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Comparative analysis of prognostic histopathologic parameters in subtypes of epithelioid pleural mesothelioma

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Comparative analysis of prognostic histopathologic parameters in subtypes of epithelioid pleural mesothelioma

Aims: Malignant pleural mesothelioma (MPM) is a rare malignancy with a dismal prognosis. While the epithelioid type is associated with a more favourable outcome, additional factors are needed to further stratify prognosis and to identify patients who can benefit from multimodal treatment. As epithelioid MPM shows remarkable morphological variability, the prognostic role of the five defined morphologies, the impact of the nuclear grading system and the mitosis-necrosis score were investigated in this study.
Methods and results: Tumour specimens of 192 patients with epithelioid MPM from five European centres were histologically subtyped. Nuclear grading and mitosis–necrosis score were determined and correlated with clinicopathological parameters and overall survival (OS). Digital slides of 55 independent

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cases from The Cancer Genome Atlas (TCGA) database were evaluated for external validation. Histological subtypes were collapsed into three groups based on their overlapping survival curves. The tubulopapillary/microcystic group had a significantly longer OS than the solid/trabecular group (732 days versus 397 days, $P = 0.0013$). Pleomorphic tumours had the shortest OS (173 days). The solid/trabecular variants showed a significant association with high nuclear grade and mitosis–necrosis score. The mitosis–necrosis score was a robust and independent prognostic factor in our patient cohort. The prognostic significance of all three parameters was externally validated in the TCGA cohort. Patients with tubulopapillary or microcystic tumours showed a greater improvement in OS after receiving multimodal therapy than those with solid or trabecular tumours.
Conclusions: Histological subtypes of epithelioid MPM have a prognostic impact, and might help to select patients for intensive multimodal treatment approaches.

Keywords: epithelioid, grading, histological subtypes, mesothelioma, prognosis

Introduction

Malignant pleural mesothelioma (MPM) is the most common primary malignancy of the pleura. Due to its highly aggressive clinical behaviour it confers a dismal prognosis.[1] MPM is divided into three major histological types; namely, epithelioid, sarcomatoid and biphasic.[2] Histological type is an important prognostic factor, also playing a substantial role in treatment decision-making.[3–5] The epithelioid type is the most common type of MPM with the most favourable prognosis.[6] However, it is a heterogeneous entity and there is a lack of morphological prognostic factors for further stratification of epithelioid MPM (eMPM).

The presence of necrosis, the degree of nuclear atypia and mitotic count have been shown to have a prognostic role in eMPM.[7–9] Furthermore, the presence of prominent nucleoli and atypical mitotic figures was found to be of prognostic value, while intranuclear inclusions or a high nuclear/cytoplasmic ratio was not associated with worse patients' outcomes, and the prognostic impact of chromatin structure alterations remains unclear.[7,9] Immunohistochemical assessment of proliferation by Ki67 labelling was also shown to have a prognostic role in eMPM when using 10% or 15% as cut-off values.[7,10] Furthermore, composite scores have been proposed and regarded as robust tools in the stratification of patient outcome.[11] The nuclear grade is based on a three-tier assessment of nuclear atypia and mitotic count.[7,8] Rosen *et al.* developed the mitosis–necrosis score (M/N score), which includes the presence of necrosis and a two-tier scoring of mitotic counts.[8] A more complex pathological grading system based on the presence of necrosis, the main histological subtype, Ki67 proliferation index and a four-tier evaluation of the mitotic count has also been developed for risk stratification of eMPM.[12]

There are limited data on the prognostic implications of histological subtypes in eMPM, but it is a promising emerging marker for predicting patient outcome, similarly to peritoneal mesothelioma[13,14] and other malignancies, such as lung,[15–17] gastric[18] and bladder cancer.[19] The pleomorphic subtype has been shown to be a significant predictor of negative clinical outcome,[20–24] while the microcystic/myxoid variant might have a positive impact on overall survival (OS) for eMPM patients.[22] A predominantly solid morphological subtype has been associated with worse outcome compared to non-solid subtypes; however, it was not found to be an independent prognostic factor.[8] The role of other individual architectural subtypes such as trabecular, tubulopapillary, microcystic and micropapillary are yet to be determined.[2]

Accordingly, we investigated the prognostic impact of five histological subtypes of eMPM and their association with the other proposed histopathological prognosticators, namely nuclear grade composed of scores for nuclear atypia and mitotic count[7] and the M/N score based on the presence of necrosis and mitotic count.[8] Finally, we examined the association between OS and histological subtypes in subgroups of patients receiving multimodal therapy versus chemotherapy or best supportive care-only regimens.

Materials and methods

STUDY COHORT

Our multicentre cohort consisted of a total of 192 patients diagnosed with eMPM between 1994 and 2015 at the Medical University of Vienna, Vienna, Austria ($n = 54$), between 2000 and 2007 at the National Korányi Institute of TB and Pulmonology, Budapest, Hungary ($n = 30$), between 2007 and 2012 at the University Clinic of Respiratory and Allergic

Diseases, Golnik, Slovenia (n = 67), between 2013 and 2014 at the University of Zagreb, School of Medicine, Jordanovac, Croatia (n = 9) and between 2016 and 2018 at the University Medicine Essen – Ruhrlandklinik, Essen, Germany (n = 32). The pathological diagnosis of eMPM was made by expert pulmonary pathologists following international histological and immunohistochemical criteria requiring a minimum of two positive mesothelial markers (calretinin, WT-1, D2-40, CK5/6) and at least two negative markers for carcinoma (such as Ber-EP4, TTF-1, CEA). Clinical data, including patients' age, gender, date of diagnosis and date of death or last contact, were collected in accordance with each institute's ethical guidelines and the latest Declaration of Helsinki. The retrospective analysis of MPM patients was approved in all participating centres by the local ethics committees at the Medical University of Vienna (no. 904/2009), the University Hospital Center Zagreb (no. 02/21AG) and at the University Medicine Essen (17-7773-BO). The Institutional Review Boards of the University Clinic Golnik and the National Koranyi Institute of Tuberculosis and Pulmonology granted a waiver for the retrospective analyses. Samples were obtained by video-assisted thoracoscopy (n = 106), percutaneous pleural needle core biopsy (n = 28) or pleurectomy (n = 28). In 30 cases the exact surgical sampling method was not specified. All tissue samples were formalin-fixed and paraffin-embedded (FFPE).

VALIDATION COHORT

We analysed 55 digital images of eMPMs openly available at the Cancer Digital Slide Archive (CDSA), which correspond to diagnostic sections of specimens submitted by tissue source sites of The Cancer Genome Atlas (TCGA).[25] Of these 55 sections, six were frozen sections and 49 were FFPE specimens. Corresponding clinical variables and survival data collected by TCGA Research Network[26] were downloaded from the cBioPortal.[27]

EVALUATION OF HISTOPATHOLOGICAL FEATURES

One haematoxylin and eosin (H&E)-stained slide was provided for each case by expert pathologists from the participating centres and was classified by Á.B. and L.B. Eventual discrepant cases were discussed together and consensus was reached. Classification was based on the predominant growth pattern and on the presence of pleomorphic cytological features (Figure 1).[2,20,28] A sample was assigned pleomorphic if at least 10% of the tumour area consisted of anaplastic or giant tumour cells.[20]

Briefly, the following morphological features were used for the classification.[28] Tubulopapillary pattern was defined by a combination of either tubular and/or papillary structures consisting of cuboidal, slightly enlarged tumour cells arranged around connective tissue cores. Solid pattern was characterised by tumour cells of epithelioid appearance forming larger sheets or nests. Samples consisting of one or two layers of relatively small, monomorphic cells forming thin cords often spreading within abundant desmoplastic stroma were assigned trabecular pattern. Microcystic pattern was characterised by tumour cells forming small cyst-like structures, while micropapillary pattern exhibited small papillae without the fibrovascular cores seen in tubulopapillary pattern.

Mitotic figures were counted in hot-spots at ×400 magnification and given as an average of mitotic figures per 10 high-power fields (Figure 2A,B).[8] The presence or absence of necrosis in the sample was also evaluated and used to calculate M/N score (0–2), as proposed earlier (Figure 2C).[8] A composite nuclear grade was calculated for each case based on nuclear atypia and mitotic count scores evaluated according to previous studies (Figure 2D–F).[7,8]

STATISTICAL ANALYSIS

OS was defined as the time from diagnosis to date of death or for censored patients as the time between diagnosis and date of last contact. The Kaplan–Meier method and log-rank (Mantel–Cox) tests were used to estimate OS and to calculate survival differences between groups, respectively. A multivariate Cox regression model including histology, M/N score and nuclear grade as variables was calculated to identify independent prognostic factors, hazard ratios (HR) and their corresponding 95% confidence intervals (CI). Fisher's exact and χ^2 tests were used to evaluate associations between histological subtypes, nuclear grade and M/N score, as well as between clinicopathological variables and histological subtypes and treatment received. For all data comparisons, results were considered as statistically significant if P was <0.05 based on two-sided tests. All calculations were performed using GraphPad Prism version 8.0 (GraphPad Inc., San Diego, CA, USA) and SPSS Statistics version 23.0 package (SPSS Inc., Chicago, IL, USA).

Results

We analysed tumour samples of 192 patients who had histopathologically confirmed diagnosis of eMPM

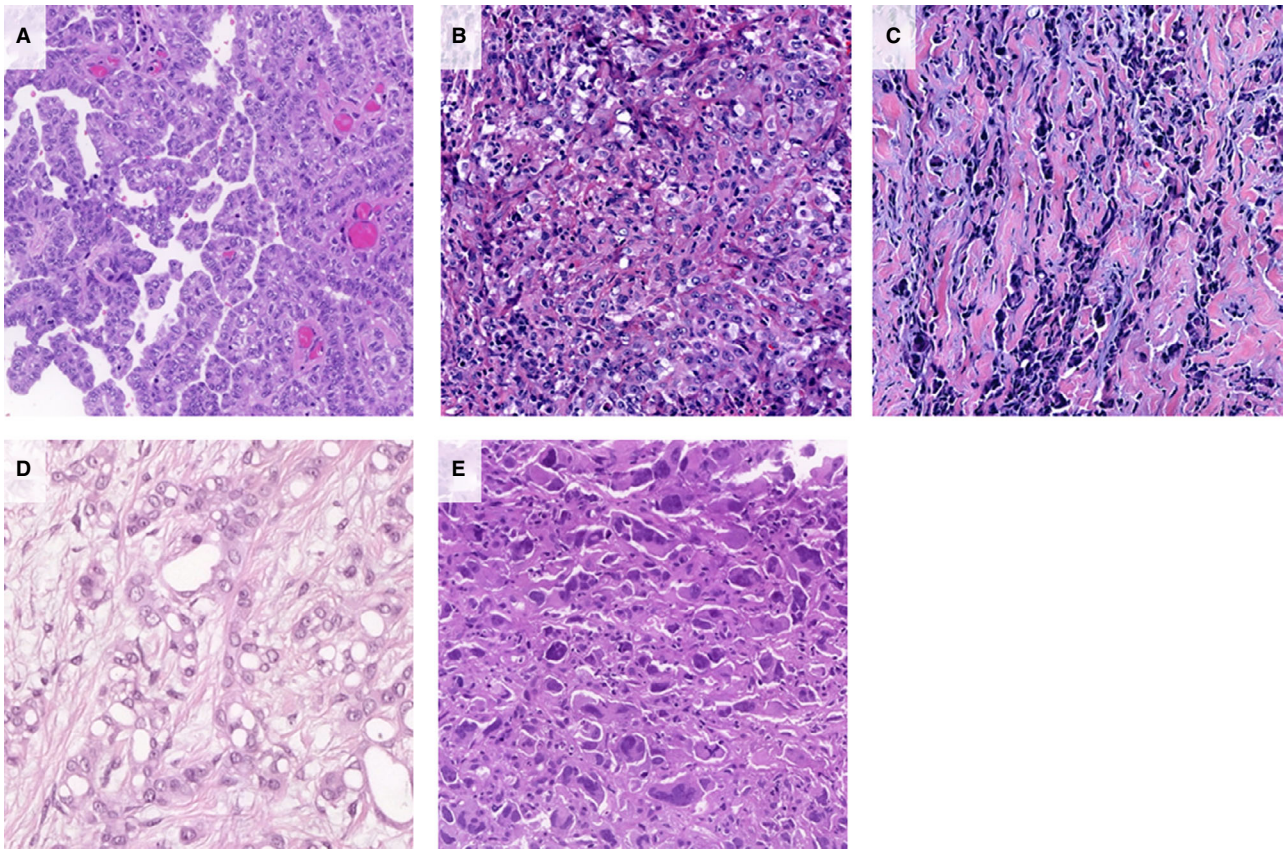

Figure 1. Histological subtypes of epithelioid malignant pleural mesothelioma (eMPM). **A,** Tubulopapillary pattern [haematoxylin and eosin (H&E)]. **B,** Solid pattern (H&E). **C,** Trabecular pattern (H&E). **D,** Microcystic pattern (H&E). **E,** Pleomorphic features (H&E).

and for whom OS data were available. Median follow-up was 423 days. Median age of patients was 65.0 ± 10.8 years; 143 (74.5%) of the patients were male. International Mesothelioma Interest Group (IMIG) stage at the time of diagnosis was available for 126 patients, 48.4% (61 of 126) of whom had early stage (IMIG I/II) disease, while 51.6% (65 of 126) had advanced stage (IMIG III/IV) disease. Among the 192 eMPM cases, the solid variant was the most common predominant pattern accounting for 52.1% (100 if 192) of all samples, while 28.6% (55 of 192) were predominantly tubulopapillary, 10.4% (20 of 192) trabecular, 4.7% (nine of 192) microcystic, 3.1% (six of 192) pleomorphic and 1.0% (two of 192) micropapillary. According to the nuclear grading system, 54.7% (105 of 192) were grouped in grade 1, 32.3% (62 of 192) in grade 2 and 13.0% (25 of 192) in grade 3. Based on the presence of necrosis and mitotic counts, an M/N score of 0 was assigned to 45.8% (88 of 192) of the cases, score 1 to 40.1% (77 of 192) and score 2 to 14.1% (27 of 192) of the samples (Table 1).

Next, we analysed the OS of each histological subtype of eMPM. Due to the very low number of micropapillary variants ($n = 2$) in our cohort, those were excluded from the survival analysis. We found that tubulopapillary and microcystic subtypes associated with better prognosis (median OS 727 and 936 days, respectively), whereas patients with solid and trabecular patterns had a shorter median OS (397 and 394 days, respectively). The pleomorphic eMPM patients had the shortest median OS of 173 days, which was significantly worse than median OS of tubulopapillary, microcystic and solid subtypes ($P < 0.0001$, 0.0085 and 0.0277, respectively) and showed a trend for worse outcomes in comparison to predominant trabecular variant ($P = 0.0906$, Figure 3A).

Due to the rarity of microcystic and trabecular patterns the four subtypes, except for the pleomorphic variant, were collapsed into two groups. Based on their overlapping survival curves, specimens showing a predominantly microcystic pattern were merged with tubulopapillary variants, while trabecular

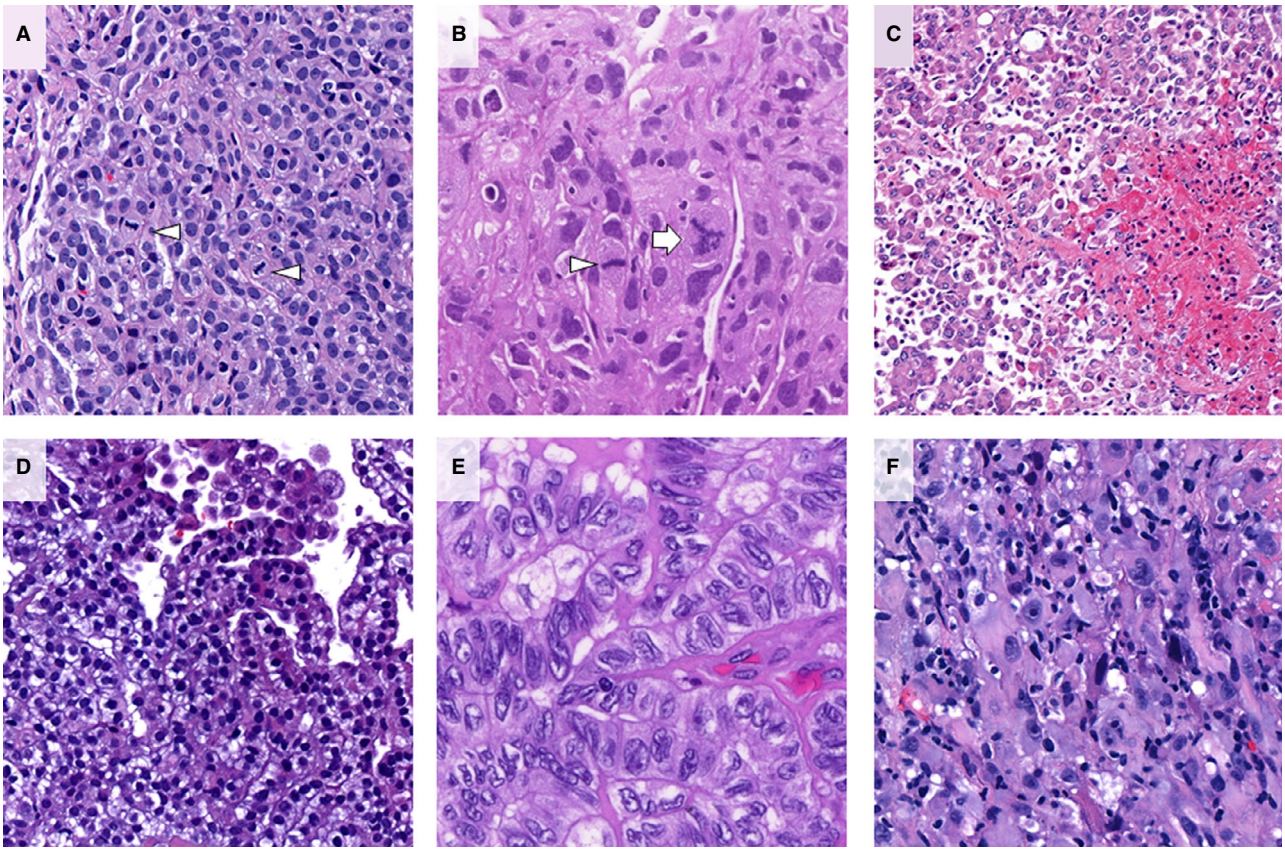

Figure 2. Mitosis, necrosis and nuclear grading in epithelioid malignant pleural mesothelioma (eMPM). **A,** Bipolar mitoses [arrowheads, haematoxylin and eosin (H&E)]. **B,** Bipolar (arrowhead) and atypical (arrow) mitoses (H&E). **C,** Coagulative necrosis (H&E). **D,** Mild nuclear atypia (H&E). **E,** Moderate nuclear atypia (H&E). **F,** Severe nuclear atypia (H&E).

patterns were merged with solid pattern tumours for further analyses. The pleomorphic subtype was analysed as a separate group. In univariate analyses, we found that patients with tubulopapillary/microcystic features had a significantly better OS than patients with solid/trabecular variants (medians 732 days versus 397 days, $P = 0.003$, Table 2, Figure 3B).

Pleomorphic tumours were associated with significantly worse outcome when compared to solid/trabecular variants (median OS 173 days versus 397 days, $P = 0.039$ Table 2, Figure 3B). As the pleomorphic variant showed a dramatically shorter OS than all other subtypes in our cohort, and several earlier studies suggested its exclusion from eMPM based on its very poor prognosis, we did not include it in our further survival analyses.[20–23]

Stage I/II disease was associated with a significantly better OS than stage III/IV (medians 650 days versus 421 days, $P = 0.015$, Table 2). The distribution of tubulopapillary/microcystic and solid/trabecular variants was similar among early (I/II) and advanced stages (III/IV) of disease ($P = 0.999$, Supporting information, Figure S1A). Early-stage cases with tubulopapillary/microcystic features showed a tendency for longer OS (Mantel–Cox test, $P = 0.194$; Grehan–Breslow–Wilcoxon test $P = 0.041$; Supporting information, Figure S1B), while among the advanced-stage patients the tubulopapillary/microcystic variants were associated with a significantly longer OS compared to the solid/trabecular variants ($P = 0.047$, Supporting information, Figure S1C).

Patients with tumours of M/N scores 1, 2 and 3 had significantly different OS of 720 days, 386 days ($P = 0.0004$) and 165 days ($P = 0.0036$), respectively (Figure 4A, Table 2). There was no significant difference in OS between nuclear grades 1 and 2. However, patients with nuclear grade 3 had significantly worse OS when compared to patients with nuclear grade 2: median OS 123 versus 486 days ($P = 0.0002$) (Figure 4B, Table 2).

Regarding the distribution of M/N scores as well as nuclear grades among the histological subtypes we

Table 1. Clinicopathological characteristics of the eMPM patient cohort

	Total (n = 192)
Gender	
Male	143
Female	49
Age (years)	
Mean ± SD	65.0 ± 10.8
Histology	
Solid	100
Tubulopapillary	55
Trabecular	20
Microcystic	9
Pleomorphic	6
Micropapillary	2
Nuclear atypia	
Mild	13
Moderate	132
Severe	47
Mitotic count	
Low (≤1)	117
Intermediate (2–4)	41
High (≥5)	34
Necrosis	
Yes	94
No	98
Nuclear grade	
1	105
2	62
3	25
M/N score	
0	88
1	77
2	27
IMIG stage (NA = 66)	
I/II	61
III/IV	65

NA, not available; SD, standard deviation; M/N, mitosis/necrosis; eMPM, epithelioid malignant pleural mesothelioma; IMIG, International Mesothelioma Interest Group.

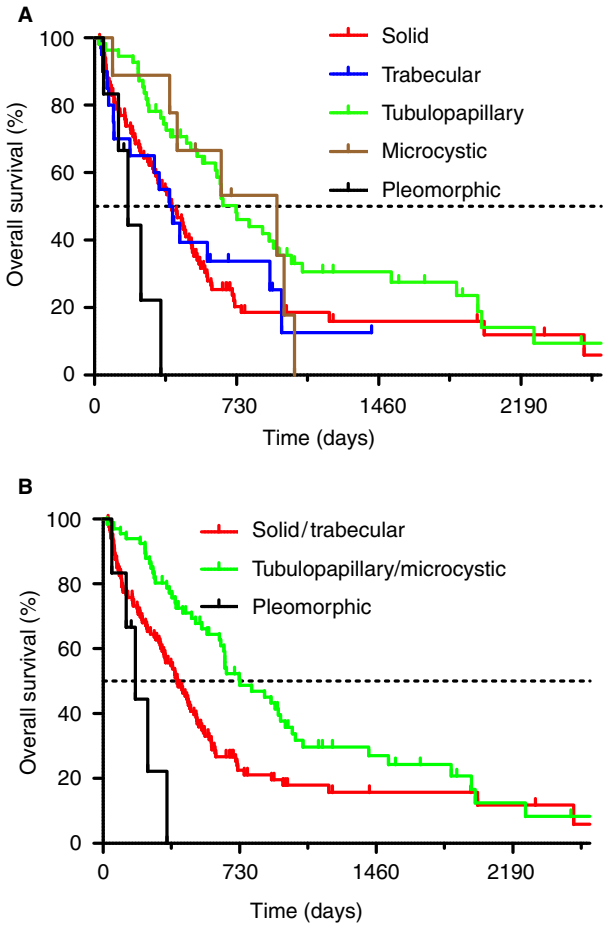

Figure 3. Histological subtypes and patient outcomes. **A,** Overall survival (OS) of the five histological subtypes: tubulopapillary, microcystic, solid, trabecular and pleomorphic (P = 0.0019, log-rank test). **B,** OS of collapsed groups: solid/trabecular [hazard ratio (HR) = Ref.], tubulopapillary/microcystic [HR = 0.57, 95% confidence interval (CI) = 0.41–0.80] and the pleomorphic subtype (HR = 4.72, 95% CI = 1.15–19.42). For all three curves: P < 0.0001, log-rank test. [Colour figure can be viewed at wileyonlinelibrary.com]

found a significant association of solid/trabecular patterns with both higher M/N scores (P < 0.0001) and higher nuclear grades (P = 0.007) in comparison to tubulopapillary/microcystic variants (Figure 4C,D).

In multivariate analysis, including histological subtype, M/N score and nuclear grading as parameters, we found M/N score to be an independent prognostic factor in our MPM cohort (Table 3). Histological subtype did not reach significance (P = 0.095).

We analysed the impact on OS of each individual factor – namely nuclear atypia, mitotic rate, presence of necrosis – used to calculate composite grades. Patients with tumours exhibiting mild atypia (median

Table 2. Univariate survival analyses in the eMPM patient cohort

	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; tubulopap., tubulopapillary; microcyst., microcystic; M/N, mitosis/necrosis; OS, overall survival; eMPM, epithelioid malignant pleural mesothelioma; MMT, multimodal therapy; CHT, chemotherapy; BSC, best supportive care.

OS 1197 days) had a significantly longer OS in comparison to those with moderate or severe atypia (median OS 501 days, $P = 0.027$ and 306 days, $P < 0.001$, respectively, Table 2, Supporting information, Figure S2A). High mitotic counts were associated with shorter median OS in comparison to low mitotic rate (239 days, $P < 0.001$), while low and intermediate mitotic counts did not show a significant difference in median OS (545 and 501 days, respectively, $P = 0.470$, Table 2, Supporting information, Figure S2B). The presence of necrosis was also associated with a significantly shorter OS in comparison to cases without necrosis (281 days versus 727 days, respectively, $P < 0.0001$, Table 2, Supporting information, Figure S2C).

We also performed a multivariate analysis of histological variants and individual components of the composite scores. We found the presence of necrosis to be a strong independent prognostic factor ($P < 0.0001$, Supporting information, Table S1).

HISTOPATHOLOGICAL ANALYSIS OF EMPM SAMPLES FROM THE TCGA COHORT

For external validation, we analysed an additional set of eMPM samples derived from the TCGA for which scanned H&E-stained sections were available. The corresponding clinicopathological variables of these 55 patients are detailed in Supporting information, Table S2. We found 50.9% (28 of 55) of the samples to be of tubulopapillary pattern, 30.9% solid (17 of 55), 5.5% microcystic (three of 55), 5.5% trabecular (three of 55) and 7.2% micropapillary (four of 55). No sample with pleomorphic features was identified. In agreement with the results obtained in our multicenter MPM patient cohort, univariate analysis of the OS data (Supporting information, Table S3) showed a

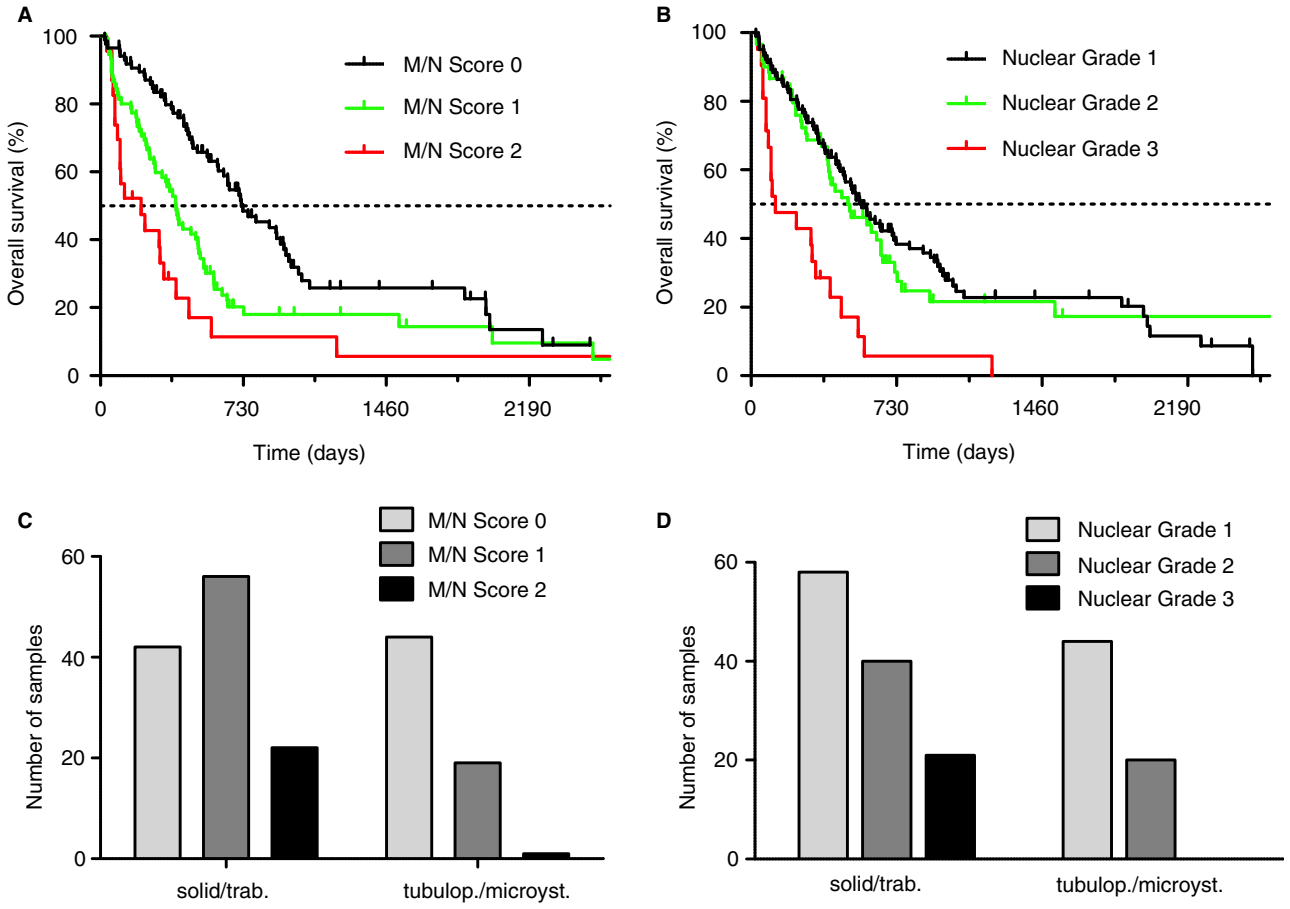

Figure 4. Mitosis/necrosis (M/N) score and nuclear grading. **A,** M/N score is a significant prognostic factor in eMPM. In comparison to M/N score 0 [720 days, hazard ratio (HR) = Ref.], M/N score 1 [386 days, HR = 2.01, 95% confidence interval (CI) = 1.37–2.95, $P < 0.0001$, log-rank test] and M/N score 2 (208 days, HR = 5.03, 95% CI = 2.43–10.46, $P < 0.0001$, log-rank test) are associated with shorter overall survival (OS). B, Nuclear grading is a significant prognostic factor in epithelioid malignant pleural mesothelioma (eMPM). Nuclear grade 1 (555 days, HR = Ref.) is associated with longer OS in comparison to nuclear grade 3 (123 days, HR = 5.64, 95% CI = 2.69–11.83, $P = 0.0002$, log-rank test), while nuclear grade 2 was not associated with significantly worse outcomes (486 days, HR = 1.10, 95% CI = 0.75–1.62, $P = 0.531$, log-rank test). **C,** M/N score significantly associates with histological subtypes of eMPM, solid/trabecular variants are associated with higher M/N scores ($P < 0.0001$, χ^2 test). **D,** Histological subtypes of eMPM show a significant association with nuclear grades, solid/trabecular variants show a higher frequency of higher nuclear grades ($P = 0.0008$, χ^2 test). [Colour figure can be viewed at wileyonlinelibrary.com]

significantly inferior OS associated with solid/trabecular subtypes in comparison to tubulopapillary/microcystic patterns (median 406 days versus 795 days, $P = 0.01$, Figure 5C). Histological grading was performed on 49 FFPE samples, while the six samples for which fresh frozen samples were only available were not included in grade analysis. The solid/trabecular subtypes showed a significant association with higher M/N scores ($P < 0.0001$, Figure 5D). Nuclear grade 3 and M/N score 2 cases were associated with significantly inferior OS than nuclear grade 1 ($P = 0.01$) and M/N score 0 ($P = 0.023$), respectively (Figure 5A,B). However, the low number of patients ($n = 55$) in the TCGA validation cohort limited the feasibility of a multivariate analysis.

THE IMPACT OF MORPHOLOGICAL SUBTYPES IN THE MULTIMODAL THERAPEUTIC SETTING

In order to identify whether tubulopapillary/microcystic and solid/trabecular epithelioid subtypes are associated with distinct outcomes after therapy, we evaluated differences in OS of patients with the treatment information available ($n = 109$, Supporting information, Table S4). Forty per cent of patients (44 of 109) received multimodal therapy (MMT) consisting of radical surgery plus chemo- and/or

Table 3. Multivariate Cox regression analysis in the MPM patient cohort

	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; MPM, malignant pleural mesothelioma.

radiotherapy, while 60% (65 of 109) received chemotherapy only (CHT) or best supportive care (BSC). Accordingly, we stratified the cohort into four subgroups based on solid/trabecular pattern eMPMs versus tubulopapillary/microcystic pattern eMPMs and MMT versus CHT/BSC treatment. We compared the distribution of several clinicopathological variables between solid/trabecular and tubulopapillary/microcystic subtypes treated with MMT or CHT/BSC (Supporting information, Table S5). Among the two subgroups with MMT there was no significant difference in patients' age, gender, stage or the tumours' nuclear grade and M/N scores. Comparing the two subgroups with CHT/BSC we found that tubulopapillary/microcystic tumours were significantly associated with younger age, lower nuclear grades and M/N scores, but we did not identify any significant differences in patients' gender or stage.

Interestingly, we found that among patients who received MMT those with tubulopapillary/microcystic pattern MPMs showed a trend for OS superior to patients with solid/trabecular pattern tumours (HR = 2.29, 95% CI = 0.95–5.12, $P = 0.066$, Figure 6). Among those not receiving MMT there was no significant difference in OS between the two main groups of histological variants (HR = 1.16, 95% CI = 0.65–2.07, $P = 0.617$, Figure 6). Furthermore, both in the tubulopapillary/microcystic as well as in the solid/trabecular subcohorts, MMT provided a significant survival benefit (tubulopapillary/microcystic: MMT versus CHT/BSC: HR = 2.67, 95% CI = 2.18–3.08, $P = 0.0006$; solid/trabecular: MMT versus CHT/BSC: HR = 1.77, 95% CI = 1.24–2.31, $P = 0.0018$, Figure 6).

Discussion

In the current study, tumour samples from 192 patients with epithelioid MPM were re-analysed from the archives of five large central European thoracic centres. To the best of our knowledge, this is the second largest study so far to evaluate the prognostic role of different histological patterns of eMPM. Moreover, this is the first study to directly compare the prognostic impact of morphological growth pattern, the nuclear grade and the M/N score. The three main histological types, epithelioid, biphasic and sarcomatoid, are recognised as distinct categories of MPM by the most recent World Health Organisation classification[2] and are a mandatory part of the final diagnosis.[4] According to the International Mesothelioma Interest Group's 2017 update, the histological pattern of eMPMs is an optional part of a pathological report but – in the light of emerging data – is currently considered a potentially important prognostic feature,[29] and is also recommended to be part of reporting by the 2020 guideline of the European Network for Rare Adult Solid Cancers and the International Association for the Study of Lung Cancer.[28] The frequency of the individual histological subtypes varies substantially in the literature; nevertheless, we observed their frequencies in our eMPM cohort to be within the range of previous studies.[20,22,23]

In the daily practice of MPM diagnostics, the amount of tissue available for histological work-up, subtyping and grading is often an issue. In our opinion, sampling heterogeneity might be an important factor regarding variable subtype frequencies among recent studies. Our cohort mainly consisted of surgical biopsies. However, was not pre-selected based on sample size and included percutaneous core needle biopsies. This is partly a limitation of this study but also the reflection of a real-life situation from which our samples come.

Regarding outcome, we found that among eMPMs those of predominantly microcystic or tubulopapillary pattern were associated with the longest OS. This finding is similar to that of Brcic *et al.*[23] and Alchami *et al.*[22] We found that the trabecular variant conferred a relatively poor prognosis, similar to the solid pattern. Regarding the trabecular variant, conflicting data are available in the current literature.[20,23]

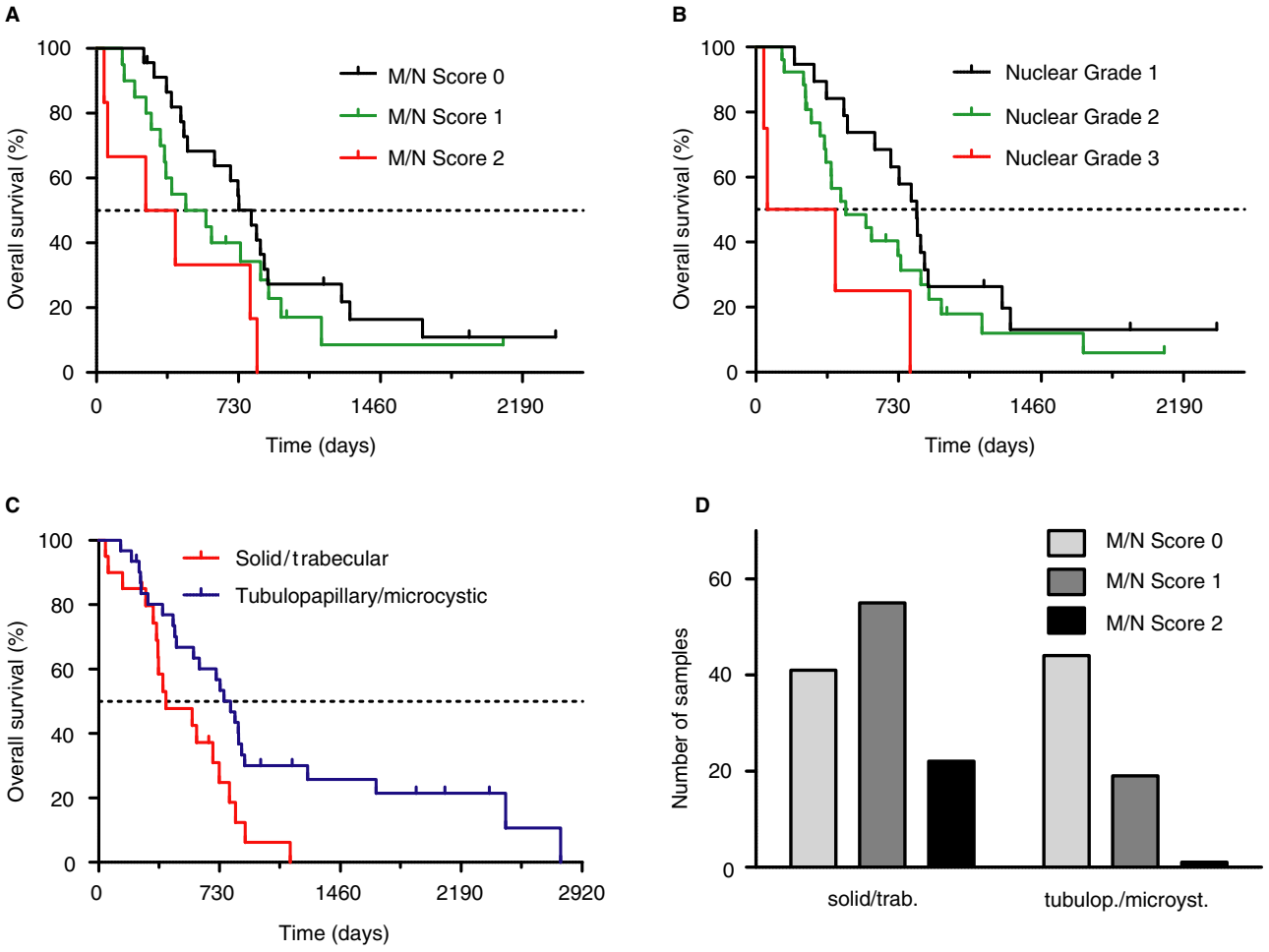

Figure 5. Analysis of the The Cancer Genome Atlas (TCGA) epithelioid malignant pleural mesothelioma (eMPM) cohort. **A,** Mitosis/necrosis (M/N) score is a significant prognosticator in eMPM. Compared to M/N score 0 [795 days, hazard ratio (HR) = Ref.], M/N score 1 (511 days, HR = 1.47, 95% confidence interval (CI) = 0.76–2.86, $P = 0.251$, log-rank test] and M/N score 2 (330 days, HR = 3.11, 95% CI = 1.17–8.23, $P = 0.023$, log-rank test) was associated with shorter overall survival (OS). **B,** Higher nuclear grade showed a tendency towards shorter OS: nuclear grade 1 (823 days, HR = Ref.), nuclear grade 2 (459 days, HR = 1.53, 95% CI = 0.80–2.92, $P = 0.200$, log-rank test) nuclear grade 3 (232 days, HR = 4.91, 95% CI = 1.45–16.59, $P = 0.010$, log-rank test). **C,** Tubulopapillary/microcystic subtype (795 days, HR = Ref.) is associated with longer OS than solid/trabecular variants (406 days, HR = 2.24, 95% CI = 1.17–4.29, $P = 0.01$, log-rank test). **D,** Solid/trabecular subtypes were associated with higher M/N scores ($P < 0.0001$, χ^2 test). [Colour figure can be viewed at wileyonlinelibrary.com]

Furthermore, we were able to confirm the previously reported dismal prognosis of MPM exhibiting pleomorphic features.[20–23] Based on overlapping survival curves, we merged the tubulopapillary and microcystic variants, as well as solid and trabecular variants. We found these two groups to have significantly different OS. In an external validation cohort consisting of 55 digitised eMPM sections of the TCGA project we confirmed a similar significant difference in OS between these two groups.

The newly proposed M/N score aiming to further stratify patients with eMPM was a robust marker in our patient population. In multivariate analysis, we found M/N score to be the single independent prognostic factor. On analysing the individual components of the composite grades, we identified the presence of necrosis to be an independent factor defining prognosis. This result may be partially explained by the significant association we observed between solid/trabecular pattern and higher M/N scores and higher nuclear grades. While nuclear grade 3 tumours showed a significantly shorter OS, we found no significant OS difference between nuclear grade 1 and 2 tumours. This finding further supports the new EURACAN/IASLC proposal on the use of preferentially two-tier grading of eMPM.[28]

Predominant histological subtypes[30] of invasive lung adenocarcinomas have been shown to have a stage-

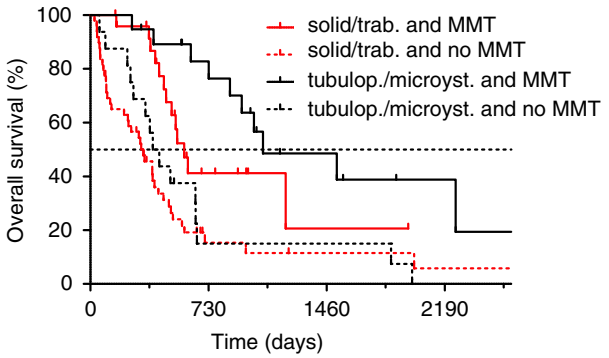

Figure 6. Histological subtypes and multimodal therapy (MMT). Histological subtypes showed a tendency for association with overall survival (OS) in patients treated with multimodal approaches. Within the patient subgroup who received MMT, tubulopapillary/microcystic subtypes [1068 days, hazard ratio (HR) = Ref.] were associated with longer OS than solid/trabecular subtypes [580 days, HR = 2.29, 95% confidence interval (CI) = 0.95–5.12, $P = 0.066$, log-rank test]. Among patients who did not receive MMT, there was no significant difference in OS between tubulopapillary/microcystic (406 days, HR = Ref.) and solid/trabecular subtypes (327 days, HR = 1.16, 95% CI = 0.65–2.07, $P = 0.617$). Within histological subtypes, MMT was associated with significantly longer OS; however, the benefit was more pronounced in the tubulopapillary/microcystic subgroup (MMT: 1068 days, HR = Ref. versus no MMT: 406 days, HR = 2.67, 95% CI = 2.18–3.08, $P = 0.0006$, log-rank test) in comparison to the solid/trabecular subgroup (MMT: 580 days, HR = Ref. versus no MMT: 327 days, HR = 1.77, 95% CI = 1.24–2.31, $P = 0.0018$, log-rank test). [Colour figure can be viewed at wileyonlinelibrary.com]

independent prognostic impact[31] and to be of predictive value for determining the patients' subgroup that might benefit from adjuvant chemotherapy after complete surgical resection.[32] In our mesothelioma subcohort analysis, we investigated if histological patterns might be a useful marker for identifying patients who might benefit more from a more aggressive treatment approach. In this regard, we found a more pronounced OS difference between patients receiving multimodal therapy versus chemotherapy only or best supportive care in case of tubulopapillary/microcystic compared to solid/trabecular MPM. These findings suggest that histological subtypes might be useful to risk-stratify eMPM patients prior to therapeutic decisions in multimodal treatment settings. Nevertheless, this observation needs further independent confirmation and prospective validation.

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Conflict of interest

None of the authors declare any conflict of interest.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. (A) Distribution of histologic variants among early and advanced disease stage patients. (P = 0.999, Fisher's exact test). (B) OS of tubulopapillary/microcystic and solid/trabecular histologic subtypes in the early stage subgroup of patients (median OS: 897 days, HR = Ref. versus median OS: 510 days, HR = 1.55, 95% CI [0.80–2.98], P = 0.194 by Mantel-Cox test; P = 0.041 by Gehan-Breslow-Wilcoxon test) (C) OS of tubulopapillary/microcystic and solid/trabecular histologic subtypes in the advanced stage subgroup of patients (median OS: 660 days, HR = Ref. versus median OS: 401 days, HR = 1.75, 95% CI [1.00–3.06], P = 0.047, Mantel-Cox test).

Figure S2. (A) OS of tumors exhibiting mild (HR = Ref.), moderate (HR = 2.29, 95% CI [1.32–3.97], P = 0.027) and severe nuclear atypia (HR = 3.47, 95% CI [1.88–6.42], P < 0.001). (B) OS and mitotic counts. Low (HR = Ref.) and intermediate (HR = 1.17, 95% CI [0.75–1.87], P = 0.470) numbers of mitotic figures are associated with a significantly better OS in comparison to high mitotic counts (HR = 2.48, 95% CI [1.45–4.25], P < 0.001) (C) The presence of necrosis is associated with significantly shorter OS (HR = 2.38, 95% CI [1.68–3.38], P < 0.0001).

Table S1. Multivariate Cox regression analysis of histologic variants and individual components of composite scores nuclear grade and mitosis-necrosis score in the eMPM patient cohort.

Table S2. Clinicopathological characteristics of the TCGA MPM patient cohort.

Table S3. Univariate survival analyses in the TCGA MPM patient cohort.

Table S4. Summary of therapeutic regimens patients in our exploratory subcohort received.

Table S5. Clinicopathologic characteristics of the exploratory subcohort in which we analysed differences in OS in the context of morphologic subtypes and therapy received. P-values were calculated by Fisher's exact tests except for *where Chi-squared tests were used and †where unpaired, two-tailed t-tests.