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Amber Louw
Edith Cowan University

Vasiliki Panou

Weronika Maria Szejniuk

Christos Meristoudis

Siaw Ming Chai

See next page for additional authors

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ORIGINAL ARTICLE

BAP1 Loss by Immunohistochemistry Predicts Improved Survival to First-Line Platinum and Pemetrexed Chemotherapy for Patients With Pleural Mesothelioma: A Validation Study

Amber Louw, BSc, M.B.B.S.,[a,b,c,d] Vasiliki Panou, MD, PhD,[e,f,g] Weronika Maria Szejniuk, MD, PhD,[h,i] Christos Meristoudis, MD,[j] Siaw Ming Chai, M.B.B.S., MSc,[a] Chris van Vliet, M.B.B.S.,[a] Y. C. Gary Lee, MbChB., PhD,[c,k,l] Ian M. Dick, PhD,[d] Tina Firth, PhD,[c,d] Louise Andersen Lynggaard, MD,[j] Azadeh Birbaneh Asghari, MD,[j] Mogens Vyberg, MD, PhD,[m] Johnni Hansen, PhD,[n] Jenette Creaney, PhD,[c,d,k,*] Oluf Dimitri Røe, MD, PhD[h,i,o,p]

[a]*Department of Anatomical Pathology, PathWest Laboratory Medicine, QEII Medical Centre, Perth, Australia*
[b]*School of Medical and Health Sciences, Edith Cowan University, Perth, Australia*
[c]*Institute for Respiratory Health, Perth, Australia*
[d]*National Centre for Asbestos Related Disease, University of Western Australia, Perth, Australia*
[e]*Department of Respiratory Medicine, Odense University Hospital, Odense, Denmark*
[f]*Department of Respiratory Diseases, Aalborg University Hospital, Aalborg, Denmark*
[g]*Department of Clinical Research, University of Southern Denmark, Odense, Denmark*
[h]*Department of Oncology & Clinical Cancer Research Center, Aalborg University Hospital, Aalborg, Denmark*
[i]*Department of Clinical Medicine, Aalborg University Hospital, Aalborg, Denmark*
[j]*Institute of Pathology, Aalborg University Hospital, Aalborg, Denmark*
[k]*Department of Respiratory Medicine, Sir Charles Gairdner Hospital, Perth, Australia*
[l]*Medical School, University of Western Australia, Perth, Australia*
[m]*Center for RNA Medicine, Department of Clinical Medicine, Aalborg University, Copenhagen, Denmark*
[n]*Danish Cancer Society, Research Center, Copenhagen, Denmark*
[o]*Department of Oncology, Levanger Hospital, Nord-Trøndelag Hospital Trust, Levanger, Norway*
[p]*Department of Clinical and Molecular Medicine, Norwegian University of Science and Technology, Trondheim, Norway*

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ABSTRACT

Introduction: Pleural mesothelioma (PM) is an aggressive malignancy with no identified predictive biomarkers. We assessed whether tumor BAP1 status is a predictive biomarker for survival in patients receiving first-line combination platinum and pemetrexed therapy.

Methods: PM cases (n = 114) from Aalborg, Denmark, were stained for BAP1 on tissue microarrays. Demographic, clinical, and survival data were extracted from registries and medical records. Surgical cases were excluded. BAP1 status was associated with overall survival (OS) by Cox regression and Kaplan-Meier methods. Results were validated in an independent cohort from Perth, Australia (n = 234).

Results: BAP1 loss was found in 62% and 60.3% of all Danish and Australian samples, respectively. BAP1 loss was an independent predictor of OS in multivariate analyses corrected for histological subtype, performance status, age, sex, and treatment (hazard ratio = 2.49, $p < 0.001$, and 1.48, $p = 0.01$, respectively). First-line platinum and pemetrexed-treated patients with BAP1 loss had significantly longer median survival than those with retained BAP1 in both the Danish (20.1 versus 7.3 mo, $p < 0.001$)

*Corresponding author.

Drs. Louw and Panou contributed equally to this work.

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Address for correspondence: Jenette Creaney, PhD, National Centre for Asbestos Related Disease, University of Western Australia, Level 5 QQ Block, 6 Verdun Street, Nedlands, Perth 6009, Australia. E-mail: jenette.creaney@uwa.edu.au

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and Australian cohorts (19.6 versus 11.1 mo, $p < 0.01$). Survival in patients with BAP1 retained and treated with platinum and pemetrexed was similar as in those with best supportive care. There was a higher OS in patients with best supportive care with BAP1 loss, but it was significant only in the Australian cohort (16.8 versus 8.3 mo, $p < 0.01$).

Conclusions: BAP1 is a predictive biomarker for survival after first-line combination platinum and pemetrexed chemotherapy and a potential prognostic marker in PM. BAP1 in tumor is a promising clinical tool for treatment stratification.

Keywords: Pleural mesothelioma; BAP1; Chemotherapy; Predictive biomarkers; Pemetrexed

Introduction

Mesothelioma is a malignancy of the serosal linings, involving the pleural cavity in more than 70% of cases.[1] The disease is most often caused by asbestos inhalation resulting in malignancy decades after exposure.[1,2] Pleural mesothelioma (PM) is aggressive, with treatment generally being palliative and overall survival (OS) from diagnosis less than 12 months.[3,4]

Combination chemotherapy with a platinum agent and pemetrexed has been the standard-of-care first-line treatment for patients with PM for almost two decades. This treatment has a 40% response rate, extending survival over platinum therapy alone by 2 to 3 months.[5] To date, no clinically meaningful predictive biomarkers have been established.[6] Recently, dual immunotherapy with ipilimumab and nivolumab has been approved as a first-line treatment in PM, revealing an increase in OS of 18.1 versus 14.1 months in comparison to platinum and pemetrexed. The overall response rate was 40%, with a more modest effect in the epithelioid subtype.[7] Because immunotherapy is a relatively costly treatment, it is likely that chemotherapy will continue to be used in the years to come, either in the first- or second-line setting. Therefore, predictive biomarkers remain relevant for this treatment modality and would provide a way to aid in targeting the correct population and avoid overtreatment.

BAP1 is a multifunctional tumor suppressor that is expressed in all normal mesothelial cells. Nevertheless, it is frequently altered in PM, with abnormalities observed at the genetic, epigenetic, and protein levels.[8–11] BAP1 plays a role in DNA repair and other mechanisms that may affect response to platinum and pemetrexed treatment.[12] BAP1 immunohistochemistry (IHC) is considered a reliable method of determining BAP1 status, indicating biallelic loss of functional protein, with BAP1 loss occurring in approximately 60% of tumors.[4,9,13–19] Less than 5% of patients with PM harbor germline abnormalities in *BAP1*; these patients were found to have longer survival than those without germline mutations and to have benefit from platinum-based therapy.[20–24]

Few studies have specifically evaluated the association of BAP1 IHC status with platinum and pemetrexed treatment.[14,25,26] On the basis of the biological function of BAP1 and longer survival in treated patients with *BAP1* germline mutation, we postulated that loss of BAP1 in the tumor may predict survival after platinum and pemetrexed treatment in a general PM cohort.

The primary aim of this study was to investigate whether patients with PM with tumors harboring BAP1 loss as determined by IHC have improved survival to a first-line chemotherapeutic regimen of platinum and pemetrexed. The secondary aim was to investigate the prognostic role of BAP1 loss in untreated patients with PM. These associations were evaluated in a Danish cohort of patients, before validating the findings in a larger Australian cohort. This is the first independently validated study to assess the relationship between BAP1 status and survival in treated and untreated patients with PM.

Materials and Methods

Study Cohorts

Danish Cohort. Patients diagnosed with having PM between 1976 and 2018 were identified at the Institute of Pathology, Aalborg University Hospital, Denmark. Pathology specimens, both biopsy and fluid, were confirmed as PM by two independent pathologists, according to international guidelines. From eligible biopsies, tissue microarrays (TMAs) were created. TMAs were constructed semiautomatically using three manually premarked representative areas (three cores) of each tumor, with a punch diameter of 2 mm. Construction was performed using the TMA Grand Master (3DHistech Ltd., Hungary). BAP1 IHC was performed on 3-μm formalin-fixed, paraffin-embedded sections using the anti-BAP1 C4 mouse monoclonal antibody (Santa Cruz Biotechnology) at a dilution of 1:50 using the OptiView DAB IHC Detection Kit (Ventana) on the Ventana BenchMark ULTRA platform (Roche Diagnostics). Any nuclear positivity was considered retained staining; stromal cells and lymphocytes were used as a positive internal control. The individuals performing and interpreting the stain were blinded as to the clinical outcome of the patients.

This project was approved by the Regional Ethical Committee of North Jutland, Denmark (registration number N-20140032) and the Danish Protection Data Agency (number 2008-58-0028).

Australian Cohort. PM cases diagnosed at PathWest Laboratory Medicine, QEII Medical Centre, Perth, Western Australia, between 2010 and 2018 were identified. Cases where patients had given consent for research were reviewed. All cases were confirmed as PM by pathologists according to diagnostic criteria current to the time period. This includes diagnosis based on review of cytologic specimens, where no histology sample was available. BAP1 IHC was performed as previously described.[27] Briefly, BAP1 IHC was performed on 4-μm formalin-fixed, paraffin-embedded sections using the same antibody clone at the same dilution as mentioned previously using the UltraView DAB Detection Kit on the same platform. The individuals performing and interpreting the stain were blinded as to the clinical outcome of the patients.

This project was approved by the Sir Charles Gairdner and Osborne Park Health Care Group Human Research Ethics Committee, number 2005-038 (RGS0000001517).

Clinical Information

For both the Danish and Australian cohorts, the following information was retrospectively collected from patient records and hospital databases: demographic information (age, sex), performance status (using the Eastern Cooperative Oncology Group Performance Status [ECOG PS]), PM subtype (on histology), treatment information, and survival data (date of death or date of censor). Patient management was decided by the treating clinician as part of routine care. Patients who underwent surgical management were excluded from the study. For classification purposes, the subtype was categorized as follows: epithelioid PM, sarcomatoid PM, biphasic PM, and PM diagnosed by cytology only (with no histologic confirmation). Treatment was categorized as either first-line platinum and pemetrexed or other systemic antineoplastic first-line therapy. The latter subgroup comprised a heterogeneous mixture of patients who received various treatments appropriate at the time or as part of a clinical trial; although this group was included in the OS analysis, they were not included in the platinum and pemetrexed analysis. Second-line or subsequent treatments received were recorded but not included in the analysis owing to their diverse nature. Patients who did not receive any systemic antineoplastic or novel therapies were categorized as having received best supportive care (BSC). Cases with incomplete treatment histories or missing follow-up data were excluded.

Statistical Analysis

Patients of both cohorts were stratified into two groups on the basis of BAP1 status, either BAP1 retained or lost. Primary outcome was OS defined as time elapsed between the date of PM diagnosis to the date of death or last follow-up. Difference in OS between groups was estimated by the Kaplan-Meier method using a log-rank test. Univariate Cox regression analysis was performed to compare the risk of death between the groups. Multivariate Cox regression analysis was performed with adjustment for age, sex, ECOG PS, histopathologic subtype, and treatment. Individuals with missing values were excluded from the analysis. For statistical purposes, age was categorized as less than or equal to 70 years and more than 70 years, ECOG PS as less than or equal to 1 and more than or equal to 2, whereas subtype was classified as epithelioid and nonepithelioid. *t* test or Fisher's exact tests were used to evaluate variances in clinical factors of patients between the groups, as appropriate. Tests of statistical significance were two sided, and p values less than 0.05 were considered statistically significant. Analyses were performed using the STATA statistical software program (STATA version 16.0; StataCorp., College Station, TX).

Results

Danish and Australian Cohorts

In the Danish cohort, a total of 149 cases diagnosed with having PM were identified during 1983 to 2018, with 114 cases meeting the inclusion criteria (Supplementary Fig. 1*A*). There were 42 who received platinum and pemetrexed in the first-line setting and 49 who received BSC. The median number of cycles of platinum and pemetrexed received in the Danish cohort was three (mean 3.48 $\pm$ 1.3 cycles). All participants were deceased at the time of the study (Table 1). Most of the tumors had loss of BAP1 (62.3%), and this was most common in the epithelioid subtype (67%) (Table 2).

In the Australian cohort, there were 262 cases available during the 8-year time period evaluated, with a total of 234 cases meeting the specified criteria (Supplementary Fig. 1*B*). Platinum and pemetrexed was administered to 108 patients, whereas 96 received BSC. A median of four cycles of platinum and pemetrexed was received in this cohort (mean 3.98 $\pm$ 1.7 cycles). Nine patients were alive at the end of the follow-up period; median follow-up for these patients was 39 months (range: 31–75 mo). In comparison to the Danish cohort, this cohort was significantly older at diagnosis, had a better performance status, had a lower frequency of the biphasic histologic subtype, and 41% were diagnosed by cytology (Table 1). BAP1 loss was detected in most of the tumors (60.7%) (Table 1).

Median survival from diagnosis was 11 and 14.3 months in the Danish and Australian cohorts, respectively (Table 1). Median survival was longest in the

Table 1. Comparison of the Demographic and Clinical Characteristics in the Danish and Australian Cohorts

Characteristics	Danish Cohort (n = 114)	Australian Cohort (n = 234)	*p* Value
Age, y, median (95% CI)	68.4 (66-72)	72.3 (70.5-74)	0.02[a]
Sex, male, n (%)	102 (89.4)	203 (86.8)	0.61[b]
Mesothelioma subtype			<0.0001[c]
Epithelioid	55	181	
Nonepithelioid	55	53	
Epithelioid, n (%)	55 (48.2)	85 (36.3)	
Sarcomatoid, n (%)	12 (10.5)	23 (9.8)	
Biphasic, n (%)	43 (37.7)	30 (12.8)	
Cytology, n (%)	Nil	96 (41.0)	
Not classified, n (%)	4 (3.5)	Nil	
ECOG PS, n (%)			0.05[b]
0-1	82 (71.9)	201 (85.9)	
2-4	22 (19.3)	29 (12.4)	
Missing	10 (8.8)	4 (1.7)	
Lines of treatment received, n (%)			0.0047[b]
One	19 (16)	67 (29)	
Two	29 (25)	32 (14)	
Three or more	17 (15)	39 (16)	
Treatment, n (%)			0.31[f]
BSC	49 (42.9)	96 (41.0)	
Platinum and pemetrexed[d]	42 (36.8)	108 (46.2)	
Other systemic treatment[e]	23 (20.2)	30 (12.8)	
BAP1			0.448[b]
Retained	43 (37.7)	92 (39.3)	
Lost	71 (62.3)	142 (60.7)	
Survival, mo, median (95% CI)	11.3 (8.5-14.2)	14.3 (12.5-17)	0.001[g]

[a]Difference between groups determined by unpaired *t* test.
[b]Difference between groups determined by Fisher's exact test or chi-square; missing data excluded from analysis.
[c]Difference between epithelioid (including cases diagnosed by cytology) and nonepithelioid (biphasic and sarcomatoid) determined by Fisher's exact test.
[d]Four patients went on to receive pembrolizumab in subsequent treatment.
[e]Three patients received pembrolizumab.
[f]Difference between BSC and platinum and pemetrexed treatment groups determined by Fisher's exact test.
[g]Difference between groups determined by log-rank test.
BSC, basic supportive care; CI, confidence interval; ECOG PS, Eastern Cooperative Oncology Group Performance Status.

epithelioid in comparison to the nonepithelioid subgroup in both cohorts (12.9 versus 6.9 mo, $p < 0.05$, and 17.9 versus 8.3 mo, $p < 0.0001$, respectively). Patients who received BSC had significantly higher risk of death compared with those receiving platinum and pemetrexed treatment in the first line, in both cohorts (hazard ratio = 2.84, $p < 0.001$, and 1.46, $p = 0.012$, respectively) on multivariate analysis (Table 3). Not unexpectedly, those patients in the Australian cohort who received less than four cycles of treatment survived for a significantly shorter period of time than those who received four to six cycles (median survival 314 d versus 625 d, $p < 0.0001$). In the Danish cohort, only four patients had less than three cycles of therapy.

Table 2. Rates of BAP1 Loss by Mesothelioma Subtype

Mesothelioma Subtype	Danish Cohort BAP1 Loss n (%)	Australian Cohort BAP1 Loss n (%)
Epithelioid mesothelioma	37/55 (67)	58/85 (68.2)
Biphasic mesothelioma	27/43 (63)	6/23 (26.1)
Sarcomatoid mesothelioma	4/12 (33)	4/30 (13.3)
Cytology diagnosis of mesothelioma	–	74/96 (77.1)
Not classified	3/4 (75)	–

BAP1 Status Is Associated With Survival in Platinum and Pemetrexed-Treated Patients

Survival analysis of the total cohort revealed a significant survival advantage in those with BAP1 loss in tumors, in both populations (14.2 versus 6.3 mo, $p < 0.0001$, and 18.9 versus 10.8 mo, $p < 0.001$, respectively) (Fig. 1*A* and *B*). Median survival in the Danish patients treated with platinum and pemetrexed in the first line was significantly longer in those with BAP1 loss compared with those with retained staining (20.2 versus 7.3 mo, $p < 0.001$), and this was validated in the Australian cohort (19.6 versus 11.1 mo, $p < 0.01$)

Table 3. Variables Associated With Overall Survival in the Danish and Australian Cohorts on Univariate and Multivariate Cox Regression Analyses

Variable	Univariate Analysis (HR, 95% CI, *p* value)	Multivariate Analysis (HR, 95% CI, *p* value)
Danish cohort		
BAP1 retained	**2.3, 1.53-3.43, $p < 0.001$**	**2.49, 1.54-4.0, $p < 0.001$**
Best supportive care	**1.77, 1.16-2.7, $p = 0.008$**[a] **1.93, 1.17-3.19, $p = 0.011$**[b]	**2.84, 1.61-5, $p < 0.001$**[a] **5.13, 2.45-10.75, $p < 0.001$**[b]
ECOG PS ≥2	**2.82, 1.74-4.59, $p < 0.001$**	1.65, 0.96-2.83, $p = 0.69$
Nonepithelioid mesothelioma	1.4, 0.95-2.07, $p = 0.91$	**2.1, 1.27-3.47, $p = 0.004$**
Age >70 y	1.23, 0.83-1.80, $p = 0.304$	**1.8, 1.09-2.97, $p = 0.023$**
Female sex	1.42, 0.79-2.54, $p = 0.242$	1.18, 0.62-2.23, $p = 0.618$
Australian cohort		
BAP1 retained	**1.90, 1.44-2.50, $p < 0.001$**	**1.48, 1.099-2, $p = 0.01$**
Best supportive care	**1.59, 1.2-2.11, $p < 0.001$**[a] **2.2, 1.43-3.38, $p < 0.001$**[b]	**1.46, 1.09-1.96, $p = 0.012$**[a] **1.65, 1.04-2.66, $p = 0.035$**[b]
ECOG PS ≥ 2	**2.19, 1.48-3.25, $p < 0.001$**	**2.03, 1.35-3.07, $p = 0.001$**
Nonepithelioid mesothelioma	**2.52, 1.82-3.48, $p < 0.001$**	**2.26, 1.59-3.23, $p < 0.001$**
Age >70 y	**1.63, 1.25-2.14, $p < 0.001$**	1.27, 0.94-1.72, $p = 0.13$
Female sex	0.90, 0.62-1.32, $p = 0.59$	1.17, 0.79-1.73, $p = 0.43$

All statistically significant results are highlighted in bold.
[a]Platinum and pemetrexed treatment.
[b]Other treatment.
CI, confidence interval; ECOG PS, Eastern Cooperative Oncology Group Performance Status; HR, hazard ratio

(Fig. 1*C* and *D*). Importantly, median survival of patients with retained BAP1 after platinum and pemetrexed treatment was no different than that of the BSC group as a whole (Fig. 2*A* and *B*).

Survival analysis of the Danish BSC group did not reveal a significant association of BAP1 status and OS (BAP1 loss 6.2 versus retained 3.8 mo, $p = 0.12$), but it was significant in the Australian cohort (BAP1 loss 16.8 versus retained 8.3 mo, $p < 0.01$) (Fig. 1*E* and *F*). BAP1 loss was positively predictive of survival in those diagnosed with having epithelioid disease but also nonepithelioid in both cohorts (Supplementary Fig. 2*A*–*D*). Posthoc analysis was performed and determined that survival outcomes, relative to BAP1 status, did not differ between cases classed as predominantly epithelioid, on the basis of histology or cytology. The group diagnosed on the basis of cytologic samples only had a higher rate of BAP1 loss (77%) than the epithelioid group (68%), although this was not significantly different ($p = 0.24$). There was no significant difference in survival by BAP1 status between cases diagnosed on the basis of histology or cytology (Supplementary Fig. 3*A* and *B*).

Discussion

To date, no predictive biomarkers for survival after primary chemotherapy in PM have been identified. In our study, we reveal and validate that tumor BAP1 loss predicts a more than 8-month increase in OS in patients treated with platinum and pemetrexed in the first-line setting, in comparison to those with retained staining. In contrast, patients with retained BAP1 who received dual chemotherapy had a similar median OS to those who received no active treatment. The results imply that all PM tumors should be stained for BAP1, and caution should be used when considering this chemotherapy doublet in patients with PM with BAP1-retained tumor.

Platinum and pemetrexed has been the primary first-line treatment of patients with PM considered fit for chemotherapy since the pivotal work of Vogelzang et al.[5] in 2003, with surgery offered to a few patients. This is reflected in our Danish and Australian cohorts, where less than 10% and 5% underwent surgery, respectively. In the cohorts, 34% and 41%, respectively, of the patients received BSC only owing to their frailty, patient preference, comorbidities, advanced disease, or a combination of these factors.

The main rationale in using a predictive biomarker is to allow targeting of the correct population and to avoid overtreatment. In mesothelioma, such a marker has not yet been identified. Tumor subtype is perhaps thus far the most consistent prognostic indicator, though it is not regarded as a predictive biomarker for chemotherapy. Previous studies attempting to identify biomarkers predictive of response to platinum and pemetrexed in PM have focused on candidates involved in drug transport, metabolism, and DNA repair, such as thymidylate synthase and excision repair cross complementing 1.[28] Despite many promising single-center studies, a lack of robust validation and difficulties in standardizing detection methods have meant that no such markers have yet been incorporated in the clinical practice.

BAP1 is located in 3p21, and it is one of the most frequently altered genes in PM.[14,29,30] Recent studies

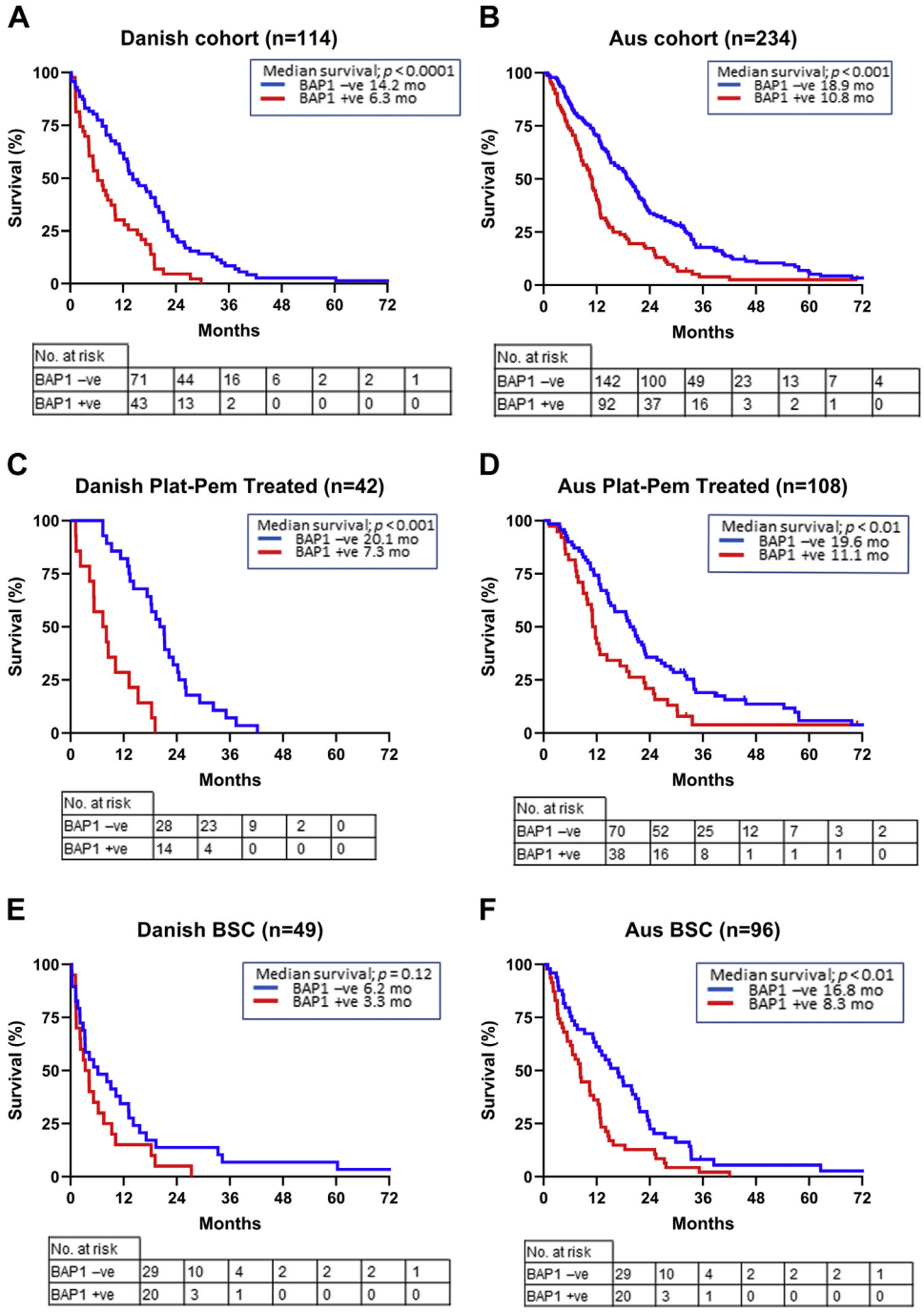

Figure 1. Kaplan-Meier survival curves for the Danish cohort (left panel) and Aus cohort (right panel) dichotomized by BAP1 status. (*A* and *B*) The total cohort; (*C* and *D*) the cohort that received first-line Plat and Pem; (*E* and *F*) the cohort that received BSC. Aus, Australian; BAP1 –ve, loss of nuclear staining; BAP1 +ve, retained nuclear staining; BSC, best supportive care; Pem, pemetrexed; Plat, platinum.

have revealed that loss of 3p21 or mutation is an early event in evolution of PM.[31] *BAP1* encodes a deubiquitinase involved in a range of cellular processes, including homologous recombination, DNA repair, cell cycle control, chromatin modification, and apoptosis.[12,32–34] The exact mechanism by which BAP1 loss provides a survival advantage to chemotherapy-treated patients is not entirely clear. The most plausible mechanism is that of a hampered DNA repair system unable to repair platinum-induced DNA damage, increasing cancer cell sensitivity, leading to apoptosis.[34] Similar findings have been observed in patients with BRCA1 or 2-deficient breast and ovarian malignancies post-platinum therapy.[35,36]

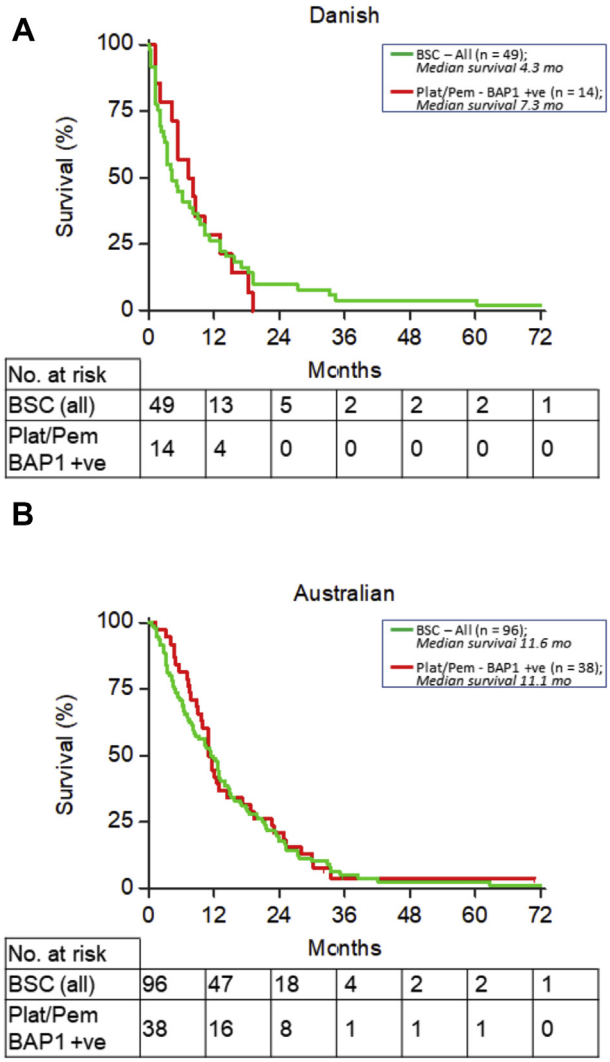

Figure 2. Kaplan-Meier curves for the (*A*) Danish and (*B*) Australian cohorts, comparing survival in those who had BSC (green) and those who received first-line Plat and Pem and had retained BAP1 staining (red). BAP1 +ve, BAP1 retained nuclear staining; BSC, best supportive care; Plat and Pem, platinum and pemetrexed.

There has been no large-scale assessment of the relationship between survival after standard therapy and BAP1 status as assessed by IHC. Many studies have evaluated the correlation of BAP1 status by IHC and prognosis and have had mixed results, although most have found no significant association with survival after adjusting for known prognostic indicators.[4,16,18,25,37–40] This lack of consistency may be due to many factors, including small number of patients, lack of validation, heterogeneity in patient selection, study size, patient management, and the adjustment, or lack thereof, within these studies for known prognostic indicators. In this study, we have revealed that BAP1 is a predictive marker in platinum and pemetrexed, nonsurgical PM patients in both the Danish and Australian cohorts, independent of other prognostic markers such as age, sex, ECOG PS, and histological subtype. The difference in survival on the basis of BAP1 status was more than 8 months (20.1 versus 7.3 mo, $p < 0.001$, and 19.6 versus 11.1 months, $p < 0.01$, in Aalborg and Perth, respectively), which is clinically meaningful, given the normally poor survival time.

Therefore, this is the first validated study to assess the association between BAP1 status and survival after first-line platinum and pemetrexed treatment. Importantly, we have also revealed the relative lack of effect of chemotherapy in those with retained BAP1 in tumors, by comparing this group with the BSC group in each cohort. The Kaplan-Meier curves of the chemotherapy-treated patients with BAP1 retained are similar to the BSC curves and reveal that this is clearly a subgroup of patients who minimally benefit from combined platinum and pemetrexed chemotherapy (Fig. 2).

As the distinction between prognostic and predictive biomarkers can be ambiguous, we performed subgroup analysis comparing the BSC patients in the two cohorts. Using this approach, differing results were observed, with a significant OS difference in BAP1 loss patients in the Australian, but not in the Danish, cohort. Although this heterogeneous finding occurring in our single study may be due to small sample numbers, it is nevertheless reflective of the wider mixed results of BAP1 prognostic value in the literature.

After the results of the CheckMate 743 study that revealed OS benefit of immunotherapy compared with platinum and pemetrexed as front-line therapy, one can question the clinical value of BAP1 as a biomarker.[7] Although dual immunotherapy will likely be the standard of care, there is a fact that in patients with epithelioid mesothelioma the median survival difference with dual immunotherapy is 2.2 months whereas in the nonepithelioid it is almost 9.3 months. Thus, there is no doubt that dual immunotherapy is the best option in nonepithelioid, but the possible fatal side effects and the high cost may push clinicians to choose chemotherapy in the epithelioid subtype. In Denmark, the combination of ipilimumab and nivolumab has recently been approved in the first-line treatment exclusively for patients with nonepithelioid disease, and consequently platinum-based combination with pemetrexed remains the first-line treatment for most patients with inoperable PM. On the basis of our results, it is tempting to treat patients with BAP1 loss and epithelioid subtype with pemetrexed and platinum as they have more than 6 months longer survival compared with those with BAP1 epithelioid with retained BAP1 in both cohorts (Supplementary Fig. 2). The dual immunotherapy could be then preserved as a second-line option in the BAP1 loss epithelioid subgroup. Because we have a lack of predictive

biomarkers for immunotherapy in epithelioid PM, BAP1 may help clinicians decide a more tailored treatment algorithm.

This study has a number of strengths and limitations. The two independent cohorts in two countries with well-defined clinical and re-evaluated pathologic data is a strength, including the relatively large number of BSC-treated patients. Patients receiving surgery were excluded from the cohorts, and patients receiving other first-line therapies, including novel therapies as part of clinical trials, were excluded from the principal predictive analysis. Although this has likely resulted in a number of younger patients with a lower ECOG PS being excluded from our study, this was done to allow more specific assessment of the association between first-line platinum and pemetrexed and BAP1 status. Interestingly, there was a difference in OS observed between the two cohorts. This may be due to several factors. First, two-thirds of the Danish cohort were diagnosed and treated before 2010, which was the start of patient inclusion for the Australian cohort. Furthermore, at the Danish site, any cytologic diagnosis of PM required biopsy confirmation, whereas at the Australian site, PM diagnosis by cytology only is commonplace. In addition to a diagnostic lead-time effect, this may affect histologic subtype proportions as we cannot exclude that some patients with a cytologic diagnosis have biphasic disease. Importantly, despite differences that may occur owing to different diagnostic or treatment traditions and time periods, the main results were recapitulated in both cohorts. Another limitation was the lack of availability of radiological response data. Although such data would be informative, it is known to be a surrogate for OS in PM and therefore should reveal similar findings. Our analyses did not account for subsequent treatment beyond the first-line setting; several patients received subsequent lines of various chemotherapies and in the Australian cohort a small number received immunotherapy. Nevertheless, to date, there is no phase 3 study revealing any survival advantage of second-line chemotherapy; thus, the effect on OS is expected to be due to the platinum and pemetrexed treatment.

BAP1 status by IHC is a simple investigation that captures loss of BAP1 protein expression occurring at multiple levels. If validated in a prospective series, BAP1 is a readily translatable biomarker as IHC is routinely used in pathology laboratories around the world as part of mesothelioma diagnostic workup and can therefore be easily adapted for use in triaging patients.

In summary, we present evidence in two independent PM cohorts that BAP1 status can predict survival after the standard first-line platinum and pemetrexed chemotherapy regimen. This may have substantial implications for patient management if confirmed in a prospective cohort as this result suggests that BAP1-retained patients receiving active chemotherapy derive limited survival benefit and likely endure treatment-associated side effects. This subgroup could therefore be considered for other types of treatment. This predictive marker can be a tool, alongside prognostic variables, such as subtype and performance status, to determine the best treatment options for patients and may represent a move toward more personalized treatment in this aggressive disease. Further prospective trials are needed to validate these findings.

CRediT Authorship Contribution Statement

Amber Louw: Investigation, Writing - original draft, Data collection, Writing - review & editing, Visualization, Project administration.

Vasiliki Panou: Project administration, Data collection, Formal analysis, Investigation, Writing - review & editing.

Weronika Maria Szejniuk: Formal analysis, Writing - review & editing.

Christos Meristoudis Siaw Ming Chai, Chris van Vliet, Louise Andersen Lynggaard, Azadeh Birbaneh Asghari, Mogens Vyberg, Johnni Hansen: Investigation, Writing - review & editing.

Y. C. Gary Lee: Supervision, Writing - review & editing.

Ian M. Dick: Formal analysis, Writing - review & editing.

Tina Firth: Data collection, Writing - review & editing.

Jenette Creaney: Supervision, Funding acquisition, Conceptualization, Writing - review & editing.

Oluf Dimitri Røe: Conceptualization, Supervision, Funding acquisition, Project administration, Investigation, Writing - review & editing.

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Supplementary Data

Note: To access the supplementary material accompanying this article, visit the online version of the *Journal of Thoracic Oncology* at www.jto.org and at https://doi.org/10.1016/j.jtho.2022.04.008.

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