as well as the recruitment of histone acetyltransferases or histone deacetylases that might cause telomere reactivation depending on the cellular context [232]. Viruses such as Epstein-Barr virus, cytomegalovirus, human papilloma virus, hepatitis B and C encode exogenous positive regulators of hTERT [233].

Telomerase-independent, recombination-based mechanisms of telomere maintenance, the so called alternative lengthening of telomeres (ALT) are reported in several human malignancies, and are associated with the loss of ATP-dependent helicase encoded by ATRX or the H3.3-specific histone chaperone DAXX both of which would otherwise repress ALT [234]. The loss of ATRX and DAXX function occurs most commonly in pancreatic neuroendocrine tumors [235] sarcomas [236, 237] and childhood glioblastomas [238].

According to recent data, rearrangements and focal amplifications of the TERT gene are relatively rare. Amplifications occur in approximately 4% of malignancies, but it is more common in lung adenocarcinomas and squamous cell carcinomas, as well as in ovarian cancer, adrenocortical and esophagus carcinomas [239]. Rearrangements have been identified in high-risk neuroblastomas, however do not seem to play a crucial role in telomerase derepression in other cancer types [240, 241].

1.7 TERT promoter mutations

1.7.1 Patomechanism of TERT promoter mutations

The TERT gene located on the short arm of chromosome 5 harbors a single proximal core promoter located at -330 to +37 upstream and downstream relative to ATG. The promoter region lacks conventional regulatory elements like CAAT and TATA boxes, however, has multiple binding sites for transcriptional factors, namely p53, c-myc, p21, SP1, ETS, E2F, HIF1 and AP1 [242]. There are three hotspots within the TERT promoter where mutations most commonly occur: at -124 (-124 C>T), -146 (-146 C>T), or -57 (-57 A>C), the mutations also frequently designated C228T, C250T and 23 A161C in the literature (Figure 14). Further recurrent TERT promoter mutations were identified in melanoma, namely tandem CC>TT mutations at -124/-125 or -138/-139 from ATG, which is likely of UV-related origin [243, 244]. All three of these point mutations create de novo ETS binding sites. Proteins GABPA and GABPB belonging to the ETS family form heterotetramers and are able to bind to the de novo ETS motif and activate the transcription of the TERT gene, thus, these non-coding mutations of the TERT promoter exert an oncogenic effect [223].

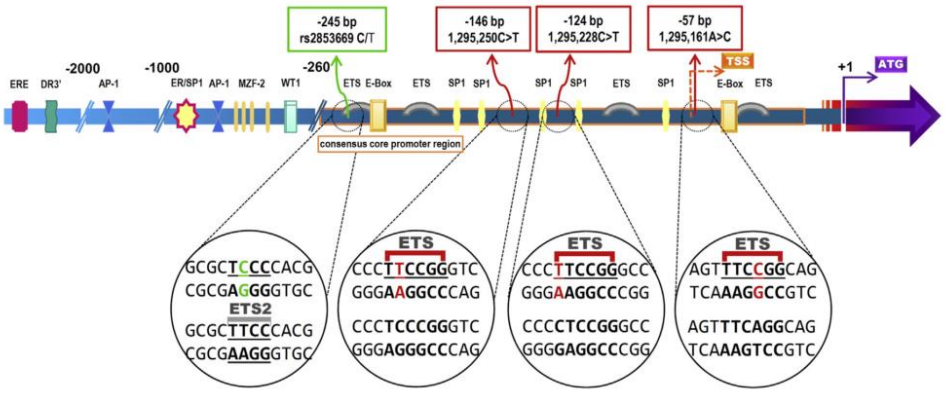

Figure 14: Hotspot mutations of the TERT promoter create de novo binding sites for members of the ETS transcription family, while T > C polymorphism rs2853669 at -245 bp disrupts a pre-existing ETS2 binding site. Reprinted with the permission of Elsevier from [245].

In reporter assays, TERT promoter mutations were associated with a two to four fold increase in promoter activity [244, 246, 247] and with a higher TERT expression in a variety of tumors [248-251] These findings indicate that these alterations in the TERT promoter are most likely to be drivers rather than passanger events in tumorigenesis [243].

1.7.2 Germline mutations

Germline mutations of hTERT may appear as autosomal dominant progeria (also known as dyskeratosis congenita) [252] or manifest in familial idiopathic pulmonary fibrosis [253]. In contrast with exon mutations, recently discovered high penetrance germline mutations of the TERT promoter region in positions -124 and -146 were found in a melanoma-prone family where patients presented with extensive melanoma history in their family and early-onset, advanced stage disease [244]. Interestingly, however, germline mutations have not been associated with any other tumor type.

1.7.3 Somatic mutations

The first studies investigating the clinicopathological relevance of TERT promoter mutations reported on its high frequency in malignant cuateneous melanomas [244, 246] The frequency of TERT promoter mutations have since been found to vary widely among malignant tumors (Figure 15). In addition to malignant melanoma the highest mutation rates are observed in basal cell carcinoma [254], glioblastoma, and urothelial bladder cancer, while hepatocellular carcinoma, thyroid cancer, head and neck squamous cell cancer [223]. The mutation rates are higher in poorly and undifferentiated thyroid carcinomas (42.9%, combined) in comparison to well differentiated papillary and follicular carcinomas of the thyroid (12.1% and 14.0%, respectively) [255]. Strikingly, in several common malignancies, such as breast, lung and colorectal cancer these genetic abnormalities occur rarely or not at all [223].

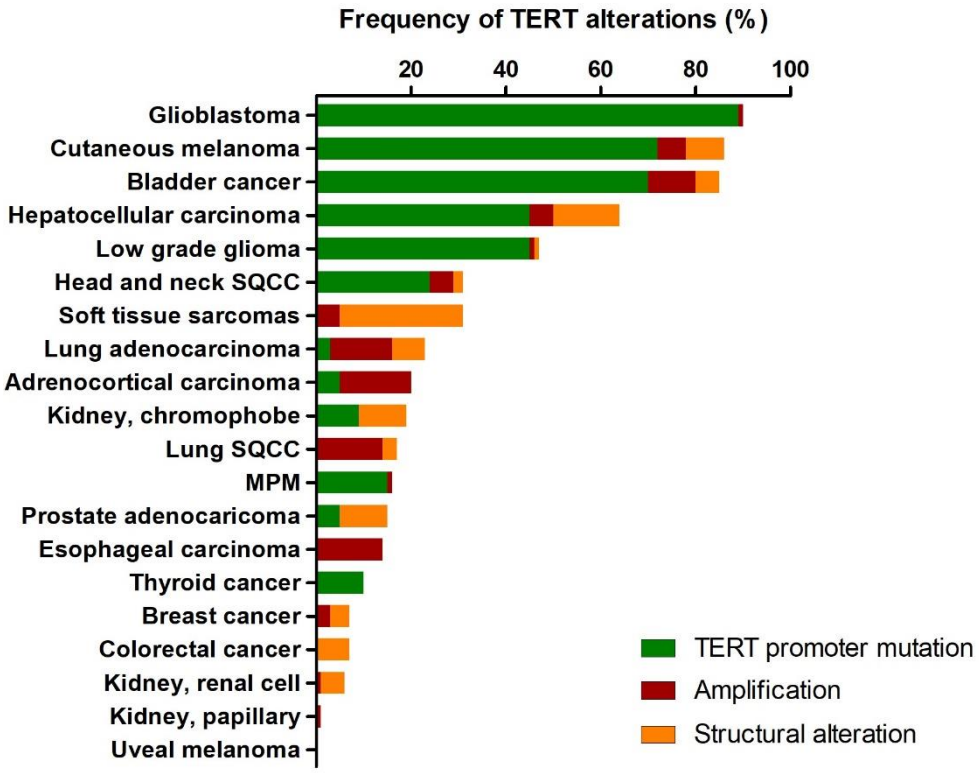

Figure 15. The frequency of TERT promoter mutations, TERT amplifications and other structural alterations in several solid malignancies based on the TCGA dataset [239] and data recently published on MPM [249].

In several malignancies TERT promoter mutations have been shown to be associated with unfavorable patient outcomes, eg., diffuse gliomas [256], primary glioblastomas [257], bladder cancer [258] and cutaneous melanoma [259]. Importantly, TERT promoter mutations are prognostic factors that help identify patients at higher risk for disease recurrence and disease specific death in potentially indolent tumors, such as papillary thyroid carcinoma [260], follicular thyroid carcinoma [261, 262] and meningioma [263].

1.7.4 Common single nucleotid polymorphism rs2853669

The common polymorphism rs2853669 C>T has been shown to modify the effect of oncogenic TERT promoter mutations through disruption of a preexisting putative Ets2 binding site in the promoter region. In cell lines carrying both the variant allele of the

SNP and the -124C>T activating TERT promoter mutation the promoter activity significantly decreased in comparison to cells carrying a mutation and the wild-type allele of the SNP, while only a moderate difference in activity was observed in cells harboring the -146C>T mutation. Regarding patient outcomes the presence of the variant allele dissolved the negative prognostic effect associated with TERT promoter mutations in bladder cancer, while noncarriers of the variant allele harboring a TERT promoter mutation showed a significantly worse overall survival and an increased risk for disease recurrence [258]. In glioblastoma patients TERT mutation only exerts a negative effect on OS in patients who do not harbor a homozygous variant allele (CC) of rs2853669 [264, 265]. Among TERT promoter mutant glioblastoma cases a significant difference in survival was observed between carriers of a homozygous variant allele (CC), heterozygous variant allele (CT) and those with the wild-type SNP (TT), the latter two being associated with hazard ratios of 4.7 and 10.7, respectively [266].

1.7.5 TERT in MPM

The reactivation of telomere function is reported to be present in virtually all MPM cases, while ALT activity is not detected in this tumor type [267]. The amplification of the 5p.15.3 chromosome region encoding TERT has been indentified in 1% of MPM samples according to TCGA database, while other studies report its frequency to be between 22 and 55%, however its association with the upregulation of TERT expression is not clear [249, 268]. There is limited data on the role of TERT promoter mutations in MPM. The sole study published so far reports the frequency of TERT promoter mutations to be 15.2% with 12 mutants identified among 61 MPM cell cultures (19.7%) and 8 promoter mutatns among 71 fresh frozen MPM tumor samples (11.3%). All the detected mutations occurred at the C228T hotspot. The TERT mRNA levels were significantly higher in cell cultures and tumor samples carrying the promoter mutation. Mechanisms of derepression of TERT in non promoter mutant cases remain unclear, however are suggested to include the expression of positive transcriptional regulator c-Myc, and epigenetic changes [249].

DOI:10.14753/SE.2020.2421

2. OBJECTIVES

We carried out comprehensive analyses of the histopathologic and molecular features of malignant pleural mesothelioma and evaluated their impact on patient outcomes. We have focused on the following objectives:

1. In a study involving five large Central European centers for the diagnosis and treatment of thoracic malignancies, we investigated the prognostic impact of nuclear grading, the newly proposed mitosis-necrosis score and the predominant growth patterns of epithelioid malignant pleural mesothelioma. We also evaluated the associations between these variables.
2. We aimed to find potential histomorphologic parameters that might be useful in recognizing patients who might benefit from a more aggressive, multimodal treatment, and those who do not benefit from such relatively high-risk therapies.
3. In a further multi-center study partially overlapping with the previous study, we evaluated the frequency of TERT promoter mutations in malignant pleural mesothelioma, its correlation with other clinicopathologic features and its power as a prognostic factor.
4. We also investigated the potential interaction between the common polymorphism rs2853669 and TERT promoter mutation in MPM.
5. We have carried out *in vitro* experiments to study the mechanisms underlying the aggressive clinical behavior of TERT promoter mutant MPMs, and determined cell line forming ability, TERT mRNA expression and in vitro cisplatin sensitivity in cell lines with wild-type or mutant TERT core promoter region.
6. We evaluated the association between the TERT promoter mutant genotype and the samples BAP1 status through immunohistochemical staining with anti-BAP1 antibody.

DOI:10.14753/SE.2020.2421

3. METHODS

3.1. Patient cohorts

3.1.1 Study cohort in histologic subtype analysis

Our multicenter study cohort consisted of 192 patients diagnosed with epithelioid MPM. The patients were diagnosed and treated in five large Central European centers: 67 patinents at the University Clinic of Respiratory and Allergic Diseases between 2007 and 2012, Golnik, Slovenia, 54 patients at the Medical University of Vienna, Vienna, Austria between 1994 and 2015, 32 patients at the University Medicine Essen - Ruhrlandklinik, Essen, Germany between 2016 and 2018, 30 patients between 2000 and 2007 at the National Korányi Institute of TB and Pulmonology, Budapest, Hungary, 9 patients at the University of Zagreb, School of Medicine, Jordanovac, Croatia between 2013 and 2014 (Table 4).

Table 4. Number of patients included from five Central European centrums, intervals of original diagnoses and median follow up time.

Center	Number of Cases	Time period of diagnoses	Median follow-up
Golnik, Slovenia	67	2007-2012	498 days
Vienna, Austria	54	1994-2015	426 days
Essen, Germany	32	2016-2018	340 days
Budapest, Hungary	30	2000-2007	326 days
Zagreb, Croatia	9	2013-2014	387 days

All 192 cases were originally diagnosed epithelioid type MPM. The diagnoses were made by pathologists experienced in thoracic malignancies, adhering to international histological and immunohistochemical criteria requiring a minimum of two positive mesothelial markers (calretinin, WT-1, D2-40, CK 5/6) and at least two negative markers for carcinoma (such as Ber-EP4, TTF-1, CEA).

Clinical data were collected in accordance with the latest Declaration of Helsinki as well as with each institute's ethical guidelines, and included patients' age, gender, date

of diagnosis, and date of death or last contact. The retrospective analysis of MPM patients was approved by the local ethic committees in each participating center: at the Medical University of Vienna (#904/2009), the University Hospital Center Zagreb (#02/21AG) and at the University Medicine Essen (17-7773-BO). The Institutional Review Boards granted a waiver for the retrospective analyses at the University Clinic Golnik and at the National Koranyi Institute of Pulmonology.

The 192 tissue samples included in the analysis were obtained through video-assisted thoracoscopy (n=106), pleurectomy (n=28) or percutaneous pleural needle core biopsy (n=28). In 30 cases the diagnostic procedure was not specified. All samples were formalin fixed, paraffin embedded and HE stained.

As a validation cohort, we also analyzed 55 virtual slides of epithelioid MPMs openly available at the Cancer Digital Slide Archive which are digitalized diagnostic sections of specimens collected by The Cancer Genome Atlas [269]. Among these 55 sections there were 6 frozen sections and 49 FFPE specimens, and all of them were HE stained. Corresponding survival data and additional clinical variables collected by TCGA Research Network [270] were downloaded from the cBioPortal [187].

3.1.2 Study cohort in the TERT promoter mutation analyses

For the evaluation of the TERT promoter mutations' impact in MPM we analyzed samples of 182 MPM patients. The cohort included 83 patients diagnosed between 1994 and 2016 at the Medical University of Vienna, 76 patients diagnosed between 2007 and 2012 at the University Clinic Golnik and 23 patients diagnosed between 2013 and 2014 at the University of Zagreb.

This patient collective was partially overlapping with the cohort analysed in the histologic subtype classification study, however, included 53 non-epithelioid tumor samples and 14 epithelioid tumor samples from Zagreb, Croatia that were excluded from our morphologic classification cohort because these were already included in a cohort published on the topic [121].

Clinical data were collected according to the Declaration of Helsinki and to each intitute's ethical guidelines as described in section 3.1.1. Clinical data included patients'

age, gender, histological diagnosis, date of diagnosis, date of last contact or date of death, Karnofsky performance score, IMIG stage, EORTC prognostic score.

3.2 Histological subtype analysis

Samples were reviewed and classified based on their predominant growth patterns. The patterns were defined based on the 4th edition of the WHO Classification of Tumours of the Lung, Pleura, Thymus and Heart [90], and detailed in chapter 1.1.2.1 of the present work (Figure 16).

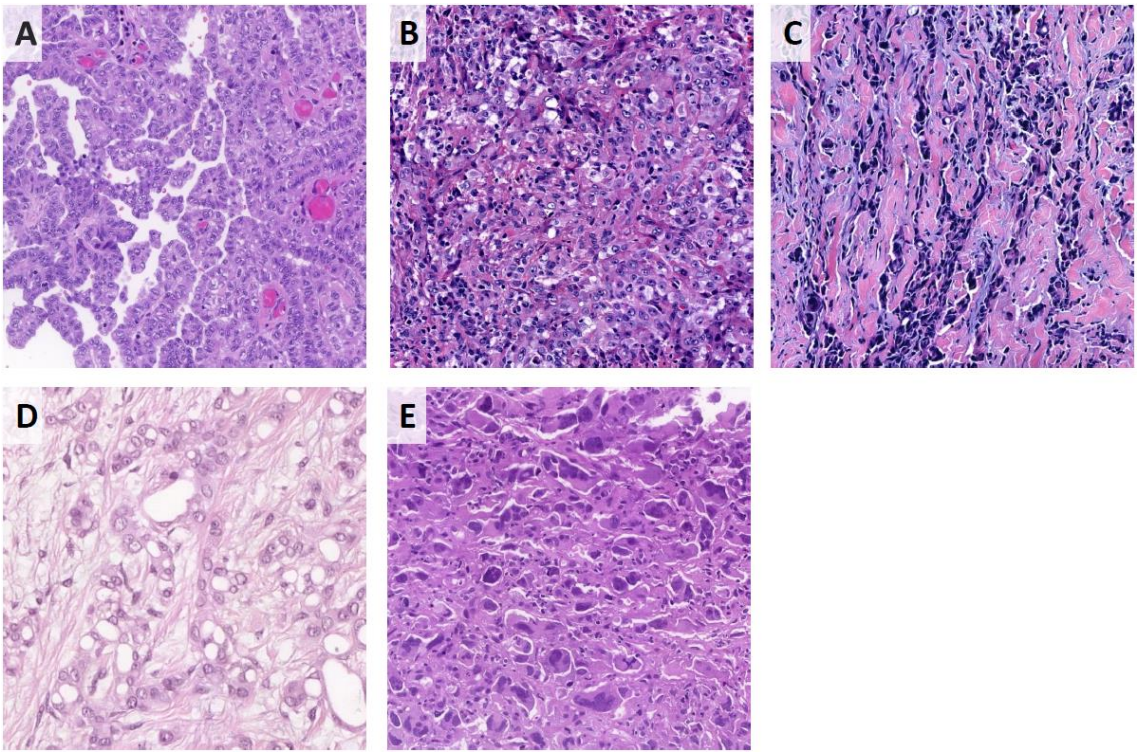

Figure 16. Main histomorphologic variants of epithelioid MPM. (A) Tubulopapillary pattern (HE, 200x). (B) Solid pattern (HE, 200x). (C) Trabecular pattern (HE, 200x). (D) Microcystic pattern (HE, 200x). (E) Pleomorphic features (HE, 200x). [271]

DOI:10.14753/SE.2020.2421

3.3 Histological grading

Nuclear atypia was assessed at 400x magnification and in the region of tumor exhibiting the highest degree of atypia adhering to the guidelines established by a recent study. Briefly, atypia was classified as mild if the tumor cell nuclei were small, uniform, lacked prominent nucleoli. If tumor cells contained nuclei of intermediate size and variable size and prominent nucleoli, atypia was considered moderate. Atypia was severe if more than 5% of the tumor cells contained macronucleoli, were multinucleated, and if nuclei were bizarre and enlarged (Figure 17) [120]. Nuclear atypia scores of 1, 2 and 3 were given to mild, moderate and sever atypia.

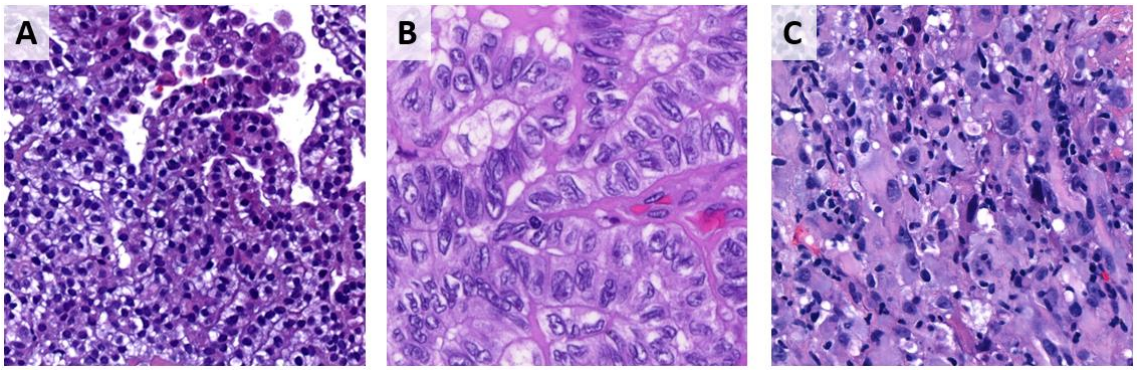

Figure 17. Different grades of nuclear atypia. (A) Mild nuclear atypia (HE, 400x). (B) Moderate nuclear atypia (HE, 400x). (C) Severe nuclear atypia (HE, 400x) [271].

Mitoses were counted at 400x magnification in hot spots and given as an average of mitotic figures per 10 high power fields (Figure 18 A and B). Mitotic counts between 0-1/HPF were considered low, intermediate if 2-4/10HPF and high if 5 or more/10 HPF. Tumors exhibiting low, intermediate and high mitotic activity were given scores of 1, 2 and 3, respectively.

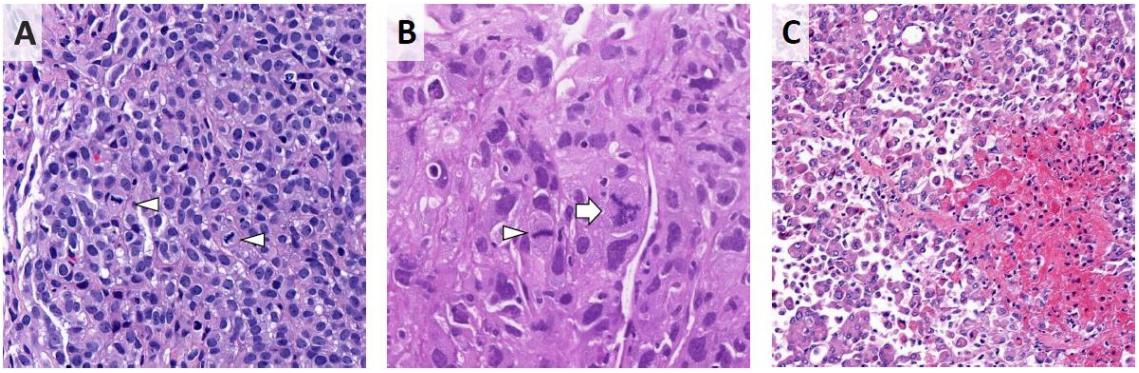

Figure 18. A) Bipolar mitoses. (arrowheads, HE, 400x) B) Typical (arrowhead) and atypical (arrow) mitoses (HE, 400x). C) Coagulative necrosis (HE, 200x) [271].

The composit nuclear grade was computed by the addition of nuclear atypia and mitotic scores. Nuclear grade I was assigned if combined scores were 2-3, nuclear grade II for scores 4-5 and to the maximum score of 6 nuclear grade III was assigned [120, 123].
The presence or absence of necrotic areas was evaluated at 400x magnification and used to compute a mitosis-necrosis score (Figure 18 C). Tumor samples with mitotic counts ≥5 were given a score of 1, those with <5 were given 0. If necrosis is present, a score of 1 was given, if absent, a score of 0. By addition of scores for mitoses and necrosis the mitosis-necrosis score was computed which thus ranges between 0 and 2 [120].

3.4 BAP1 staining

We performed BAP1 immunohistochemistry on 75 FFPE MPM samples as described previously [272]. Briefly, 4 µm tissue sections were deparaffinized and rehydrated, then heated for 10 minutes in 10 mM citrate buffer (pH 6.0). The sections were incubated at room temperature for 1 hour with the primary antibody (BAP-1, Clone C-4, sc-28383, Santa Cruz; dilution 1:200). Antibody binding was detected using the UltraVision LP detection system (Lab Vision Corporation, Fremont, California). The BAP1 staining was evaluated in the tumor cells as described previously in the literature [134]. A sample was considered negative for BAP1 expression in the absence of nuclear reactivity, regardless of the presence or absence of cytoplasmic staining (Figure 19). Nuclear BAP1 positivity detected in lymphocytes, vascular endothelium and/or stromal cells was used as internal positive control in the samples (Figure 19 B).

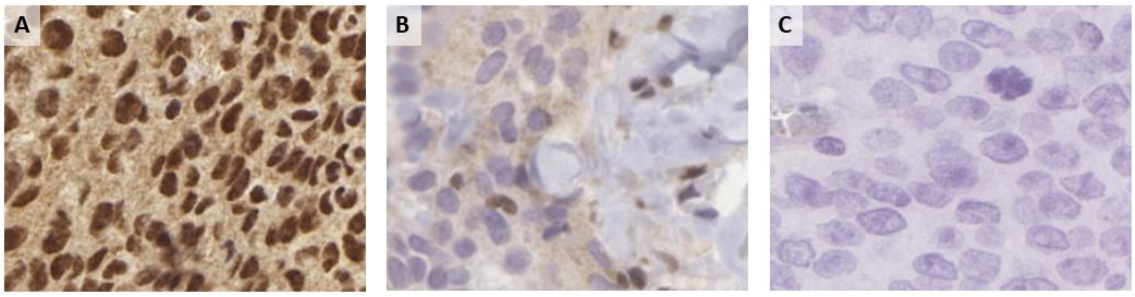

Figure 19: Nuclear expression of BAP1 is considered wild-type/ positive BAP1 reaction (A), while the absence of nuclear staining with cytoplasmic positivity (B) or without cytoplasmic reaction (C) is considered aberrant expression/ negative BAP1 reaction (HE, 400X). Reprinted with the permission of American Association for Cancer Research (AACR) from: [273].

3.5 Mesothelioma cell lines

We used 22 primary cell lines established at the Medical University of Vienna between 2009 and 2016 [274]. Additional 5 international cell lines were kindly provided by collaborators: the SPC111, SPC212 by Professor R. Stahel (University of Zurich, Zurich, Switzerland), M38K by Professor V.L. Kinnula (University of Helsinki, Helsinki, Finland), P31wt and its cisplatin resistant derivative, P31res by Professor K. Grankvist (University of Umea, Umea, Sweden). Cells were cultured in DMEM medium (Lonza, Switzerland) at 37ºC in a humified incubator with 5% CO2 atmosphere [275]. Table 5 contains details of patients and MPM tumor histology of which the cell lines were derived.

DOI:10.14753/SE.2020.2421

Table 5. Characteristics of MPM cell lines. With permission of AACR from: [273]

Cell model	Gender	Histology	TERT promoter status
VMC-6	female	epithelioid	wild-type
VMC-12	male	epithelioid	wild-type
VMC-14	male	epithelioid	wild-type
VMC-20	male	epithelioid	-124 C>T
VMC-23	male	epithelioid	wild-type
VMC-28	male	epithelioid	wild-type
VMC-40	male	biphasic	wild-type
VMC-45	male	epithelioid	wild-type
VMC-46	male	biphasic	-124 C>T
VMC-48	female	biphasic	wild-type
Meso49	male	biphasic	wild-type
Meso62	male	sarcomatoid	-124 C>T
Meso71	male	epithelioid	-57 A>C
Meso80	male	sarcomatoid	-124 C>T
Meso84	female	sarcomatoid	-124 C>T
Meso92	male	biphasic	-124 C>T
Meso103	male	epithelioid	wild-type
Meso110	male	epithelioid	wild-type
VMC-58	male	biphasic	-124 C>T
Meso189	male	epithelioid	wild-type
Meso194	male	epithelioid	-57 A>C
Meso200	male	not available	wild-type
M38K	NA	biphasic	wild-type
SPC111	NA	biphasic	wild-type
SPC212	NA	biphasic	wild-type
P31 wt	NA	epithelioid	wild-type
P31cis	NA	epithelioid	wild-type

3.6 DNA extraction and TERT promoter status analysis

Genomic DNA from FFPE MPM samples was isolated using High Pure FFPET DNA Isolation Kit (Roche Diagnostics) according to the manufacturer's protocol. Briefly, 5 µm thick sections were cut and deparaffinized using Xylol. Tissue lysis and digestion was carried out overnight in a lysis buffer containing 16% Proteinase K at 56ºC shaking at 600 rpm in a Thermomixer R Mixer, 1.5 ml Block (Eppendorf). Samples were then incubated at 90ºC for 60 minutes shaking at 600 rpm. Through the addition of 200 µl DNA Binding Buffer the DNA-content of the sample was bound to the filter compartment of the High Pure Filter Tube assembly which was then washed repeatedly. The purified, extracted DNA was eluted in 50 µl DNA Elution Buffer.

From MPM cell lines genomic DNA was extracted using the DNeasy Blood and Tissue Kit (Quiagen) according to the manufacturers' protocol. Briefly, cells were cultured in 50 ml flasks with a growth surface of 25 cm^2 until almost confluent. $3x10^6$ cells were harvested, centrifuged and the pellet was resuspended in 200 µl PBS. Upon addition of 20 µl Protein K and 200 µl Buffer AL the mixture was incubated for 10 minutes at 56 ºC. After the addition of 200 µl absolute ethanol the mixture was pipetted in a DNeasy Mini spin column and centrifuged at 8000 rpm for 1 minute. The membrane of the comlumn was washed repeatedly using the wash buffers provided by the manufacturer and centrifuged. In the end, the extracted DNA was eluted from the DNeasy membrane to in 200 µl Buffer AE.

The concentration and purity of the DNA was measured in a NanoDrop ND-1000 UV Visible Spectrophotometer (Thermo Scientific).

The core TERT promoter region between the +65 and -278 bp from the ATG start site was amplified by PCR and screened using Sanger sequencing (Figure 20). The mutation analyses were performed by Prof. Rajiv Kumar's group at the German Cancer Research Center, Heidelberg, Germany, as previously described [276].

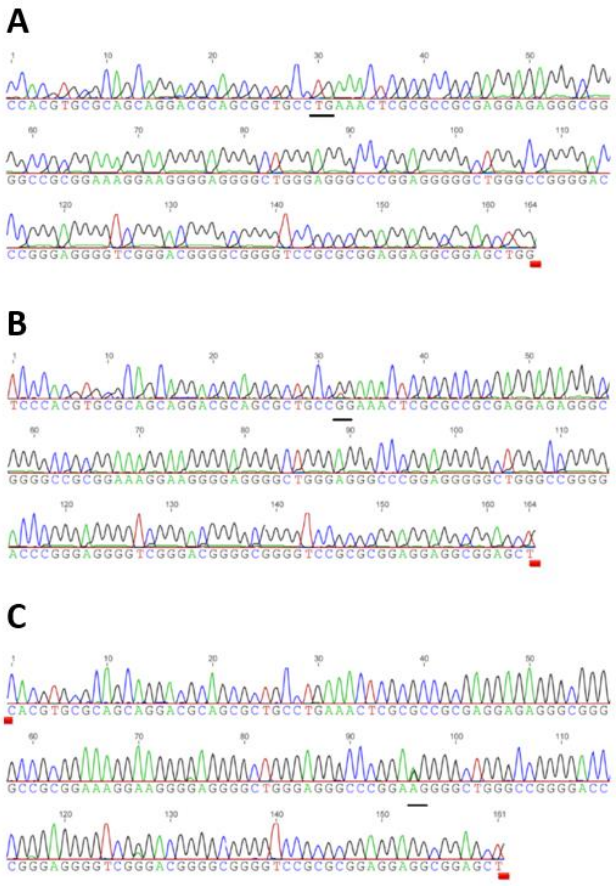

Figure 20: Sequence and chromatograms show (A) wild-type TERT promoter,(B) a promoter region harboring a -57A>C mutation or (C) harboring a -124 C>T mutantion of the TERT promoter. Reprinted with permission of AACR from: [273]

3.7 TERT mRNA expression

Using TRIzol® Reagent® (Invitrogen) total RNA was extracted from 22 MPM cell lines and purified with Turbo DNase Kit (Ambion) according to the manufacturer's protocol. From each sample a 2 µg amount of total RNA was reverse transcribed using High Capacity RNA-to-cDNA Kit kit (Applied Biosystems) according to the manufacturer's protocol [277].

Quantitative reverse transcription polymerase chain reaction (qRT-PCR was carried out using cDNA samples as templates, Maxima SYBR Green qPCR master mix (Thermo Scientific) and gene specific probes in a C1000 Touch Thermal Cycler using the CFX96 Real-Time System (Biorad) [278]. The following gene specific probes were used: *TERT* fw (5′-CCAAGTTCCTGCACTGG-3′) and TERT rev (5′-

TTCCCGATGCTGCCTGAC-3′); RPL41 fw (5′-CAAGTGGAGGAAGAAGC-3′) and RPL41 rev (5′-TTACTTGGACCTCTGCCT-3′). As endogenous reference RPL41 was used and fold changes were determined by ΔΔCt method [279].

3.8 Cell viability assay

To characterize cellular sensitivity to cisplatin, we used Sulforhodamine B (SRB) assay. Briefly, cells were plated in the inner 60 wells of a 96-well plate. After 24hs cisplatin-treatment with different drug concentrations (0, 0.5, 1, 3, 5, and 10 uM), cells were fixed with 10% trichloroacetic acid, which was followed by 15-minute staining with SRB. Excess dry was removed by repeated washing with 1% (vol/vol) acetic acid. After dissolving the protein-bound dye in 10 mM Tris OD at 570 nm was measured using a microplate reader (EL800, BioTec Instruments, Winooski, Vermont) [280].

3.9 Statistical analysis

Associations between two categorical variables such as histopathologic characteristics, clinical parameters and TERT promoter status were calculated by Fisher's exact test. Overall survival was defined as the time elapsed between the date of diagnosis and the date of death or date of last contact, and given in days. Overall survivals of subgroups within the study collectives were estimated using Kaplan-Meier method and differences between subgroup OS were computed by log-rank (Mantel-Cox) tests. Differences in hTERT mRNA expressions and in cisplatin sensitivity between TERT promoter wild-type and mutant samples were analysed by two-tailed Student`s *t* test. To identify independent prognostic factors, multivariate Cox regression tests were performed and hazard ratios and corresponding 95% confidence intervals were calculated. Results were considered statistically significant if $p<0.05$, two-sided. Softwares GraphPad Prism 8.0 (GraphPad Inc.) and SPSS Statistics 23.0 package (SPSS Inc) were used to perform all calculations.

DOI:10.14753/SE.2020.2421

4. RESULTS

4.1 Histologic grading and subtype analysis

4.1.1 Clinicopathological characteristics of the patient collective

Our study cohort consisted of 192 Central European patients, of whom 74.5% were male and 25.5% female patients. Their mean age was 65.0 years at the time of diagnosis. IMIG stage data was available for 126 patients, who had early stage disease and advanced stage disease in similar numbers (Table 6).

Table 6: Clinicopathological characteristics of the MPM patient cohort. (NA= not available, SD=standard deviation).

		Total (n= 192)	**Percentage (100%)**
gender	male	143	74.5%
	female	49	25.5%
age (years)	mean ± SD	65.0 ± 10.8	
IMIG stage (NA = 66)	I / II	61	31.8%
	III / IV	65	33.9%

4.1.2 Histopathologic characteristics

We analysed 192 MPM samples originally diagnosed as epithelioid type MPM. We classified these samples based on their predominant growth patterns. The most common subtype found among these epithelioid MPM samples was the solid pattern, accounting for 52.1% of all samples. Other common patterns were tubulopapillary accounting for 28.6% and trabecular pattern identified in 10.4% of the samples. Microcystic, pleomorphic and micropapillary subtypes were rare, accounting for 4.7, 3.1 and 1.0%, respectively (Table 7).

DOI:10.14753/SE.2020.2421

Table 7. Histologic subtypes, nuclear grade and mitosis-necrosis score groups of the study cohort. [271]

		Total (192)	**Percentage (100%)**
Histology	solid	100	52.1%
	tubulopapillary	55	28.6%
	trabecular	20	10.4%
	microcystic	9	4.7%
	pleomorphic	6	3.1%
	micropapillary	2	1.0%
Nuclear atypia	mild	13	6.7%
	moderate	132	68.8%
	severe	47	24.5%
Mitotic count	low (≤1)	117	60.9%
	intermediate (2-4)	41	21.4%
	high (≥5)	34	17.7%
Necrosis	absent	98	51.0%
	present	94	49.0%
Nuclear grade	1	105	54.7%
	2	62	32.3%
	3	25	13.0%
M/N score	0	88	45.8%
	1	77	40.1%
	2	27	14.1%

We performed histological grading of the samples using composit scores nuclear grade and mitosis-necrosis score. Nuclear grade was based on nuclear atypia and mitotic counts. Nuclear atypia was mild in 13 cases (6.7%), moderate in 132 cases (68.8%) and severe in 47 cases (24.5%). Mitotic count was low in 60.9%, intermediate in 21.4% and high in 17.7% of the cases. On calculating the scores based on these variables, 54.7% of the samples resulted nuclear grade 1, 32.3% nuclear grade 2 and 13.0% nuclear grade 3.

Necrosis was present in 49.0% of the samples and absent in 51.0%. Mitosis-necrosis score was calculated based on mitotic counts and the presence of necrosis. A mitosis-necrosis score of 0 was assigned in 45.8% of the cases, while 40.1% of the cases was given mitosis-necrosis score 1 and 14.1% mitosis-necrosis score 3 (Table 7).

4.1.3 Association between EMM subtypes and grade

We found a significant association between solid and trabecular histologic subtypes and higher nuclear grades (p=0.0008, Chi-squared test) and mitosis-necrosis scores (p<0.0001, Chi-squared test) as visualized in Figure 21.

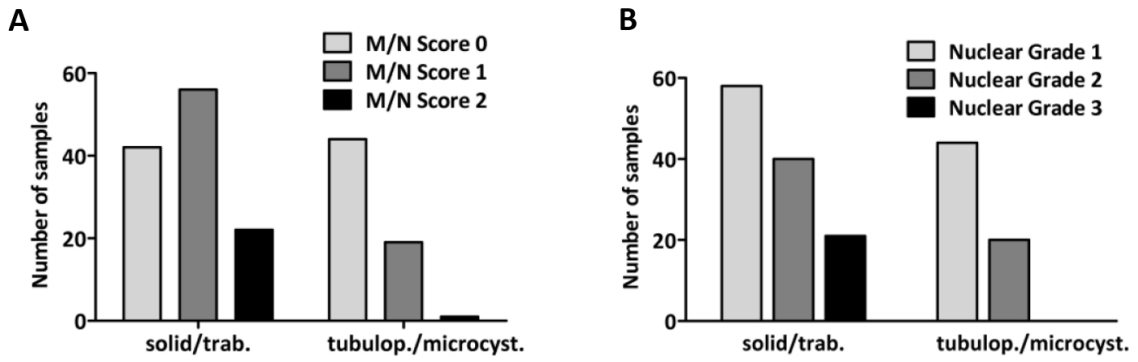

Figure 21. Distribuiton of (A) mitosis-necrosis scores and (B) nuclear grades among different EMM subtypes. [271]

4.1.4 Histopathologic parameters and disease outcome

We analyzed differnces in overall survival between histologic subtypes of EMM. Better prognosis was associated with the tubulopapillary and microcystic subtypes (median OS=727 and 936 days, respectively), while patients with a predominantly solid or trabecular pattern EMM had shorter median OS (397 and 394 days, respectively, Figure 22 A). The shortest OS (173 days) was observed among patients with pleomorphic subtype EMM, which was significantly worse compared to tubulopapillary, microcystic and solid subtypes (p<0.0001, 0.0085 and 0.0277, respectively, Figure 22 A, Table 8).
For further survival analyses we merged samples showing a predominantly microcystic pattern with tubulopapillary variants, as well as trabecular and solid pattern specimens,

because of the low frequencies of the microcystic and trabecular pattern and the pairwise overlapping survival curves. The pleomorphic variant was still included separately, because of its distinct dismal prognosis both in our study population and in literature data. (Figure 22 B, Table 8) Due to the low sample numbers, for tumors with micropapillary pattern (n=2), however, we were not able to calculate a median OS. Thus, we were not able to merge this subtype with any other patterns and these cases were excluded from further analysis.

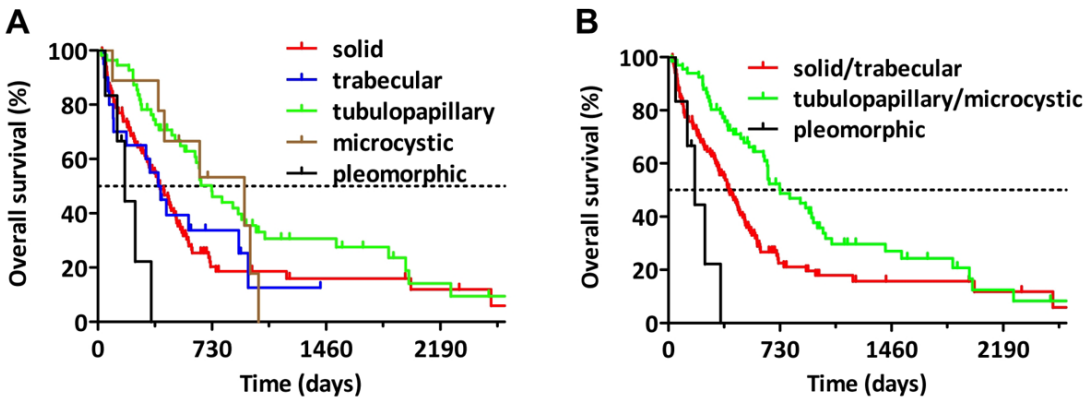

Figure 22. (A) Kaplan-Meier curves for solid, trabecular, tubulopapillary, microcystic and pleomorphic subtype EMM patients. (B) Survival curves of patients with pleomorphic, solid/trabecular or tubulopapillary/microcystic EMM. [271]

Lumped solid/trabecular and tubulopapillary/microcystic subtype groups showed significantly different outcomes (median OS: 397 vs. 732 days, respectively, p=0.003, Figure 22 B, Table 8).

We next analysed the associations between parameters of histological grading, composit grades and patient outcomes. On analysis of individual coponents of composit scores, we observed a significant difference in OS between subgroups exhibiting mild, moderate and severe nuclear atypia (median OS: 1197 days, 501 days [p=0.027] and 306 days [p<0.001], respectively, Figure 23 A, Table 8). The presence of necrosis also associated with significantly shorter median survival (281 vs. 727 days, p<0.0001, Figure 23 C, Table 8).

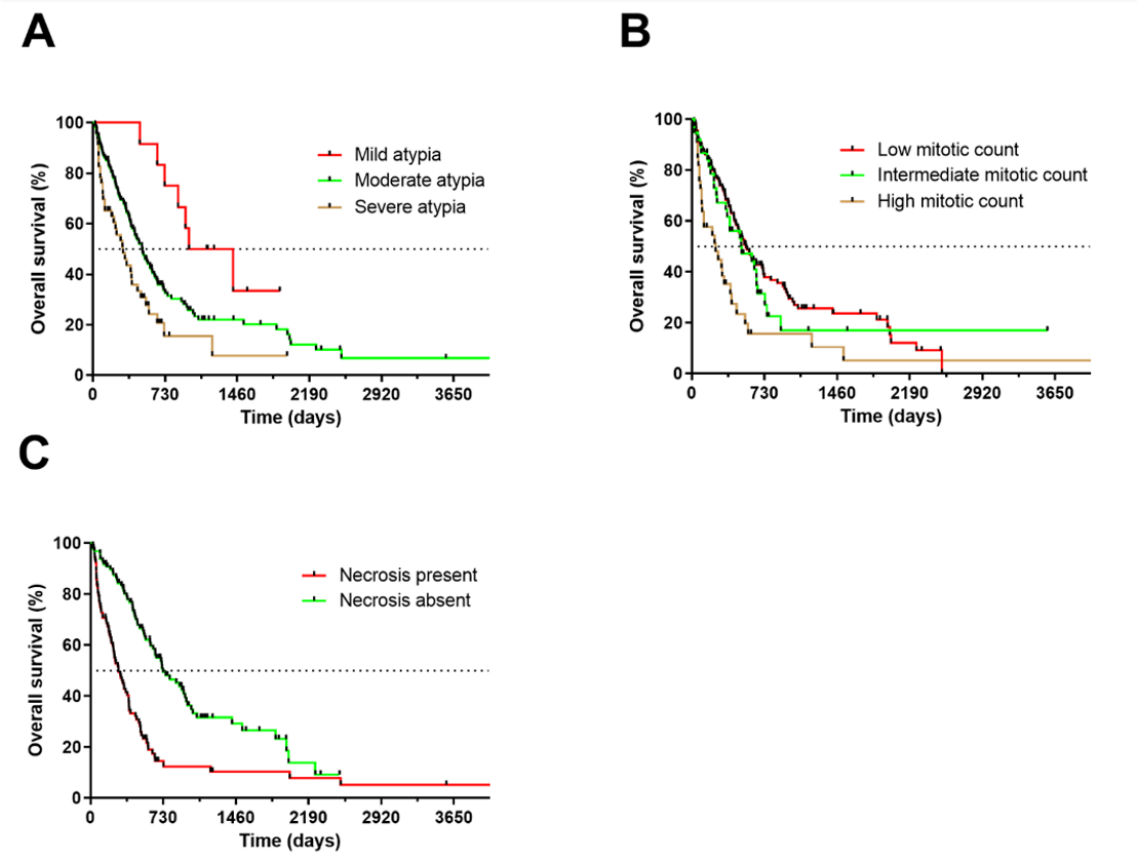

Figure 23. Survival curves associated with individual parameters included in composit grading systems: (A) nuclear atypia, (B) mitotic count and (C) presence of necrosis. [271]

We found that the composit histologic grading systems were able to predict patient outcomes. Significant survival differences were observed between patient subgroups with mitosis-necrosis scores 0, 1 and 2, median OS was 720 days, 383 days ($p<0.0001$) and 165 days ($p<0.0001$), respectively (Figure 24 A, Table 8). There was no significant difference between nuclear grade 1 and 2 patients median OS (555 days and 486 days, respectively, $p=0.531$), however, nuclear grade 3 was associated with significantly shorter median OS (123 days, $p=0.0002$, Figure 24 B, Table 8).

In additional univariate analyses, we found that age or gender did not influence patient outcome, however, we identified significant differences among patient grouped by IMIG stage at the time of diagnosis, type of treatment received.

Table 8. Univariate survival analyses in the MPM patient cohort. [271]

		Univariate analysis		
		OS (days)	**HR (95% CI)**	**p-value**
Age	<70 years	495	0.92 (0.65-1.30)	0.619
	≥70 years	463		
Gender	male	486	0.99 (0.69-1.44)	0.999
	female	469		
Histology	solid/trabecular	397	1	-
	tubulopap./microcyst.	732	0.58 (0.41-0.83)	***0.003***
	pleomorphic	173	2.65 (1.95-6.68)	***0.039***
Nuclear atypia	mild	1197	1	-
	moderate	501	2.29 (1.32-3.97)	**0.027**
	severe	306	3.47 (1.88-6.42)	**<0.001**
Mitotic count	low (≤1)	545	1	-
	intermediate (2-4)	501	1.17 (0.75-1.87)	0.470
	high (≥5)	239	2.48 (1.45-4.25)	**<0.001**
Necrosis	yes	281	2.38 (1.68-3.38)	***<0.0001***
	no	727		
M/N score	0	720	1	-
	1	383	2.01 (1.37-2.95)	***<0.0001***
	2	165	2.61 (1.39-4.97)	***<0.0001***
Nuclear grade	1	555	1	-
	2	486	1.10 (0.75-1.62)	0.531
	3	123	3.75 (1.86-7.56)	***0.0002***
IMIG stage (NA = 66)	I/II	650	0.60 (0.39-0.91)	***0.015***
	III/IV	421		
Treatment (NA = 76)	MMT	936	0.35 (0.23-0.55)	***<0.0001***
	CHT/BSC	340		

*NA, not available; SD, standard deviation; M/N, mitosis/necrosis; OS, overall survival, HR, hazard ratio.

DOI:10.14753/SE.2020.2421

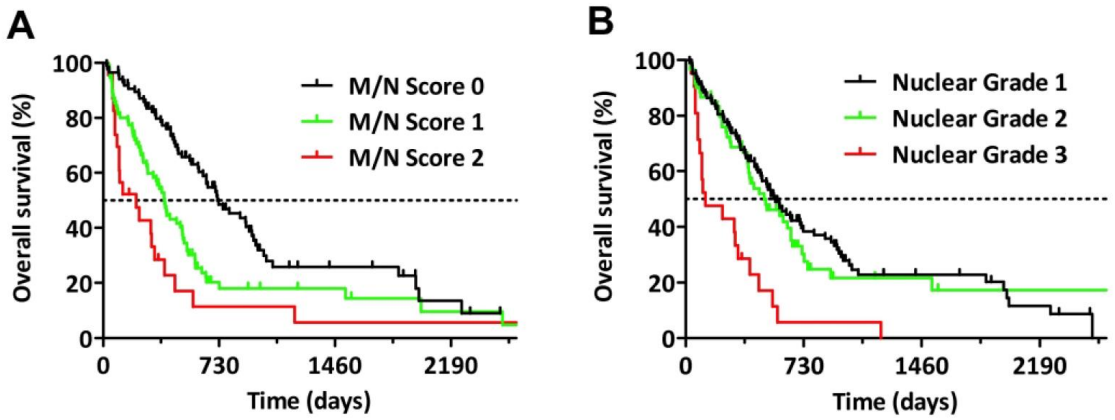

Figure 24 Patient outcomes associated with diferent histopathologic grading systems: (A) nuclear grading and (B) mitosis-necrosis score. [271]

In multivariate analyses, the mitosis-necrosis score was the single independent prognostic factor among the histopathologic variables investigated (Table 9).

Table 9. Multivariate Cox regression analysis in the MPM patient cohort. [271]

		Multivariate analysis		
		HR	**95% CI**	**p-value**
Histology	solid/trabecular tubulopap./microcyst.	0.723	0.50-1.06	*0.095*
M/N Score	0 1 2	1.56	1.13-2.16	***0.007***
Nuclear grade	1 2 3	1.08	0.78-1.48	0.648

*CI, confidence interval; HR, hazard ratio; tubulopap., tubulopapillary; microcyst., microcystic; M/N, mitosis/necrosis.

4.1.5 Histopathologic parameters in the validation cohort

The validation cohort (Table 10) consisting of 55 patients included in the TCGA mesothelioma collection exhibited similar male to female ratio (76.4% to 23.6%), while patients mean age was lower (60.3 years) and the ratio of advanced stage disease

patients was higher (69.1% vs 30.9%) than in the study collective. In the validation cohort we identified the tubulopapillary and solid subtypes as most common patterns (50.9% and 30.9%, respectively), while micropapillary, trabecular and microcystic patterns occurred with a relatively low frequency (7.3%, 5.5% and 5.5%, respectively). Since both composit grading systems require assessment of fine morphological characteristics such as mitotic count and nuclear atypia, histological grading was only carried out on FFPE specimens (n=49) and was omitted in cases where only fresh frozen sections were available (n=6). Nuclear grade 1, 2 and 3 samples were identified in 34.5% 47.3% and 7.3%, while mitosis-necrosis score 0, 1 and 2 were assigned in 41.8%, 36.4% and 10.9% of the cases.

Table 10. Clinical and histological parameters in the validation cohort. [271]

		Total (n= 55)	**Percentage (100%)**
Gender	male	42	76.4%
	female	13	23.6%
Age (years)	mean ± SD	60.3 ± 10.2	
IMIG stage (NA = 66)	I / II	17	30.9%
	III / IV	38	69.1%
Histology	solid	17	30.9%
	tubulopapillary	28	50.9%
	trabecular	3	5.5%
	microcystic	3	5.5%
	pleomorphic	0	0.0%
	micropapillary	4	7.3%
M/N score (NA = 6)	0	23	41.8%
	1	20	36.4%
	2	6	10.9%
Nuclear grading (NA = 6)	grade 1	19	34.5%
	grade 2	26	47.3%
	grade 3	4	7.3%

*IMIG, International Mesothelioma Interest Group; M/N, mitosis/necrosis.

In concordance with our findings in the study cohort, we found a significant association between high histologic grades and solid/trabecular growth patterns (Figure 25).

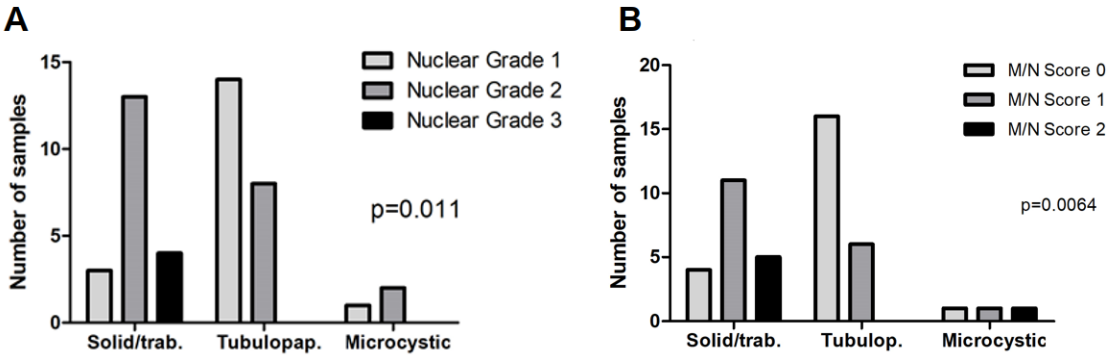

Figure 25. Distribution of (A) nuclear grades 1-3 and (B) mitosis-necrosis scores 0-2 among histologic variants of epithelioid mesothelioma. [271]

In order to allow better comparison between our study cohort and the validation cohort, we merged solid and trabecular, as well as tubulopapillary and microcystic predominant pattern subgroups for survival analyses. In univariate analysis we observed a significantly worse median OS associated with solid/trabecular growth patterns (406 vs. 795 days, respectively, p=0.01, Figure 26, Table 12),

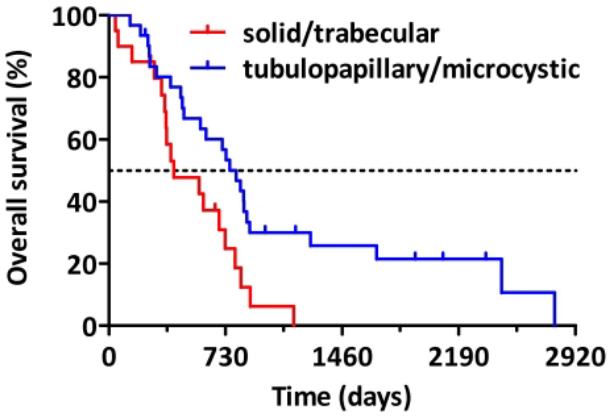

Figure 26. Kaplan-Meier curves for solid/trabecular and tubulopapillary/microcystic predominant pattern MPM in the TCGA cohort. [271]

Patients with nuclear grade 3 tumors had a significantly shorter median OS, than those with lower grade tumors (232 days vs. 823 and 459 days, respectively, Figure 27 B,

Table 12), and the same association was seen for mitosis-necrosis score 2 tumors (330 days vs. 795 and 511 days in M/Nscores 0 and 1, respectively Figure 27 A, Table 11).

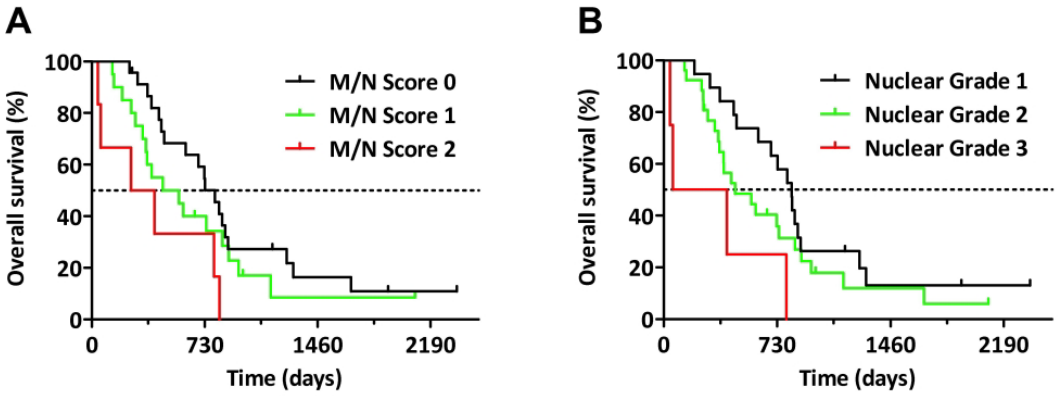

Figure 27. Survival curves for mitosis-necrosis scores (A) and nuclear grades (B). [271]

Table 11. Univariate survival analyses in the TCGA MPM patient cohort. [271]

		OS (days)	HR (95% CI)	p-value
Age	<65 years	689	1.10 (0.60-2.03)	*0.750*
	≥65 years	742		
Gender	male	709	0.92 (0.46-1.84)	*0.812*
	female	572		
Histology	solid/trabecular	406	2.24 (1.17-4.29)	***0.010***
	tubulopapillary/microcystic	795		
M/N score	0	795	1	-
	1	511	1.47 (0.76-2.86)	*0.251*
	2	330	3.11 (1.17-8.23)	***0.023***
Nuclear grade	1	823	1	-
	2	459	1.53 (0.80-2.92)	*0.200*
	3	232	4.91 (1.45-16.59)	***0.010***
IMIG stage	early (I/II)	563		
	late (III/IV)	732	1.02 (0.56-1.89)	0.936

*CI, confidence interval; HR, hazard ratio; M/N, mitosis/necrosis; IMIG, International Mesothelioma Interest Group.

The relatively small sample number of the validation cohort limited the feasibility of a multivariate analysis.

4.1.6 Histological subtypes and disease stage

We analysed the prognostic impact of histological subtypes within subcohorts of patients with early stage (IMIG I/II) or advanced (IMIG III/IV) diseases. In advanced stage disease we found a significant difference in overall survival between the two histologic subtypes (p=0.047, Mantel-Cox regression, Figure 28 B). In early stage cases the difference between the OS of the two histologic subtype groups proved significant by Gehan-Breslow-Wilcoxon (p=0.041) which is more sensitive for early events. Of note, using the Mantel-Cox regression analysis, the difference was not significant (p=0.194) due to a cross-over at the tails of the curves (Figure 28 A). We found that the solid/trabecular and tubulopapillary/microcystic histologic subtypes were evenly distributed among early and advanced stages (p=0.999, Fisher's exact test, Figure 28 C).

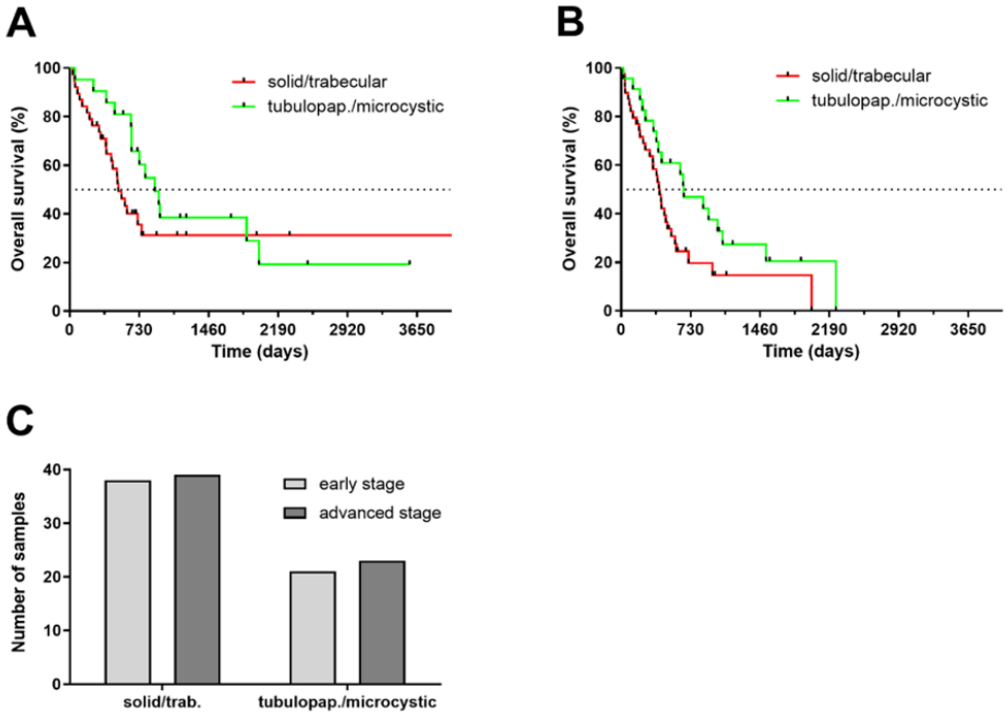

Figure 28. Survival curves of lumped groups of solid/trabecular and tubulopapillary/microcystic among early stage patients (A) and late stage patients (B). (C) Distribution of histologic subtypes among patients in early and late IMIG stages. [271]

4.1.7 Differences in response to MMT among EMM subtypes

To assess association of treatment with epithelioid MPM subtypes, we analysed the correlations and OS data in the subcohort with available treatment information (n=109).

Table 12: Therapeutic regimens received by the patients in our exploratory subcohort. [271]

Treatment modalities	**109 patients**
Multimodal therapy	**44**
EPP + platinum/pemetrexed + IMRT	**18**
Surgery + chemotherapy	**25**
- EPP + platinum/pemetrexed	10
- eP/D + platinum/pemetrexed	8
- EPP+ platinum/gemcitabine	4
- eP/D + platinum	1
- unspecified MCR surgery + platinum/taxane	1
- unspecified MCR surgery + platinum/epirubicine	1
EPP + radiation therapy	**1**
Non-multimodal therapy	**65**
Chemotherapy	**44**
- platinum/pemetrexed	28
- platinum/gemcitabine	6
- platinum/cyclophosphamide/epirubicine	3
- platinum/bortezomib	2
- pemetrexed	1
- unspecified chemotherapy	4
Radiotherapy	**1**
Chemotherapy + radiotherapy	**1**
Surgery	**5**
- EPP	3
- unspecified surgery	2
Best supportive care	**14**

In this subcohort, 59.6% (65/109) did not receive multimodal therapy. Among these patients 44 (40.4%) received chemotherapy, 5 (4.6%) surgery only, 1 (0.9%) radiotherapy only, 1 (0.9%) chemoradiotherapy. 40.4% of the patient (44/109) received multimodal therapy which included macroscopic total resection of the tumor in combination with chemoradiotherapy (Table 12).

Between the two compared subgroups solid/trabecular and tubulopapillary/microcystic tumor growth patterns we found no significant difference in the contingency neither in patients' age, gender, disease stage nor histopathologic grades of tumors among patiets who received MMT. Among patients who did not receive MMT, tubulopapillary/microcystic tumors were significantly associated with lower nuclear grades, mitosis-necrosis scores and younger age (Table 13, [271]).

DOI:10.14753/SE.2020.2421

Table 13. Clinicopathological characteristics of patients receiving MMT or non MMT.

		All histologic subtypes			Multimodal therapy (MMT)			No MMT		
		MMT	**No MMT**	**p-value**	**Solid/ trabecular**	**Tubulop./ microcystic**	**p-value**	**Solid/ trabecular**	**Tubulop./ microcystic**	**p-value**
Number of samples		44	65	-	25	19	-	49	16	-
Mean age ± SE (years)		62.1 ±1.66	68.0 ±1.31	***0.005***†	63.0 ±2.32	60.9 ±2.37	0.526†	69.5 ±1.38	63.6 ±3.07	***0.049***†
Gender	**male**	33	49	1.000	19	14	1.000	38	11	0.514
	female	11	16		6	5		11	5	
M/N score	**0**	26	17		14	12		8	9	
	1	15	31	***0.005*** *	8	7	0.294*	25	6	***0.012****
	2	3	12		3	0		11	1	
Nuclear grade	**1**	24	32		11	13		25	7	
	2	17	22	0.301 *	11	6	0.143*	13	9	***0.032****
	3	3	11		3	0		11	0	
Stage (NA=20)	**I/II**	14	27	0.663	8	6	0.304	21	6	1.000
	III/IV	19	29		7	12		23	6	

P-values calculated by Fisher's exact test, except * where Chi-squared and † where unpaired, two-tailed t-tests were applied.

MMT, multimodal therapy; tubulop., tubulopapillary; NA, not available; SE, standard error; M/N, mitosis/necrosis.

We grouped patients into four subgroups based on tumor histologic subtype (solid/trabecular or tubulopapillary/microcystic) and the treatment received (MMT or non-MMT). Interestingly, we were able to identify a tendency for better patient outcomes associated with tubulopapillary/microcystic patterns in contrast to solid/trabecular subtypes within the MMT subgroup (1068 versus 580 days, HR: 2.29 [95% CI: 0.95-5.12], p=0.066, Figure 29). MMT provided a significant benefit within both tubulopapillary/microcystic and solid/trabecular patterns, compared to a non-MMT approach, however, the difference was more pronounced in the tubulopapillary/microcystic predominant subtype (MMT versus non-MMT within the tubulopapillary/microcystic patterns: 1068 vs. 406 days, HR: 2.67 [95% CI: 2.18-3.08], p=0.0006; MMT versus non-MMT within the solid/trabecular variants: 580 vs. 327 days, HR: 1.77 [95% CI: 1.24-2.31], p=0.0018). There was no significant difference in patient outcomes among the histologic subtypes among patients who did not receive MMT (tubulopapillary/microcystic: median OS=406 days, solid/trabecular: 327 days, HR=1.16 [95%CI: 0.65-2.07], p=0.617).

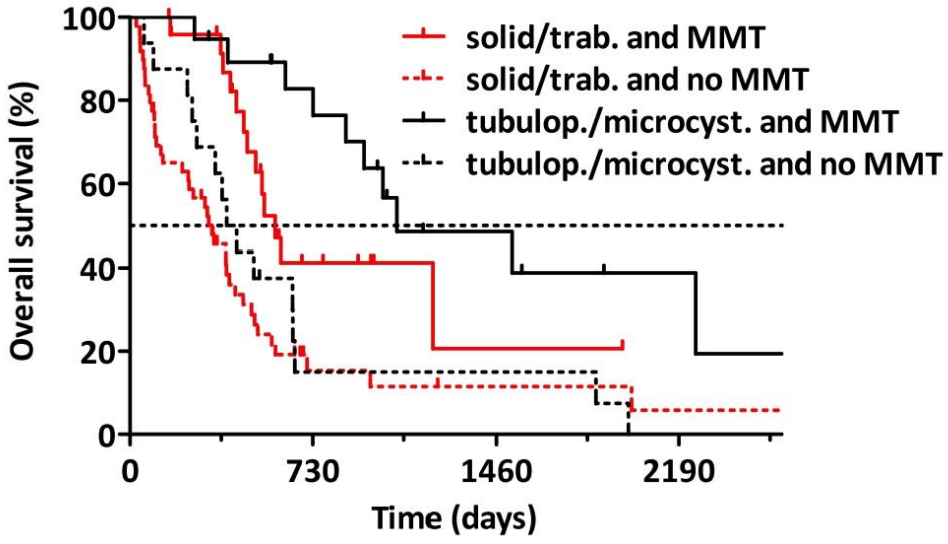

Figure 29. Kaplan-Meier curves of solid/trabecular and tubulopapillary/microcystic variants among patients who received multimodal therapy (MMT) or a non-multimodal treatment (no MMT).[271]

4.2 TERT promoter mutation

4.2.1 TERT promoter mutation and clinicopathological characteristics

We analysed 182 MPM cases including 69.8% epithelioid, 24.2% biphasic and 4.9% sarcomatoid histologies. The male to female ratio was 3.7 (78.6% and 21.4%, respectively). 29.7% of the patients were diagnosed at stage I/II and 47.3% at stage III/IV disease, IMIG stage was not available in 42 cases (23.1%). Karnofsky performance status was available in 167 cases, 83.8% (n=140) of whom were able to carry on normal activity with no or only some symptoms at the time of diagnosis. EORTC prognostic score was available in 140 cases and estimated a poor prognosis in 71.4% (n=100) of these patients (Table 14).

Table 14. Clinicopathological characteristics of MPM patients grouped based on TERT promoter mutation status and their associations estimated by Fisher's exact test. Reprinted with the permission of AACR from: [273]

		Total (N= 182)	**TERTp^{wt} (N= 163)**	**TERTp^{mut} (N= 19)**	**p-value**
Gender	male female	143 39	125 38	18 1	0.070
Age (years)	mean ± SD	64.18 ± 0.8	64.0 ± 0.8	65.3 ± 2.6	0.614
Karnofsky PS (NA = 15)	PS ≥80 PS <80	140 27	128 23	12 4	0.313
Histology (NA = 1)	Epithelioid Non-epithelioid - biphasic - sarcomatoid - NA = 1	127 54 44 9	120 42 38 3	7 12 6 6	**<0.001***
IMIG stage (NA = 42)	I / II III / IV	54 86	54 64	0 12	**0.002**
EORTC Score (NA = 42)	≤1.27 >1.27	40 100	38 87	2 13	0.232

* = p calculated for epithelioid vs. non-epithelioid

We found TERT promoter mutations in 10.4% of all samples (19/182). The mutations were detected at higher frequencies among non-epithelioid types than in epithelioid samples (22% and 5.5%, respectively). The association between non-epithelioid histologic types and TERT promoter mutant status was statistically significant ($p<0.001$). All of the identified TERT promoter mutant cases were diagnosed at an advanced stage ($p=0.002$, Table 13). Among the 19 TERT promoter mutant MPM samples 13 (68.4%) harbored the -124 C>T mutation. We detected the -146 C>T mutation in 2 (10.5%) cases and the -57 A>C mutation in 4 (21.0%).

We divided our cohort into a test cohort consisting of our Austrian patient collective (n=83, Table 15) and a validation cohort of the Slovenian and Croatian patients (n=99, Table 16).

Table 15. Clinicopathological characteristics in the Austrian patient collective and their distribution among TERT promoter wild-type and mutant cases. Reprinted with the permission of AACR from: [273]

		Total (n= 83)	**TERTp^{wt} (n= 72)**	**TERTp^{mut} (n= 11)**	**p-value**
Gender	male	63	53	10	0.212
	female	20	19	1	
Age (years)	mean ± SD	61.8 ± 1.3	61.7 ± 1.4	62.8 ± 3.7	0.780
Karnofsky PS (NA = 13)	PS ≥80	64	57	7	0.673
	PS <80	6	5	1	
Histology (NA = 1)	Epithelioid	59	55	4	**<0.001**
	Non- epithelioid	24	17	7	
	- biphasic	17	14	3	
	- sarcomatoid	6	2	4	
IMIG stage (NA = 24)	I / II	15	15	0	0.100
	III / IV	44	37	7	
EORTC Prognostic Score (NA = 16)	≤1.27	29	27	2	0.280
	>1.27	38	31	7	

Similarly to the merged cohort we found a significant association between non-epithelioid subtype and TERT promoter mutant status ($p<0.001$ in the test cohort and

p=0.041 in the validation cohort). A significant association between the mutant status and advanced IMIG stage was only observed in the validation cohort (p=0.026). TERT promoter mutation was not associated with gender, age, performance and prognostic scores in either of the cohorts.

Table 16. Clinicopathological characteristics of the Slovenian-Croatian validation cohort grouped based on TERT promoter status. Reprinted with the permission of AACR from: [273]

		Total (n= 99)	**TERTp^{wt} (n= 91)**	**TERTp^{mut} (n= 8)**	**p-value**
Gender	male	80	72	8	0.151
	female	19	19	0	
Age (years)	Mean ± SD	66.2 ± 0.9	65.9 ± 0.9	68.9 ± 3.4	0.374
Karnofsky PS (*NA = 2)	PS ≥80	76	71	5	0.256
	PS <80	21	18	3	
Histology (NA = 1)	Epithelioid	68	65	3	**0.041**
	Non-epithelioid	30	25	5	
	- biphasic	27	24	3	
	- sarcomatoid	3	1	2	
	- NA = 1				
IMIG stage (NA = 18)	I / II	39	39	0	**0.026**
	III / IV	42	37	5	
EORTC Prognostic Score (NA = 26)	≤1.27	11	11	0	0.582
	>1.27	62	56	6	

4.2.2 TERT promoter mutation and histological subtypes of epitheioid MPM

We also investigated the TERT promoter mutations' distribution among 109 epithelioid samples exhibiting different predominant growth patterns (Table 17). No significant association to TERT promoter status was identified among the solid, trabecular, tubulopapillary, microcystic and micropapillary patterns (p=0.75). Pleomorphic pattern EMM samples exhibited a TERT promoter mutation frequency similar to that of the non-epithelioid MPMs (33.3% and 22%, respectively), and in comparison to non-

pleomorphic epithelioid MPM samples, the pleomorphic pattern was significantly associated with more frequent TERT promoter mutations (p=0.035).

Table 17. TERT promoter mutations in histologic subtypes of epithelioid MPM.

		Total (n=109)	**TERTp^{wt} (n=103)**	**TERTp^{mut} (n=6)**	**p-value**
Histologic subtype	Solid	44	43	1	
	Trabecular	10	9	1	
	Tubulopapillary	40	38	2	0.875*
	Microcystic	8	8	0	
	Micropapillary	1	1	0	
	Pleomorphic	6	4	2	***0.035****
	Non-pleomorphic	103	99	4	

*: Chi- squared test, **: Fisher's exact test

4.2.3 TERT promoter mutation and BAP1 expression

We performed BAP1 immunohistochemical staining on 75 samples. Loss of nuclear BAP1 staining was observed at a frequency in line with the literature and was significantly associated with epithelioid type MPM (p=0.023, Table 18).

Table 18. BAP1 expression among the three main histologic types. Reprinted with the permission of AACR from: [273]

	Total (n= 75)	**BAP1 +**	**BAP1 –**	**p-value**
Histology				
Epithelioid	55	21 (38.2%)	34 (61.8%)	
Biphasic	15	6 (40.0%)	9 (60.0%)	**0.023***
Sarcomatoid	5	5 (100%)	0 (0%)	
TERT promoter				
Mutant	9	9	0	**0.0002****
Wild-type	66	22	44	

*Chi-squared, ** Fisher's exact test.

Strikingly, we found a strong correlation between TERT promoter mutant status and retained BAP1 expression (p=0.0002). Among all TERT promoter mutant samples, each exhibited a retained nuclear BAP1 expression, thus we found that the TERT promoter mutation and the loss of BAP1 were mutually exclusive in our cohort (Table 18).

4.2.4 TERT promoter status and patient outcomes

The prognostic impact of TERT promoter mutations was analysed by Kaplan-Meier method. Among the 182 cases of the entire patient collective, TERT promoter mutant status was associated with a significantly worse median OS when compared to TERT promoter wild-type samples (262 vs. 469 days, p<0.0001, Figure 30 A). The prognostic impact was also found when analyzing the Austrian and the Croatian-Slovenian patient collectives separately. In the Austrian cohort the TERT mutant subgroup had a 262-day median OS, while the wild-type subgroup's median OS was 524 days (p=0.0012, Figure 30 C). In the Croatian-Slovenian subcohort, where the difference in survival was also significant between TERT promoter mutant and wild-type cases (104 vs. 465 days, respectively, p=0.0024, Figure 30 D).

Histologic types of MPM are known to have a strong prognostic impact on OS, which was also confirmed in our study: patients with epithelioid type tumors had a significantly longer OS compared to those with non-epithelioid tumors (459 days vs. 353 days, p=0.01, Figure 30 B). Since the TERT promoter mutant status was significantly associated with non-epithelioid histologic type, we analysed the impact of TERT promoter mutations among epithelioid and non-epithelioid MPM cases separately. The significant negative prognostic effect of TERT promoter mutant status was identified in both histologic subgroups, median OS of mutant and wild-type tumors was 340 and 510 days among the epithelioid subgroup, and 199 days vs. 412 days among non-epithelioid tumors (Figure 30 E, 30 F).

DOI:10.14753/SE.2020.2421

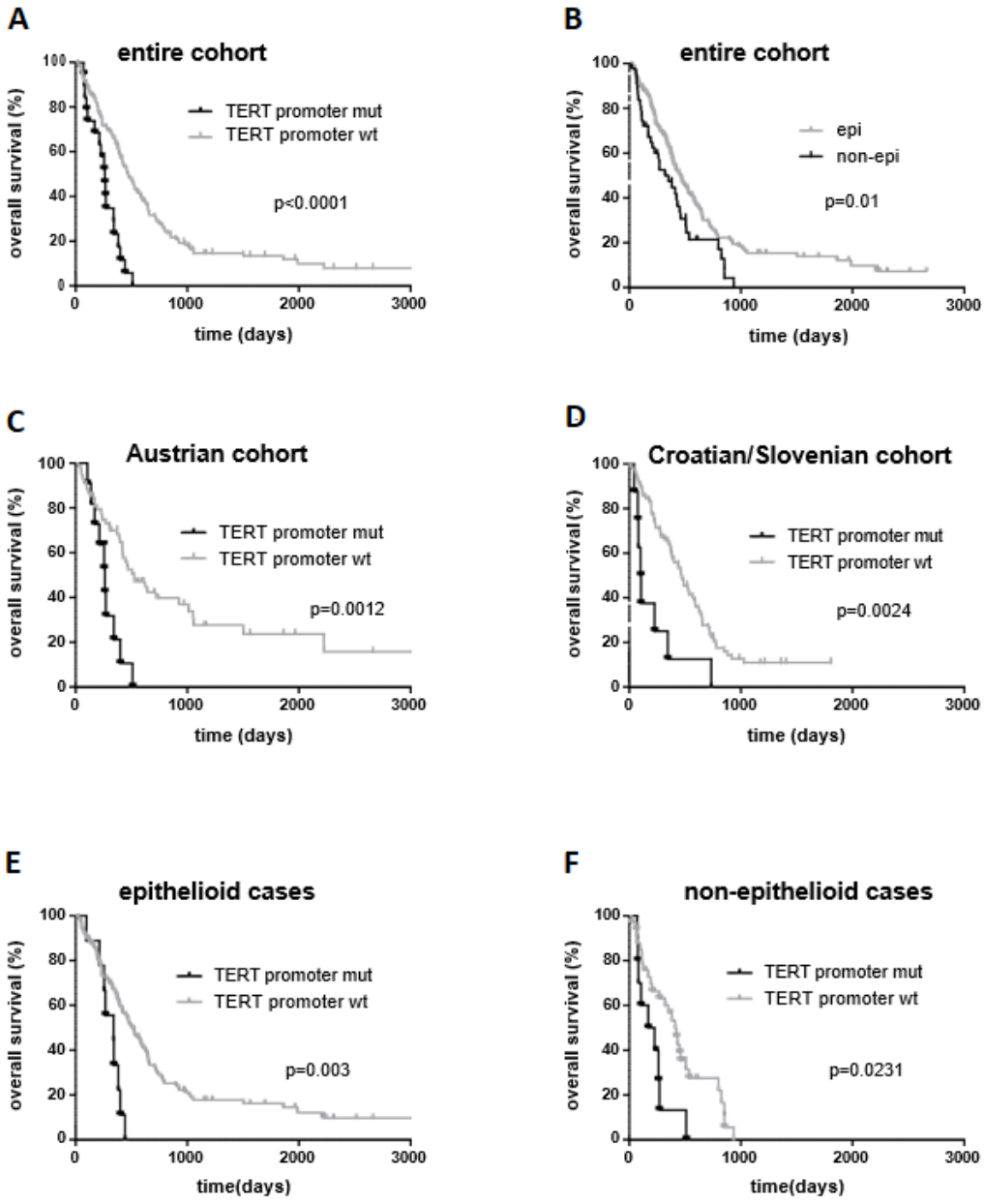

Figure 30. TERT promoter status and histologic type both have a significant impact on patient outcomes (OS). Kaplan-Meier curves for the entire cohort grouped by: (A) TERT promoter mutant or wild-type status, (B) epithelioid and non-epithelioid histologic types. Overall survival and TERT promoter status: (C) in the Austrian patient collective and (D) in the Croatian plus Slovenian validation cohort, (E) among all epithelioid histotype MPM cases and (F) all non-epithelioid MPM cases. Reprinted with the permission of AACR from: [273]

In univariate analyses, epithelioid and non-epithelioid histologic types, Karnofsky performance status, EORTC prognostic score, IMIG stage and the presence of TERT promoter mutations all had a significant prognostic impact (Table 19).

Table 19. Univariate analyses of clinicopathological variables in all MPM patients. P-values calculated by log-rank test. Reprinted with the permission of AACR from: [273].

		HR	95% CI	OS (days)	p-value
Gender	male	1.202	0.829-1.742	431	0.331
	female			528	
Age (years)	≥70	0.927	0.654-1.314	490	0.668
	<70			426	
Karnofsky PS	≥80	0.209	0.113-0.387	475	**<0.001**
	<80			189	
Histology	epithelioid	0.503	0.337-0.751	510	**<0.001**
	non-epithelioid			354	
IMIG stage	early (I/II)	0.629	0.433-0.914	650	**0.015**
	late (III/IV)			401	
TERTp status	$TERTp^{wt}$	0.116	0.051-0.267	490	**<0.001**
	$TERTp^{mut}$			257	
EORTC Prognostic Score	≤1.27	0.552	0.378-0.806	598	**0.009**
	>1.27			390	

In multivariate analyses we found that TERT promoter status and main histologic types were independent prognostic factors (p=0.011 and p=0.009, respectively, Table 20).

Table 20. Multivariate analyses of clinicopathological factors influencing OS. Reprinted with the permission of AACR from: [273]

		HR	95% CI	p-value
Gender	male female	0.923	0.580-1.470	0.737
Age (years)	<70 ≥70	1.013	0.665-1.544	0.952
Histology	epithelioid non-epithelioid	0.563	0.366-0.867	**0.009**
IMIG stage	early (I/II) late (III/IV)	0.673	0.447-1.013	0.058
TERTp status	$TERTp^{wt}$ $TERTp^{mut}$	0.427	0.220-0.826	**0.011**

The TERT promoter mutant status was found to be also an independent prognostic factor in a multivariate analysis that included the EORTC prognostic score (Table 21).

Table 21. Multivariate analysis of clinical prognostic factors and TERT promoter status influencing disease outcome. Reprinted with the permission of AACR from: [273]

		HR	95% CI	p-value
Age (years)	<70 ≥70	0.942	0.599-1.482	0.797
EORTC Prognostic Score	≤1.27 >1.27	0.545	0.333-0.894	**0.016**
IMIG stage	early (I/II) late (III/IV)	0.732	0.466-1.150	0.175
TERTp status	$TERTp^{wt}$ $TERTp^{mut}$	0.392	0.179-0.858	**0.019**

4.2.5 Patient outcomes and SNP rs2853669

Data on the rs2853669 SNP carrier status was available on 121 cases, of which 5.8% were TERT promoter mutant and carriers of the common polymorphism (7/121), 6.6% mutants and non-carriers (8/121), 47.1% TERT promoter wild-type and non-carriers (57/121) and 40.5% wild-type and SNP carriers (49/121). Median OS of the four groups were 200 days, 257 days, 622 days and 490 days, respectively. There was no significant difference between carriers and non-carriers among TERT promoter mutatns (p=0.935), thus, the presence of the SNP did not eliminate the negative prognostic impact of the promoter mutations (Figure 31).

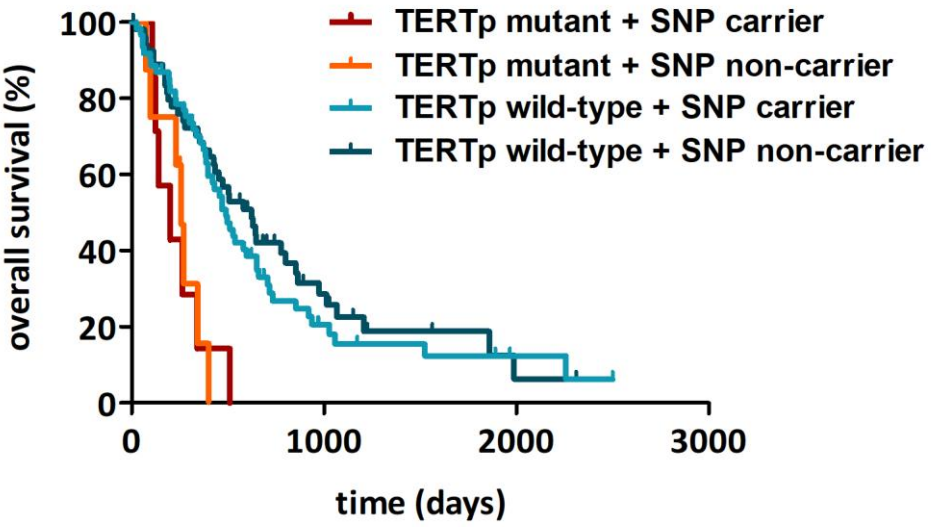

Figure 31. Kaplan-Meier curves of patients subgroups carrying and not carrying the common polymorphism rs2853669 with and without TERT promoter mutations.

4.2.6 TERT mRNA expression and TERT promoter status

We analysed mRNA expression of the hTERT gene in 22 de novo established MPM cell lines by quantitative PCR. Among the 22 cell lines, 7 harbored -124C>T and 2 -57A>C mutation of the TERT promoter region, while 13 were TERTp wild-type. We identified a significant difference in the hTERT expression of TERT promoter mutant and wild-type cell lines ($p<0.01$, Figure 32).

DOI:10.14753/SE.2020.2421

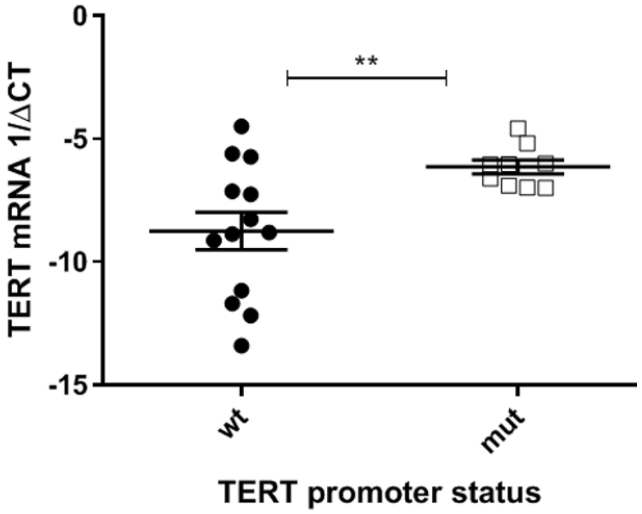

Figure 32. TERT mRNA expression relative to expression of housekeeping gene RPL41 is significantly different in cell lines harboring TERT promoter mutations (n=9) and TERT promoter wild-type cell lines (n=13). Reprinted with the permission of AACR from: [273]

4.2.7 TERT promoter status and cell line formation

To further investigate the mechanisms underlying the TERT promoter mutations negative impact on patient outcomes, we compared the ability of 45 MPM tumor samples to form de novo cell cultures based on our experience between 2009 and 2016. Of 45 primary cell cultures, 22 cell lines were successfully established (48.9%). We found that successful *in vitro* cell line formation was associated with shorter median OS (268.5 days vs. 607 days, $p<0.001$, Figure 33 A) Of the 22 immortalized tumor cell lines 9 harbored TERT promoter mutations, while neither of the 23 cultures that failed to undergo immortalization did harbor any of these non-coding mutations. Thus, TERT promoter mutant status was significantly associated with cell line formation (Figure 33 B, $p<0.001$), while IMIG stage of the original tumor or non-epithelioid histology did not confer a pro-immortalization effect ($p=0.539$ and $p=0.206$, respectively, Figure 33 C and D).

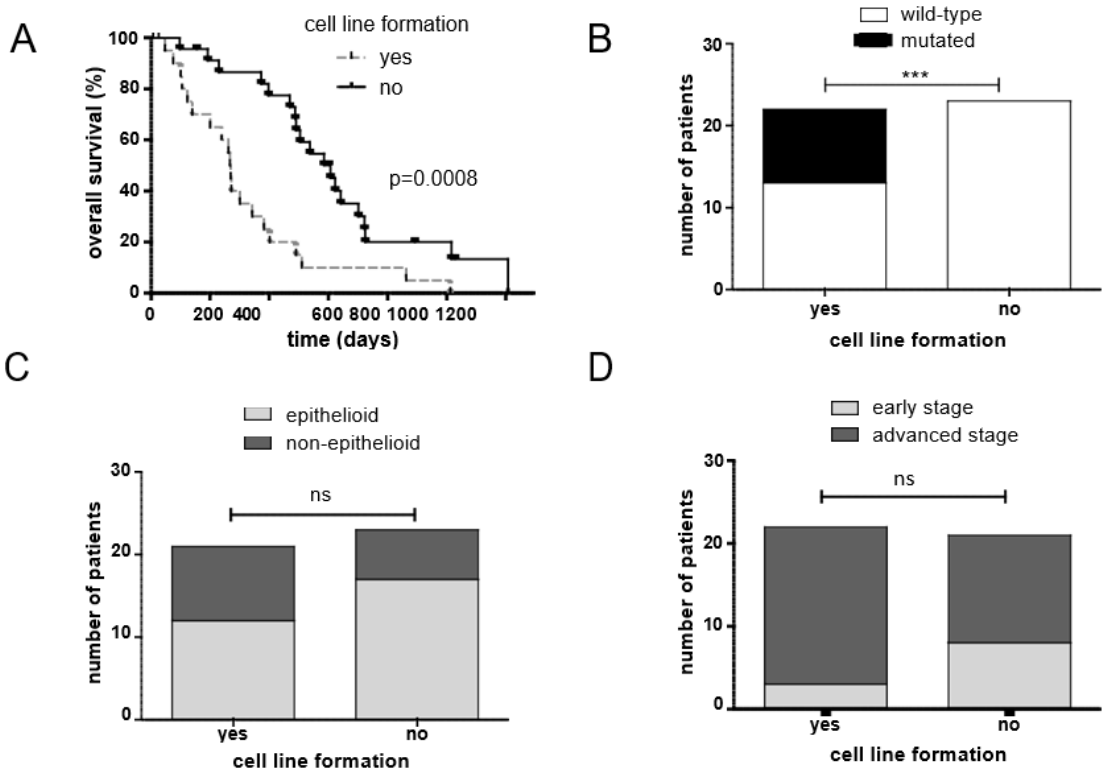

Figure 33. (A) Successful cell line formation conferred a significant negative impact on patient outcomes (p=0.0008). (B) Tumors harboring a TERT promoter mutation were significantly more likely to be immortalized (p<0.001). (C) Original tumors' histologic type and (D) stage at the time of diagnosis did not have a significant impact on cell line formation. Reprinted with the permission of AACR from: [273]

4.2.8 TERT promoter status and in vitro cisplatin sensitivity

To assess the impact of TERT promoter status on cisplatin sensitivity in MPM, we used 24 cell MPM cell lines, 5 of them international cell lines and 19 de novo cell lines and carried out SRB assays to determine their IC50 values for cisplatin. 7 of the cell lines harbored a TERT promoter mutation, while 17 were TERT promoter wild-type. We did not find a statistically significant difference in cisplatin sensitivity, however we observed that cell lines harboring TERT promoter mutations showed a tendency to have lower IC50 values (p=0.097, Figure 34).

DOI:10.14753/SE.2020.2421

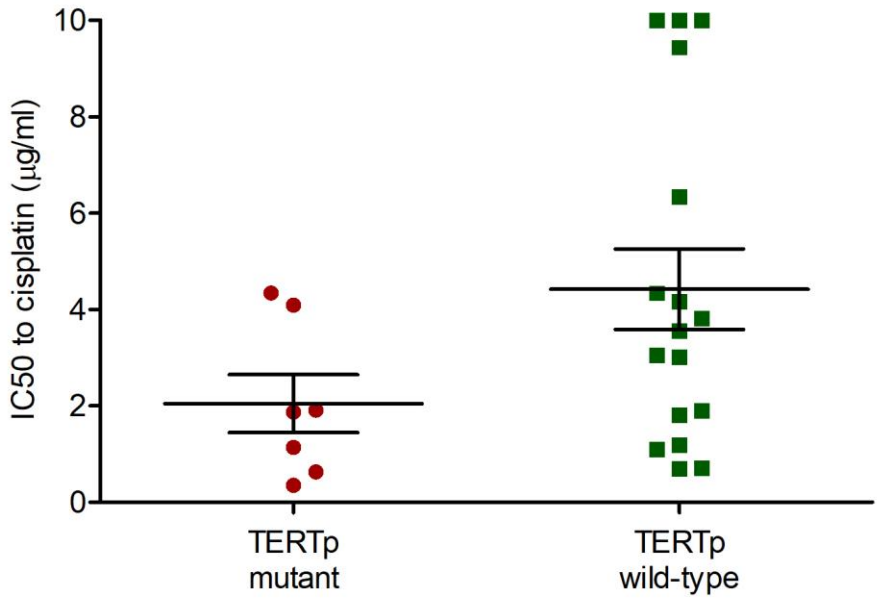

Figure 34: A tendency for lower IC50 values for cisplatin was observed in the seven cell lines harboring a TERT promoter mutation in comparison to seventeen TERTp wild-type MPM cell lines (p=0.097).

DOI:10.14753/SE.2020.2421

5. DISCUSSION

5.1 Histologic subtypes of epithelioid mesothelioma

Of the three main histologic types – epithelioid, biphasic and sarcomatoid – of MPM, epithelioid histology is associated with the longest median overall survival; however, median OS shows a wide variability among these cases. Patients with EMM are the most likely to be eligible for a more aggressive therapy consisting of macroscopic complete resection of the tumor and adjuvant chemo- and/or radiotherapy [91]. The multimodal treatment approach is, however, associated with numerous serious complications, and less than half of the patients are able to complete the MMT protocols, in part because of complications, in part because of progression during treatment. Taking all these into account, there is an urgent need for both prognostic and predictive factors that help identify patients most likely to benefit from multimodality treatment among EMM cases.

Histologic subtyping of EMM is a promising prognostic factor according to previous studies. However, data is only available on retrospective cohorts of limited size due to the rarity of the disease (Table 22). The studies available at this time show substantial variability among the different subtypes, however, in each of them solid and tubulopapillary subtypes occurred at the highest frequencies, while other growth patterns were relatively rare. The prognostic impact of most subtypes remains unclear. It is only the pleomorphic subtype which was found to be an independent prognostic factor in multivariate analyses [94]. The solid pattern compared to all non-solid subtypes was associated with shorter median OS, however not independently associated with OS [120].

To the best of our knowledge, our investigation provides data on EMM subtyping in context of patient outcomes on the second largest cohort published until this date. No definitve finding on the prognostic impact on the subtypes was provided, however, we found growth pattern analysis to be a promising histopathologic factor which can be evaluated on small biopsy samples that are available for most MPM patients in a clinical setting.

Table 22. Summary of results from studies investigating patient outcomes and histologic subtypes of EMM.

Study (No. of samples)	**Subtypes analysed**	**Frequency**	**Median OS (months)**	**p-values**
Kadota et al. [94] (232)	Solid	38%	13.7 (10.0–16.9)	0.020
	Tubulopapillary	22%	17.9 (14.4–32.5)	0.002
	Trabecular	16%	24.9 (22.9–39.9)	<0.001
	Pleomorphic	15%	8.1 (4.9–17.0)	Ref.
	Micropapillary	9%	15.8 (9.3–45.3)	0.021
Brčić et al. [121] (98)	Solid	45%	NA	NA
	Tubulopapillary	19%	NA	NA
	Acinar	18.4%	NA	NA
	Adenomatoid	6%	NA	NA
	Pleomorphic	5%	NA	NA
	Micropapillary	2%	NA	NA
Alchami et al. [119] (112)	Tubulopapillary	43%	17 (15.1-18.9)	0.084
	Solid	29%	14 (9.4-18.6)	0.040
	Microcystic/ myxoid	14%	24 (16.2-32.8)	Ref.
	Pleomorphic	9%	8 (6.0-10.0)	<0.001
	Micropapillary	5%	12 (7.2-16.8)	0.008
Bilecz et al. [271] (182)	Solid	52%	13.2	
	Tubulopapillary	29%	24.2	
	Trabecular	10%	13.1	
	Microcystic	5%	31.2	
	Pleomorphic	3%	8.0	
	Micropapillary	1%	47.3	

5.2 Pleomorphic subtype EMM confers dismal prognosis

There is consensus on the negative prognostic effect of EMM with pleomorphic features, which is an independent prognostic factor in multivariate analyses [94]. It was proposed to be reclassified as non-epithelioid MPM; however, it was not implemented by the most recent WHO classification of MPM. We provided data supporting previous findings on dismal prognosis associated with pleomorphic EMM.
We also found that pleomorphic feature tumors harbored TERT promoter mutations at a high frequency similar to that of non-epithelioid MPM, providing a compelling molecular pathomechanism that explains the aggressive clinical behavior of pleomorphic MPM.

5.3 Histopathologic grading systems are robust prognostic factors in EMM

Until recently, there was no established histologic grading of EMM. Nuclear grading is the most widely accepted method, however, still not included as mandatory part of MPM pathologic reporting. The mitosis-necrosis score showed similar prognostic power to that of the nuclear grading and was based on easier-to-assess histologic variables. We were able to confirm the prognostic value of both grading systems, however, the mitosis-necrosis score was a more robust prognosticator of EMM outcomes in our cohort. In concordance with our findings, the 2019 EURACAN/ISLC proposal also recommends the use of a two-trier grading system for MPM. We found a significant association between high histologic grades and solid/trabecular predominant growth patterns of the EMM samples.

5.4 Histologic subtyping and predicting patients' benefit from MMT

There are no established predictive factors that identify patients who are most likely to benefit from MMT. So far localized disease stage, young age and epithelioid histology were used to select patients for aggressive surgical treatment and chemoradiotherapy. Blood CRP levels are emerging biomarkers that are likely to be predictive in this clinical setting [281]. In lung adenocarcinomas, there is substantial evidence that

histologic assessment of predominant growth patterns predicts patient subgroups showing more pronounced treatment response than other histologic subtypes.

Histological biopsy samples are available in almost all MPM cases. We evaluated if histologic subtyping of HE stained sections of small biopsies would be of help to identify patient subgroups with favorable responses to multimodal treatment in an explorative subcohort analysis. We formed patient subgroups based on therapy received (MMT or no MMT) and tumor histology (lumped groups solid/trabecular or tubulopapillary/microcystic). The subgroups that received MMT were comparable regarding patients' age, gender and tumor grade distribution. Interestingly, we found a borderline significant difference in patient outcomes between the solid/trabecular and tubulopapillary/microcystic patterns, the latter being associated with longer OS after MMT. These findings might be an exciting path for further investigation; however, their feasibility is limited by the size and the retrospective nature of our study and needs independent confirmation.

5.5 TERT promoter mutation is an independent negative prognostic factor in malignant pleural mesothelioma

TERT promoter mutation is associated with shorter median OS in a variety of tumor types, e. g. in gliomas, cutaneous malignant melanoma or bladder cancer. In a previous study, these non-coding mutations were associated with shorter median OS and with sarcomatoid histologic type and caused hTERT overexpression [282]. Recently, the negative prognostic role of TERT promoter mutation has been recapitulated and ´this mutation was found to be the third most common genetic alteration in MPM [190].

We were able to confirm the TERT promoter mutation as an independent negative prognostic factor. This prognostic impact was demonstrated both in epithelioid as well as in non-epithelioid cases. The difference in OS was independent of geographic differences, too, being present in both the Austrian and the Croatian-Slovenian subcohort in our study.

5.6 TERT promoter mutation and implications in systemic treatment for MPM

Reverse transcriptase inhibitors have long been used in the setting of AIDS treatment with tolerable toxicities and telomerase inhibitors have previously been tested as anticancer agents [283, 284]. The failure of previous studies might be attributable to ineffective inclusion criteria. Although telomerase activity or alternative telomere lengthening can be proven in most malignancies, the TERT promoter mutations leading to TERT overexpression represent a distinctive therapeutic target. We identified that approximately 10% of MPMs carry the quasi oncogenic mutation in the TERT promoter region which might be a marker for telomerase inhibitor efficacy.

First line systemic therapy of MPM is platinum based in combination with antifolate agents. The efficacy of these treatments is, however, dissatisfactory: most patients experience serious side effects and disease progression during therapy or recurrence after that. To evaluate the TERT promoter mutations' interaction with cisplatin sensitivity we carried out viability assays at various cisplatin concentrations in cell cultures. Interestingly, we found increased sensitivity for cisplatin in cell lines harboring TERT promoter mutations. The potential predictive role of TERT promoter mutations has been reported in gliomas where the mutant status was associated with response to adjuvant radiotherapy and alkylating agents [285]. Furthermore, TERT promoter mutation was found to predict response to eribulin mesylate, an agent reversing epithelial-mesenchymal transition in cell lines derived from ovarian malignancies [286]. In poorly differentiated thyroid carcinomas TERT promoter mutations were associated with radioidine resistance [287].

5.7 Mechanism of increased aggressivity of TERT promoter mutant MPM

TERT promoter mutations occur more frequently in MPM derived cell lines in comparison to FFPE MPM tumor specimens. Previously, this was explained by the higher sensitivity of the mutation detection in tumor cell lines [249]. We offer a different explanation for this finding, thus, that harboring TERT promoter mutations promote cell line formation. We observed that among 45 primary cell cultures all those originating from tumors harboring a promoter mutation were successfully immortalized,

and the TERT promoter mutation frequency was 41% among cell lines. In contrast, cell line formation was not associated with the histologic type and tumor stage at the time of the diagnosis and was only moderately associated with pleomorphic features in the original tumor samples.

5.8 Impact of the SNP rs2853669 in mesothelioma

The polymorphism rs2853669 C>T is known to quenche the negative prognostic effect associated with TERT promoter mutations in bladder cancer [258], diffuse gliomas and primary glioblastoma [264-266]. We compared patient outcomes among four subgroups: harboring TERT promoter mutations with or without variant allels of the SNP and TERT promoter wild type patients with or without the variant allele. We did not observe any difference between subgroups differing in the SNP status. Thus, the quenching effect of the variant allele seen in other cancer types was not confirmed in our MPM cohort.

5.9 Association of major molecular alterations and TERT promoter mutation

Nuclear reactivity of BAP1 immunohistochemical staining is known to correlate with retained expression of the BAP1 gene and the intact functionality of the protein product. In our subcohort consisting of 75 MPM samples we observed that each TERT promoter mutant sample exhibited nuclear immunohistochemical reactivity for BAP1, while the loss of nuclear BAP1 staining only occurred in TERT promoter wild-type samples. Thus, we concluded that loss-of function mutations and deletions of the BAP1 gene were mutually exclusive with the TERT promoter mutations. This finding is in line with a very recent previous study [190].

DOI:10.14753/SE.2020.2421

6. CONCLUSIONS

Reflecting on the aims of the present study and summarizing our results, we were able to draw the following conclusions:

1. We found that the microcystic and tubulopapillary subtypes of epithelioid mesothelioma associated with the longest median overall survival, while the solid and trabecular variants with shorter OS. Our data supports the proposal to consider the pleomorphic subtype in terms of prognosis as sarcomatoid MPM. We found an association between higher histological grades and solid/trabecular subtypes. Both the nuclear grading system and mitosis-necrosis score was useful in predicting patient outcomes, however, the mitosis score composed of two-tier factors (mitoses low vs. high, necrosis present vs. absent) was more robust in our experience.

2. We found a more pronounced benefit from a multimodal treatment approach in patients with tubulopapillary/microcystic subtype tumors compared to solid/trabecular tumors.

3. TERT promoter mutant status was a strong, independent predictor of poor prognosis. The difference in OS remained significant both within histologic types (epithelioid and non-epithelioid) and in geographically different subcohorts. We found that the mutant status was strongly associated with non-epithelioid histological type and the pleomorphic subtype of EMM.

4. We found that the common polymorphism rs2853669 C>T – in contrast to literature data on bladder cancer and primary glioblastoma – did not show any interaction with the TERT promoter mutation in MPM, and the effect of TERT promoter mutation on patient outcomes was not modified by the carried allele of the SNP.

5. The probability of de novo cell line formation was significantly higher in TERT promoter mutant MPM samples. TERT mRNA expression was significantly higher among cell lines harboring a TERTp mutation. We observed a tendency of higher *in vitro* cisplatin sensitivity among cell lines with mutant TERT core promoter region in comparison to TERTp wild-type cell lines.

6. In our MPM cohort mutations of the TERT core promoter region and genetic alterations of the BAP1 locus were mutually exclusive.

DOI:10.14753/SE.2020.2421

7. ÖSSZEFOGLALÁS

Az MPM ritka, ám rendkívül rossz prognózisú betegség. Megelőzését szolgálja az azbeszthasználat tilalma, azonban a közeljövőben nem várható az MPM incidenciájának jelentős csökkenése világszerte. A jelenlegi kezelési módok nem eredményeznek jelentős javulást a betegek túlélésében és a terápiás választ előrejelző faktorok sem ismertek. Így célul tűztük ki, hogy klinikailag alkalmazható, prognosztikus és prediktív szövettani és molekuláris faktorokat azonosítsunk. 192 epithelioid MPM esetet és az ezekhez kapcsolódó klinikai adatokat gyűjtöttünk öt nagy európai mellkassebészeti centrumból, majd a mintákat szövettani növekedési mintázat és grádus szempontjából vizsgáltuk HE festett metszeteken. Az EMM szövettani altípusai és a betegek túlélése között jelentős összefüggést találtunk, ezen belül a pleomorph altípus járt a legrosszabb prognózissal. A szolid/trabekuláris altípusok között nagyobb számban voltak magas magi grádusúak. Mind a magi jellemzők, mind a mitozisszám és nekrózis megítélésére épülő grade-rendszer jelentős prognosztikus erővel bírt. A trimodális kezelésben részesült betegek két jelentősen eltérő túlélésű csoportra voltak bonthatóak a tumoraik szolid/trabekuláris vagy tubulopapilláris/mikrocisztikus növekedési mintázata alapján. 182 epithelioid és nem-epithelioid MPM szövetminta és 22 MPM eredetű sejtvonal esetében a TERT promoter (TERTp) mutációs státuszát határoztuk meg. Az esetek 10.4%-ában azonosítottunk mutációt. A mutáns státusz a betegek túlélését kedvezőtlenül befolyásoló független prognosztikus faktornak bizonyult. 75 minta esetében BAP1 elleni antitesttel immunhisztokémiai reakciót végztünk, és a BAP1-vesztés valamint a TERTp mutációk egymás kizáró voltát azonosítottuk. Primer sejtkultúrákat hoztunk létre 45 beteg mintájából, melynek során a sejtvonal létrehozás sikere és a TERTp mutáns státusz között szignifikáns összefüggést találtunk. Az elvégzett qPCR vizsgálatok alapján a mutáns státusz fokozott mRNS-expresszióval járt. Sejtvonalak életképességét különböző ciszplatin koncentrációjú médiumban vizsgálva a TERTp mutáns sejtvonalak ciszplatinnal szembeni fokozott érzékenységét figyeltük meg. Összefoglalva, eredményeink alapján az EMM szövettani típusai és szövettani gradálása prognosztikus és prediktív jelentőségű, így a patológiai leletek fontos része lehet. A TERT promoter mutációi az MPM új molekuláris csoportját definiálják és független előrejelzői a kedvezőtlen klinikai kimenetelnek.

DOI:10.14753/SE.2020.2421

8. SUMMARY

Malignant pleural mesothelioma (MPM) is a rare disease with dismal prognosis. Although there are efforts to prevent MPM mostly through government bans on asbestos production and use, its worldwide incidence is not expected to diminish soon. The treatment options available for MPM patients fail to achieve long-term survival and there is an urgent need for predictive biomarkers. Therefore, we aimed to identify molecular and histological prognostic and predictive factors that can be employed in the clinical setting. We collected 192 epithelioid MPM (EMM) samples and corresponding clinical data from five large European thoracic oncology centers. Next, we evaluated the histological subtypes and performed grading on HE stained sections. We found histological subtypes of EMM to have significant prognostic impact, and we confirmed the dismal prognosis associated with the pleomorphic subtype. The solid/trabecular patterns were associated with higher nuclear grades. Both the nuclear grading system and the mitosis-necrosis score showed significant prognostic power. Among patients who received a more aggressive, multimodal treatment those with tubulopapillary/microcystic subtype tumors showed a borderline significant tendency for more pronounced improvement in median OS. We also tested 182 samples including both epithelioid and non-epithelioid cases, as well as 22 novel patient-derived MPM cell lines for TERT promoter mutation. Mutations were found in 10.4% of the cases. The promoter mutations significantly associated with poor patient outcomes and were found to be an independent prognostic factor. Through BAP1 immunohistochemistry on 75 specimens we found TERT promoter mutation and BAP1 loss to be mutually exclusive genetic alterations. Primary cell cultures were established in 45 patients and TERT promoter mutation conferred an increased probability of de novo cell line formation. Harboring a promoter mutation led to significant increase in TERT mRNA expression as quantified by qPCR in our 22 MPM cell lines. By measuring cell viability following cisplatin treatment we found increased cisplatin sensitivity in TERT promoter mutant cells. In conclusion, we provided data supporting the inclusion of histological subtypes and histological grading in pathological reporting on epithelioid MPM due to their prognostic and potentially predictive role. TERT promoter mutations independently predict poor outcomes and identify a distinct molecular subgroup of MPM.

9. REFERENCES

1. García-Fadrique A, Mehta A, Mohamed F, Dayal S, Cecil T, and Moran BJ. (2017) Clinical presentation, diagnosis, classification and management of peritoneal mesothelioma: a review. Journal of gastrointestinal oncology, 8: 915-924.
2. McGehee E, Gerber DE, Reisch J, and Dowell JE. (2019) Treatment and outcomes of primary pericardial mesothelioma: A contemporary review of 103 published cases. Clinical lung cancer, 20: e152-e157.
3. Arda E, Arıkan MG, Cetin G, Kuyumcuoğlu U, and Usta U. (2017) Malignant Mesothelioma of Tunica Vaginalis Testis: Macroscopic and Microscopic Features of a Very Rare Malignancy. Cureus, 9: e1860-e1860.
4. Attanoos RL and Gibbs AR. (2000) Primary malignant gonadal mesotheliomas and asbestos. Histopathology, 37: 150-9.
5. Shavelle R, Vavra-Musser K, Lee J, and Brooks J. (2017) Life Expectancy in Pleural and Peritoneal Mesothelioma. Lung cancer international, 2017: 2782590-2782590.
6. Delgermaa V, Takahashi K, Park E-K, Le GV, Hara T, and Sorahan T. (2011) Global mesothelioma deaths reported to the World Health Organization between 1994 and 2008. Bulletin of the World Health Organization, 89: 716-724.
7. 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, and 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.
8. Baas P, Fennell D, Kerr KM, Van Schil PE, Haas RL, and Peters S. (2015) Malignant pleural mesothelioma: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. Ann Oncol, 26 Suppl 5: v31-9.
9. Bianchi C and Bianchi T. (2014) Global mesothelioma epidemic: Trend and features. Indian J Occup Environ Med, 18: 82-8.
10. Järvholm B and Burdorf A. (2015) Emerging evidence that the ban on asbestos use is reducing the occurrence of pleural mesothelioma in Sweden. Scandinavian Journal of Public Health, 43: 875-881.
11. Hemminki K and Li X. (2003) Time trends and occupational risk factors for pleural mesothelioma in Sweden. Journal of occupational and environmental medicine, 45: 456-461.
12. Stayner L, Welch LS, and Lemen R. (2013) The worldwide pandemic of asbestos-related diseases. Annual review of public health, 34: 205-216.
13. Buikhuisen WA, Hiddinga BI, Baas P, and van Meerbeeck JP. (2015) Second line therapy in malignant pleural mesothelioma: A systematic review. Lung Cancer, 89: 223-231.
14. Boffetta P, Malvezzi M, Pira E, Negri E, and La Vecchia C. (2018) International Analysis of Age-Specific Mortality Rates From Mesothelioma on the Basis of the International Classification of Diseases, 10th Revision. J Glob Oncol, 4: 1-15.

15. Marsili D, Terracini B, Santana VS, Ramos-Bonilla JP, Pasetto R, Mazzeo A, Loomis D, Comba P, and Algranti E. (2016) Prevention of Asbestos-Related Disease in Countries Currently Using Asbestos. International journal of environmental research and public health, 13: 494.
16. Holmes D. (2013) IARC in the dock over ties with asbestos industry. The Lancet, 381: 359-361.
17. Kameda T, Takahashi K, Kim R, Jiang Y, Movahed M, Park EK, and Rantanen J. (2014) Asbestos: use, bans and disease burden in Europe. Bull World Health Organ, 92: 790-7.
18. *http://www.ibasecretariat.org/alpha_ban_list.php*. Updated: July 15 2019.
19. Thomas A, Chen Y, Yu T, Gill A, and Prasad V. (2015) Distinctive clinical characteristics of malignant mesothelioma in young patients. Oncotarget, 6: 16766-16773.
20. Goldberg M, Imbernon E, Rolland P, Ilg AGS, Savés M, de Quillacq A, Frenay C, Chamming's S, Arveux P, and Boutin C. (2006) The French national mesothelioma surveillance program. Occupational and environmental medicine, 63: 390-395.
21. Liu B, van Gerwen M, Bonassi S, and Taioli E. (2017) Epidemiology of environmental exposure and malignant mesothelioma. Journal of Thoracic Oncology, 12: 1031-1045.
22. Taioli E, Wolf AS, Camacho-Rivera M, and Flores RM. (2014) Women With Malignant Pleural Mesothelioma Have a Threefold Better Survival Rate Than Men. The Annals of Thoracic Surgery, 98: 1020-1024.
23. Robinson BM. (2012) Malignant pleural mesothelioma: an epidemiological perspective. Annals of cardiothoracic surgery, 1: 491.
24. Montanaro F, Bray F, Gennaro V, Merler E, Tyczynski JE, and Parkin DM. (2003) Pleural mesothelioma incidence in Europe: evidence of some deceleration in the increasing trends. Cancer causes & control, 14: 791-803.
25. Carbone M, Ly BH, Dodson RF, Pagano I, Morris PT, Dogan UA, Gazdar AF, Pass HI, and Yang H. (2012) Malignant mesothelioma: facts, myths, and hypotheses. J Cell Physiol, 227: 44-58.
26. Yang H, Rivera Z, Jube S, Nasu M, Bertino P, Goparaju C, Franzoso G, Lotze MT, Krausz T, Pass HI, Bianchi ME, and Carbone M. (2010) Programmed necrosis induced by asbestos in human mesothelial cells causes high-mobility group box 1 protein release and resultant inflammation. Proc Natl Acad Sci U S A, 107: 12611-6.
27. Roggli V, Gibbs AR, Attanoos R, Churg A, Popper H, Corrin B, Franks T, Galateau-Salle F, Galvin J, Hasleton P, and Honma K. (2016) Pathology of Asbestosis: An Update of the Diagnostic Criteria Response to a Critique. Archives of pathology & laboratory medicine, 140: 950-952.
28. Carbone M, Adusumilli PS, Alexander Jr HR, Baas P, Bardelli F, Bononi A, Bueno R, Felley-Bosco E, Galateau-Salle F, Jablons D, Mansfield AS, Minaai M, de Perrot M, Pesavento P, Rusch V, Severson DT, Taioli E, Tsao A, Woodard G, Yang H, Zauderer MG, and Pass HI. (2019) Mesothelioma: Scientific clues for prevention, diagnosis, and therapy. CA: A Cancer Journal for Clinicians, 69: 402-429.
29. Lin R-T, Takahashi K, Karjalainen A, Hoshuyama T, Wilson D, Kameda T, Chan C-C, Wen C-P, Furuya S, and Higashi T. (2007) Ecological association

between asbestos-related diseases and historical asbestos consumption: an international analysis. The lancet, 369: 844-849.
30. Hodgson JT and Darnton A. (2000) The quantitative risks of mesothelioma and lung cancer in relation to asbestos exposure. Annals of Occupational Hygiene, 44: 565-601.
31. Reid A, De Klerk N, Magnani C, Ferrante D, Berry G, Musk A, and Merler E. (2014) Mesothelioma risk after 40 years since first exposure to asbestos: a pooled analysis. Thorax, 69: 843-850.
32. Nuyts V, Nawrot T, Nemery B, and Nackaerts K. (2018) Hotspots of malignant pleural mesothelioma in Western Europe. Translational lung cancer research, 7: 516.
33. Mak V, Davies E, Putcha V, Choodari-Oskooei B, and Møller H. (2008) The epidemiology and treatment of mesothelioma in South East England 1985–2002. Thorax, 63: 160-166.
34. Marinaccio A, Binazzi A, Marzio DD, Scarselli A, Verardo M, Mirabelli D, Gennaro V, Mensi C, Riboldi L, and Merler E. (2012) Pleural malignant mesothelioma epidemic: incidence, modalities of asbestos exposure and occupations involved from the Italian National Register. International journal of cancer, 130: 2146-2154.
35. Fazzo L, De Santis M, Minelli G, Bruno C, Zona A, Marinaccio A, Conti S, and Comba P. (2012) Pleural mesothelioma mortality and asbestos exposure mapping in Italy. American journal of industrial medicine, 55: 11-24.
36. Rolland P, Gramond C, Lacourt A, Astoul P, Chamming's S, Ducamp S, Frenay C, Galateau-Salle F, Ilg AG, Imbernon E, Le Stang N, Pairon JC, Goldberg M, and Brochard P. (2010) Occupations and industries in France at high risk for pleural mesothelioma: A population-based case-control study (1998-2002). Am J Ind Med, 53: 1207-19.
37. Peretz A, Van Hee VC, Kramer MR, Pitlik S, and Keifer MC. (2008) Pleural plaques related to "take-home" exposure to asbestos: An international case series. International journal of general medicine, 1: 15-20.
38. Baumann F, Ambrosi JP, and Carbone M. (2013) Asbestos is not just asbestos: an unrecognised health hazard. Lancet Oncol, 14: 576-8.
39. Schinwald A, Murphy FA, Prina-Mello A, Poland CA, Byrne F, Movia D, Glass JR, Dickerson JC, Schultz DA, Jeffree CE, Macnee W, and 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: 461-70.
40. Teta MJ, Lau E, Sceurman BK, and Wagner ME. (2007) Therapeutic radiation for lymphoma: risk of malignant mesothelioma. Cancer, 109: 1432-8.
41. Carbone M, Pass HI, Rizzo P, Marinetti M, Di Muzio M, Mew DJ, Levine AS, and Procopio A. (1994) Simian virus 40-like DNA sequences in human pleural mesothelioma. Oncogene, 9: 1781-90.
42. Yap TA, Aerts JG, Popat S, and Fennell DA. (2017) Novel insights into mesothelioma biology and implications for therapy. Nat Rev Cancer, 17: 475-488.
43. Testa JR, Cheung M, Pei J, Below JE, Tan Y, Sementino E, Cox NJ, Dogan AU, Pass HI, and Trusa S. (2011) Germline BAP1 mutations predispose to malignant mesothelioma. Nature genetics, 43: 1022.

44. Masoomian B, Shields JA, and Shields CL. (2018) Overview of BAP1 cancer predisposition syndrome and the relationship to uveal melanoma. Journal of current ophthalmology, 30: 102-109.
45. Wiesner T, Obenauf AC, Murali R, Fried I, Griewank KG, Ulz P, Windpassinger C, Wackernagel W, Loy S, Wolf I, Viale A, Lash AE, Pirun M, Socci ND, Rütten A, Palmedo G, Abramson D, Offit K, Ott A, Becker JC, Cerroni L, Kutzner H, Bastian BC, and Speicher MR. (2011) Germline mutations in BAP1 predispose to melanocytic tumors. Nature Genetics, 43: 1018-1021.
46. Wiesner T, Murali R, Fried I, Cerroni L, Busam K, Kutzner H, and Bastian BC. (2012) A distinct subset of atypical Spitz tumors is characterized by BRAF mutation and loss of BAP1 expression. Am J Surg Pathol, 36: 818-30.
47. Harbour JW, Onken MD, Roberson EDO, Duan S, Cao L, Worley LA, Council ML, Matatall KA, Helms C, and Bowcock AM. (2010) Frequent Mutation of BAP1 in Metastasizing Uveal Melanomas. Science, 330: 1410-1413.
48. Popova T, Hebert L, Jacquemin V, Gad S, Caux-Moncoutier V, Dubois-d'Enghien C, Richaudeau B, Renaudin X, Sellers J, Nicolas A, Sastre-Garau X, Desjardins L, Gyapay G, Raynal V, Sinilnikova OM, Andrieu N, Manié E, de Pauw A, Gesta P, Bonadona V, Maugard CM, Penet C, Avril M-F, Barillot E, Cabaret O, Delattre O, Richard S, Caron O, Benfodda M, Hu H-H, Soufir N, Bressac-de Paillerets B, Stoppa-Lyonnet D, and Stern M-H. (2013) Germline BAP1 mutations predispose to renal cell carcinomas. American journal of human genetics, 92: 974-980.
49. De La Fouchardiere A, Cabaret O, Savin L, Combemale P, Schvartz H, Penet C, Bonadona V, Soufir N, and Bressac-de Paillerets B. (2015) Germline BAP1 mutations predispose also to multiple basal cell carcinomas. Clinical genetics, 88: 273-277.
50. Rai K, Pilarski R, Cebulla CM, and Abdel-Rahman MH. (2016) Comprehensive review of BAP1 tumor predisposition syndrome with report of two new cases. Clinical Genetics, 89: 285-294.
51. Carbone M, Flores EG, Emi M, Johnson TA, Tsunoda T, Behner D, Hoffman H, Hesdorffer M, Nasu M, and Napolitano A. (2015) Combined genetic and genealogic studies uncover a large BAP1 cancer syndrome kindred tracing back nine generations to a common ancestor from the 1700s. PLoS genetics, 11: e1005633.
52. Baumann F, Flores E, Napolitano A, Kanodia S, Taioli E, Pass H, Yang H, and Carbone M. (2015) Mesothelioma patients with germline BAP1 mutations have 7-fold improved long-term survival. Carcinogenesis, 36: 76-81.
53. Hassan R, Morrow B, Thomas A, Walsh T, Lee MK, Gulsuner S, Gadiraju M, Panou V, Gao S, Mian I, Khan J, Raffeld M, Patel S, Xi L, Wei JS, Hesdorffer M, Zhang J, Calzone K, Desai A, Padiernos E, Alewine C, Schrump DS, Steinberg SM, Kindler HL, King MC, and Churpek JE. (2019) Inherited predisposition to malignant mesothelioma and overall survival following platinum chemotherapy. Proc Natl Acad Sci U S A, 116: 9008-9013.
54. Betti M, Aspesi A, Biasi A, Casalone E, Ferrante D, Ogliara P, Gironi LC, Giorgione R, Farinelli P, Grosso F, Libener R, Rosato S, Turchetti D, Maffe A, Casadio C, Ascoli V, Dianzani C, Colombo E, Piccolini E, Pavesi M, Miccoli S,

Mirabelli D, Bracco C, Righi L, Boldorini R, Papotti M, Matullo G, Magnani C, Pasini B, and Dianzani I. (2016) CDKN2A and BAP1 germline mutations predispose to melanoma and mesothelioma. Cancer Lett, 378: 120-30.

55. Kondola S, Manners D, and Nowak AK. (2016) Malignant pleural mesothelioma: an update on diagnosis and treatment options. Therapeutic Advances in Respiratory Disease, 10: 275-288.
56. van Zandwijk N, Clarke C, Henderson D, Musk AW, Fong K, Nowak A, Loneragan R, McCaughan B, Boyer M, and Feigen M. (2013) Guidelines for the diagnosis and treatment of malignant pleural mesothelioma. J Thorac Dis, 5: E254.
57. Bibby AC, Tsim S, Kanellakis N, Ball H, Talbot DC, Blyth KG, Maskell NA, and Psallidas I. (2016) Malignant pleural mesothelioma: an update on investigation, diagnosis and treatment. European Respiratory Review, 25: 472.
58. Woolhouse I, Bishop L, Darlison L, de Fonseka D, Edey A, Edwards J, Faivre-Finn C, Fennell DA, Holmes S, and Kerr KM. (2018) BTS guideline for the investigation and management of malignant pleural mesothelioma. BMJ open respiratory research, 5: e000266.
59. Sinha S, Swift AJ, Kamil MA, Matthews S, Bull MJ, Fisher P, De Fonseka D, Saha S, Edwards JG, and Johns CS. (2020) The role of imaging in malignant pleural mesothelioma: an update after the 2018 BTS guidelines. Clinical Radiology.
60. Kim YK, Kim JS, Lee KW, Yi CA, Goo JM, and Jung S-H. (2016) Multidetector CT Findings and Differential Diagnoses of Malignant Pleural Mesothelioma and Metastatic Pleural Diseases in Korea. Korean J Radiol, 17: 545-553.
61. Kindler HL, Ismaila N, Armato SG, Bueno R, Hesdorffer M, Jahan T, Jones CM, Miettinen M, Pass H, Rimner A, Rusch V, Sterman D, Thomas A, and Hassan R. (2018) Treatment of Malignant Pleural Mesothelioma: American Society of Clinical Oncology Clinical Practice Guideline. Journal of Clinical Oncology, 36: 1343-1373.
62. Whitaker D. (2000) Invited review The cytology of malignant mesothelioma. Cytopathology, 11: 139-151.
63. Aliya Noor Husain, Thomas V. Colby, Nelson G. Ordóñez, Timothy Craig Allen, Richard Luther Attanoos, Mary Beth Beasley, Kelly Jo Butnor, Lucian R. Chirieac, Andrew M. Churg, Sanja Dacic, Françoise Galateau-Sallé, Allen Gibbs, Allen M. Gown, Thomas Krausz, Leslie Anne Litzky, Alberto Marchevsky, Andrew G. Nicholson, Victor Louis Roggli, Anupama K. Sharma, William D. Travis, Ann E. Walts, and Mark R. Wick. (2018) Guidelines for Pathologic Diagnosis of Malignant Mesothelioma 2017 Update of the Consensus Statement From the International Mesothelioma Interest Group. 142: 89-108.
64. Ikeda K, Tate G, Suzuki T, Kitamura T, and Mitsuya T. (2011) Diagnostic usefulness of EMA, IMP3, and GLUT-1 for the immunocytochemical distinction of malignant cells from reactive mesothelial cells in effusion cytology using cytospin preparations. Diagnostic cytopathology, 39: 395-401.
65. Lonardi S, Manera C, Marucci R, Santoro A, Lorenzi L, and Facchetti F. (2011) Usefulness of Claudin 4 in the cytological diagnosis of serosal effusions. Diagn Cytopathol, 39: 313-7.

66. Ikeda K, Tate G, Suzuki T, Kitamura T, and Mitsuya T. (2010) IMP3/L523S, a novel immunocytochemical marker that distinguishes benign and malignant cells: the expression profiles of IMP3/L523S in effusion cytology. Hum Pathol, 41: 745-50.
67. Sheaff M. (2011) Should cytology be an acceptable means of diagnosing malignant mesothelioma? Cytopathology, 22: 3-4.
68. Hjerpe A, Ascoli V, Bedrossian CW, Boon ME, Creaney J, Davidson B, Dejmek A, Dobra K, Fassina A, Field A, Firat P, Kamei T, Kobayashi T, Michael CW, Onder S, Segal A, and Vielh P. (2015) Guidelines for the cytopathologic diagnosis of epithelioid and mixed-type malignant mesothelioma. Complementary statement from the International Mesothelioma Interest Group, also endorsed by the International Academy of Cytology and the Papanicolaou Society of Cytopathology. Acta Cytol, 59: 2-16.
69. Scherpereel A, Astoul P, Baas P, Berghmans T, Clayson H, de Vuyst P, Dienemann H, Galateau-Salle F, Hennequin C, Hillerdal G, Le Pechoux C, Mutti L, Pairon JC, Stahel R, van Houtte P, van Meerbeeck J, Waller D, and Weder W. (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: 479-95.
70. Geltner C, Errhalt P, Baumgartner B, Ambrosch G, Machan B, Eckmayr J, Klikovits T, Hoda MA, Popper H, and Klepetko W. (2016) Management of malignant pleural mesothelioma – part 1: epidemiology, diagnosis, and staging: Consensus of the Austrian Mesothelioma Interest Group (AMIG). Wiener klinische Wochenschrift, 128.
71. Desai NR and Lee HJ. (2017) Diagnosis and management of malignant pleural effusions: state of the art in 2017. J Thorac Dis, 9: S1111-s1122.
72. Pereyra MF, San-José E, Ferreiro L, Golpe A, Antúnez J, González-Barcala F-J, Abdulkader I, Álvarez-Dobaño JM, Rodríguez-Núñez N, and Valdés L. (2013) Role of blind closed pleural biopsy in the managment of pleural exudates. Can Respir J, 20: 362-366.
73. Rice DC, Steliga MA, Stewart J, Eapen G, Jimenez CA, Lee JH, Hofstetter WL, Marom EM, Mehran RJ, Vaporciyan AA, Walsh GL, and Swisher SG. (2009) Endoscopic ultrasound-guided fine needle aspiration for staging of malignant pleural mesothelioma. Ann Thorac Surg, 88: 862-8; discussion 868-9.
74. Czarnecka-Kujawa K, de Perrot M, Keshavjee S, and Yasufuku K. (2019) Endobronchial ultrasound-guided transbronchial needle aspiration mediastinal lymph node staging in malignant pleural mesothelioma. J Thorac Dis, 11: 602-612.
75. Richards WG. (2017) Malignant pleural mesothelioma: predictors and staging. Ann Transl Med, 5: 243-243.
76. Péus D, Newcomb N, and Hofer S. (2013) Appraisal of the Karnofsky Performance Status and proposal of a simple algorithmic system for its evaluation. BMC Med Inform Decis Mak, 13: 72-72.
77. Sørensen JB, Klee M, Palshof T, and Hansen HH. (1993) Performance status assessment in cancer patients. An inter-observer variability study. Br J Cancer, 67: 773-775.
78. Curran D, Sahmoud T, Therasse P, van Meerbeeck J, Postmus PE, and Giaccone G. (1998) Prognostic factors in patients with pleural mesothelioma: the

European Organization for Research and Treatment of Cancer experience. Journal of Clinical Oncology, 16: 145-152.

79. Sandri A, Guerrera F, Roffinella M, Olivetti S, Costardi L, Oliaro A, Filosso PL, Lausi PO, and Ruffini E. (2016) Validation of EORTC and CALGB prognostic models in surgical patients submitted to diagnostic, palliative or curative surgery for malignant pleural mesothelioma. J Thorac Dis, 8: 2121-2127.
80. Fennell DA, Parmar A, Shamash J, Evans MT, Sheaff MT, Sylvester R, Dhaliwal K, Gower N, Steele J, and Rudd R. (2005) Statistical Validation of the EORTC Prognostic Model for Malignant Pleural Mesothelioma Based on Three Consecutive Phase II Trials. Journal of Clinical Oncology, 23: 184-189.
81. 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, and Rusch VW. (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. Journal of thoracic oncology : official publication of the International Association for the Study of Lung Cancer, 11: 2100-2111.
82. 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, Goldstraw P, Rami-Porta R, Asamura H, Ball D, Beer D, Beyruti R, Bolejack V, Chansky K, Crowley J, Detterbeck FC, Eberhardt WEE, Edwards J, Galateau-Sallé F, Giroux D, Gleeson F, Groome P, Huang J, Kennedy C, Kim J, Kim YT, Kingsbury L, Kondo H, Krasnik M, Kubota K, Lerut T, Lyons G, Marino M, Marom EM, van Meerbeeck JP, Mitchell A, Nakano T, Nicholson AG, Nowak A, Peake M, Rice TW, Rosenzweig K, Ruffini E, Rusch VW, Saijo N, Van Schil P, Sculier J-P, Shemanski L, Stratton K, Suzuki K, Tachimori Y, Thomas CF, Travis WD, Tsao MS, Turrisi A, Vansteenkiste J, Watanabe H, Wu Y-L, Baas P, Erasmus J, Hasegawa S, Inai K, Kernstine K, Kindler H, Krug L, Nackaerts K, Pass H, Rice D, Falkson C, Filosso PL, Giaccone G, Kondo K, Lucchi M, Okumura M, Blackstone E, Asamura H, Batirel H, Bille A, Pastorino U, Call S, Cangir A, Cedres S, Friedberg J, Galateau-Sallé F, Hasagawa S, Kernstine K, Kindler H, McCaughan B, Nakano T, Nowak A, Ozturk CA, Pass H, de Perrot M, Rea F, Rice D, Rintoul R, Ruffini E, Rusch V, Spaggiari L, Galetta D, Syrigos K, Thomas C, van Meerbeeck JP, Nafteux P, Vansteenkiste J, Weder W, Optiz I and Yoshimura M. (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. Journal of Thoracic Oncology, 11: 2112-2119.
83. Bonomi M, De Filippis C, Lopci E, Gianoncelli L, Rizzardi G, Cerchiaro E, Bortolotti L, Zanello A, and Ceresoli GL. (2017) Clinical staging of malignant pleural mesothelioma: current perspectives. Lung Cancer (Auckl), 8: 127-139.
84. Pass HI and Carbone M. (2009) Current Status of Screening for Malignant Pleural Mesothelioma. Seminars in Thoracic and Cardiovascular Surgery, 21: 97-104.
85. Lamote K, Vynck M, Thas O, Van Cleemput J, Nackaerts K, and van Meerbeeck JP. (2017) Exhaled breath to screen for malignant pleural mesothelioma: a validation study. European Respiratory Journal, 50: 1700919.

86. Roberts HC, Patsios DA, Paul NS, dePerrot M, Teel W, Bayanati H, Shepherd F, and Johnston MR. (2009) Screening for Malignant Pleural Mesothelioma and Lung Cancer in Individuals with a History of Asbestos Exposure. Journal of Thoracic Oncology, 4: 620-628.
87. van Meerbeeck JP and Hillerdal G. (2008) Screening for Mesothelioma. American Journal of Respiratory and Critical Care Medicine, 178: 781-782.
88. Brusselmans L, Arnouts L, Millevert C, Vandersnickt J, van Meerbeeck JP, and Lamote K. (2018) Breath analysis as a diagnostic and screening tool for malignant pleural mesothelioma: a systematic review. Translational lung cancer research, 7: 520-536.
89. Woolhouse I, Bishop L, Darlison L, De Fonseka D, Edey A, Edwards J, Faivre-Finn C, Fennell DA, Holmes S, Kerr KM, Nakas A, Peel T, Rahman NM, Slade M, Steele J, Tsim S, and Maskell NA. (2018) British Thoracic Society Guideline for the investigation and management of malignant pleural mesothelioma. Thorax, 73: i1.
90. Travis WD, Brambilla E, Burke AP, Marx A, Nicholson AJT, and Heart. (2015) WHO classification of Tumours of the Lung, Pleura. 4: 78-9.
91. Verma V, Ahern CA, Berlind CG, Lindsay WD, Shabason J, Sharma S, Culligan MJ, Grover S, Friedberg JS, and Simone CB. (2018) Survival by Histologic Subtype of Malignant Pleural Mesothelioma and the Impact of Surgical Resection on Overall Survival. Clinical Lung Cancer, 19: e901-e912.
92. Yang C, Mayne N, Deng J, Commander S, D'amico T, and Berry M. (2018) The Impact of Malignant Pleural Mesothelioma Histology on the Use of Surgery and Survival in a Population-Based Analysis. Journal of Thoracic Oncology, 13.
93. Meyerhoff RR, Yang C-FJ, Speicher PJ, Gulack BC, Hartwig MG, D'Amico TA, Harpole DH, and Berry MF. (2015) Impact of mesothelioma histologic subtype on outcomes in the Surveillance, Epidemiology, and End Results database. Journal of Surgical Research, 196: 23-32.
94. Kadota K, Suzuki K, Sima CS, Rusch VW, Adusumilli PS, and Travis WD. (2011) Pleomorphic Epithelioid Diffuse Malignant Pleural Mesothelioma: A Clinicopathological Review and Conceptual Proposal to Reclassify as Biphasic or Sarcomatoid Mesothelioma. Journal of Thoracic Oncology, 6: 896-904.
95. Galateau Salle F, Le Stang N, Nicholson AG, Pissaloux D, Churg A, Klebe S, Roggli VL, Tazelaar HD, Vignaud JM, Attanoos R, Beasley MB, Begueret H, Capron F, Chirieac L, Copin MC, Dacic S, Danel C, Foulet-Roge A, Gibbs A, Giusiano-Courcambeck S, Hiroshima K, Hofman V, Husain AN, Kerr K, Marchevsky A, Nabeshima K, Picquenot JM, Rouquette I, Sagan C, Sauter JL, Thivolet F, Travis WD, Tsao MS, Weynand B, Damiola F, Scherpereel A, Pairon JC, Lantuejoul S, Rusch V, and Girard N. (2018) New Insights on Diagnostic Reproducibility of Biphasic Mesotheliomas: A Multi-Institutional Evaluation by the International Mesothelioma Panel From the MESOPATH Reference Center. Journal of Thoracic Oncology, 13: 1189-1203.
96. Ordóñez NG. (2012) Deciduoid mesothelioma: report of 21 cases with review of the literature. Modern Pathology, 25: 1481.
97. Galateau-Sallé F, Attanoos R, Gibbs AR, Burke L, Astoul P, Rolland P, soit Ilg AG, Pairon JC, Brochard P, and Begueret HJTAjosp. (2007) Lymphohistiocytoid variant of malignant mesothelioma of the pleura: a series of 22 cases. 31: 711-716.

98. Ordonez NG. (2012) Mesotheliomas with small cell features: report of eight cases. Mod Pathol, 25: 689-98.
99. Nicholson AG, Sauter JL, Nowak AK, Kindler HL, Gill RR, Remy-Jardin M, Armato SG, Fernandez-Cuesta L, Bueno R, Alcala N, Foll M, Pass H, Attanoos R, Baas P, Beasley MB, Brcic L, Butnor KJ, Chirieac LR, Churg A, Courtiol P, Dacic S, De Perrot M, Frauenfelder T, Gibbs A, Hirsch FR, Hiroshima K, Husain A, Klebe S, Lantuejoul S, Moreira A, Opitz I, Perol M, Roden A, Roggli V, Scherpereel A, Tirode F, Tazelaar H, Travis WD, Tsao MS, van Schil P, Vignaud JM, Weynand B, Cree I, Rusch VW, Girard N, and Galateau-Salle F. (2019) EURACAN/IASLC proposals for updating the histologic classification of pleural mesothelioma: towards a more multidisciplinary approach. Journal of Thoracic Oncology.
100. Gullo I, Carneiro F, Oliveira C, and Almeida GMJP. (2018) Heterogeneity in gastric cancer: from pure morphology to molecular classifications. 85: 50-63.
101. Fernández MI, Williams SB, Willis DL, Slack RS, Dickstein RJ, Parikh S, Chiong E, Siefker-Radtke AO, Guo CC, Czerniak BA, McConkey DJ, Shah JB, Pisters LL, Grossman HB, Dinney CPN, and Kamat AM. (2017) Clinical risk stratification in patients with surgically resectable micropapillary bladder cancer. 119: 684-691.
102. Chang S-J, Ryu H-S, Chang K-H, Yoo S-C, and Yoon J-H. (2008) Prognostic significance of the micropapillary pattern in patients with serous borderline ovarian tumors. Acta obstetricia et gynecologica Scandinavica, 87: 476-481.
103. Travis WD, Brambilla E, Noguchi M, Nicholson AG, Geisinger KR, Yatabe Y, Beer DG, Powell CA, Riely GJ, and Van Schil PE. (2011) International association for the study of lung cancer/american thoracic society/european respiratory society international multidisciplinary classification of lung adenocarcinoma. Journal of thoracic oncology, 6: 244-285.
104. Borczuk ACJERR. (2016) Prognostic considerations of the new World Health Organization classification of lung adenocarcinoma. 25: 364-371.
105. Tsao AS, Scagliotti GV, Bunn PA, Carbone DP, Warren GW, Bai C, de Koning HJ, Yousaf-Khan AU, McWilliams A, Tsao MS, Adusumilli PS, Rami-Porta R, Asamura H, Van Schil PE, Darling GE, Ramalingam SS, Gomez DR, Rosenzweig KE, Zimmermann S, Peters S, Ignatius Ou S-H, Reungwetwattana T, Jänne PA, Mok TS, Wakelee HA, Pirker R, Mazières J, Brahmer JR, Zhou Y, Herbst RS, Papadimitrakopoulou VA, Redman MW, Wynes MW, Gandara DR, Kelly RJ, Hirsch FR, and Pass HI. (2016) Scientific Advances in Lung Cancer 2015. Journal of Thoracic Oncology, 11: 613-638.
106. Yu Y, Jian H, Shen L, Zhu L, and Lu S. (2016) Lymph node involvement influenced by lung adenocarcinoma subtypes in tumor size≤ 3 cm disease: A study of 2268 cases. European Journal of Surgical Oncology (EJSO), 42: 1714-1719.
107. Kadota K, Villena-Vargas J, Yoshizawa A, Motoi N, Sima CS, Riely GJ, Rusch VW, Adusumilli PS, and Travis WD. (2014) Prognostic significance of adenocarcinoma in situ, minimally invasive adenocarcinoma, and nonmucinous lepidic predominant invasive adenocarcinoma of the lung in patients with stage I disease. The American journal of surgical pathology, 38: 448.
108. Kamiya K, Hayashi Y, Douguchi J, Hashiguchi A, Yamada T, Izumi Y, Watanabe M, Kawamura M, Horinouchi H, and Shimada N. (2008)

Histopathological features and prognostic significance of the micropapillary pattern in lung adenocarcinoma. Modern Pathology, 21: 992.

109. Cha MJ, Lee HY, Lee KS, Jeong JY, Han J, Shim YM, and Hwang HS. (2014) Micropapillary and solid subtypes of invasive lung adenocarcinoma: clinical predictors of histopathology and outcome. The Journal of thoracic and cardiovascular surgery, 147: 921-928. e2.
110. Yanagawa N, Shiono S, Abiko M, Katahira M, Osakabe M, and Ogata S-y. (2016) The Clinical Impact of Solid and Micropapillary Patterns in Resected Lung Adenocarcinoma. Journal of Thoracic Oncology, 11: 1976-1983.
111. Ujiie H, Kadota K, Chaft JE, Buitrago D, Sima CS, Lee M-C, Huang J, Travis WD, Rizk NP, and Rudin CM. (2015) Solid predominant histologic subtype in resected stage I lung adenocarcinoma is an independent predictor of early, extrathoracic, multisite recurrence and of poor postrecurrence survival. Journal of clinical oncology, 33: 2877.
112. Tsuta K, Kawago M, Inoue E, Yoshida A, Takahashi F, Sakurai H, Watanabe SI, Takeuchi M, Furuta K, Asamura H, and Tsuda H. (2013) The utility of the proposed IASLC/ATS/ERS lung adenocarcinoma subtypes for disease prognosis and correlation of driver gene alterations. Lung Cancer, 81: 371-376.
113. Kadota K, Yeh Y-C, D'Angelo SP, Moreira AL, Kuk D, Sima CS, Riely GJ, Arcila ME, Kris MG, Rusch VW, Adusumilli PS, and Travis WD. (2014) Associations between mutations and histologic patterns of mucin in lung adenocarcinoma: invasive mucinous pattern and extracellular mucin are associated with KRAS mutation. The American journal of surgical pathology, 38: 1118-1127.
114. Yoshizawa A, Sumiyoshi S, Sonobe M, Kobayashi M, Fujimoto M, Kawakami F, Tsuruyama T, Travis WD, Date H, and Haga H. (2013) Validation of the IASLC/ATS/ERS lung adenocarcinoma classification for prognosis and association with EGFR and KRAS gene mutations: analysis of 440 Japanese patients. Journal of Thoracic Oncology, 8: 52-61.
115. Tsao M-S, Marguet S, Le Teuff G, Lantuejoul S, Shepherd FA, Seymour L, Kratzke R, Graziano SL, Popper HH, and Rosell RJJoco. (2015) Subtype classification of lung adenocarcinoma predicts benefit from adjuvant chemotherapy in patients undergoing complete resection. 33: 3439.
116. Riely GJ and Travis WD. (2014) Can IASLC/ATS/ERS subtype help predict response to chemotherapy in small biopsies of advanced lung adenocarcinoma? European Respiratory Journal, 43: 1240.
117. Luo J, Huang Q, Wang R, Han B, Zhang J, Zhao H, Fang W, Luo Q, Yang J, Yang YJJocr, and oncology c. (2016) Prognostic and predictive value of the novel classification of lung adenocarcinoma in patients with stage IB. 142: 2031-2040.
118. Campos-Parra AD, Aviles A, Contreras-Reyes S, Rojas-Marin CE, Sanchez-Reyes R, Borbolla-Escoboza RJ, and Arrieta O. (2014) Relevance of the novel IASLC/ATS/ERS classification of lung adenocarcinoma in advanced disease. Eur Respir J, 43: 1439-47.
119. Alchami FS, Attanoos RL, and Bamber AR. (2017) Myxoid variant epithelioid pleural mesothelioma defines a favourable prognosis group: an analysis of 191 patients with pleural malignant mesothelioma. 70: 179-182.

120. Rosen LE, Karrison T, Ananthanarayanan V, Gallan AJ, Adusumilli PS, Alchami FS, Attanoos R, Brcic L, Butnor KJ, Galateau-Salle F, Hiroshima K, Kadota K, Klampatsa A, Stang NL, Lindenmann J, Litzky LA, Marchevsky A, Medeiros F, Montero MA, Moore DA, Nabeshima K, Pavlisko EN, Roggli VL, Sauter JL, Sharma A, Sheaff M, Travis WD, Vigneswaran WT, Vrugt B, Walts AE, Tjota MY, Krausz T, and Husain AN. (2018) Nuclear grade and necrosis predict prognosis in malignant epithelioid pleural mesothelioma: a multi-institutional study. Mod Pathol, 31: 598-606.
121. Brčić L, Jakopović M, Brčić I, Klarić V, Milošević M, Šepac A, Samaržija M, and Seiwerth SJVA. (2014) Reproducibility of histological subtyping of malignant pleural mesothelioma. 465: 679-685.
122. Ordóñez NG. (2012) Pleomorphic mesothelioma: report of 10 cases. Modern Pathology, 25: 1011.
123. Kadota K, Suzuki K, Colovos C, Sima CS, Rusch VW, Travis WD, and Adusumilli PS. (2012) A nuclear grading system is a strong predictor of survival in epitheloid diffuse malignant pleural mesothelioma. Mod Pathol, 25: 260-71.
124. Habougit C, Trombert-Paviot B, Karpathiou G, Casteillo F, Bayle-Bleuez S, Fournel P, Vergnon J-M, Tiffet O, Péoc'h M, and Forest F. (2017) Histopathologic features predict survival in diffuse pleural malignant mesothelioma on pleural biopsies. Virchows Archiv, 470: 639-646.
125. Zhang YZ, Brambilla C, Molyneaux PL, Rice A, Robertus JL, Jordan S, Lim E, Lang-Lazdunski L, Begum S, Dusmet M, Anikin V, Beddow E, Finch J, Asadi N, Popat S, Cookson WOC, Moffatt MF, and Nicholson AG. (2020) Utility of Nuclear Grading System in Epithelioid Malignant Pleural Mesothelioma in Biopsy-heavy Setting: An External Validation Study of 563 Cases. The American journal of surgical pathology, 44: 347-356.
126. Pulford E, Huilgol K, Moffat D, Henderson DW, and Klebe S. (2017) Malignant Mesothelioma, BAP1 Immunohistochemistry, and VEGFA: Does BAP1 Have Potential for Early Diagnosis and Assessment of Prognosis? Disease markers, 2017: 1310478-1310478.
127. McGregor SM, Dunning R, Hyjek E, Vigneswaran W, Husain AN, and Krausz T. (2015) BAP1 facilitates diagnostic objectivity, classification, and prognostication in malignant pleural mesothelioma. Human Pathology, 46: 1670-1678.
128. Galateau-Salle F, Churg A, Roggli V, and Travis WD. (2016) The 2015 World Health Organization Classification of Tumors of the Pleura: Advances since the 2004 Classification. Journal of Thoracic Oncology, 11: 142-154.
129. Lo Iacono M, Monica V, Righi L, Grosso F, Libener R, Vatrano S, Bironzo P, Novello S, Musmeci L, Volante M, Papotti M, and Scagliotti GV. (2015) Targeted next-generation sequencing of cancer genes in advanced stage malignant pleural mesothelioma: a retrospective study. J Thorac Oncol, 10: 492-9.
130. Cigognetti M, Lonardi S, Fisogni S, Balzarini P, Pellegrini V, Tironi A, Bercich L, Bugatti M, Rossi G, Murer B, Barbareschi M, Giuliani S, Cavazza A, Marchetti G, Vermi W, and Facchetti F. (2015) BAP1 (BRCA1-associated protein 1) is a highly specific marker for differentiating mesothelioma from reactive mesothelial proliferations. Modern Pathology, 28: 1043-1057.

131. Berg KB, Dacic S, Miller C, Cheung S, and Churg A. (2018) Utility of Methylthioadenosine Phosphorylase Compared With BAP1 Immunohistochemistry, and CDKN2A and NF2 Fluorescence In Situ Hybridization in Separating Reactive Mesothelial Proliferations From Epithelioid Malignant Mesotheliomas. Arch Pathol Lab Med, 142: 1549-1553.
132. Vigneswaran WT, Kircheva DY, Ananthanarayanan V, Watson S, Arif Q, Celauro AD, Kindler HL, and Husain AN. (2017) Amount of Epithelioid Differentiation Is a Predictor of Survival in Malignant Pleural Mesothelioma. The Annals of Thoracic Surgery, 103: 962-966.
133. Klebe S, Brownlee NA, Mahar A, Burchette JL, Sporn TA, Vollmer RT, and Roggli VL. (2010) Sarcomatoid mesothelioma: a clinical–pathologic correlation of 326 cases. Modern Pathology, 23: 470.
134. Righi L, Duregon E, Vatrano S, Izzo S, Giorcelli J, Rondón-Lagos M, Ascoli V, Ruffini E, Ventura L, and Volante M. (2016) BRCA1-associated protein 1 (BAP1) immunohistochemical expression as a diagnostic tool in malignant pleural mesothelioma classification: a large retrospective study. Journal of Thoracic Oncology, 11: 2006-2017.
135. Wu D, Hiroshima K, Yusa T, Ozaki D, Koh E, Sekine Y, Matsumoto S, Nabeshima K, Sato A, and Tsujimura T. (2017) Usefulness of p16/CDKN2A fluorescence in situ hybridization and BAP1 immunohistochemistry for the diagnosis of biphasic mesothelioma. Annals of diagnostic pathology, 26: 31-37.
136. Klebe S, Mahar A, Henderson DW, and Roggli VL. (2008) Malignant mesothelioma with heterologous elements: clinicopathological correlation of 27 cases and literature review. Modern Pathology, 21: 1084-1094.
137. Carbone M, Shimizu D, Napolitano A, Tanji M, Pass HI, Yang H, and Pastorino S. (2016) Positive nuclear BAP1 immunostaining helps differentiate non-small cell lung carcinomas from malignant mesothelioma. Oncotarget, 7: 59314.
138. Marchevsky AM, LeStang N, Hiroshima K, Pelosi G, Attanoos R, Churg A, Chirieac L, Dacic S, Husain A, and Khoor A. (2017) The differential diagnosis between pleural sarcomatoid mesothelioma and spindle cell/pleomorphic (sarcomatoid) carcinomas of the lung: evidence-based guidelines from the International Mesothelioma Panel and the MESOPATH National Reference Center. Human pathology, 67: 160-168.
139. Berg KB and Churg A. (2017) GATA3 immunohistochemistry for distinguishing sarcomatoid and desmoplastic mesothelioma from sarcomatoid carcinoma of the lung. The American journal of surgical pathology, 41: 1221-1225.
140. Wu D, Hiroshima K, Matsumoto S, Nabeshima K, Yusa T, Ozaki D, Fujino M, Yamakawa H, Nakatani Y, and Tada Y. (2013) Diagnostic usefulness of p16/CDKN2A FISH in distinguishing between sarcomatoid mesothelioma and fibrous pleuritis. American journal of clinical pathology, 139: 39-46.
141. Cinausero M, Rihawi K, Sperandi F, Melotti B, and Ardizzoni A. (2018) Chemotherapy treatment in malignant pleural mesothelioma: a difficult history. J Thorac Dis, 10: S304-S310.
142. Vogelzang NJ, Rusthoven JJ, Symanowski J, Denham C, Kaukel E, Ruffie P, Gatzemeier U, Boyer M, Emri S, and Manegold C. (2003) Phase III study of pemetrexed in combination with cisplatin versus cisplatin alone in patients with malignant pleural mesothelioma. Journal of clinical oncology, 21: 2636-2644.

143. van Meerbeeck JP, Gaafar R, Manegold C, Van Klaveren RJ, Van Marck EA, Vincent M, Legrand C, Bottomley A, Debruyne C, and Giaccone G. (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: 6881-9.
144. Nicolini F, Bocchini M, Bronte G, Delmonte A, Guidoboni M, Crinò L, and Mazza M. (2020) Malignant Pleural Mesothelioma: State-of-the-Art on Current Therapies and Promises for the Future. Frontiers in Oncology, 9.
145. 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, and Manegold C. (2008) Pemetrexed Plus Cisplatin or Pemetrexed Plus Carboplatin for Chemonaïve Patients with Malignant Pleural Mesothelioma: Results of the International Expanded Access Program. Journal of Thoracic Oncology, 3: 756-763.
146. Damhuis RA, Schroten C, and Burgers JA. (2012) Population-based survival for malignant mesothelioma after introduction of novel chemotherapy. European Respiratory Journal, 40: 185-189.
147. Zalcman G, Mazieres J, Margery J, Greillier L, Audigier-Valette C, Moro-Sibilot D, Molinier O, Corre R, Monnet I, and Gounant V. (2016) Bevacizumab for newly diagnosed pleural mesothelioma in the Mesothelioma Avastin Cisplatin Pemetrexed Study (MAPS): a randomised, controlled, open-label, phase 3 trial. The Lancet, 387: 1405-1414.
148. David SE, Douglas EW, Wallace A, Lyudmila AB, Hossein B, David Ross C, Richard TC, Lucian RC, Thomas ADA, Thomas D, Michael D, Ramaswamy G, Mark H, Leora H, Thierry MJ, Ritsuko K, Rudy PL, Michael L, Rogerio L, Jules L, Billy WL, Renato M, Gregory AO, Jyoti DP, Katherine MP, Karen R, Gregory JR, Steven ES, Theresa AS, Neelesh S, Scott JS, James S, Kurt T, Stephen CY, Kristina G, and Miranda H. (2016) NCCN Guidelines Insights: Malignant Pleural Mesothelioma, Version 3.2016. Journal of the National Comprehensive Cancer Network J Natl Compr Canc Netw, 14: 825-836.
149. Muers MF, Stephens RJ, Fisher P, Darlison L, Higgs CM, Lowry E, Nicholson AG, O'Brien M, Peake M, and Rudd R. (2008) Active symptom control with or without chemotherapy in the treatment of patients with malignant pleural mesothelioma (MS01): a multicentre randomised trial. The Lancet, 371: 1685-1694.
150. Di Noia V, Vita E, Ferrara M, Strippoli A, Basso M, Schinzari G, Cassano A, Bria E, Barone C, and D'Argento E. (2019) Malignant Pleural Mesothelioma: Is Tailoring the Second-Line Therapy Really "Raising the Bar?". Current treatment options in oncology, 20: 23.
151. Stebbing J, Powles T, McPherson K, Shamash J, Wells P, Sheaff MT, Slater S, Rudd RM, Fennell D, and Steele JP. (2009) The efficacy and safety of weekly vinorelbine in relapsed malignant pleural mesothelioma. Lung Cancer, 63: 94-97.
152. Mutlu H, Gündüz Ş, Karaca H, Büyükçelik A, Cihan YB, Erden A, Akca Z, and Coşkun HŞ. (2014) Second-line gemcitabine-based chemotherapy regimens improve overall 3-year survival rate in patients with malignant pleural mesothelioma: a multicenter retrospective study. Medical Oncology, 31: 74.

153. Jassem J, Ramlau R, Santoro A, Schuette W, Chemaissani A, Hong S, Blatter J, Adachi S, Hanauske A, and 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. Journal of Clinical Oncology, 26: 1698-1704.
154. Ceresoli GL, Zucali PA, De Vincenzo F, Gianoncelli L, Simonelli M, Lorenzi E, Ripa C, Giordano L, and Santoro A. (2011) Retreatment with pemetrexed-based chemotherapy in patients with malignant pleural mesothelioma. Lung Cancer, 72: 73-77.
155. Price A. (2011) What is the role of radiotherapy in malignant pleural mesothelioma? The oncologist, 16: 359-365.
156. Gomez DR, Hong DS, Allen PK, Welsh JS, Mehran RJ, Tsao AS, Liao Z, Bilton SD, Komaki R, and Rice DC. (2013) Patterns of failure, toxicity, and survival after extrapleural pneumonectomy and hemithoracic intensity-modulated radiation therapy for malignant pleural mesothelioma. J Thorac Oncol, 8: 238-45.
157. Nelson DB, Rice DC, Mitchell KG, Tsao AS, Vaporciyan AA, Antonoff MB, Hofstetter WL, Walsh GL, Swisher SG, Roth JA, Gomez DR, Mehran RJ, and Sepesi B. (2019) Defining the role of adjuvant radiotherapy for malignant pleural mesothelioma: a propensity-matched landmark analysis of the National Cancer Database. J Thorac Dis, 11: 1269-1278.
158. Lewis GD, Dalwadi SM, Farach A, Brian Butler E, and Teh BS. (2019) The Role of Adjuvant Radiotherapy in the Treatment of Pleural Mesothelioma. Annals of Surgical Oncology, 26: 1879-1885.
159. Shaaban SG, Verma V, Choi JI, Shabason J, Sharma S, Glass E, Grover S, Badiyan SN, and Simone CB, 2nd. (2018) Utilization of Intensity-Modulated Radiation Therapy for Malignant Pleural Mesothelioma in the United States. Clin Lung Cancer, 19: e685-e692.
160. Cao C, Yan TD, Bannon PG, and McCaughan BC. (2011) Summary of prognostic factors and patient selection for extrapleural pneumonectomy in the treatment of malignant pleural mesothelioma. Ann Surg Oncol, 18: 2973-9.
161. Rice D, Rusch V, Pass H, Asamura H, Nakano T, Edwards J, Giroux DJ, Hasegawa S, Kernstine KH, Waller D, and Rami-Porta R. (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. Journal of Thoracic Oncology, 6: 1304-1312.
162. Flores RM, Pass HI, Seshan VE, Dycoco J, Zakowski M, Carbone M, Bains MS, and Rusch VW. (2008) Extrapleural pneumonectomy versus pleurectomy/decortication in the surgical management of malignant pleural mesothelioma: results in 663 patients. The Journal of thoracic and cardiovascular surgery, 135: 620-626. e3.
163. Treasure T, Lang-Lazdunski L, Waller D, Bliss JM, Tan C, Entwisle J, Snee M, O'Brien M, Thomas G, and Senan S. (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. The lancet oncology, 12: 763-772.

164. Cao CQ, Yan TD, Bannon PG, and McCaughan BC. (2010) A systematic review of extrapleural pneumonectomy for malignant pleural mesothelioma. Journal of Thoracic Oncology, 5: 1692-1703.
165. Sharkey AJ, Tenconi S, Nakas A, and Waller DA. (2015) The effects of an intentional transition from extrapleural pneumonectomy to extended pleurectomy/decortication. European Journal of Cardio-Thoracic Surgery, 49: 1632-1641.
166. Taioli E, Wolf AS, and Flores RM. (2015) Meta-analysis of survival after pleurectomy decortication versus extrapleural pneumonectomy in mesothelioma. The Annals of thoracic surgery, 99: 472-480.
167. Rena O and Casadio C. (2012) Extrapleural pneumonectomy for early stage malignant pleural mesothelioma: a harmful procedure. Lung Cancer, 77: 151-5.
168. Ricciardi S, Cardillo G, Zirafa CC, Carleo F, Facciolo F, Fontanini G, Mutti L, and Melfi F. (2018) Surgery for malignant pleural mesothelioma: an international guidelines review. J Thorac Dis, 10: S285-S292.
169. Casiraghi M, Maisonneuve P, Brambilla D, Solli P, Galetta D, Petrella F, Piperno G, De Marinis F, and Spaggiari L. (2017) Induction chemotherapy, extrapleural pneumonectomy and adjuvant radiotherapy for malignant pleural mesothelioma. Eur J Cardiothorac Surg, 52: 975-981.
170. Rimner A, Zauderer MG, Gomez DR, Adusumilli PS, Parhar PK, Wu AJ, Woo KM, Shen R, Ginsberg MS, and Yorke ED. (2016) Phase II study of hemithoracic intensity-modulated pleural radiation therapy (IMPRINT) as part of lung-sparing multimodality therapy in patients with malignant pleural mesothelioma. Journal of Clinical Oncology, 34: 2761.
171. Van Schil P, Baas P, Gaafar R, Maat A, Van de Pol M, Hasan B, Klomp H, Abdelrahman A, Welch J, and Van Meerbeeck J. (2010) Trimodality therapy for malignant pleural mesothelioma: results from an EORTC phase II multicentre trial. European Respiratory Journal, 36: 1362-1369.
172. Scagliotti GV, Gaafar R, Nowak AK, Nakano T, van Meerbeeck J, Popat S, Vogelzang NJ, Grosso F, Aboelhassan R, Jakopovic M, Ceresoli GL, Taylor P, Orlandi F, Fennell DA, Novello S, Scherpereel A, Kuribayashi K, Cedres S, Sorensen JB, Pavlakis N, Reck M, Velema D, von Wangenheim U, Kim M, Barrueco J, and Tsao AS. (2019) Nintedanib in combination with pemetrexed and cisplatin for chemotherapy-naive patients with advanced malignant pleural mesothelioma (LUME-Meso): a double-blind, randomised, placebo-controlled phase 3 trial. Lancet Respir Med, 7: 569-580.
173. Grosser R, Cherkassky L, Chintala N, and Adusumilli PS. (2019) Combination Immunotherapy with CAR T Cells and Checkpoint Blockade for the Treatment of Solid Tumors. Cancer Cell, 36: 471-482.
174. Hassan R, Kindler HL, Jahan T, Bazhenova L, Reck M, Thomas A, Pastan I, Parno J, O'Shannessy DJ, and Fatato P. (2014) Phase II clinical trial of amatuximab, a chimeric antimesothelin antibody with pemetrexed and cisplatin in advanced unresectable pleural mesothelioma. Clinical cancer research, 20: 5927-5936.
175. Blumenschein GR, Hassan R, Moore KN, Santin A, Kindler HL, Nemunaitis JJ, Seward SM, Rajagopalan P, Walter A, Sarapa N, and Bendell JC. (2016) Phase I study of anti-mesothelin antibody drug conjugate anetumab ravtansine (AR). Journal of Clinical Oncology, 34: 2509-2509.

176. Zauderer M, Dao T, Rusch V, Ginsberg M, Tsao A, Panageas K, Stergiou A, Scheinberg D, and Krug L. (2016) Randomized phase II study of adjuvant WT1 vaccine (SLS-001) for malignant pleural mesothelioma (MPM) after multimodality therapy. Journal of Clinical Oncology, 34: 8519-8519.
177. Szlosarek PW, Steele JP, Nolan L, Gilligan D, Taylor P, Spicer J, Lind M, Mitra S, Shamash J, Phillips MM, Luong P, Payne S, Hillman P, Ellis S, Szyszko T, Dancey G, Butcher L, Beck S, Avril NE, Thomson J, Johnston A, Tomsa M, Lawrence C, Schmid P, Crook T, Wu B-W, Bomalaski JS, Lemoine N, Sheaff MT, Rudd RM, Fennell D, and Hackshaw A. (2017) Arginine Deprivation With Pegylated Arginine Deiminase in Patients With Argininosuccinate Synthetase 1–Deficient Malignant Pleural Mesothelioma: A Randomized Clinical Trial. JAMA Oncology, 3: 58-66.
178. Aerts J, de Goeje PL, Cornelissen R, Kaijen-Lambers MEH, Bezemer K, van der Leest CH, Mahaweni NM, Kunert A, Eskens F, Waasdorp C, Braakman E, van der Holt B, Vulto AG, Hendriks RW, Hegmans J, and Hoogsteden HC. (2018) Autologous Dendritic Cells Pulsed with Allogeneic Tumor Cell Lysate in Mesothelioma: From Mouse to Human. Clin Cancer Res, 24: 766-776.
179. Maio M, Scherpereel A, Calabro L, Aerts J, Cedres Perez S, Bearz A, Nackaerts K, Fennell DA, Kowalski D, Tsao AS, Taylor P, Grosso F, Antonia SJ, Nowak AK, Taboada M, Puglisi M, Stockman PK, and 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.
180. Scherpereel A, Mazieres J, Greillier L, Lantuejoul S, Do P, Bylicki O, Monnet I, Corre R, Audigier-Valette C, Locatelli-Sanchez M, Molinier O, Guisier F, Urban T, Ligeza-Poisson C, Planchard D, Amour E, Morin F, Moro-Sibilot D, and Zalcman G. (2019) Nivolumab or nivolumab plus ipilimumab in patients with relapsed malignant pleural mesothelioma (IFCT-1501 MAPS2): a multicentre, open-label, randomised, non-comparative, phase 2 trial. The Lancet. Oncology, 20: 239-253.
181. Nowak A, Kok P, Lesterhuis W, Hughes B, Brown C, Kao S, Karikios D, John T, Pavlakis N, O'Byrne K, Yip S, Lam W, Briscoe K, Karapetis C, and Stockler M. (2018) OA08.02 DREAM - A Phase 2 Trial of Durvalumab with First Line Chemotherapy in Mesothelioma: Final Result. Journal of Thoracic Oncology, 13: S338-S339.
182. Fennell DA, Baas P, Taylor P, Nowak AK, Gilligan D, Nakano T, Pachter JA, Weaver DT, Scherpereel A, Pavlakis N, van Meerbeeck JP, Cedres S, Nolan L, Kindler H, and Aerts J. (2019) Maintenance Defactinib Versus Placebo After First-Line Chemotherapy in Patients With Merlin-Stratified Pleural Mesothelioma: COMMAND-A Double-Blind, Randomized, Phase II Study. Journal of clinical oncology : official journal of the American Society of Clinical Oncology, 37: 790-798.
183. Murthy V, Katzman D, and Sterman DH. (2019) Intrapleural immunotherapy: An update on emerging treatment strategies for pleural malignancy. Clin Respir J, 13: 272-279.
184. Mutti L, Peikert T, Robinson BWS, Scherpereel A, Tsao AS, de Perrot M, Woodard GA, Jablons DM, Wiens J, Hirsch FR, Yang H, Carbone M, Thomas

A, and Hassan R. (2018) Scientific Advances and New Frontiers in Mesothelioma Therapeutics. Journal of Thoracic Oncology, 13: 1269-1283.

185. Weinstein JN, Collisson EA, Mills GB, Shaw KRM, Ozenberger BA, Ellrott K, Shmulevich I, Sander C, Stuart JM, and Network CGAR. (2013) The cancer genome atlas pan-cancer analysis project. Nature genetics, 45: 1113.
186. Guo G, Chmielecki J, Goparaju C, Heguy A, Dolgalev I, Carbone M, Seepo S, Meyerson M, and Pass HI. (2015) Whole-exome sequencing reveals frequent genetic alterations in BAP1, NF2, CDKN2A, and CUL1 in malignant pleural mesothelioma. Cancer Res, 75: 264-9.
187. Gao J, Aksoy BA, Dogrusoz U, Dresdner G, Gross B, Sumer SO, Sun Y, Jacobsen A, Sinha R, Larsson E, Cerami E, Sander C, and Schultz N. (2013) Integrative analysis of complex cancer genomics and clinical profiles using the cBioPortal. Sci Signal, 6: pl1.
188. Sage A, Martinez V, Minatel B, Pewarchuk M, Marshall E, MacAulay G, Hubaux R, Pearson D, Goodarzi A, and Dellaire G. (2018) Genomics and epigenetics of malignant mesothelioma. High-throughput, 7: 20.
189. Lindholm P, Salmenkivi K, Vauhkonen H, Nicholson A, Anttila S, Kinnula VL, and Knuutila S. (2007) Gene copy number analysis in malignant pleural mesothelioma using oligonucleotide array CGH. Cytogenetic and genome research, 119: 46-52.
190. Quetel L, Meiller C, Assie JB, Blum Y, Imbeaud S, Montagne F, Tranchant R, de Wolf J, Caruso S, Copin MC, Hofman V, Gibault L, Badoual C, Pintilie E, Hofman P, Monnet I, Scherpereel A, Le Pimpec-Barthes F, Zucman-Rossi J, Jaurand MC, and Jean D. (2020) Genetic alterations of malignant pleural mesothelioma: association with tumor heterogeneity and overall survival. Mol Oncol.
191. Kettunen E, Savukoski S, Salmenkivi K, Böhling T, Vanhala E, Kuosma E, Anttila S, and Wolff H. (2019) CDKN2A copy number and p16 expression in malignant pleural mesothelioma in relation to asbestos exposure. BMC cancer, 19: 507-507.
192. Sherr CJ. (2006) Divorcing ARF and p53: an unsettled case. Nature Reviews Cancer, 6: 663-673.
193. Kim MP, Zhang Y, and Lozano G. (2015) Mutant p53: Multiple Mechanisms Define Biologic Activity in Cancer. Frontiers in Oncology, 5.
194. 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, and Ladanyi M. (2011) The nuclear deubiquitinase BAP1 is commonly inactivated by somatic mutations and 3p21.1 losses in malignant pleural mesothelioma. Nat Genet, 43: 668-72.
195. Chiosea S, Krasinskas A, Cagle PT, Mitchell KA, Zander DS, and Dacic S. (2008) Diagnostic importance of 9p21 homozygous deletion in malignant mesotheliomas. Modern Pathology, 21: 742.
196. Wong L, Zhou J, Anderson D, and Kratzke RA. (2002) Inactivation of p16INK4a expression in malignant mesothelioma by methylation. Lung Cancer, 38: 131-6.
197. Hmeljak J, Sanchez-Vega F, Hoadley KA, Shih J, Stewart C, Heiman D, Tarpey P, Danilova L, Drill E, Gibb EA, Bowlby R, Kanchi R, Osmanbeyoglu HU, Sekido Y, Takeshita J, Newton Y, Graim K, Gupta M, Gay CM, Diao L, Gibbs

DL, Thorsson V, Iype L, Kantheti H, Severson DT, Ravegnini G, Desmeules P, Jungbluth AA, Travis WD, Dacic S, Chirieac LR, Galateau-Sallé F, Fujimoto J, Husain AN, Silveira HC, Rusch VW, Rintoul RC, Pass H, Kindler H, Zauderer MG, Kwiatkowski DJ, Bueno R, Tsao AS, Creaney J, Lichtenberg T, Leraas K, Bowen J, Felau I, Zenklusen JC, Akbani R, Cherniack AD, Byers LA, Noble MS, Fletcher JA, Robertson AG, Shen R, Aburatani H, Robinson BW, Campbell P, and Ladanyi M. (2018) Integrative Molecular Characterization of Malignant Pleural Mesothelioma. Cancer Discovery, 8: 1548.

198. Dacic S, Kothmaier H, Land S, Shuai Y, Halbwedl I, Morbini P, Murer B, Comin C, Galateau-Salle F, and Demirag F. (2008) Prognostic significance of p16/cdkn2a loss in pleural malignant mesotheliomas. Virchows Archiv, 453: 627-635.
199. Lopez-Rios F, Chuai S, Flores R, Shimizu S, Ohno T, Wakahara K, Illei PB, Hussain S, Krug L, Zakowski MF, Rusch V, Olshen AB, and Ladanyi M. (2006) Global gene expression profiling of pleural mesotheliomas: overexpression of aurora kinases and P16/CDKN2A deletion as prognostic factors and critical evaluation of microarray-based prognostic prediction. Cancer Res, 66: 2970-9.
200. Kato S, Tomson BN, Buys TP, Elkin SK, Carter JL, and Kurzrock R. (2016) Genomic landscape of malignant mesotheliomas. Molecular cancer therapeutics, 15: 2498-2507.
201. Yu H, Mashtalir N, Daou S, Hammond-Martel I, Ross J, Sui G, Hart GW, Rauscher FJ, 3rd, Drobetsky E, Milot E, Shi Y, and Affar el B. (2010) The ubiquitin carboxyl hydrolase BAP1 forms a ternary complex with YY1 and HCF-1 and is a critical regulator of gene expression. Mol Cell Biol, 30: 5071-85.
202. Wang A, Papneja A, Hyrcza M, Al-Habeeb A, and Ghazarian D. (2016) Gene of the month: BAP1. Journal of Clinical Pathology, 69: 750-753.
203. Bononi A, Giorgi C, Patergnani S, Larson D, Verbruggen K, Tanji M, Pellegrini L, Signorato V, Olivetto F, Pastorino S, Nasu M, Napolitano A, Gaudino G, Morris P, Sakamoto G, Ferris LK, Danese A, Raimondi A, Tacchetti C, Kuchay S, Pass HI, Affar EB, Yang H, Pinton P, and Carbone M. (2017) BAP1 regulates IP3R3-mediated Ca(2+) flux to mitochondria suppressing cell transformation. Nature, 546: 549-553.
204. Bononi A, Yang H, Giorgi C, Patergnani S, Pellegrini L, Su M, Xie G, Signorato V, Pastorino S, and Morris P. (2017) Germline BAP1 mutations induce a Warburg effect. Cell death and differentiation, 24: 1694.
205. Cercek A, Zauderer MG, Rimner A, Rusch VW, Adusumili PS, Nash GM, Hmeljak J, Ladanyi M, and Krug LM. (2015) Confirmation of high prevalence of BAP1 inactivation in mesothelioma. Journal of Clinical Oncology, 33: 7564-7564.
206. Bueno R, Stawiski EW, Goldstein LD, Durinck S, De Rienzo A, Modrusan Z, Gnad F, Nguyen TT, Jaiswal BS, and Chirieac LR. (2016) Comprehensive genomic analysis of malignant pleural mesothelioma identifies recurrent mutations, gene fusions and splicing alterations. Nature genetics, 48: 407.
207. Nasu M, Emi M, Pastorino S, Tanji M, Powers A, Luk H, Baumann F, Zhang Y-a, Gazdar A, Kanodia S, Tiirikainen M, Flores E, Gaudino G, Becich MJ, Pass HI, Yang H, and Carbone M. (2015) High Incidence of Somatic BAP1 Alterations in Sporadic Malignant Mesothelioma. Journal of Thoracic Oncology, 10: 565-576.

208. Zheng Y and Pan D. (2019) The Hippo Signaling Pathway in Development and Disease. Developmental Cell, 50: 264-282.
209. Chapel DB, Churg A, Santoni-Rugiu E, Tsujimura T, Hiroshima K, and Husain AN. (2019) Molecular pathways and diagnosis in malignant mesothelioma: A review of the 14th International Conference of the International Mesothelioma Interest Group. Lung Cancer, 127: 69-75.
210. Piccolo S, Dupont S, and Cordenonsi M. (2014) The Biology of YAP/TAZ: Hippo Signaling and Beyond. Physiological Reviews, 94: 1287-1312.
211. Shreberk-Shaked M and Oren M. (2019) New insights into YAP/TAZ nucleo-cytoplasmic shuttling: new cancer therapeutic opportunities? Molecular oncology, 13: 1335-1341.
212. Hylebos M, Van Camp G, van Meerbeeck JP, and de Beeck KO. (2016) The genetic landscape of malignant pleural mesothelioma: results from massively parallel sequencing. Journal of Thoracic Oncology, 11: 1615-1626.
213. Schroeder RD, Angelo LS, and Kurzrock R. (2014) NF2/merlin in hereditary neurofibromatosis 2 versus cancer: biologic mechanisms and clinical associations. Oncotarget, 5: 67-77.
214. Murakami H, Mizuno T, Taniguchi T, Fujii M, Ishiguro F, Fukui T, Akatsuka S, Horio Y, Hida T, and Kondo Y. (2011) LATS2 is a tumor suppressor gene of malignant mesothelioma. Cancer research, 71: 873-883.
215. Miyanaga A, Masuda M, Tsuta K, Kawasaki K, Nakamura Y, Sakuma T, Asamura H, Gemma A, and Yamada T. (2015) Hippo Pathway Gene Mutations in Malignant Mesothelioma: Revealed by RNA and Targeted Exon Sequencing. Journal of Thoracic Oncology, 10: 844-851.
216. Gomez DE, Armando RG, Farina HG, Menna PL, Cerrudo CS, Ghiringhelli PD, and Alonso DF. (2012) Telomere structure and telomerase in health and disease. International journal of oncology, 41: 1561-1569.
217. Meyne J, Ratliff RL, and MoYzIs RK. (1989) Conservation of the human telomere sequence (TTAGGG) n among vertebrates. Proceedings of the National Academy of Sciences, 86: 7049-7053.
218. Wright WE, Tesmer VM, Huffman KE, Levene SD, and Shay JW. (1997) Normal human chromosomes have long G-rich telomeric overhangs at one end. Genes & development, 11: 2801-2809.
219. Hansel R, Lohr F, Foldynova-Trantirkova S, Bamberg E, Trantirek L, and Dotsch V. (2011) The parallel G-quadruplex structure of vertebrate telomeric repeat sequences is not the preferred folding topology under physiological conditions. Nucleic Acids Res, 39: 5768-75.
220. Zvereva M, Shcherbakova D, and Dontsova O. (2010) Telomerase: structure, functions, and activity regulation. Biochemistry (Moscow), 75: 1563-1583.
221. Martínez P and Blasco MA. (2011) Telomeric and extra-telomeric roles for telomerase and the telomere-binding proteins. Nature Reviews Cancer, 11: 161.
222. Soudet J, Jolivet P, and Teixeira Maria T. (2014) Elucidation of the DNA End-Replication Problem in Saccharomyces cerevisiae. Molecular Cell, 53: 954-964.
223. Yuan X, Larsson C, and Xu D. (2019) Mechanisms underlying the activation of TERT transcription and telomerase activity in human cancer: Old actors and new players. Oncogene: 1.
224. Chiodi I and Mondello C. (2012) Telomere-independent functions of telomerase in nuclei, cytoplasm, and mitochondria. Frontiers in Oncology, 2.

225. Kour S and Rath PC. (2016) Long noncoding RNAs in aging and age-related diseases. Ageing Research Reviews, 26: 1-21.
226. Shay JW. (2016) Role of Telomeres and Telomerase in Aging and Cancer. Cancer Discov, 6: 584-93.
227. Hanahan D and Weinberg RA. (2011) Hallmarks of cancer: the next generation. cell, 144: 646-674.
228. Bretones G, Delgado MD, and León J. (2015) Myc and cell cycle control. Biochimica et Biophysica Acta (BBA) - Gene Regulatory Mechanisms, 1849: 506-516.
229. Wang J, Xie LY, Allan S, Beach D, and Hannon GJ. (1998) Myc activates telomerase. Genes & development, 12: 1769-1774.
230. Lee DD, Leao R, Komosa M, Gallo M, Zhang CH, Lipman T, Remke M, Heidari A, Nunes NM, Apolonio JD, Price AJ, De Mello RA, Dias JS, Huntsman D, Hermanns T, Wild PJ, Vanner R, Zadeh G, Karamchandani J, Das S, Taylor MD, Hawkins CE, Wasserman JD, Figueiredo A, Hamilton RJ, Minden MD, Wani K, Diplas B, Yan H, Aldape K, Akbari MR, Danesh A, Pugh TJ, Dirks PB, Castelo-Branco P, and Tabori U. (2019) DNA hypermethylation within TERT promoter upregulates TERT expression in cancer. J Clin Invest, 129: 223-229.
231. Liu C, Fang X, Ge Z, Jalink M, Kyo S, Björkholm M, Gruber A, Sjöberg J, and Xu D. (2007) The telomerase reverse transcriptase (hTERT) gene is a direct target of the histone methyltransferase SMYD3. Cancer research, 67: 2626-2631.
232. Liu T, Yuan X, and Xu D. (2016) Cancer-specific telomerase reverse transcriptase (TERT) promoter mutations: biological and clinical implications. Genes (Basel), 7: 38.
233. Bellon M and Nicot C. (2008) Regulation of telomerase and telomeres: human tumor viruses take control. Journal of the National Cancer Institute, 100: 98-108.
234. Pickett HA and Reddel RR. (2015) Molecular mechanisms of activity and derepression of alternative lengthening of telomeres. Nature Structural &Amp; Molecular Biology, 22: 875.
235. Chan CS, Laddha SV, Lewis PW, Koletsky MS, Robzyk K, Da Silva E, Torres PJ, Untch BR, Li J, Bose P, Chan TA, Klimstra DS, Allis CD, and Tang LH. (2018) ATRX, DAXX or MEN1 mutant pancreatic neuroendocrine tumors are a distinct alpha-cell signature subgroup. Nature Communications, 9: 4158.
236. Lee J-C, Jeng Y-M, Liau J-Y, Tsai J-H, Hsu H-H, and Yang C-Y. (2015) Alternative lengthening of telomeres and loss of ATRX are frequent events in pleomorphic and dedifferentiated liposarcomas. Modern Pathology, 28: 1064.
237. Henson JD and Reddel RR. (2010) Assaying and investigating Alternative Lengthening of Telomeres activity in human cells and cancers. FEBS letters, 584: 3800-3811.
238. Schwartzentruber J, Korshunov A, Liu X-Y, Jones DT, Pfaff E, Jacob K, Sturm D, Fontebasso AM, Quang D-AK, and Tönjes M. (2012) Driver mutations in histone H3. 3 and chromatin remodelling genes in paediatric glioblastoma. Nature, 482: 226.
239. Barthel FP, Wei W, Tang M, Martinez-Ledesma E, Hu X, Amin SB, Akdemir KC, Seth S, Song X, Wang Q, Lichtenberg T, Hu J, Zhang J, Zheng S, and

Verhaak RGW. (2017) Systematic analysis of telomere length and somatic alterations in 31 cancer types. Nature Genetics, 49: 349.
240. Peifer M, Hertwig F, Roels F, Dreidax D, Gartlgruber M, Menon R, Krämer A, Roncaioli JL, Sand F, and Heuckmann JM. (2015) Telomerase activation by genomic rearrangements in high-risk neuroblastoma. Nature, 526: 700.
241. Colebatch AJ, Dobrovic A, and Cooper WA. (2019) TERT gene: its function and dysregulation in cancer. Journal of Clinical Pathology, 72: 281-284.
242. Akincilar SC, Unal B, and Tergaonkar V. (2016) Reactivation of telomerase in cancer. Cellular and Molecular Life Sciences, 73: 1659-1670.
243. Heidenreich B, Rachakonda PS, Hemminki K, and Kumar R. (2014) TERT promoter mutations in cancer development. Current Opinion in Genetics & Development, 24: 30-37.
244. Horn S, Figl A, Rachakonda PS, Fischer C, Sucker A, Gast A, Kadel S, Moll I, Nagore E, and Hemminki K. (2013) TERT promoter mutations in familial and sporadic melanoma. Science, 339: 959-961.
245. Heidenreich B and Kumar R. (2017) TERT promoter mutations in telomere biology. Mutation Research/Reviews in Mutation Research, 771: 15-31.
246. Huang FW, Hodis E, Xu MJ, Kryukov GV, Chin L, and Garraway LA. (2013) Highly recurrent TERT promoter mutations in human melanoma. Science, 339: 957-959.
247. Rachakonda PS, Hosen I, de Verdier PJ, Fallah M, Heidenreich B, Ryk C, Wiklund NP, Steineck G, Schadendorf D, Hemminki K, and Kumar R. (2013) TERT promoter mutations in bladder cancer affect patient survival and disease recurrence through modification by a common polymorphism. Proceedings of the National Academy of Sciences, 110: 17426.
248. Vinagre J, Almeida A, Pópulo H, Batista R, Lyra J, Pinto V, Coelho R, Celestino R, Prazeres H, and Lima L. (2013) Frequency of TERT promoter mutations in human cancers. Nature communications, 4.
249. Tallet A, Nault JC, Renier A, Hysi I, Galateau-Salle F, Cazes A, Copin MC, Hofman P, Andujar P, Le Pimpec-Barthes F, Zucman-Rossi J, Jaurand MC, and Jean D. (2014) Overexpression and promoter mutation of the TERT gene in malignant pleural mesothelioma. Oncogene, 33: 3748-3752.
250. Nault JC, Mallet M, Pilati C, Calderaro J, Bioulac-Sage P, Laurent C, Laurent A, Cherqui D, Balabaud C, and Zucman-Rossi J. (2013) High frequency of telomerase reverse-transcriptase promoter somatic mutations in hepatocellular carcinoma and preneoplastic lesions. Nature communications, 4: 2218.
251. Arita H, Narita Y, Fukushima S, Tateishi K, Matsushita Y, Yoshida A, Miyakita Y, Ohno M, Collins VP, and Kawahara N. (2013) Upregulating mutations in the TERT promoter commonly occur in adult malignant gliomas and are strongly associated with total 1p19q loss. Acta neuropathologica, 126: 267-276.
252. Armanios M, Chen J-L, Chang Y-PC, Brodsky RA, Hawkins A, Griffin CA, Eshleman JR, Cohen AR, Chakravarti A, Hamosh A, and Greider CW. (2005) Haploinsufficiency of telomerase reverse transcriptase leads to anticipation in autosomal dominant dyskeratosis congenita. Proceedings of the National Academy of Sciences of the United States of America, 102: 15960-15964.
253. Armanios MY, Chen JJ, Cogan JD, Alder JK, Ingersoll RG, Markin C, Lawson WE, Xie M, Vulto I, Phillips JA, 3rd, Lansdorp PM, Greider CW, and Loyd JE.

(2007) Telomerase mutations in families with idiopathic pulmonary fibrosis. N Engl J Med, 356: 1317-26.

254. Scott GA, Laughlin TS, and Rothberg PG. (2014) Mutations of the TERT promoter are common in basal cell carcinoma and squamous cell carcinoma. Modern Pathology, 27: 516-523.
255. Liu X, Bishop J, Shan Y, Pai S, Liu D, Murugan AK, Sun H, El-Naggar AK, and Xing M. (2013) Highly prevalent TERT promoter mutations in aggressive thyroid cancers. Endocrine-related cancer, 20: 603-610.
256. Eckel-Passow JE, Lachance DH, Molinaro AM, Walsh KM, Decker PA, Sicotte H, Pekmezci M, Rice T, Kosel ML, Smirnov IV, Sarkar G, Caron AA, Kollmeyer TM, Praska CE, Chada AR, Halder C, Hansen HM, McCoy LS, Bracci PM, Marshall R, Zheng S, Reis GF, Pico AR, O'Neill BP, Buckner JC, Giannini C, Huse JT, Perry A, Tihan T, Berger MS, Chang SM, Prados MD, Wiemels J, Wiencke JK, Wrensch MR, and Jenkins RB. (2015) Glioma Groups Based on 1p/19q, IDH, and TERT Promoter Mutations in Tumors. New England Journal of Medicine, 372: 2499-2508.
257. Simon M, Hosen I, Gousias K, Rachakonda S, Heidenreich B, Gessi M, Schramm J, Hemminki K, Waha A, and Kumar R. (2014) TERT promoter mutations: a novel independent prognostic factor in primary glioblastomas. Neuro-Oncology, 17: 45-52.
258. Rachakonda PS, Hosen I, de Verdier PJ, Fallah M, Heidenreich B, Ryk C, Wiklund NP, Steineck G, Schadendorf D, Hemminki K, and Kumar R. (2013) TERT promoter mutations in bladder cancer affect patient survival and disease recurrence through modification by a common polymorphism. Proc Natl Acad Sci U S A, 110: 17426-31.
259. Nagore E, Heidenreich B, Rachakonda S, Garcia-Casado Z, Requena C, Soriano V, Frank C, Traves V, Quecedo E, Sanjuan-Gimenez J, Hemminki K, Landi MT, and Kumar R. (2016) TERT promoter mutations in melanoma survival. International Journal of Cancer, 139: 75-84.
260. Kim TH, Kim Y-E, Ahn S, Kim J-Y, Ki C-S, Oh YL, Kim K, Yun JW, Park W-Y, and Choe J-H. (2016) TERT promoter mutations and long-term survival in patients with thyroid cancer. Endocrine-related cancer, 23: 813-823.
261. Liu T, Wang N, Cao J, Sofiadis A, Dinets A, Zedenius J, Larsson C, and Xu D. (2014) The age- and shorter telomere-dependent TERT promoter mutation in follicular thyroid cell-derived carcinomas. Oncogene, 33: 4978-4984.
262. Bournaud C, Descotes F, Decaussin-Petrucci M, Berthiller J, de la Fouchardière C, Giraudet A-L, Bertholon-Gregoire M, Robinson P, Lifante J-C, Lopez J, and Borson-Chazot F. (2019) TERT promoter mutations identify a high-risk group in metastasis-free advanced thyroid carcinoma. European Journal of Cancer, 108: 41-49.
263. Spiegl-Kreinecker S, Lötsch D, Neumayer K, Kastler L, Gojo J, Pirker C, Pichler J, Weis S, Kumar R, and Webersinke G. (2018) TERT promoter mutations are associated with poor prognosis and cell immortalization in meningioma. Neuro-oncology, 20: 1584-1593.
264. Spiegl-Kreinecker S, Lötsch D, Ghanim B, Pirker C, Mohr T, Laaber M, Weis S, Olschowski A, Webersinke G, Pichler J, and Berger W. (2015) Prognostic quality of activating TERT promoter mutations in glioblastoma: interaction with

the rs2853669 polymorphism and patient age at diagnosis. Neuro-Oncology, 17: 1231-1240.

265. Batista R, Cruvinel-Carloni A, Vinagre J, Peixoto J, Catarino TA, Campanella NC, Menezes W, Becker AP, de Almeida GC, Matsushita MM, Clara C, Neder L, Viana-Pereira M, Honavar M, Castro L, Lopes JM, Carvalho B, Vaz RM, Máximo V, Soares P, Sobrinho-Simões M, Reis RM, and Lima J. (2016) The prognostic impact of TERT promoter mutations in glioblastomas is modified by the rs2853669 single nucleotide polymorphism. International Journal of Cancer, 139: 414-423.
266. Mosrati MA, Malmström A, Lysiak M, Krysztofiak A, Hallbeck M, Milos P, Hallbeck A-L, Bratthäll C, Strandéus M, Stenmark-Askmalm M, and Söderkvist P. (2015) TERT promoter mutations and polymorphisms as prognostic factors in primary glioblastoma. Oncotarget, 6: 16663-16673.
267. Au AY, Hackl T, Yeager TR, Cohen SB, Pass HI, Harris CC, and Reddel RR. (2011) Telomerase activity in pleural malignant mesotheliomas. Lung Cancer, 73: 283-288.
268. Jean D, Daubriac J, Le Pimpec-Barthes F, Galateau-Salle F, and Jaurand M-C. (2012) Molecular changes in mesothelioma with an impact on prognosis and treatment. Archives of pathology & laboratory medicine, 136: 277-293.
269. Gutman DA, Khalilia M, Lee S, Nalisnik M, Mullen Z, Beezley J, Chittajallu DR, Manthey D, and Cooper LAD. (2017) The Digital Slide Archive: A Software Platform for Management, Integration, and Analysis of Histology for Cancer Research. Cancer Res, 77: e75-e78.
270. https://www.cancer.gov/tcga.
271. Bilecz A, Stockhammer P, Theegarten D, Kern I, Jakopovic M, Samarzija M, Klikovits T, Hoda MA, Dome B, Oberndorfer F, Muellauer L, Fillinger J, Kovacs I, Pirker C, Schuler M, Plones T, Aigner C, Klepetko W, Berger W, Brcic L, Laszlo V, and Hegedus B. (2020) Comparative analysis of prognostic histopathologic parameters in subtypes of epithelioid pleural mesothelioma. Histopathology.
272. Hoda MA, Mohamed A, Ghanim B, Filipits M, Hegedus B, Tamura M, Berta J, Kubista B, Dome B, and Grusch M. (2011) Temsirolimus inhibits malignant pleural mesothelioma growth in vitro and in vivo: synergism with chemotherapy. Journal of Thoracic Oncology, 6: 852-863.
273. Pirker C, Bilecz A, Grusch M, Mohr T, Heidenreich B, Laszlo V, Stockhammer P, Lotsch-Gojo D, Gojo J, Gabler L, Spiegl-Kreinecker S, Doeme B, Steindl A, Klikovits T, Hoda MA, Jakopovic M, Samarzija M, Mohorcic K, Kern I, Kiesel B, Brcic L, Oberndorfer F, Mullauer L, Klepetko W, Schmidt WM, Kumar R, Hegedus B, and Berger W. (2020) Telomerase reverse transcriptase promoter mutations identify a genomically defined and highly aggressive human pleural mesothelioma subgroup. Clin Cancer Res.
274. Laszlo V, Valko Z, Kovacs I, Ozsvar J, Hoda MA, Klikovits T, Lakatos D, Czirok A, Garay T, Stiglbauer A, Helbich TH, Groger M, Tovari J, Klepetko W, Pirker C, Grusch M, Berger W, Hilberg F, Hegedus B, and Dome B. (2018) Nintedanib Is Active in Malignant Pleural Mesothelioma Cell Models and Inhibits Angiogenesis and Tumor Growth In Vivo. Clin Cancer Res, 24: 3729-3740.

275. Garay T, Juhasz E, Molnar E, Eisenbauer M, Czirok A, Dekan B, Laszlo V, Hoda MA, Dome B, Timar J, Klepetko W, Berger W, and Hegedus B. (2013) Cell migration or cytokinesis and proliferation? - Revisiting the "go or grow" hypothesis in cancer cells in vitro. Exp Cell Res.
276. Hosen I, Rachakonda PS, Heidenreich B, Sitaram RT, Ljungberg B, Roos G, Hemminki K, and Kumar R. (2015) TERT promoter mutations in clear cell renal cell carcinoma. International Journal of Cancer, 136: 2448-2452.
277. Berta J, Hoda MA, Laszlo V, Rozsas A, Garay T, Torok S, Grusch M, Berger W, Paku S, Renyi-Vamos F, Masri B, Tovari J, Groger M, Klepetko W, Hegedus B, and Dome B. (2014) Apelin promotes lymphangiogenesis and lymph node metastasis. Oncotarget, 5: 4426-4437.
278. Spiegl-Kreinecker S, Lotsch D, Neumayer K, Kastler L, Gojo J, Pirker C, Pichler J, Weis S, Kumar R, Webersinke G, Gruber A, and Berger W. (2018) TERT promoter mutations are associated with poor prognosis and cell immortalization in meningioma. Neuro Oncol, 20: 1584-1593.
279. Pfaffl MW. (2001) A new mathematical model for relative quantification in real-time RT-PCR. Nucleic Acids Res, 29: e45.
280. Garay T, Kenessey I, Molnár E, Juhász É, Réti A, László V, Rózsás A, Dobos J, Döme B, Berger W, Klepetko W, Tóvári J, Tímár J, and Hegedűs B. (2015) Prenylation Inhibition-Induced Cell Death in Melanoma: Reduced Sensitivity in BRAF Mutant/PTEN Wild-Type Melanoma Cells. PLOS ONE, 10: e0117021.
281. 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, and 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: 357-62.
282. Tallet A, Nault J, Renier A, Hysi I, Galateau-Salle F, Cazes A, Copin M, Hofman P, Andujar P, and Le Pimpec-Barthes F. (2014) Overexpression and promoter mutation of the TERT gene in malignant pleural mesothelioma. Oncogene, 33: 3748.
283. Leeansyah E, Cameron PU, Solomon A, Tennakoon S, Velayudham P, Gouillou M, Spelman T, Hearps A, Fairley C, Smit DV, Pierce AB, Armishaw J, Crowe SM, Cooper DA, Koelsch KK, Liu J-P, Chuah J, and Lewin SR. (2013) Inhibition of Telomerase Activity by Human Immunodeficiency Virus (HIV) Nucleos(t)ide Reverse Transcriptase Inhibitors: A Potential Factor Contributing to HIV-Associated Accelerated Aging. The Journal of Infectious Diseases, 207: 1157-1165.
284. Jafri MA, Ansari SA, Alqahtani MH, and Shay JW. (2016) Roles of telomeres and telomerase in cancer, and advances in telomerase-targeted therapies. Genome Medicine, 8: 69.
285. Gao K, Li G, Qu Y, Wang M, Cui B, Ji M, Shi B, and Hou P. (2016) TERT promoter mutations and long telomere length predict poor survival and radiotherapy resistance in gliomas. Oncotarget, 7: 8712-8725.
286. Yamaguchi S, Maida Y, Yasukawa M, Kato T, Yoshida M, and Masutomi K. (2014) Eribulin mesylate targets human telomerase reverse transcriptase in ovarian cancer cells. PloS one, 9: e112438-e112438.

287. de la Fouchardière C, Decaussin-Petrucci M, Berthiller J, Descotes F, Lopez J, Lifante J-C, Peix J-L, Giraudet A-L, Delahaye A, Masson S, Bournaud-Salinas C, and Borson Chazot F. (2018) Predictive factors of outcome in poorly differentiated thyroid carcinomas. European Journal of Cancer, 92: 40-47.

DOI:10.14753/SE.2020.2421

10. BIBLIOGRAPHY OF THE CANDIDATE'S PUBLICATIONS

10.1 Publications related to the thesis

Bilecz A, Stockhammer P, Theegarten D, Kern I, Jakopovic M, Samarzija M, Klikovits T, Hoda MA, Dome B, Oberndorfer F, Muellauer L, Fillinger J, Kovacs I, Pirker C, Schuler M, Plones T, Aigner C, Klepetko W, Berger W, Brcic L, Laszlo V, and Hegedus B. (2020) Comparative analysis of prognostic histopathologic parameters in subtypes of epithelioid pleural mesothelioma. Histopathology. [Epub ahead of print]

Pirker C, Bilecz A, Grusch M, Mohr T, Heidenreich B, Laszlo V, Stockhammer P, Lotsch-Gojo D, Gojo J, Gabler L, Spiegl-Kreinecker S, Doeme B, Steindl A, Klikovits T, Hoda MA, Jakopovic M, Samarzija M, Mohorcic K, Kern I, Kiesel B, Brcic L, Oberndorfer F, Mullauer L, Klepetko W, Schmidt WM, Kumar R, Hegedus B, and Berger W. (2020) Telomerase reverse transcriptase promoter mutations identify a genomically defined and highly aggressive human pleural mesothelioma subgroup. Clin Cancer Res. [Epub ahead of print]

10.2 Publications not related to the thesis

Laengle J, Stift J, Bilecz A, Wolf B, Beer A, Hegedus B, Stremitzer S, Starlinger P, Tamandl D, Pils D, and Bergmann M. (2018) DNA damage predicts prognosis and treatment response in colorectal liver metastases superior to immunogenic cell death and T cells. Theranostics, 8: 3198-3213.

Bridgeman VL, Vermeulen PB, Foo S, Bilecz A, Daley F, Kostaras E, Nathan MR, Wan E, Frentzas S, Schweiger T, Hegedus B, Hoetzenecker K, Renyi-Vamos F, Kuczynski EA, Vasudev NS, Larkin J, Gore M, Dvorak HF, Paku S, Kerbel RS, Dome B, and Reynolds AR. (2017) Vessel co-option is common in human lung metastases and mediates resistance to anti-angiogenic therapy in preclinical lung metastasis models. J Pathol, 241: 362-374.

Hegedus L, Garay T, Molnar E, Varga K, Bilecz A, Torok S, Padanyi R, Paszty K, Wolf M, Grusch M, Kallay E, Dome B, Berger W, Hegedus B, and Enyedi A. (2017) The plasma membrane Ca(2+) pump PMCA4b inhibits the migratory and metastatic activity of BRAF mutant melanoma cells. Int J Cancer, 140: 2758-2770.

DOI:10.14753/SE.2020.2421

11. ACKNOWLEDGMENTS

During my PhD studies I had the opportunity to work in an inspiring environment and with great colleagues who helped and educated me during the time we spent together.

I am grateful to Professor József Tímár and to Professor András Kiss who supported my work in their departments.

I am thankful to my supervisor Balázs Hegedűs, who has been an inexhaustible source of knowledge, support and positivity ever since I started to work in his group. Under his guidance I was able to learn the fundamentals of clinical and translational research and absorb his hard-working attitude.

In his group I was lucky enough to work with Tamás Garay, Eszter Molnár, Anikó Gaál, Marcell Baranyi and Dominika Rittler, impressive young scientists, who are also fun to be around. In Balázs's Vienna working group I was lucky to get to know Paul Stockhammer who has been a bright colleague, and a very nice person to work with.

I am also grateful for the opportunity to work in Ágnes Enyedi's lab and to collaborate with Luca Hegedűs, Karolina Varga and Krisztina Szendrefyné Lór who taught me about molecular methods and inspired me with their professional approach to science.

During my PhD studies I had the opportunity to participate in international collaborations. I was able to be a visiting scholar at the Medical University of Vienna thanks to Professor Michael Bergman, and to meet and collaborate with Professor Walter Berger, Christine Pirker and Brigitte Wolf. I am thankful for the opportunity to work with Balázs Döme and to participate in the very exciting vessel co-option project coordinated by him and Andrew Reynolds at The Institute of Cancer Research in London, UK. I am grateful for the opportunity to visit the Medical University of Graz and to learn from Luka Brčić who taught me a lot about mesothelioma pathology.

I would like to thank Judit Pápay for her valuable insights that helped to improve my thesis.

In the end, I would like to express my gratitude toward my good friend Benedek and my manager Tekla for their invaluable support and to my loving family – Anyu, Apu, Papa, Gábor and Tomi – who have been there for me and provided the safe space where both joy and sorrow could be shared.