ORIGINAL ARTICLE

Ki67 (MIB-1) as a Prognostic Marker for Clinical Decision Making Before Extended Pleurectomy Decortication in Malignant Pleural Mesothelioma

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ABSTRACT

Introduction: The role of surgery for early stage malignant pleural mesothelioma (MPM) remains controversial. Current expert opinion is only to treat patients surgically as part of multimodality therapy. It is still challenging to identify patients who will not benefit from surgery. We specifically evaluated tumor-related parameters in combination with clinical parameters to identify prognostic markers for survival.

Methods: Clinical data of 27 consecutive patients with MPM treated with extended pleurectomy and decortication within a multimodality approach were collected and analyzed. Several tumor (immuno-)histopathologic characteristics were determined on resected tumor material, among which MTAP and Ki67 (MIB-1). Univariable and multivariable analyses served to correlate clinical and tumor-related parameters to overall survival (OS) and progression-free survival (PFS).

Results: The median PFS (mPFS) was 15.3, and the median OS (mOS) was 26.5 months. Patients with a Ki67 score greater than 10% had a significantly shorter PFS (mPFS = 8.81 versus 25.35 mo, $p = 0.001$) and OS (mOS 19.7 versus 44.5 mo, $p = 0.002$) than those with a Ki67 score less than or equal to 10. Receiver operating characteristic curve analysis for Ki67 revealed an area under the curve of 0.756 with a sensitivity of 90% and specificity of 71% for a cutoff of 10% for Ki67. Patients with loss of MTAP had a significantly shorter mPFS (9 versus 21.1 mo, $p = 0.014$) and mOS (19.7 versus 42.6 mo, $p = 0.047$) than those without MTAP loss.

Conclusions: In our study, Ki67 was prognostic for OS and PFS in patients with MPM treated with extended pleurectomy/decortication in a multimodality approach. Determination of Ki67 before surgery combined with specific clinical parameters could assist in clinical decision making by identifying patients, with high Ki67, who are unlikely to benefit from surgery.

Keywords: Malignant pleural mesothelioma; ki-67; MTAP; Prognostic marker; Extended pleurectomy decortication

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Drs. Belderbos and Maat contributed equally to this work.

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Introduction

Malignant pleural mesothelioma (MPM) is an aggressive cancer of the pleural linings of the lung with a median overall survival (mOS) of approximately 12 months from diagnosis.[1] Current first-line treatment consisting of platinum/antifolate combination chemotherapy has revealed a 3-month increase in OS compared with cisplatin alone.[2] Addition of bevacizumab to first-line chemotherapy increases the mOS with 2.8 months, but it is currently only accepted as standard of care in France.[3] Very recently, the CheckMate 743 phase 3 trial, comparing first-line nivolumab combined with ipilimumab to combination chemotherapy, yielded an improvement in OS of 4 months for patients treated with combination immunotherapy leading to a mOS of 18.1 months.[4]

For early stage MPM, the role of surgery remains controversial. Current expert opinion is only to treat patients surgically in a clinical trial setting as part of multimodality treatment.[5] Two different surgical techniques for complete macroscopic resection of the visceral and parietal pleura can be discerned: extrapleural pneumonectomy (EPP) and extended pleurectomy/decortication (eP/D). Historically, EPP was advocated as the standard operation of choice in patients eligible for surgery, but at the cost of considerable perioperative mortality and morbidity.[6] The MARS trial has revealed that EPP versus no EPP in patients treated with chemoradiation is not beneficial.[7] Because a large multicenter study failed to reveal a survival difference between EPP and eP/D, most mesothelioma centers have shifted to eP/D.[8–10] For eP/D, the mOS ranges from 10.4 to 32 months.[6] Surgically treated patients seem to have a longer OS than those who are not operated. Nevertheless, this comparison is likely to be confounded by the selection of patients for surgery who have a high-performance status, low tumor stage, and limited comorbidities.[11,12] Surgery might severely affect the quality of life in patients with a relatively short expected OS. If surgery is not effective, the impact on quality of life can be detrimental.[13] Therefore, there is an unmet need to identify who will or will not benefit from surgery within a multimodality approach.

Several factors that determine prognosis in MPM are the following: histologic subtype, sex, age, TNM stage, performance score (PS), weight loss, and several peripheral blood values (e.g., albumin, CRP, lymphocytes). Prognostic score models combine some of these parameters, such as the European Organization for Research and Treatment of Cancer (EORTC) score,[14–16] the modified Glasgow Prognostic Score (mGPS), and the neutrophil-to-lymphocyte ratio (NLR).[17] These models have proven to be prognostic for MPM, and the EORTC score has been validated in surgically treated patients with MPM. Unfortunately, it remains difficult to identify patients who will benefit from surgery on the basis of these parameters.

Several tumor-related parameters have been found to determine prognosis in MPM in general, such as the following: nuclear atypia, mitotic rate, necrosis, percentage of solid growth, BAP1 loss, and Ki67 (MIB-1Ki67).[18–26] Ki67 antigen is exclusively expressed by cells in the active phases (G1, G2, S, and mitosis) of the cell cycle and is used as a proliferation marker in analogy to the mitosis score. Ki67 is used as a predictive marker in several types of cancer including peritoneal mesothelioma.[24] In MPM, however, Ki67 or other tumor markers are not used for clinical decision making although many of these parameters have a prognostic value in MPM.

In this article, we report the clinical outcomes of patients with MPM treated in a multimodality treatment setting including eP/D. In addition, we report the results of our evaluation of the prognostic value of both tumor-related and clinical parameters to identify patients who might benefit from surgical treatment.

Materials and Methods

We retrospectively collected data from all patients surgically treated with eP/D at the Erasmus University Medical Center (Rotterdam, NL) from May 2010 to May 2019. Our research proposal was submitted to the medical research and ethical committee, and no formal approval was required according to Dutch guidelines owing to the retrospective nature of the study (MEC-2020-0546). These patients had all been treated with a multimodality approach consisting of eP/D with (neo-) adjuvant chemotherapy or adjuvant radiotherapy. Chemotherapy consisted of platinum-based chemotherapy and pemetrexed. All patients had a histologically proven diagnosis of MPM and stages cT1 to 3, N0 to 2, M0 according to TNM classification available at time of diagnosis. If available, fluorodeoxyglucose positron emission tomography–computed tomography (CT) scans were used to evaluate the presence of M1, supraclavicular, and coeliac node involvement. All eP/D procedures had been performed according to the surgical technique reported by Maat et al.[9] Follow-up was done at Erasmus MC or in referring peripheral hospitals. For patients with a follow-up at Erasmus MC, radiologic tumor assessment was done retrospectively according to modified Response Evaluation Criteria In Solid Tumors version 1.1. CT scans had been made every 3 to 4 months. For patients with clinical follow-up in peripheral centers, CT scans were requested from the peripheral centers and reassessed at the Erasmus MC as stated

previously. If CT scans for patients with follow-up in referring centers were not available, the response that was evaluated by the treating pulmonologist is according to modified Response Evaluation Criteria In Solid Tumors and communicated with Erasmus MC. OS was defined as time from surgery to death of any cause and censored at the last contact date for patients who were alive at time of data cutoff. Progression-free survival (PFS) was measured from time of surgery until radiologic progression or recurrence of disease or death of any cause.

Data Collection

Patient characteristics were collected from the electronic patient files. The following clinical variables were collected: age, sex, PS, weight loss (defined as >5% in last 3 mo), number of treatment lines received (only first-line versus more than one line of treatment), neoadjuvant chemotherapy, adjuvant chemotherapy, adjuvant radiotherapy, inhospital mortality, and 90-day postoperative mortality. The following peripheral blood parameters were collected at baseline (0–6 wk before surgery): CRP, leucocytes, platelets, neutrophils, lymphocytes, monocytes, and albumin.

In addition, the following tumor (immuno-)histopathologic characteristics were determined on resected tumor material during eP/D: histologic subtype, extent of solid pattern, grading of nuclear atypia, mitotic rate, mitosis score, necrosis, combined mitosis/necrosis score, BAP-1 expression, MTAP expression, percentage of Ki67 expression, and nuclear grading.

Both tumor (immuno-) histopathologic characteristics and patient characteristics were evaluated in the statistical analysis.

Immunohistochemistry

The tumor material was processed by the pathology laboratory at the Erasmus MC according to routine procedures. A 4-μm section of formalin-fixed, paraffin-embedded tissue was mounted serially on adhesive glass slides. Deparaffinization was performed according to the Ventana BenchMark Ultra protocol. Antigen retrieval was performed by CC1 antigen retrieval solution (referent [ref.] 950-124, Ventana Medical Systems, Inc., Oro Valley, AZ). Specimens were incubated with the primary antibody, followed by detection with OptiView DAB (ref. 760-700, Ventana Medical Systems, Inc.), UltraView-DAB (ref. 760-500, Ventana Medical Systems, Inc.), or UltraView-AP (ref. 760-501, Ventana Medical Systems, Inc.) with amplification (amplification kit ref: 760-080 or OptiView amplification kit ref: 760-099, Ventana Medical Systems, Inc.). Next, the specimens were counterstained with hematoxylin II (ref: 790-2208, Ventana Medical Systems, Inc.) and coverslipped.

Each slide contained a positive control. All stains were performed on the Ventana BenchMark Ultra (Ventana Medical Systems, Inc.). Primary antibodies, detection, and amplification methods used are mentioned in Supplementary Table 1.

(Immuno-)Histopathologic Assessment

Morphologic assessment was done on hematoxylin-eosin stains. It included scoring of growth pattern (epithelioid, mixed [mesenchymal and epithelioid] mesenchymal [which can be divided into sarcomatoid and transitional]), grading of nuclear atypia on a three-tier scale (1–3), presence of necrosis (<50% [0] versus >50% [1]), mitotic rate (mitoses/10 high-power fields [HPFs]), mitosis score (≤4/10 HPFs [0] versus >4/10 HPFs [1]), and combined mitosis plus necrosis score (sum of necrosis and mitosis score; 0–2). Scoring was done according to previously published methods.[23,26] Nuclear grading was scored in three categories as described by Kadota et al.[23]

Scoring of immunohistochemistry (IHC) for Ki67, BAP-1, and MTAP was performed as follows: Ki67 was scored as percentage-positive tumor cells; BAP1 and MTAP were scored as either present or absent in tumor cells (using surrounding stroma as a positive internal control).

Homogeneity of Ki67 Expression Within the Tumor of Patients With MPM

Overall analysis of IHC-derived, tumor-related parameters was initially done on whole slides of selected blocks of tumor material resected during eP/D. Although expression of Ki67 in breast cancer is known to be heterogeneous, in MPM, the heterogeneity of Ki67 expression has never been analyzed.[27] Implementation of Ki67 in clinical decision making requires homogeneity to prevent unreliable outcomes of Ki67 expression determined on small tissue samples, such as CT-guided needle biopsies. To evaluate the homogeneity of expression of Ki67 within the tumor of patients with MPM, we analyzed randomly selected circular areas of 2 mm matching the size of needle biopsies and tissue microarray (TMA) samples. We excluded samples of patients for whom fewer than three 2-mm regions were available for analysis. If possible, 10 randomly picked regions within the tumor were assessed for levels of Ki67. These were determined by digitally selecting, coding, and randomly presenting these areas to the scoring pathologist to avoid scoring bias. Results of scoring these regions were compared (1) with other regions selected from the same specimen and (2) with

the originally determined overall Ki67 expression. Finally, we checked if patients would fall into the same category (high or low Ki67 expression) on the basis of 2-mm region Ki67 scores.

To test the reproducibility of Ki67 expression through time, sequentially collected tumor material was also stained for Ki67 and MTAP. The availability of biopsies taken before surgery within the Erasmus MC from surgically treated patients with MPM was checked. Analysis on sequential biopsies was not done if any form of therapy (chemotherapy, radiotherapy, immunotherapy) was given between the biopsy and the operation, as (chemo)therapy can influence the expression levels of Ki67.[18]

Statistical Analysis

Patient and tumor characteristics are presented as count and percentage for categorical variables and as median and range for continuous variables. Median follow-up time was calculated by reversed Kaplan-Meier analysis for OS. mOS and PFS were estimated with a Kaplan-Meier curve. Differences in probability of survival between the strata were evaluated by log-rank (Mantel-Cox) test. The hazard ratios (HRs) of progression and death and their associated 95% confidence intervals (95% CIs) for clinically important factors (e.g., adjuvant radiotherapy, adjuvant chemotherapy, and sex) were calculated using univariable Cox proportional hazard model. For all continuous variables, except postoperative admission time, a cutoff was chosen to identify comparable groups. For Ki67 and age, the cutoff was set at the median; for mitosis, the cutoff was on the basis of literature describing a cutoff of 0 to 4 versus greater than 4.[23] For lymphocyte count, leucocyte count, monocyte count, platelet count, and neutrophil count, the cutoff was set at the median. For albumin, the cutoff was set on 35 g/liter, and for CRP, the cutoff was set to 10 mg/liter according to literature. The cutoff for the EORTC score was set to 1.27 according to a validation study in MPM performed by Fennel et al.[16] Patients with an NLR above 3 were considered to have a high NLR, in accordance with recent literature.[28]

Parameters that had a p value less than 0.1 on the basis of the univariable Cox regression model for PFS and OS were then evaluated in a Cox multivariable proportional hazard regression model. Because of the low number of events, only two coefficients could be estimated in the same model. The Cox multivariable proportional hazard regression model was repeated until all parameters that met the requirements had been tested with each other. Ki67 and mitosis score are both parameters by which the proliferative capacity of tumor cells is measured. Therefore, the parameter representing proliferation with the highest HR and lowest p value was applied in multivariable analysis.

A significance level of 0.05 with a two-sided α was chosen to determine statistical significance. Statistical analyses were performed using R 3.6.0 (R foundation for statistical computing).

Results

From May 2010 to May 2019, a total of 27 patients had been treated surgically with eP/D in a multimodality approach. Data of all patients were available for analysis. Histological subtyping showed that epithelial MPM was found in 74% of all MPM tumors. Neither inhospital mortality nor 90-day mortality was reported. All baseline characteristics are summarized in Table 1. At

Table 1. Patient Baseline Characteristics

Characteristics		
Total = 27	N	%
Median age	60	Range: 36–73
Sex, male	22	81
Histologic subtype		
Epithelioid	20	74
Nonepithelioid	7	26
Mixed	6	22
Mesenchymal:transitional	1	4
Performance score (ECOG)		
0	15	56
1	9	33
2	3	11
Weight loss		
Yes	3	11
No	23	85
Data unavailable	1	4
Perioperative treatment		
Previous first-line of chemotherapy	2	7
Neoadjuvant chemotherapy	9	33
Adjuvant chemotherapy	16	59
Neoadjuvant or adjuvant chemotherapy + radiotherapy	8	30
Adjuvant radiotherapy	9	33
Median admission time (d)	11	Range 5–37
Tumor markers		
BAP-1 loss	12	44
MTAP	16	59
Median Ki67	10	Range 0–70
Ki67 > 10	13	48
Peripheral blood parameters (median)		
Albumin, g/liter	42	Range 32–48
CRP, mg/liter	17	Range 0.5–240
Hemoglobin, mmol/liter	8.4	Range 5.7–10.6

Note: Data are presented as absolute numbers (%), unless stated otherwise. Weight loss: >5% weight loss in the last 3 months. Previous first-line of chemotherapy: chemotherapy administered more than 3 months before surgery.
ECOG, Eastern Cooperative Oncology Group.

median follow-up time of 60.7 months, 21 patients had progressed, of whom 17 had died. The mOS was 26.5 months (95% CI: 22.0–not applicable), and median PFS (mPFS) was 15.3 (95% CI: 9.6–31.5) (Fig. 1).

Association of Clinically Important Factors With Survival Outcomes

Log-rank test and univariable Cox proportional hazard regression analysis of clinical parameters revealed that PFS and OS were significantly longer in female patients, for whom the mPFS and mOS were not reached during follow-up, whereas the mPFS for male patients was 11 months (log-rank $p = 0.01$, [HR = 8.389, 95% CI: 1.113–63.258, $p = 0.039$]) and mOS was 25.4 months (log-rank $p = 0.03$, [HR = 7.105, 95% CI: 0.930–55.86, $p = 0.062$]) (Fig. 2*A* and *B*). Other clinical parameters, such as weight loss before surgery, (neo-)adjuvant chemotherapy, and PS or age (>60), did not significantly correlate with PFS or OS (Table 2). Hemoglobin, albumin, and CRP did not correlate with PFS and OS, just as the number of leucocytes, lymphocytes, monocytes, platelets, and neutrophils (data not found). Prognostic score indexes such as the EORTC score, mGPS, and NLR did not correlate with survival (Table 2).

Association of Tumor Markers With Survival Outcomes

The median score for Ki67 was 10%, which was determined to be the cutoff value. A tumor with a score higher than 10% was considered a highly proliferating tumor. Log-rank tests and univariable Cox proportional hazard analysis of tumor-associated parameters revealed that patients with a high Ki67 score had a significantly shorter PFS (mPFS = 8.81 versus 25.35 mo, log-rank $p = 0.00017$, [HR = 6.301, 95% CI: 2.200–18.047, $p = 0.001$]) and OS (mOS = 19.7 versus 44.5 mo, log-rank $p = 0.0007$, [HR = 6.594, 95% CI: 1.998–21.754, $p = 0.002$]) than those with a low Ki67 score (Fig. 2*C* and *D*). A high mitosis score also was significantly associated with shorter PFS and OS, but with a lower HR and higher p value than Ki67 (Table 2). In multivariable analysis, only Ki67 retained its significance as a prognostic marker (data not shown). As both markers are used to quantify proliferating cells, Ki67 was used in further analysis as the marker for proliferation.

MTAP loss was correlated with worse clinical outcome. The mPFS for patients with MTAP loss was 9 months versus 21.1 months for patients with MTAP expression (log-rank $p = 0.0095$, [HR = 0.313, 95% CI: 0.124–0.788; $p = 0.014$]). There was also a significant difference in mOS between patients with and without MTAP loss (mOS = 19.7 versus 42.6 mo, log-rank $p = 0.047$, [HR = 0.373, 95% CI: 0.136–1.021; $p = 0.055$]) (Fig. 2*E* and *F*). In Figure 3, we report the Ki67 expression and MTAP expression of one patient with a long OS and PFS (Fig. 3*A*–*D*) and one patient with a short OS and PFS (Fig. 3*E* and *F*). MTAP and Ki67 did not seem to correlate with each other, as the mean Ki67 score did

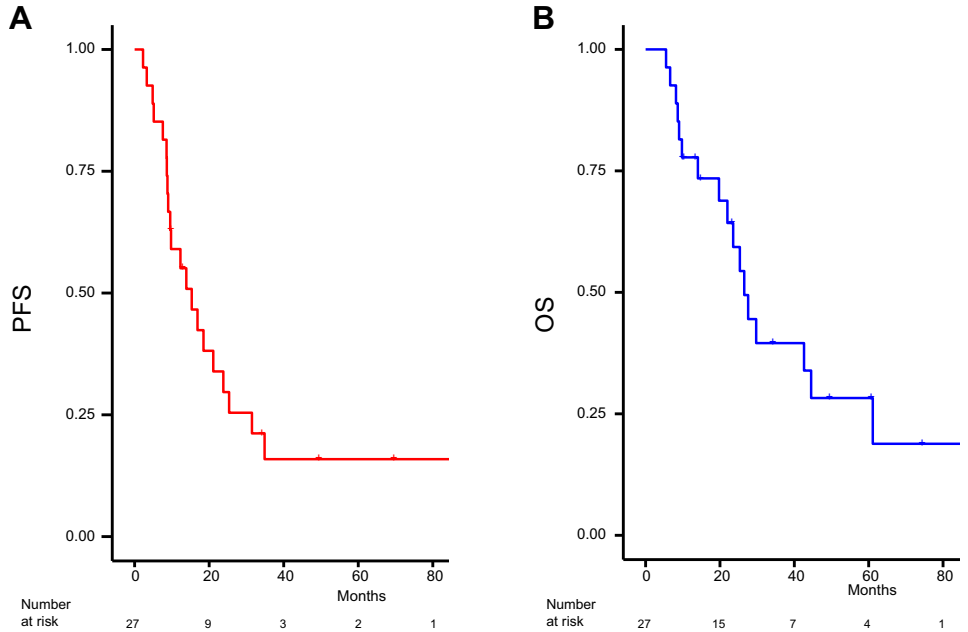

Figure 1. Kaplan-Meier curves of survival in the entire cohort of patients with MPM treated within a multimodality approach including eP/D. (*A*) PFS for the entire cohort. (*B*) OS for the entire cohort. eP/D, extended pleurectomy/decortication; MPM, malignant pleural mesothelioma; OS, overall survival; PFS, progression-free survival.

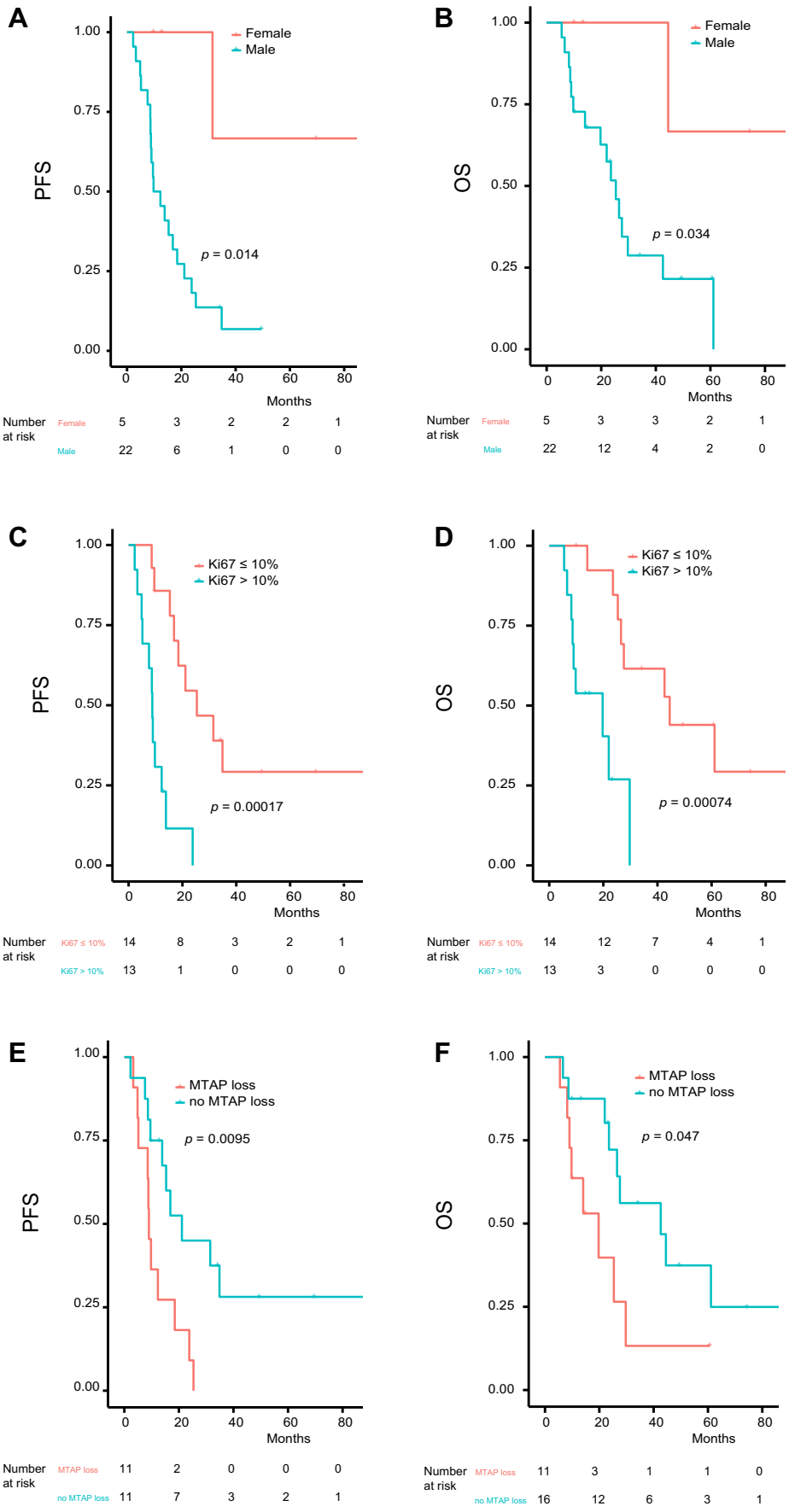

Figure 2. Kaplan-Meier curves of survival for subgroups on the basis of KI67, MTAP, and sex. (*A*) PFS and (*B*) OS by sex. (*C*) PFS and (*D*) OS in patients with a KI67 expression >10% versus those with a KI67 expression ≤10%. (*E*) PFS and (*F*) OS in patients with MTAP loss versus patients without loss of MTAP expression. OS, overall survival; PFS, progression-free survival.

Table 2. Univariable Analysis of PFS and OS for Clinical and Tumor-Related Parameters

	PFS			OS		
Parameter	HR	95% CI	*p* Value	HR	95% CI	*p* Value
Age (> 60 vs. <60)	0.904	0.382-2.142	0.819	0.8799	0.3367-2.3	0.794
Sex (male vs. female)	8.389	1.113-63.258	0.039	7.105	0.930-55.86	0.062
ECOG PS	0.6693	0.3313-1.352	0.263	0.6258	0.2777-1.411	0.258
Weight loss (yes vs. no)	1.544	0.4415-5.399	0.497	1.521	0.4252-5.441	0.519
Histology (epithelioid vs. nonepithelioid)	0.338	0.132-0.868	0.024	0.7287	0.2308-2.3	0.589
Postoperative admission time (d)[a]	1.002	0.964-1.041	0.928	1.019	0.976-1.064	0.385
Neoadjuvant chemotherapy (yes vs. no)	1.260	0.498-3.190	0.625	1.295	0.461-3.642	0.624
Adjuvant chemotherapy[b] (yes vs. no)	0.509	0.213-1.220	0.130	0.467	0.178-1.230	0.123
Adjuvant radiotherapy (yes vs. no)	0.382	0.139-1.055	0.063	0.568	0.197-1.636	0.295
Solid pattern (yes vs. no)	2.061	0.855-4.967	0.107	1.977	0.685-5.702	0.207
Atypia 2 vs. 1	2.070	0.724-5.919	0.175	2.793	0.862-9.056	0.087
Atypia 3 vs. 1	6.800	1.434-32.257	0.016	4.954	0.768-31.941	0.092
Mitosis score	3.216	1.217-8.497	0.018	3.881	1.284-11.73	0.016
Necrosis (yes vs. no)	2.040	0.706-5.900	0.188	1.026	0.290-3.624	0.968
Mitosis-necrosis score 1 vs. 0	3.052	1.127-8.268	0.028	1.677	0.555-5.073	0.360
Mitosis-necrosis score 2 vs. 0	4.373	0.864-22.127	0.075	3.796	0.734-19.641	0.112
BAP1 (yes vs. no)	1.298	0.548-3.075	0.553	2.364	0.896-6.236	0.082
MTAP loss (no vs. yes)	0.313	0.124-0.788	0.014	0.373	0.136-1.021	0.055
Ki67 (>10 vs. <10)	6.301	2.200-18.047	0.001	6.594	1.998-21.754	0.002
Nuclear grade 2 vs. 1	1.362	0.536-3.455	0.516	1.400	0.504-3.890	0.518
Nuclear grade 3 vs. 1	4.753	1.151-19.620	0.031	2.731	0.511-14.600	0.240
EORTC score[a]	0.961	0.3962-2.333	0.93	1.239	1.452-3.395	0.677
mGPS (0 vs. 1 vs. 2)	1.274	0.551-2.944	0.572	0.928	0.3715-2.319	0.873
NLR[a]	1.937	0.7681-4.884	0.161	1.512	0.5469-4.179	0.426

Note: The univariable Cox proportional hazard model was used to calculate the HRs of tumor progression or death, and the univariable logistic regression was used to calculate the ORs of response. Weight loss: >5% weight loss in the last 3 months.

[a]Continuous variable.

[b]n = 26, data of a patient who received adjuvant dendritic cell therapy removed for this analysis.

CI, confidence interval; ECOG PS, Eastern Cooperative Oncology Group Performance Status; EORTC, European Organization for Research and Treatment of Cancer; HR, hazard ratio; mGPS, modified Glasgow Prognostic Score; NLR, neutrophil-to-lymphocyte ratio; OS, overall survival; PFS, progression-free survival.

not significantly differ between MTAP positive and negative tumors (data not found). Combining Ki67 and MTAP in a prognostic index by scoring 1 point for Ki67greater than 10 and 1 point for a MTAP-negative tumor did not improve the identification of patients who benefit from surgical treatment compared with Ki67 alone (Supplementary Fig. 1). Patients with an epithelioid histology had a longer PFS (mPFS = 16.9 versus 8.6 mo, log-rank $p = 0.02$, [HR = 0.338, 95% CI: 0.132–0.868; $p = 0.024$) but a not significant difference in OS (mOS = 29.7 versus 22.0 mo, log-rank $p = 0.6$ [HR = 0.464, 95% CI: 0.182–1.184; $p = 0.108$]) compared with patients with a nonepithelioid histology. Nuclear grading, nuclear atypia, and the mitosis–necrosis score all revealed strong relations with the survival outcome and were significantly correlated with PFS, but not with OS (Table 2).

Multivariable Analysis and Receiver Operating Characteristic Curve

Sex, nuclear atypia, MTAP loss, and Ki67, as marker for proliferation, were the only markers with *p* values less than 0.1 for both PFS and OS. Because nuclear atypia is a categorical variable with three categories, two coefficients are needed to estimate this covariate and the lack of power prohibits adding this covariate to a multivariable model. Multivariable Cox proportional hazard regression analysis with the three remaining variables (Table 3) revealed that only Ki67 retained its prognostic value, indicating Ki67 with a cutoff of 10% remains an independent prognostic marker, conditional on sex and MTAP loss. Therefore, we performed a receiver operating characteristic curve to test the sensitivity and specificity of detecting death before mOS of 26.5 months, which revealed an area under the curve of 0.756 (Fig. 4). With the Youden index, an optimal cutoff was determined to be 12.5% (sensitivity 90%, specificity 71%). The sensitivity and specificity with a Ki67 cutoff of 10% were the same, as none of the patients had a Ki67 expression between 10% and 12.5%. A sensitivity of 100% with a specificity of 47% was reached when the Ki67 cutoff is set to 20%, implying patients with a Ki67 expression above 20% before surgery do not reach the mOS of 26.5 months.

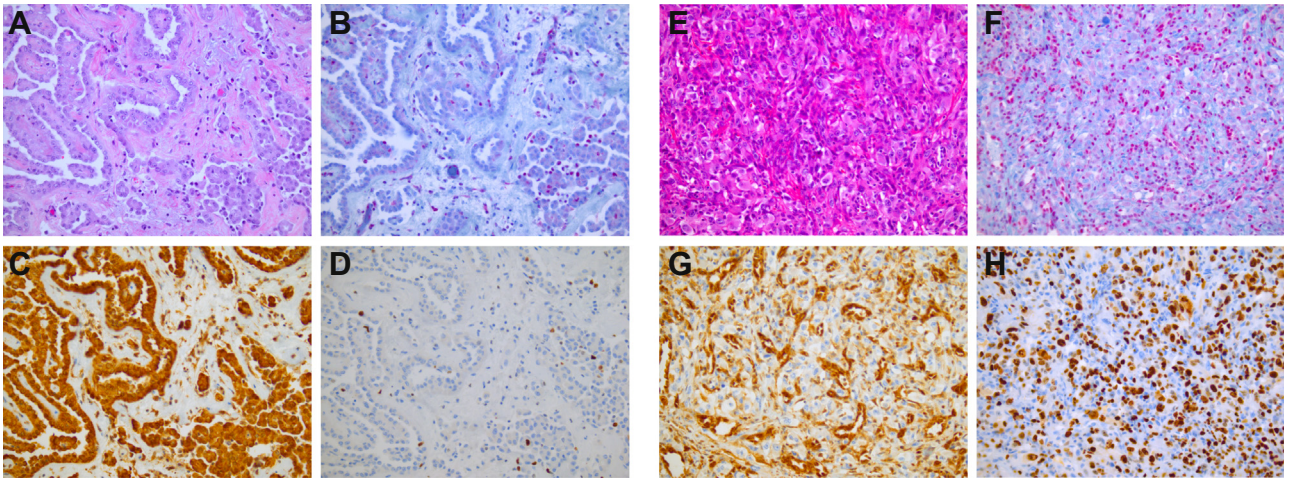

Figure 3. Illustrative stains of representative cases. (*A-D*) Stains of a malignant pleural epithelioid mesothelioma with relatively long patient survival (PFS = 69.6 mo, OS = 74.4, patient still has no progression of disease): (*A*) H&E stain revealing tubulopapillary growth pattern with limited nuclear atypia (grade 1) and low mitotic rate (<1 mitoses/10 HPFs), lacking necrosis; (*B*) absent nuclear BAP1 staining in tumor cells; (*C*) retained, strong cytoplasmic, and nuclear MTAP staining in tumor cells; (*D*) low proliferative activity (Ki-67, <1%). (*E-H*) Stains of a malignant pleural mixed-type mesothelioma with relatively short patient survival (PFS = 4.8 mo, OS = 10.3 mo, patient has progressed but is still alive with stable disease on nivolumab treatment): (*E*) H&E stain revealing predominantly solid growth pattern with evident nuclear atypia (grade 3) and high mitotic rate (26 mitoses/10 HPFs), but lacking necrosis; (*F*) absent nuclear BAP1 staining in tumor cells; (*G*) absent cytoplasmic and nuclear MTAP staining in tumor cells with positive staining in stromal cells; (*H*) high proliferative activity (Ki67, 60%). H&E, hematoxylin and eosin; HPF, high-power field; OS, overall survival; PFS, progression-free survival.

Homogeneity of Ki67 Expression in MPM Tumors

In total, 24 biopsies were available for additional IHC analysis to check for the similarity between the originally determined Ki67 expression on the resected material and the Ki67 expression in 2-mm-wide, randomly selected circular areas of the tumor (Supplementary Table 2). In 22 of 24 cases, nine or ten 2-mm spots were available for analysis, and in the two remaining cases, five and eight 2-mm spots were analyzed, respectively. In 19 of 24 patients, determination of Ki67 on these 2-mm spots resulted in a congruent categorization of high (>10%) or low (≤10%) Ki67 expression compared with overall scoring of the specimen block (Fig. 5). In five patients, however, results of the pseudo-

Table 3. Multivariable Analysis of PFS and OS for Sex and Ki67

	PFS			OS		
Parameter	HR	95% CI	*p* Value	HR	95% CI	*p* Value
Sex (M vs. F)	6.394	0.829-49.33	0.075	5.373	0.649-44.48	0.119
Ki67 (>10 vs. <10)	5.040	1.773-14.33	0.002	5.196	1.578-17.11	0.007
	PFS			OS		
Parameter	HR	95% CI	*p* Value	HR	95% CI	*p* Value
	HR	95% CI	*p* Value	HR	95% CI	*p* Value
MTAP loss (no vs. yes)	0.622	0.214-1.81	0.348	0.748	0.232-2.415	0.628
Ki67 (>10 vs. <10)	1.578	1.469-15.96	0.009	5.581	1.436-21.68	0.013
	PFS			OS		
Parameter	HR	95% CI	*p* Value	HR	95% CI	*p* Value
	HR	95% CI	*p* Value	HR	95% CI	*p* Value
Sex (M vs. F)	6.246	0.786-49.62	0.083	5.726	0.689-47.585	0.106
MTAP loss (no vs. yes)	0.457	0.178-1.17	0.103	0.528	0.19-1.465	0.220

Note: Variables with *p* values less than 0.1 for OS and PFS in univariable analysis were included in the multivariable models. The multivariable Cox proportional hazard model was used to calculate the HRs of progression or death, and the univariable logistic regression was used to calculate the ORs of response.
CI, confidence interval; F, female; HR, hazard ratio; M, male; OS, overall survival; PFS, progression-free survival.

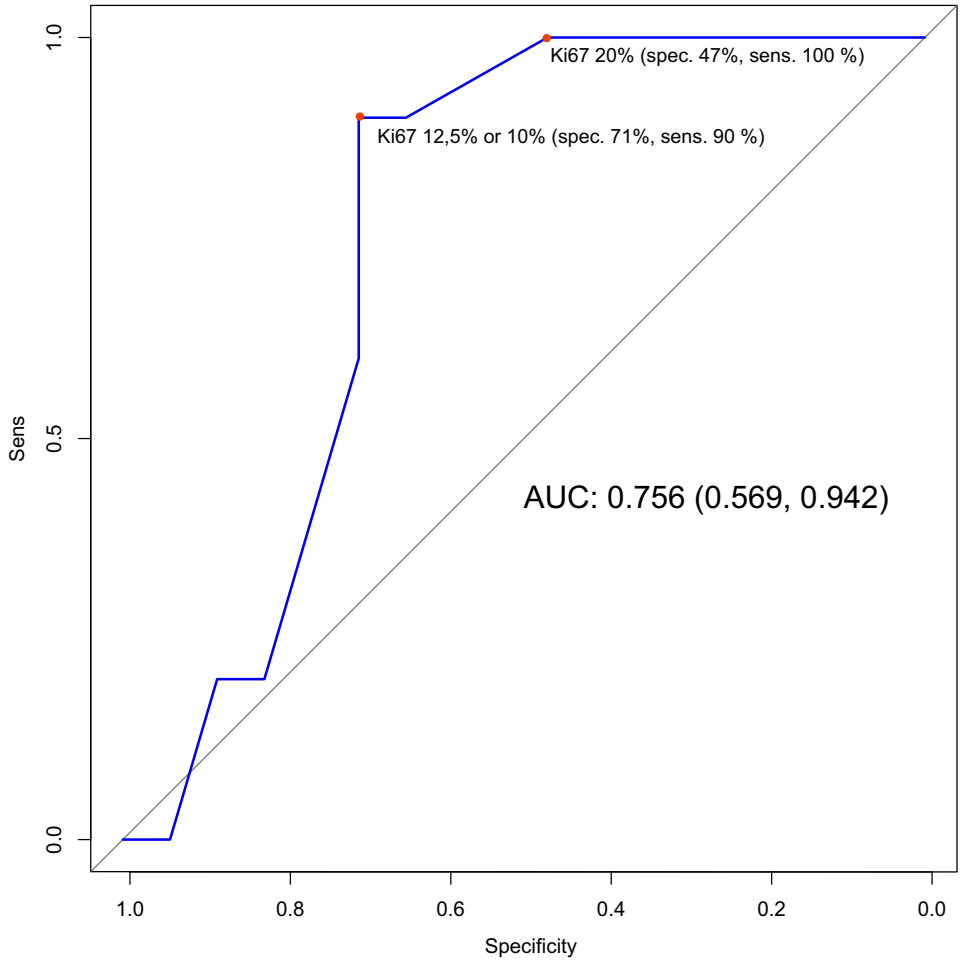

Figure 4. A receiver operating characteristic curve revealing the sens. and spec. of Ki67 for detecting death before median OS of 26.5 months. AUC, area under the curve; OS, overall survival; sens., sensitivity; spec., specify.

TMA analysis were not congruent with originally determined Ki67 expression on the resection material. For patients 3, 18, and 24, a subselection of the additional Ki67 analyses resulted in a different risk profile for the relevant patient. In patient 18, a total of 10% of the additional staining resulted in a different risk profile. For patients 3 and 24, this was 30% and 70%, respectively. The originally determined Ki67 expression for patient 7 was 15%, but all additional focal analyses were below 10%. For patient 21, with an original Ki67 expression of 25%, all additional focal analyses revealed a Ki67 expression below 10%. Importantly, patients 21 and 7, who both had a high Ki67 expression after the original Ki67 analysis on the resected tumor material, had a respectable mPFS of 13.9 and 12.3 months, respectively. To conclude, 19 of 24 patients would have been scored in the correct overall Ki67 expression category on the basis of determination of Ki67 on randomly selected 2-mm wide spots in the tumor. Two of 24 patients would have been scored in a different risk category on the basis of these analyses, and 3 of 24 patients would have potentially been scored in a different risk category. In total, 229 additional IHC subanalyses were done and 199 had a Ki67 expression that was congruent with the overall Ki67 expression determined originally on the resected tumor material, leading to a true positive score of 87%.

For the analysis of sequentially collected tumor samples, 11 patients were excluded because they had received chemotherapy between their biopsy and eP/D. Unfortunately, from the remaining patients, only two had sequentially collected tumor material that was available at the Erasmus MC. One patient (patient 21) was also included in the overall analysis. The other patient (patient A) was excluded from the overall analysis as this patient underwent a pleuropneumonectomy. Analysis of the biopsies and surgically resected material from eP/D revealed a variability of maximally 5% (Table 4). More importantly, patients with a low Ki67 expression (<10%) did not have high expression of Ki67 (>10%) in the tumor material resected during eP/D and vice versa. MTAP expression remained the same in these patients.

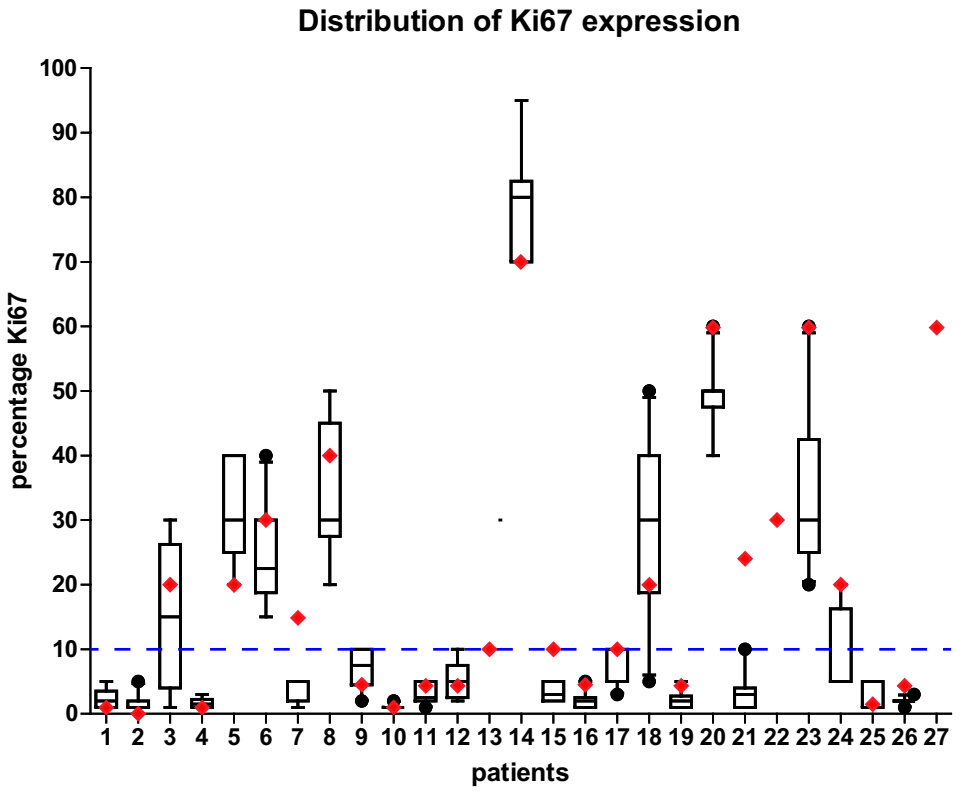

Figure 5. Distribution of Ki67 expression per patient. Boxplots of additional analysis of Ki67 expression on randomly selected 2-mm-wide parts of tumor tissue per patient. The red diamonds report the expression of Ki67 determined originally on the resected tumor material.

Discussion

In this retrospective study of 27 patients with MPM treated with eP/D in a multimodality approach, we found a mPFS of 15.3 months and a mOS of 26.5 months, in line with currently reported PFS and OS in surgically treated patients. Although Ki67 expression has been correlated with clinical outcome in patients with epithelioid MPM and MPM treated with chemotherapy and EPP, we hereby report for the first time a correlation between high Ki67 expression and poor survival in patients with MPM treated with eP/D in a multimodality approach irrespective of histology. All patients with a high expression of Ki67 (>10%) had died within 30 months, whereas the mOS for the patient group with a low expression of Ki67 was 44.5 months. Preoperative tumor sampling and immunohistochemical staining in MPM could therefore be of great value for clinical decision making in a multidisciplinary setting. In this retrospective analysis, we were able to identify Ki67 as a prognostic marker. The predictive value of Ki67 is unfortunately not assessable, as a control group was lacking. A validation study of patients treated with either eP/D in a multimodality approach or chemotherapy alone could identify the potential predictive value of Ki67 for eP/D.

In several types of cancer, proliferation and thus Ki67 expression, is related to tumor growth and progression.[29,30] In MPM, chemotherapy is known to reduce Ki67 expression and thus proliferation of cancer cells. In a study in which patients were treated with neoadjuvant chemotherapy in combination with EPP, the median Ki67 expression before chemotherapy was 20% and 11.25% after chemotherapy.[18] The survival analysis revealed a significant relation between shorter mOS and high Ki67 expression (>20%) in tumor samples collected before chemotherapy and for high Ki67 expression (>11.25%) in tumor samples collected after chemotherapy. In our study, some patients had received neoadjuvant chemotherapy. As all samples were collected during surgery, tumor samples of these patients were collected after chemotherapy, whereas for patients receiving adjuvant chemotherapy, tumor samples were collected before chemotherapy. In patients treated with neoadjuvant chemotherapy, Ki67 expression might have been higher before chemotherapy. Although we did not correct for this, there is still a significant relation between Ki67 expression and clinical outcome. This implies that irrespective of previous treatment, a cutoff of 10% for Ki67 before eP/D is prognostic and clinically relevant for patients treated within a multimodality approach. In a

Table 4. Expression of Ki67 and MTAP in Sequentially Collected Tumor Material

Patient	Material	Date of Material Collection	MTAP	Ki67 (%)
21	VATS biopsies	December 19, 2017	Pos	20
21	eP/D	March 6, 2018	Pos	25
A	Thorascopical biopsies	July 23, 2001	Pos	5
A	Pleurapneumonectomy	February 6, 2002	Pos	1

Note: Patient 21 is a patient who was in our original analysis cohort. Patient A was excluded from the original cohort because this patient got a pleurapneumonectomy. In both patients, a similar Ki67 expression in sequential biopsies is observed and MTAP expression is constant.
eP/D, extended pleurectomy/decortication; Pos, positive; VATS, video-assisted thoracic surgery.

study by Verma et al.,[31] the survival time for neoadjuvant chemotherapy was similar to that for adjuvant chemotherapy in combination with surgery, but neoadjuvant chemotherapy was related to longer hospitalization and higher 30-day mortality. This finding may be a reason to limit the clinical implementation of neoadjuvant chemotherapy. Nevertheless, considering that chemotherapy decreases Ki67 expression, neoadjuvant chemotherapy might still be of benefit to patients with high Ki67 before surgery. Analysis of biopsies before and after chemotherapy in the EORTC1205 trial might elucidate whether neoadjuvant chemotherapy enhances the prognosis of patients whose Ki67 expression has been decreased by chemotherapy before surgery.

Several studies have revealed the prognostic value of Ki67 in peritoneal and pleural mesothelioma, but with different cutoff values, ranging from 10% to 25%.[18,22,24] In individual studies, the cutoff value is determined on the basis of the available data and results. For example, low Ki67 expression (<15%) has already been reported to have a prognostic value in epithelioid MPM irrespective of treatment, except for patients treated with surgery alone.[22] In peritoneal mesothelioma, preoperative determination of Ki67 has already been implemented in clinical decision making.[32] In our study, receiver operating characteristic curve analysis indicated an optimal cutoff value of 10% with a sensitivity of 90% and a specificity of 71%. In addition, our data revealed that patients with a Ki67 expression above 20% will definitely not benefit from a multimodality approach including eP/D. For clinical implementation, a universally accepted cutoff value for Ki67 should be determined in future research on a large data set derived from multiple centers. Determination of this cutoff value is essential for the use of Ki67 as a selection criterion for surgery in MPM. From our analysis, it seems that a high Ki67 can be best used as a negative selection criterion. Given that the survival in these patients is short, the adverse effects associated with surgery are not justified in patients with high Ki67.

If Ki67 is to be used as a negative selection marker in the future, determination of Ki67 in needle biopsy material should represent the Ki67 expression in the tumor. The expression throughout the tumor should thus be homogeneous. To check if Ki67 expression in needle biopsies was congruent with the Ki67 expression throughout the tumor, we determined Ki67 expression in randomly selected 2-mm-wide circular areas of the tumor. Here, we found that in 80% of the patients, all of the additional focal analyses classified patients in their originally determined Ki67 expression category. Furthermore, in 87% of all 229 additional Ki67 analyses, the score was congruent with the originally determined Ki67 expression. In all cases in which the score of the TMA did not match the originally determined score, the pseudo-TMA score was lower than suspected. From these results, we can conclude the following two things: (1) If a patient has a high (>10%) Ki67 expression in their needle biopsy, the patient is probably not going to have a benefit from eP/D. (2) If a patient has a low Ki67 score in their needle biopsy material and is a potential candidate for surgery, additional tumor material could be collected during video-assisted thoracic surgery pleurodesis before eP/D to confirm the Ki67 expression levels.

In our analysis, Ki67 has a stronger relation to PFS and OS than mitosis score. Mitoses are determined by counting of the number of mitoses per 10 HPFs by the pathologist who selects the regions of interest. By contrast, Ki67 staining is less dependent on morphologic interpretation and is relatively easy to recognize in a whole slide, which lowers the probability of a sampling error owing to analysis of different regions of the biopsy. A meta-analysis has revealed that assessment of proliferation by Ki67 leads to a higher interobserver agreement than for mitotic count.[33] Ki67 seems to be a better prognostic factor in our study and has been proven to be more reliable for multicenter use in clinical practice as well.

MTAP is located adjacent to CDKN2A and is codeleted in 90% of mesothelioma tumors with a CDKN2A homozygous deletion. CDKN2A is a gene located on chromosome 9 which encodes for the p16 protein. Homozygous deletion of CDKN2A detected by fluorescence in situ hybridization is a diagnostic marker for malignancy in mesothelioma. Immunohistochemical staining with an

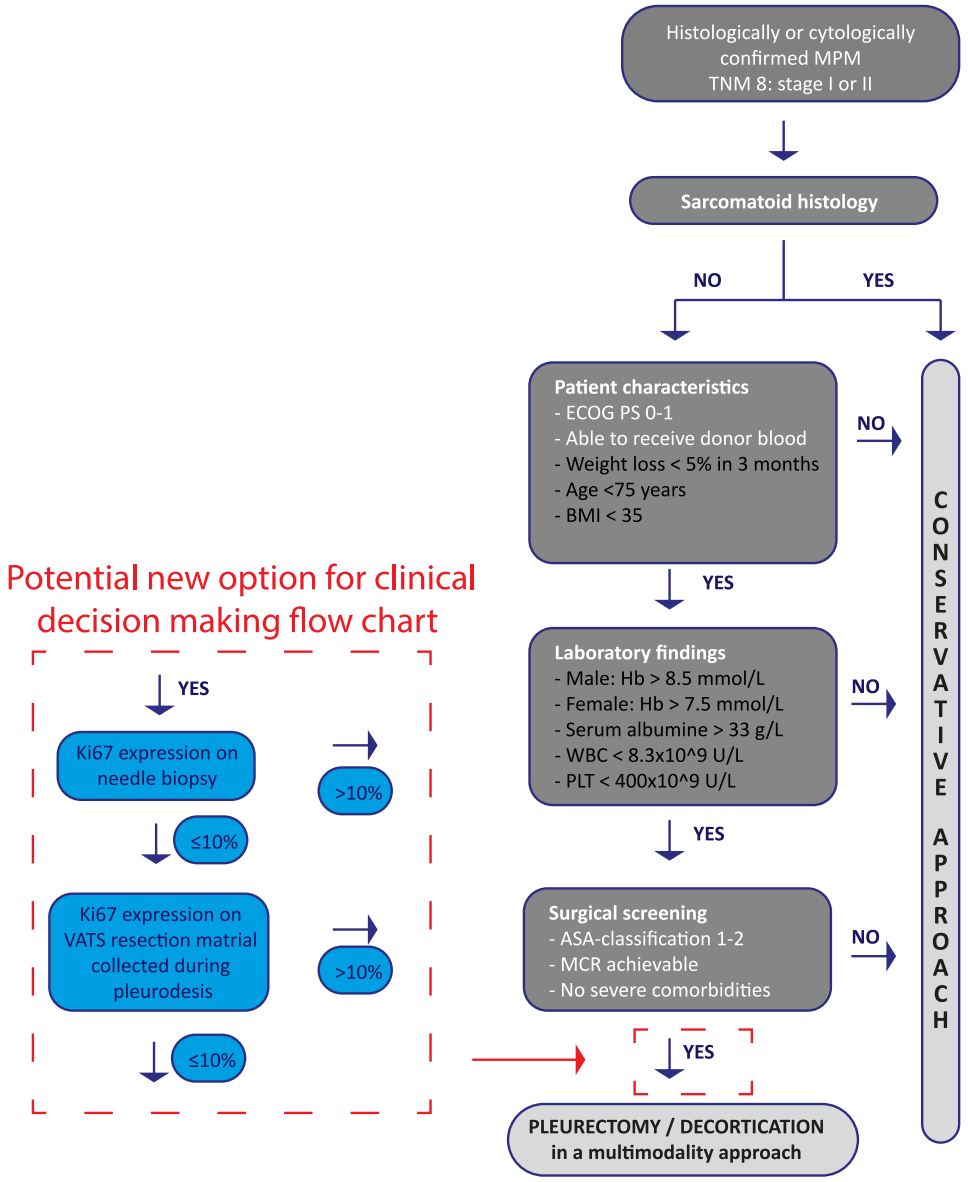

Figure 6. Flow diagram revealing different steps in clinical decision making with inclusion of Ki67. The parameters written in white are mandatory. The proposed parameters in black should provide guidance, but are not absolute cut-off values, e.g. a patient aged 76 that meets all other criteria should still be eligible for surgery. Within the red dotted line: Ki67 as a proposed new parameter for clinical decision making. Based on our data, a cut-off value of >10% is considered as Ki67 high expression. ASA, American Society of Anesthesiologists; BMI, body mass index; COPD, chronic obstructive pulmonary disease; ECOG PS, Eastern Cooperative Oncology Group–performance score; Hb, hemoglobin; MCR, macroscopic complete resection; MPM, malignant pleural mesothelioma; PLT, platelet; WBC, white blood cell count.

MTAP antibody is less labor-intensive and has lower costs than CDKN2A fluorescence in situ hybridization. The interobserver agreement and interlaboratory reproducibility for MTAP were excellent, and MTAP loss was 78% sensitive and 96% specific for CDKN2A homozygous deletion.[19] CDKN2A homozygous deletion has been correlated with poor survival in MPM, but MTAP has not yet been evaluated as a prognostic marker in MPM. For malignant peritoneal mesothelioma, MTAP has been proven to be significantly associated with OS and disease-specific survival.[34] Importantly, we hereby describe for the first time a significant positive relation between loss of MTAP expression and shorter PFS and OS in MPM. In the Cox regression models, no significant association was found with OS, although the effect size was substantial (HR = 0.373), which can be caused by a power issue with our small data set. In the multivariable model combined with Ki67, the effect of MTAP seemed attenuated (HR = 0.748) and also not significant. Larger cohorts are needed to further investigate the usefulness

of this marker. In our study, MTAP and Ki67 did not correlate with each other, and therefore the hypothesis was that combining both parameters might have an additive prognostic effect. Unfortunately, this was not found in our data.

An obvious limitation of our study is the low number of patients, which can be explained by the fact that eP/D in MPM is not standard of care in the Netherlands. eP/D is rarely carried out, only in selected hospitals and after discussion in a multidisciplinary team or within a clinical trial. An extra parameter that can support the clinical decision for or against eP/D would be helpful. Because of the low number of events, a multivariable analysis for all collected parameters was not possible. In addition, the low number of events resulted in low power for the analyses, resulting in covariates with high HRs and corresponding statistically not significant p values. We were, however, able to identify Ki67 as an independent prognostic factor for OS and PFS in patients treated with eP/D within a multimodality approach. Histologic subtype is the only tumor-related parameter that is currently taken into account in clinical decision making. In our analysis, however, histology was related only to PFS, not to OS. Furthermore, clinical parameters that are prognostic for mesothelioma, such as sex, significantly correlated with survival in univariable analysis. But, in a multivariable analysis with Ki67, sex no longer significantly correlated with survival. This possibly indicates that Ki67 is a more accurate prognostic factor than the currently used and accepted prognostic parameters.

Clinical Implication and Surgical Decision Making

Patients eligible for surgery need to be strictly selected as only few may benefit from surgical treatment.[35] Patients with stages I to II according to the latest TNM eighth version[36] and non-sarcomatoid tumor histology are potential candidates for surgery.[35,37,38] However, their physical condition and frailty need to be evaluated before major thoracic surgery.[35,37,38] Several laboratory prognostic factors, such as serum hemoglobin level,[39] serum albumin level,[39] white blood cell count,[40] and platelet count,[40,41] have been described which can have significant implication on surgical outcome,

Screening of the patient by a dedicated surgeon and anesthesiologist is an indispensable part of the preoperative evaluation. On the basis of the radiologic findings, which are often combined with a diagnostic video-assisted thoracic surgery procedure, the surgeon should determine the likelihood of macroscopic complete resection that should be the goal of the operation.[35,37,38] The use of validated comorbidity scores, as described in the literature, is recommended for surgical risk stratification.[42,43]

Surgery is not recommended in the presence of severe comorbidities such as the following: congestive cardiac failure, severe (peripheral) vascular disease, cerebrovascular disease (hemiplegia or paraplegia, dementia), chronic pulmonary disease, severe rheumatologic disease, liver disease, diabetes mellitus with end-organ damage, renal disease, any other malignancy, obesity body mass index greater than 35, previous heart/lung surgery, immobility, or severe mental disorders. In this study, we, therefore, present our patient selection flowchart for surgical decision making combining the already available prognostic factors with Ki67 to define the best surgical candidates (Fig. 6). Especially, when it proves difficult to reach a consensus within a multidisciplinary meeting, Ki67 might provide the decisive factor. Clinical implementation of this flowchart can only be justified after validation of Ki67 expression and its correlation to prognosis after surgery in larger cohorts.

Conclusions

Ki67 is prognostic for PFS and OS in patients with MPM treated with eP/D in a multimodality approach and is likely to be reliably assessable from needle biopsy-collected tumor material. Determination of Ki67 before surgery combined with the already available clinical prognostic factors could be helpful in clinical decision making by identifying patients with high Ki67 ($>$10%) who are unlikely to benefit from surgery. Owing to the low number of patients in our study and no consensus for the cutoff of Ki67 in the literature, a definitive cutoff for Ki67 in clinical decision making has to be validated. Large retrospective studies on tumor material from surgically treated patients might lead to consensus for a cutoff of Ki67. Furthermore, future cohort studies can evaluate the effect of combining currently existing prognostic scores, such as the EORTC score, mGPS, and NLR, with tumor-related parameters such as Ki67.

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Supplementary Data

Note: To access the supplementary material accompanying this article, visit the online version of the *JTO Clinical and Research Reports* at www.jtocrr.org and at https://doi.org/10.1016/j.jtocrr.2021.100155.

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