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High BIN1 expression has a favorable prognosis in malignant pleural mesothelioma and is associated with tumor infiltrating lymphocytes

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ABSTRACT

Objectives: A number of key immune regulators show prognostic value in malignant pleural mesothelioma (MPM), but the association between Bridging integrator 1 (BIN1), indoleamine 2,3 dioxygenase 1 (IDO1) and patient outcome has not been investigated. We aimed to determine the expression of BIN1 and IDO1, their association with other markers and impact on overall survival (OS) in MPM.

Materials and methods: The expression of BIN1, IDO1, CD3, CD20 and CD68 were evaluated by immunohistochemistry in 67 patients who underwent pleurectomy/decortication. Survival analyses were performed using the Kaplan Meier method and significant biomarkers were entered into a Cox Regression multivariate model, accounting for known prognostic factors such as age, gender, histological subtype, PD-L1 expression and neutrophil-to-lymphocyte ratio.

Results: Immune markers were variably expressed in tumor cells, ranging from 0% to 100% for BIN1 (median: 89%), and 0% to 77.5% for IDO1 (median: 0%). Expression of markers of tumor-infiltrating lymphocytes (TILs) and macrophages ranged from 0% to more than 50%. BIN1 expression was high in 35 patients (51%) and was associated with increased OS (median: 12 vs 6 months for high and low BIN1 respectively,$p = 0.03$). Multivariate analysis showed BIN1 remained an independent prognostic indicator (HR 0.39; 95% CI: 0.18-0.82, $p = 0.01$). The majority of patients had immune inflamed tumors (77%) and there was a significant association between TILs and BIN1 ($p = 0 < 0.01$), PD-L1 ($p = 0.04$) and CD68+ macrophages in the tumor ($p < 0.01$). There were no significant associations between PD-L1 and BIN1 or IDO1.

Conclusion: High BIN1 expression is a favorable prognostic biomarker and is associated with TILs in MPM.

1. Introduction

Malignant pleural mesothelioma (MPM) is an aggressive and invariably fatal cancer of the pleural surface that is associated with asbestos exposure [1]. MPM is usually detected at an advanced stage, therefore treatment options are limited to systemic chemotherapy with cisplatin and pemetrexed as the mainstay of palliative treatment for most patients [2]. Prognosis is generally poor for MPM, with a median survival of 12 months in patients treated with pemetrexed and cisplatin chemotherapy [3] but can be very variable, with around 5% of patients surviving more than 5 years [4]. There is an urgent clinical need for better prognostic biomarkers to aid prognostication, guide treatment timing and improve MPM patient outcomes [5].

It is widely recognized that the immune system plays a key role in the pathogenesis of several cancers, including MPM [6,7]. Under normal physiological conditions, the immune system helps maintain a

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tightly controlled balance of cell proliferation and cell apoptosis [8]. Cancer can escape the immune system through several mechanisms, such as by attenuating the expression of tumor suppressor proteins such as Bridging integrator 1 (BIN1), or by producing immunosuppressive enzymes such as Indoleamine 2,3 dioxygenase 1 (IDO1) [9,10]. BIN1, also called Amphiphysin 2, is a member of BAR domain superfamily involved in endocytosis, organelle biogenesis, cell division, and cell migration [11]. BIN1 can interact with c-myelocytomatosis (c-Myc) oncoprotein in cancer to induce cell death and inhibit its oncogenic functions [12]. IDO1, on the other hand, is a metabolic immunosuppressive enzyme that can suppress T cell function by consuming and depleting tryptophan, an amino acid that is essential for their function [13]. The level of expression of these markers can help us understand their role in the tumor microenvironment and in the prognosis of MPM. These dysregulated proteins have the potential to serve as prognostic biomarkers in MPM, as demonstrated in esophageal cancer, hepatocellular carcinoma and melanoma [14–17].

Immune escape can also be facilitated by cancer cells expressing receptors or checkpoints that can inhibit the function of T cells, resulting in immune system exclusion or ignorance. The most recognized immune checkpoint is the programmed death protein 1 (PD-1) [18]. PD-1 checkpoints are found on activated T cells, B cells, and myeloid cells, while its natural ligand and corresponding receptor, programmed death ligand 1 (PD-L1), can be expressed on cancer cells but it may also be expressed by host immune cells [1,19]. When PD-L1 binds to PD-1, it transmits an inhibitory signal that suppresses T cell function, leading to immune tolerance and escape of the cancer cell.

It has also been shown, in in vivo studies as well as in patients, that inhaled asbestos fibers can trigger a chronic inflammatory response, which results in the infiltration of a range of immune cells, stromal cells and inflammatory cells, including neutrophils, macrophages (CD68+), B lymphocytes (CD20+) and T cells (CD3+) [7,10,20–25]. The extent of their infiltration can help us better understand the prognostic value of these cells in the tumor microenvironment [26,27].

The aim of our study was to evaluate and characterize the tumor expression of BIN1, IDO1 and tumor infiltrating lymphocytes (TILs) (CD3 + T cells and CD20 + B cells) and macrophages (CD68+) located in tumor and stroma in surgically resected MPM patients. We also aimed to determine their association with overall survival (OS) and relate these to our previously published PD-L1 expression data [28].

2. Materials and methods

2.1. Patients

MPM patients who had pleurectomy with decortication at Royal Prince Alfred Hospital in Sydney between 1992 and 2007 were examined, and those with assessable tumor sample cores were included in this study. Diagnosis and subtyping were conducted in accordance with the WHO classification (epithelioid, sarcomatoid and biphasic) [29]. Sarcomatoid and biphasic histological subtypes were grouped as non-epithelioid. Neutrophil to lymphocyte ratio (NLR) was calculated by dividing the absolute neutrophil count by the absolute lymphocyte count in the peripheral blood counts prior to the operation. The study was approved by the Human Research Ethics Committee at the Sydney Local Health District (Concord Repatriation General Hospital Zone).

2.2. Immunohistochemistry

The tissue microarray (TMA) used in this study and its method of construction were described previously [28]. The expression of biomarkers was evaluated by immunohistochemistry. IDO1 (mIDO-48, Santa Cruz Biotechnology, sc-53978) and BIN1 (polyclonal, Proteintech Group, 14647-1-AP) immunohistochemistry was performed on 3 µm sections at antibody dilutions of 1:100 and 1:200 respectively. Staining was performed on an automated Leica Bond III instrument (Leica Microsystems, Australia) using a Bond Polymer Refine Detection Kit (Leica Biosystems, UK). Heat-induced epitope retrieval was performed on all slides in Bond Epitope Retrieval Solution 2 for 20 min (Leica Biosystems, UK). Primary antibody was applied and incubated for 20 min at room temperature. With 3,3′-diaminobenzidine (DAB) chromogenic detection and hematoxylin counterstaining. For quantitative evaluation of IDO1 and BIN1, the expression level, defined as the percentage of positive tumor cells in the cores of each patient (0%–100%) was assessed, irrespective of intensity. An average score was derived by averaging the scores from assessable cores for each patient. The assessors were blinded to the other clinical details, including survival.

Tumor infiltrating lymphocytes and macrophages were stained in the intra-tumoral component and tumor-associated stroma, which was defined as the cells adjacent to the tumor nest and which has been shown to play an important role as an independent predictor of survival in MPM [30,31]. Markers tested were CD3+ (LN10 clone, 1:200 dilution, Novocastra, Newcastle Upon Tyne, UK), CD20+ (L26 clone, 1:100 dilution, Novocastra) and CD68+ (KP1 clone, 1:8000, Dako, Carpinteria, CA, USA). They were scored as: 0 (no staining); 1 (1–10% staining); 2 (11–50% staining); 3 (> 50% staining). The scores were then categorized as positive/negative or high/low based on the distributions to ensure sufficient numbers in each group (Table 1).

CD3 + T lymphocyte infiltration was further categorized into three immune phenotypes as described previously [32,33] (Table 1): immune desert (lacking CD3+ in both the tumor and stroma); immune excluded (high expression of CD3+ in the stroma but not in the tumor); or immune inflamed (positive or high expression of CD3 + T lymphocytes in the tumor).

2.3. Statistical analysis

The minimal-p approach was used to investigate the optimal value of each biomarker with regard to overall survival. The value which

Table 1
Baseline demographics for the study of patients, N(%).

PATIENT DEMOGRAPHICS		
Age (years)	Median	65
	Range	42-83
Gender	Female	12 (18%)
	Male	55 (82%)
Subtype	Epithelioid	33 (49%)
	Non-epithelioid	34 (51%)
BIOMARKERS		
IDO1	Negative	47 (70%)
	Positive (> 0%)	20 (30%)
BIN1	Low (< 89%)	33 (49%)
	High (≥89%)	34 (51%)
PD-L1	Negative (< 5%)	50 (75%)
	Positive (≥5%)	17 (25%)
NLR	Low (< 4)	24 (36%)
	High (≥4)	27 (40%)
	Missing	16
LYMPHOCYTES AND MACROPHAGES		
CD3+ Stroma	Low (≤10%)	30 (45%)
	High (> 10%)	37 (55%)
CD3 + Tumor	Negative (< 1%)	15 (22%)
	Positive (≥1%)	50 (75%)
	Missing	2
CD20+ Stroma	Negative (< 1%)	37 (55%)
	Positive (≥1%)	30 (45%)
CD20+ Tumor	Negative (< 1%)	60 (90%)
	Positive (≥1%)	6(9%)
	Missing	1
CD68+ Stroma	Low (≤10%)	24 (36%)
	High (> 10%)	43 (64%)
CD68+ Tumor	Low (≤10%)	33 (49%)
	High (> 10%)	32 (48%)
Immune phenotypes	Immune desert	2 (3%)
	Immune excluded	13 (20%)
	Immune inflamed	50 (77%)

minimized the p-value obtained from the log-rank test was taken as the optimal cut-point to dichotomize into low and high-risk groups. Overall survival was measured from date of pleurectomy until date of death. Survival curves were generated using the Kaplan-Meier method and compared using a log-rank test. Cox proportional hazards models were used to investigate the association of each marker with overall survival. Models were then adjusted for known prognostic factors including age, gender, histological subtype, PD-L1 expression ($< 5\%$ vs $\geq 5\%$ as per the previous publication [28]) and the blood-based NLR (< 4 vs ≥ 4 as determined by the minimal-p approach), which has previously been shown to be prognostic in MPM [34]. Using the optimal cut-points of each biomarker, 2×2 frequency tables were constructed and a Fisher's exact test applied to determine the relationship between the biomarkers and the immune phenotypes. A *p*-value of 0.05 or less was considered statistically significant. Stata/SE 14.2 software was used for the statistical analysis.

3. Results

3.1. Baseline demographics

A total of 67 patients were included in the study. Table 1 details the baseline patient characteristics. The majority of MPM patients were male (82%), with a median age of 65 years (range 42–83 years) and an equal distribution of patients with epithelioid and non-epithelioid subtype (49% versus 51% respectively). One patient remained alive by the end of our study. The median overall survival time was 7.7 months (95% confidence interval (CI): 5.6–9.8).

3.2. Biomarker expression and immune cell infiltrates

Both IDO1 and BIN1 showed cytoplasmic staining in mesothelioma tumor cells (Fig. 1). Stromal fibroblasts were negative. IDO1 and BIN1 were variably expressed in tumor cells, ranging from 0% to 77.5% for IDO1 (median: 0%, IQR: 0 to 15%) and 0% to 100% for BIN1 (median: 89%, IQR 74 to 95%). When positive, the intensity of BIN1 expression was almost always strong. In contrast, IDO1 was often negative and when positive, the cytoplasmic staining was weak. Both IDO1 and BIN1 are strongly positive in lymphocytes and served as a positive internal control when the inflammatory cells were present amongst the mesothelioma tumor cells. Using the minimal-p approach, the optimal cut-point for IDO1 and BIN1 were 0% and 89%, respectively. A total of 20 patients (30%) had positive IDO1 expression ($> 0\%$) and 34 patients (51%) had a high BIN1 expression ($\geq 89\%$). Only 4 patients had no BIN1 expression. Fig. 1A and B show positive IDO1 expression and positive BIN1 expression, respectively. The pattern of staining for IDO1 and BIN1 showed an inverse relationship between the two biomarkers. The majority (77%) had tumors with the immune inflamed phenotype (Fig. 1C), while the tumors in some patients had the immune excluded phenotype (20%; Fig. 1D) and only two patients had the immune desert phenotype (3%; Fig. 1E).

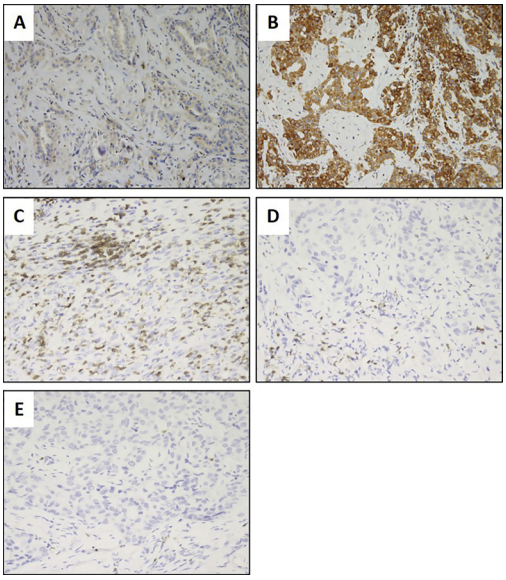

Fig. 1. Expression of immune markers in MPM tumor section. Examples of MPM tumor sections stained for IDO1 (A), BIN1 (B) and CD3 (C–E) are shown (all 200x magnification). Positive IDO1 expression is weakly cytoplasmic, whereas positive BIN1 expression is strongly cytoplasmic. CD3+ cell positivity was variable, and found in both tumor and stroma components (C), in stroma only (D) or absent in both tumor and stroma components (E).

3.3. High expression of BIN1 was significantly associated with overall survival

On univariate analysis, age and gender were not associated with OS in our cohort of patients (Table 2). Histological subtype and PD-L1 were significantly associated with OS, as established previously [28]. The median OS was longer in patients with high BIN1 compared to low (median: 12 months vs 6 months, Fig. 2A) and high BIN1 was significantly associated with better OS (HR: 0.56; 95% CI: 0.34-0.93; $p = 0.03$; Table 2). There was no significant association between IDO1 expression and OS (Fig. 2B and Table 2). In contrast, the presence of CD68+ macrophages in tumor sections was significantly associated with poor overall survival (median: 6 vs 11 months, $p = 0.04$; Fig. 2C), as was NLR (median: 6 vs 9 months, $p = 0.03$; Fig. 2D). In multivariable analysis, BIN1 remained an independent prognostic factor (HR = 0.39; 95% CI: 0.18-0.82, $p = 0.01$; Table 2), when adjusted for age, gender, histological subtype, PD-L1, NLR and CD68+ macrophages in the tumor. CD20 + B cells and CD68+ macrophages in the stroma, and TILs (CD3 + T cells) were not significantly associated with overall survival (Supplementary Fig. 1 and 2).

3.4. MPM was associated with tumor infiltrating lymphocytes irrespective of biomarker expression

Our results showed a significant association between CD3 + TILs and BIN1 ($p < 0.01$), PD-L1 ($p = 0.04$) and CD68+ macrophages in the tumor ($p < 0.001$) (Table 3). The majority of patients (93%) who expressed high BIN1 had immune inflamed phenotype. Also, all tumors with observable PD-L1 expression (n = 16), previously reported as an adverse prognostic indicator [28], had the immune inflamed phenotype. Interestingly, for tumors with negative PD-L1 expression (n = 49) and low BIN1 expression (n = 21), the inflamed phenotype was still most common (69% and 64% respectively). Similarly, almost all tumors with high CD68+ expression (n = 32) had inflamed phenotype (97%), with the inflamed phenotype also most common (58%) for tumors with low CD68+ marker expression (n = 33). Further analysis did not reveal a significant association between PD-L1 and BIN1 ($p = 0.78$) or IDO1 ($p = 0.56$). We also did not find any significant associations between IDO1 and BIN1 ($p = 0.60$).

4. Discussion

Immune escape is one of the hallmarks of cancer [35]. It is known that immunosuppressive markers such as IDO1, T cell inhibitory signals such as PD-L1, and the extent of tumor infiltration by lymphocytes, macrophages and neutrophils all play a key role in facilitating immune escape of cancer cells [32,33]. However, there is currently limited knowledge of the mechanisms involved in the interaction between the different immune markers that lead to immune escape.

BIN1 is an important tumor suppressor protein, which plays a role in the induction of apoptosis when abnormal cell behavior is detected during the cell cycle. An association between BIN1 and cancer was

Table 2
Association of biomarkers with overall survival.

Characteristic		*Univariate analysis*			*Multivariate analysis*[*]		
		HR	95% CI	*p*-value	HR	95% CI	*p*-value
Age	≤65						
	> 65	1.43	0.87-2.34	0.16	1.66	0.82-3.37	0.16
Gender	Female						
	Male	1.67	0.84-3.29	0.14	2.44	0.93-6.38	0.07
Histological Subtype	Epithelioid						
	Non-epithelioid	4.11	2.33-7.26	< **0.01**	3.12	1.41-6.92	**0.01**
PD-L1	Negative (< 5)						
	Positive (≥5%)	2.80	1.55-5.03	< 0.01	2.57	1.21-5.45	**0.01**
IDO Expression	Negative						
	Positive (> 0%)	0.73	0.43-1.25	0.25	–	–	–
BIN1 Expression	Low (< 89%)						
	High (≥89%)	0.56	0.34-0.93	**0.03**	0.39	0.18-0.82	**0.01**
NLR	Low (< 4)						
	High (≥4)	1.84	1.03-3.29	**0.04**	0.68	0.30-1.52	0.34
CD68+ Tumor	Low (≤10%)						
	High (> 10%)	1.70	1.02-2.82	**0.04**	1.77	0.81-3.85	0.15

* Adjusted for age, gender, subtype, PD-L1, NLR, BIN1 and CD68+ tumor.

originally reported by Sakamuro and colleagues in 1996, where they found that BIN1 expression was either greatly reduced or undetectable in half of carcinoma cell lines and primary breast tumors [36]. BIN1 was identified as a tumor suppressor and prognostic marker in esophageal squamous cell cancer and in hepatocellular carcinoma [16,17]. We show for the first time that BIN1 expression was a significant favorable prognostic indicator in MPM. It is interesting to note that there are contrasting reports on the level of BIN1 expression in different cancers. In our study, BIN1 expression was detectable in almost all of the MPM samples we analysed. This contrasts greatly with previous studies showing substantial downregulation or complete loss of BIN1 expression in carcinoma cell lines and primary breast tumors [17,36,37]. In a study in melanoma, BIN1 was elevated in some of the malignant and metastatic cell lines, but was inappropriately expressed

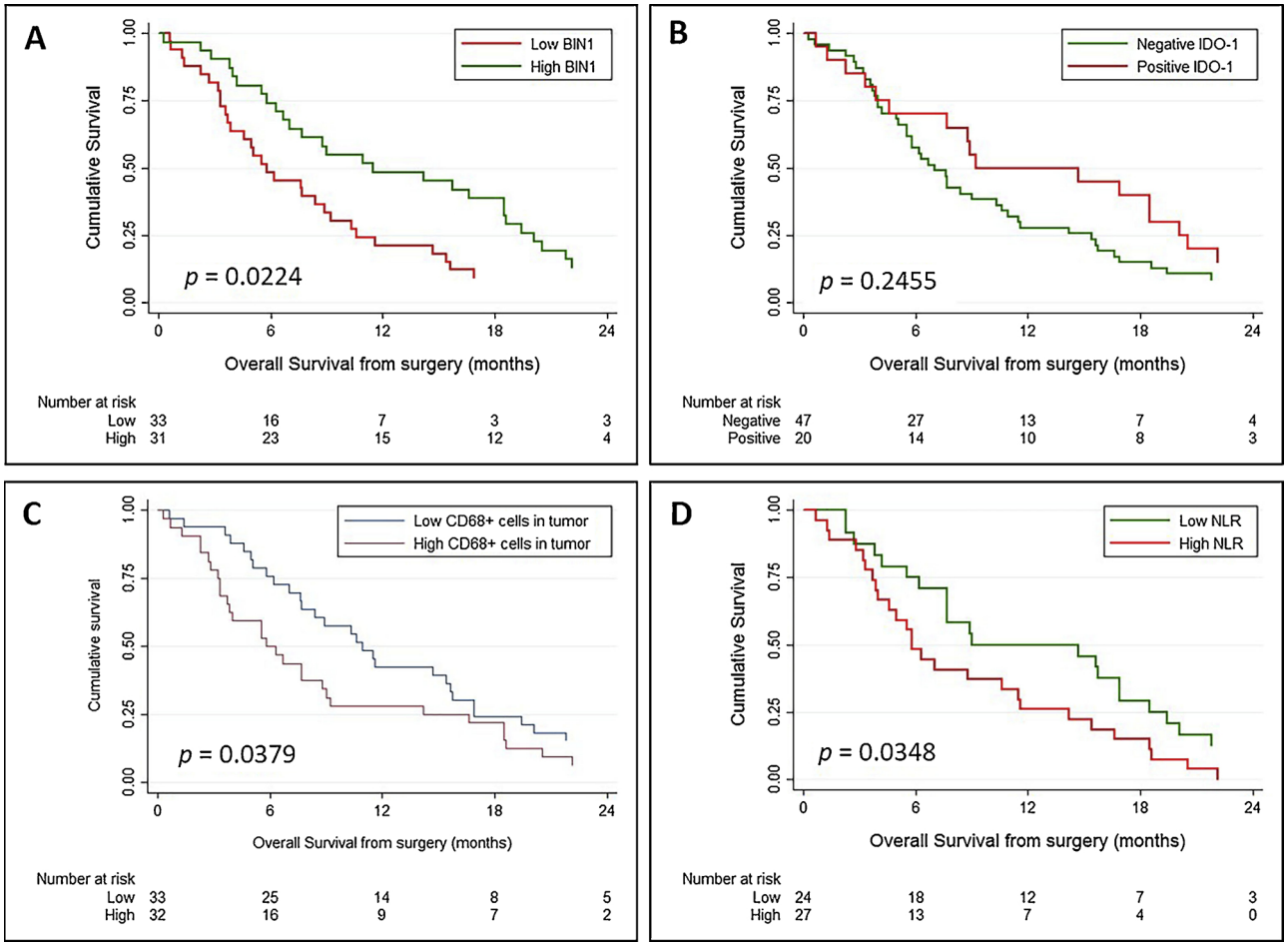

Fig. 2. Association between immune marker expression and survival in MPM. Kaplan-Meier curves show that survival of MPM patients was significantly associated with high BIN1 expression (A), low NLR (C) and low CD68+ cells in the tumor (D), but not IDO1 expression (B).

Table 3
Association between biomarkers and immune phenotypes.

	BIN-1		PD-L1		CD68 in tumor	
	Low (< 89%)	High (≥89%)	Negative	Positive (≥5%)	Low (≤10%)	High (> 10%)
Immune desert[1]	1 (3%)	1 (3%)	2 (4%)	0	2 (6%)	0 (0%)
Immune excluded[2]	11 (33%)	1 (3%)	13 (27%)	0	12 (36%)	1 (3%)
Immune inflamed[3]	21 (64%)	28 (93%)	34 (69%)	16 (100%)	19 (58%)	31 (97%)
	P < 0.01		**p = 0.04**		**p < 0.001**	

[1] no CD3 lymphocytes in stroma or tumour.
[2] CD3 lymphocytes in stroma but not tumour.
[3] CD3 lymphocytes in tumour and stroma.

through an aberrant splicing event that resulted in loss of its tumor suppressor function [38]. These results suggest that BIN1 is likely silenced or misspliced during cancer progression and it is possible for this to have occurred in the MPM tumor samples we analyzed. It is important to note that tumor suppressor genes are not always acting to suppress tumor progression. For example, the BRCA1-associated protein 1 gene (BAP1) is a tumor suppressor gene that is frequently mutated and is frequently lost in MPM. However counterintuitively, Artz et al. 2014 [39] found that high BAP1 expression (non-mutated BAP1), as detected by immunohistochemistry, correlated with shorter overall survival.

The significance of our results is that we show BIN1 might play an important role in suppressing tumor growth in MPM (hence the prolonged overall survival in patients expressing high levels of BIN1), but it is likely that other immune factors in the tumor microenvironment eventually abrogate its tumor suppressive functions, resulting in MPM progression. Our study encourages further research to explore the mechanisms that govern BIN1 expression and that inhibit its functional role in apoptosis, particularly in association to other established prognostic markers in MPM, such as PD-L1. Once these mechanisms are identified, it could open up new avenues in cancer therapy, where BIN1 expression could be modulated, such as through gene therapy or small molecule inhibitors that reactivate tumor suppressor function, in order to restore its tumor suppressive functions [40,41].

Whether BIN1 influences PD-L1 expression directly in MPM remains to be determined. In our study, we did not observe a significant association between PD-L1 and BIN1. While a role for BIN1 in control of PD-L1 expression via c-MYC and EGFR signaling was demonstrated in non-small cell lung cancer [42], this was not observed in melanoma [38], so the mechanisms involved are likely to be complex and cancer specific. We have previously shown an involvement for microRNAs in the regulation of PD-L1 in MPM [28] and other reports suggest that the Yes-associated protein (YAP) and PTEN/PI3K pathway are both implicated in the control of PD-L1 in MPM [43,44], suggesting multiple mechanisms control PD-L1 expression. In contrast, BIN1 does not contain target sites for microRNAs known to be dysregulated in MPM. Our study provides rationale for targeting BIN1 as a key immune marker in prospective studies in MPM, in order to better understand mechanisms involved in immune evasion of oncogenic cells in MPM.

In relation to IDO1, previous reports have indicated that BIN1 can inhibit cancer cell growth by suppressing expression of IDO1 [45], however we did not observe a relationship between the expression of these two proteins in our study. IDO1 is an immunosuppressive enzyme that suppresses the function of T cells to kill their target cells by converting tryptophan (an amino acid that is essential for the proper functioning and survival of T cells [46,47]) into kynurenine. It is not fully understood how IDO1 is regulated, however it is postulated that IDO1 may be induced by inflammatory cytokines such as interferon gamma (IFN-γ) in a variety of immune cells, tumor cells and stromal cells [9,45]. High IDO1 expression has been reported in prostate, colorectal, pancreatic and cervical cancer, and has been associated with poor prognosis [9]. Contrary to reports in other tumor types [9,45], our results did not show a significant difference in OS in relation to IDO1 level in our patients and we did not obtain an inverse relationship between IDO1 and BIN1. Whether this was due to our small number of IDO1-positive samples, or to differences in the regulation of BIN1 in different tumors, requires further investigation.

Although PD-L1 expression is common in mesothelioma [48,49], not all patients with positive PD-L1 expression respond to the anti-PD-L1 antibody, pembrolizumab [50], as seen in the KEYNOTE-028 trial [51], where only a small percentage of MPM patients (20%) were sensitive to pembrolizumab. Similar results were found in subsequent clinical trials of pembrolizumab [52], avelumab [53] and nivolumab [54], among others. Why response rates in MPM are lower than in other tumour types such as NSCLC and melanoma remains unknown. It has been suggested that the lower mutational burden in MPM [55] contributes to these observations, but the tumour microenvironment is also likely to play a role, as is clearly the case in other tumour types [30].

We note that the median overall survival in our study was lower than the median survival commonly reported in the literature (7.7 months compared to 12 months respectively). This is possibly because we had a relatively higher proportion of patients with non-epithelioid subtype in our study (51%), compared to a much lower incidence of non-epithelioid subtype generally reported in the literature for MPM (around 30%) [56].

We also note that the majority of MPM patients in our study had immune inflamed tumors, characterized by infiltration of CD3 + T cells into the tumor (75%). Our results are consistent with another larger study involving 230 MPM patients [24], where high expressions of CD3 + T cells in the tumor was also a dominant characteristic for most MPM patients (75%). CD3 + T cells play opposing roles to PD-L1 and CD68+ macrophages. The role of T cells is to kill their target cancer cell and their infiltration has long been associated with better prognosis and longer survival in MPM [57,58]. Macrophages (CD68+) on the other hand, can have dual roles in the tumor microenvironment. Classically activated macrophages have pro-inflammatory and anti-tumor activity, whereas alternatively activated macrophages can have pro-tumor activity [26]. In MPM, activated macrophages can promote chronic asbestos-induced inflammation in a process of 'frustrated phagocytosis' [8,25]. It was therefore interesting to note that almost all patients (97%) who had tumors with high CD68 expression were associated with TILs. Similarly, all of our patients who had tumors staining positive for PD-L1 were still associated with TILs. These results suggest that while MPM is characterized by T cell infiltration, these T cells are possibly suppressed or "exhausted" by the presence of other immune suppressive cells in the tumor microenvironment, such as regulatory T cells, myeloid-derived suppressor cells and macrophages, resulting in loss of T cell function [24–26]. Continuing research will be necessary to realise the prognostic and/or predictive value of the immune microenvironment in MPM.

5. Summary and conclusion

In this retrospective study we demonstrate that high BIN1

expression was a favorable prognostic factor in MPM, and that a proportion of our patients had IDO1 expression in tumor cells, providing the rationale for investigating IDO1 in MPM. We found that our tumor samples mostly had tumor inflamed phenotype regardless of the positive expressions of PD-L1 and CD68+ macrophages, suggesting that T cells are possibly suppressed by other factors or other suppressive immune cells. Finally, we showed that the MPM tumor microenvironment is characterized by a complex interaction of pro- and anti-tumor inflammatory proteins, enzymes and checkpoints, which must be investigated as a whole system in order to better understand the prognostic role of different immune markers and assist in the optimal and rational use of immunotherapeutic combinations in the future.

Conflicts of interest

SK served on advisory boards for AstraZeneca, Pfizer and Boehringer Ingelheim and received honoraria (paid to his institution) from Astra Zeneca, Roche, Bristol-Myers Squibb and Merck Sharp & Dohme. WC has served on advisory boards for AstraZeneca, BMS and MSD.

TA, KL, CC, AL, BM, RA, SC and GR declare no conflicts of interest.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.lungcan.2019.02.005.

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