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Prognosis of patients with malignant mesothelioma by expression of programmed cell death 1 ligand 1 and mesothelin in a contemporary cohort in Finland

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ABSTRACT

Objectives: We aimed to describe mesothelin (MSLN) and programmed cell death 1 ligand 1 (PD-L1) tumour overexpression amongst patients with malignant mesothelioma (MM), and their associations with survival, amongst a cohort of patients with MM in Finland.

Methods: Between 2004 and 2017, 91 adults with histologically confirmed MM were identified from the Auria Biobank in Finland and followed-up using linked data from electronic health records and national statistics. Biomarker content in tumour cell membranes was determined using automated Immunohistochemistry on histological sections. Stained tumour sections were scored for MSLN and PD-L1 intensity. Adjusted associations between MSLN/PD-L1 co-expression and mortality were evaluated by estimating hazard ratios (HRs) with 95% confidence intervals (CIs) using Cox regression.

Results: Biomarker overexpression occurred in 52 patients for MSLN and 34 patients for PD-L1 and was associated with tumour histology and certain comorbidities. Fifteen per cent of patients had a tumour that overexpressed both biomarkers; r =-0.244, p-value: 0.02. Compared with MSLN+/PD-L1+ patients, HRs (95% CIs) for death were 4.18 (1.71–10.23) for MSLN-/PD-L1+ patients, 3.03 (1.35–6.77) for MSLN-/PD-L1- patients, and 2.13 (0.97–4.67) for MSLN+/PD-L1- patients.

Conclusions: Both MSLN and PD-L1 markers were independent prognostic indicators in patients with MM. Overexpression of MSLN was associated with longer survival; yet their combined expression gave a better indication of survival. The risk of death was four times higher amongst MSLN-/PD-L1+ patients than in MSLN+/PD-L1+ patients.

1. Introduction

Malignant mesothelioma (MM) is a devastating cancer arising mainly in the cells lining the pleura [1]. In most cases, it is caused by past exposure to asbestos fibres in workplace environments, and has an average latency period between exposure and onset of symptoms of 35–45 years[2-4]. According to recent registry-based data, the incidence of mesothelioma in Europe has a male:female ratio of approximately 3:1 [5]. There is considerable inter-country variation in the incidence and prevalence of mesothelioma [6] and, for some countries, under-reporting of this cancer may hide a much higher burden of disease [7, 8].

Mesothelioma is a fatal disease with patients having a median overall survival of 15 months [9]. This poor prognosis is further affected by tumour histology – the sarcomatoid type having shorter median survival compared with epithelioid and mixed types [10]. Differential diagnosis of MM is difficult not only because of its heterogeneity, but also because pleura is a common site for metastatic disease, and MM is often

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confounded by other tumour types (e.g. other tumours of the pleura, ovarian cancer, etc.) [11-13]. To overcome these challenges, recent years have seen extensive research into biomarkers with good specificity and sensitivity profiles for identifying MM. Some of the most promising are mesothelin (MSLN) and programmed cell death 1 ligand 1 (PD-L1) [14-16], both of which are current therapeutic targets in the treatment of MM [17-19].

MSLN is a protein expressed in the membrane surface in the majority (around 75–80%) of mesothelioma tumour cells [20, 21]. It has good specificity but poor sensitivity in the diagnosis of malignant pleural mesothelioma [14, 20]. In addition, MSLN has been studied as a potential indicator of treatment response and prognosis, but despite better performance than other biomarkers, results have been inconsistent and study sizes often small [14, 22-26]. PD-L1 is expressed in approximately 20% of the tumour cells in patients with MM [27]. Notwithstanding extensive clinical research, the prognostic role of PD-L1 in mesothelioma patients remains controversial [28-32]. The differential clinical course of pleural mesothelioma with and without overexpression of MSLN and PD-L1 is not well understood [23, 27] and there has been little research into the co-expression of these two biomarkers in mesothelioma tumours and its clinical implications. To this end, we performed a retrospective observational study among a cohort of patients with MM in Finland that aimed to describe MSLN and PD-L1 tumour expression and their associations with the natural history of the disease.

2. Materials and methods

2.1. Data sources and study population

We used data from the Auria Biobank in Finland with linkage to hospital-based electronic health records (EHRs) and to Statistics Finland. The Biobank is situated in Turku, Finland and is a joint institution between the hospital districts of Southwest Finland, Satakunta and Vaasa, VSSHP, and Turku University. It was established in 2012, and in Spring 2014 it received its operating license from the National Supervisory Authority for Welfare and Health. The Biobank's activities adhere to the 2013 Finnish Biobank Act, which came into force in September 2013. Donor samples and associated information are collected and saved in the Biobank for research purposes either with the donor's direct consent or through a notification process. A total of 146 individuals aged ≥18 years were identified from the Biobank with a diagnosis of mesothelioma (International Classification of Diseases [ICD] version 10: code C45.x) between 2004 and 2017. Among these individuals, 140 had at least one Formalin-Fixed Paraffin-Embedded (FFPE) tumour sample available from the pathology archives, 91 of whom had MM confirmed following histopathology and were included in the study. All but two mesothelioma tumour samples used in this study were collected either before, or at the time of, mesothelioma diagnosis. Histology and molecular characteristics of the tumours, including the level of MSLN and PD-L1 expression, were determined by immunohistochemistry (IHC) and histopathology expert evaluation. Clinical and demographic characteristics of patients, comorbidities and treatments administered were obtained from linked hospital-based EHRs. Further patient data including socio-economic status, employment history, asbestos exposure, and cause and date of death was obtained through linkage to Statistics Finland using a non-trackable unique identifier. Use of these tumour samples and other patient data were approved by Auria Biobank's Scientific Steering Committee under Decision AB17–8946 and by Statistics Finland under application number TK-53–124–18 and project ID U1118_a. All patients included in the study were followed from the date of mesothelioma diagnosis to either death or the end of follow-up at 31st December 2017, whichever came first.

2.2. Patient characteristics

We obtained information from the patients' electronic health records on demographics (age at diagnosis and socioeconomic status), exposure to asbestos, treatment (chemotherapy, radiotherapy) and comorbidities. We calculated the Charlson comorbidity score for each patient and categorised them as scores of 0–3, 4–5 and ≥5. To identify the start and end dates of a treatment, and therefore differentiate the lines of treatment, for each patient we identified any longitudinal change in the treatment. According to ESMO guidelines [11], the recommended first-line therapy is pemetrexed plus platinum-based treatment, which may be followed by maintenance on pemetrexed as monotherapy. Currently, there is no standard of care for second-line therapy, thus any treatment regime after the first administered was considered to be second-line treatment.

2.3. Analysis of MSLN and PD-L1 in tumour and classification of patients according to their expression levels

Automated IHC was performed on 4 μm histological FFPE sections on a Ventana Discovery autostainer using DAB detection chemistry with anti-PD-L1 antibody (clone SP263) [33] and anti-MSLN antibody (clone SP74) [34] from Ventana Medical Systems Inc. (Tucson, AZ, USA), respectively. Stained sections were transferred physically to the same board of certified pathologist (Provitro AG, Germany) for scoring. In addition, one H&E stained slide per sample was provided to assess the tumour content. The result of the analysis included the following information for each sample: the tumour content as a percentage, the percentage of tumour cells showing membrane staining with intensity scores of 0, 1, 2 and 3 for PD-L1 and MSLN, and the calculated H-score for both biomarkers. The H-score is a measure of biomarker expression that ranges from 0 to 300 and considers the number of cells expressing the marker of interest as well as the intensity of such expression. It is calculated using the following formula [35, 36]

$$1 \times (\%cells1+) + 2 \times (\%cells2+) + 3 \times (\%cells3+)$$

For analysis, we classified the study population into four mutually exclusive categories according to the level of MSLN and PD-L1 expression. First, we assigned a threshold to identify overexpression of each biomarker. For MSLN, we considered overexpression (MSLN+) as an expression at moderate or strong membrane intensity (2 or 3) on ≥30% of tumour cells in the FFPE section [37, 38]. For PD-L1 we considered overexpression (PD-L1+) as an expression showing a Tumour Proportion Score (TPS) ≥ 1% [39]. TPS is defined as the percentage of viable tumour cells showing partial or complete membrane staining in the FFPE section related to all tumour cells on the slide [27, 39]. Patients were subsequently assigned to one of the following four groups: MSLN+/PD-L1+, MSLN+/PD-L1-, MSLN-/PD-L1+, and MSLN-/PD-L1-.

2.4. Statistical analysis

Baseline characteristics were described for each biomarker expression group. Categorical variables were summarised using frequency counts and percentages, and continuous variables were summarised using medians with inter-quartile range (IQR). The H-score, as a continuous variable, was used to calculate the Pearson's correlation coefficient (r) as a measure of the linear association between MSLN and PD-L1 expression levels. Associations between patient characteristics and MSLN and PD-L1 overexpression were identified using multinomial logistic regression to calculate crude odds ratios (ORs) with 95% confidence intervals (CI). To explore the effects of MSLN/PD-L1 overexpression, and other patient characteristics on mortality, we produced Kaplan–Meier survival curves and used Cox regression to calculate hazard ratios (HRs) with 95% CIs adjusted for confounders. Tumour overexpression was evaluated independently for the two biomarkers (i.e. two categories for each; MSLN+ or MSLN-, and separately PD-L1+ or

PD-L1-), as well as according to their co-expression (i.e. the four biomarker categories). For the latter, we also evaluated the risk of death for MSLN+/PD-L1-, MSLN-/PD-L1+ and MSLN-/PD-L1- patients, using MSLN+/PD-L1+ patients as the reference group. Statistical Analysis Software version 9.4 (SAS Institute, Cary, NC) was used for all data management and statistical analysis.

3. Results

3.1. Baseline characteristics

Baseline characteristics of the 91 mesothelioma patients in the study are described according to MSLN and PD-L1 co-expression in Table 1, and according to MSLN and PD-L1 expression separately, in the **Appendix Table**. Eighty-three (91%) patients had pleural mesothelioma. There were 39 (43%) MSLN- patients, of whom 20 were PD-L1- and 19 were PD-L1+, and 52 (57%) MSLN+ patients of whom 37 were PD-L1- and 15 were PD-L1+. The H-score of MSLN and PD-L1 showed an inverse non-adjusted correlation (Pearson coefficient -0.244, *p*-value: 0.02). Demographic characteristics were generally similar irrespective of MSLN or PD-L1 overexpression, although PD-L1- patients were slightly younger than PD-L1+ patients. Of the four biomarker expression subgroups, the MSLN-/PD-L1+ group had the highest frequency of males (94.7%); this group was also the oldest with a mean age of 70.1 years. Most MSLN+ patients and most PD-L1- patients had epithelioid tumour histology (88.5% and 82.5%; **Appendix Table**). Epithelioid tumour histology was seen in the majority of MSLN+ patients irrespective of PD-L1 overexpression (91.9% for MSLN+/PD-L1- patients and 80.0% for MSLN+/PD-L1+ patients; Table 1). The MSLN-/PD-L1+ biomarker subgroup was the most heterogeneous in terms of tumour histology: 37% epithelioid, 42% sarcomatoid and 21% mixed histology.

MSLN- patients were treated with chemotherapy less frequently than MSLN+ patients (79.5% vs 90.4%, respectively; **Appendix Table**). Administration of second-line treatment following pemetrexed + platinum-based therapy was commonly received by PD-L1 patients: 52.9% of MSLN+/PD-L1- patients, 50.0% of MSLN-/PD-L1- patients compared with 30.8% of MSLN+/PD-L1+ patients, and 13.3% of MSLN-/PD-L1+ patients (Table 1). No differences between biomarker subgroups were seen in respect to treatment with radiotherapy (in total, between approximately 36% to 40% of patients received at least one dose; **Appendix Table**).

Table. 1
Characteristics of the 91 patients with malignant mesothelioma by MSLN and PD-L1 co-expression levels.

	MSLN- (*n* = 39)				MSLN+ (*n* = 52)			
	PD-L1- (*n* = 20)		PD-L1+ (*n* = 19)		PD-L1- (*n* = 37)		PD-L1+ (*n* = 15)	
	n	%	n	%	n	%	n	%
Male,	17	85.0	18	94.7	30	81.1	12	80.0
Age at diagnosis (years)								
<65	8	40.0	5	26.3	15	40.5	4	26.7
65–74	8	40.0	8	42.1	14	37.8	8	53.3
≥75	4	20.0	6	31.6	8	21.6	3	20.0
Socio-economic status (1990–2010)								
Self-employed	2	10.0	3	15.8	8	21.6	3	20.0
Upper-level employee	1	5.0	3	15.8	5	13.5	1	6.7
Lower-level employee	9	45.0	2	10.5	6	16.2	2	13.3
Manual worker	4	20.0	9	47.4	15	40.5	7	46.7
Others	4	20.0	2	10.5	3	8.1	2	13.3
Exposed to asbestos according to medical records	10	50.0	9	47.4	22	59.5	6	40.0
Pleura anatomical site	18	90.0	18	94.7	34	91.9	13	86.7
Histology								
Missing	2	10.0	0	0.0	2	5.4	1	6.7
Epithelioid	13	65.0	7	36.8	34	91.9	12	80.0
Mixed	1	5.0	4	21.1	1	2.7	2	13.3
Sarcomatoid	4	20.0	8	42.1	0	0.0	0	0.0
Death	19	95.0	19	100.0	36	97.3	14	93.3
Follow-up*median ± IQR	245±435		155±230		345±520		435±595	
Chemotherapy	16	80.0	15	78.9	34	91.9	13	86.7
Pemetrexed & platinum-based therapy								
Maintained	8	50.0	12	80.0	13	38.2	7	53.8
Followed by other therapies	8	50.0	2	13.3	18	52.9	4	30.8
Sole monotherapy	0	0.0	1	6.7	3	8.8	2	15.4
Radiotherapy								
1	4	20.0	4	21.1	9	24.3	5	33.3
2–5	4	20.0	2	10.5	4	10.8	3	20.0

* Rounded to the nearest 5 days. IQR, inter-quartile range; MM, malignant mesothelioma; MSLN, mesothelin; PD-L1, programmed cell death 1 ligand 1.

3.2. Patient characteristics and MSLN/PD-L1 overexpression

Associations between patients' baseline characteristics and MSLN/PD-L1 overexpression are shown in Table 2. PD-L1 overexpression (OR 0.43, 95% CI: 0.18–1.02) and comorbidities of the circulatory system (OR: 0.28, 95% CI: 0.08–0.98) were both associated with a reduced likelihood of MSLN overexpression. There was some evidence that chemotherapy was associated with more than twice the likelihood of MSLN overexpression compared with no chemotherapy (OR 2.43, 95% CI: 0.73–8.10) although the wide CI meant the estimate was imprecise. Having either mixed or sarcomatoid tumour histology was associated with an increased likelihood of PD-L1 overexpression when compared with epithelioid histology: (OR 7.42, 95% CI: 1.37–40.09) for mixed histology and OR 4.95 (95% CI: 1.33–18.39) for sarcomatoid histology. Other factors for which there was some evidence that they may be associated with PD-L1 overexpression were: older age (OR 1.92, 95% CI: 0.60–6.10 for ≥75 years vs 18–64 years, and OR 1.86, 95% CI: 0.68–5.07 for 65–74 years vs. 18–64 years), a Charlson comorbidity score of ≥six (OR 2.36, 95% CI: 0.71–7.90 vs. a score of 0–3), and having a circulatory system comorbidity (OR 3.20, 95% CI: 0.95–10.76).

3.3. Survival

Eighty-eight patients (97%) died over a total follow-up of 53,265 person-days (median of 285 days, IQR 150–570). The three surviving patients had an observational time-at-risk of 2545 (patient belonging to the MSLN-/PD-L1- group), 1945 (MSLN+PD-L1+) and 140 days (MSLN+/PD-L1-), and none were MSLN-/PD-L1+. Among all 91 patients, survival time was shortest for MSLN- patients (median of 195 days), and longest for MSLN+ patients (median of 375 days). The reverse pattern, although less marked, was seen for PD-L1 expression, i.e. PD-L1+ patients had a shorter survival time (median of 228 days) than PD-L1- patients (median of 330 days). The better survival of MSLN+ patients (vs. MSLN- patients) and of PD-L1- patients (vs. PD-L1+ patients) is shown in the Kaplan–Meier survival curves (Fig. 1**a** and **1b**, respectively). Furthermore, the survival curves in relation to the biomarker H-scores show the increasingly better survival with increasing MSLN intensity (**Figure 1c**), and the increasingly worse survival with increasing PD-L1 intensity (**Figure 1d**). In the Cox regression analysis, MSLN+ status (vs. MSLN-) was associated with a reduced risk of death after adjusting for confounders, including tumour histology (HR 0.53, 95% CI: 0.31–0.91) (Table 3). The age-and gender adjusted HR for PD-L1+ (vs. PD-L1-) was 1.15 (95% CI: 0.73–1.80) and this changed substantially to HR 0.56 (95% CI: 0.32–0.99) in the fully-adjusted model, being driven predominantly by adjustment for histology and chemotherapy sequence.

Of the four biomarker subgroups, the MSLN-/PD-L1+ group (the oldest group with the highest percentage of males) had the worst prognosis. All 19 of these patients died, their median time between

Table. 2
Crude odds ratios (95% CI) for the association between patient characteristics and of overexpression of MSLN or PD-L1.

	MSLN			PD-L1		
	n (%) MSLN-	n (%) MSLN+	OR (95% CI)	n (%) PD-L1-	n (%) PD-L1+	OR (95% CI)
PD-L1+	19 (48.7)	15 (28.8)	0.43 (0.18–1.02)			–
Exposure to asbestos	19 (48.7)	28 (53.8)	1.23 (0.54–2.82)	32 (56.1)	15 (44.1)	0.62 (0.26–1.45)
Age at diagnosis, years						
18–64	13 (33.3)	19 (36.5)	1.00 (reference)	23 (40.4)	9 (26.5)	1.00 (reference)
65–74	16 (41.0)	22 (42.3)	0.94 (0.36–2.45)	22 (38.6)	16 (47.1)	1.86 (0.68–5.07)
≥75	10 (25.6)	11 (21.2)	0.75 (0.25–2.28)	12 (21.1)	9 (26.5)	1.92 (0.60–6.10)
Histology						
Epithelioid	20 (51.3)	46 (88.5)	1.00 (reference)	47 (82.5)	19 (55.9)	1.00 (reference)
Mixed	5 (12.8)	3 (5.8)	–	2 (3.5)	6 (17.6)	7.42 (1.37–40.09)
Sarcomatoid	12 (30.8)	0 (0)	–	4 (7.0)	8 (23.5)	4.95 (1.33–18.39)
Comorbidities						
ICD-10 chapter						
II. Neoplasm	5 (12.8)	13 (25.0)	2.27 (0.73–7.01)	12 (21.1)	6 (17.7)	0.80 (0.27–2.38)
IV. Endocrine	2 (5.1)	7 (13.5)	2.88 (0.56–14.70)	5 (8.8)	4 (11.8)	1.39 (0.35–5.56)
IX. Circulatory	9 (23.1)	4 (7.7)	0.28 (0.08–0.98)	5 (8.8)	8 (22.5)	3.20 (0.95–10.76)
X. Respiratory	31 (79.5)	37 (71.2)	0.64 (0.24–1.70)	44 (77.2)	24 (70.6)	0.71 (0.27–1.86)
XVIII. Signs, symptoms	9 (23.1)	7 (13.5)	0.52 (0.17–1.54)	11 (19.3)	5 (14.7)	0.72 (0.23–2.29)
Charlson comorbidity score						
0–3	17 (43.6)	20 (38.5)	1.00 (reference)	26 (45.6)	11 (32.4)	1.00 (reference)
4–5	13 (33.3)	25 (48.1)	1.64 (0.64–4.15)	23 (40.4)	15 (44.1)	1.54 (0.59–4.02)
≥6	9 (23.1)	7 (13.5)	0.66 (0.20–2.15)	8 (14.0)	8 (23.5)	2.36 (0.71–7.90)
Chemotherapy	31 (79.5)	47 (90.4)	2.43 (0.73–8.10)	50 (87.7)	28 (82.4)	0.65 (0.20–2.14)
Radiotherapy	14 (35.9)	21 (40.4)	1.21 (0.51–2.85)	21 (36.8)	14 (41.2)	1.20 (0.50–2.86)

CI, confidence interval; MSLN, mesothelin; OR, odds ratio; PD-L1, programmed cell death 1 ligand 1.

diagnosis and death was 155 days, and only 13.3% reached second-line treatment. PD-L1+/MSLN+ patients had the longest survival (median of 435 days), even though 14 (93%) of these patients died during follow-up. Age-adjusted associations of death with baseline patient characteristics stratified by overexpression of MSLN (MSLN+ or MSLN-) and separately by overexpression of PD-L1 (PD-L1+ or PD-L1-), are shown in Table 4. Regardless of overexpression of each biomarker, patients aged 75 years or more had higher risk of death. Likewise, an increased risk of death was seen among patients with sarcomatoid or mixed histology tumours compared to those with epithelioid histology. However, this association did not exist in patients with MSLN+ tumour. A high co-morbidity index was also associated with higher risk of death. Not receiving chemotherapy or radiotherapy was also associated with a higher risk of death. Overexpression of MSLN was associated with a reduced risk of death especially among PD-L1+ patients (age-adjusted HR 0.26, 95% CI: 0.11–0.58). Interestingly, among MSLN- patients, overexpression of PD-L1 was associated with an increased risk of death (age-adjusted HR, 1.88, 95% CI: 0.96–3.71), whereas among MSLN+ patients, overexpression of PD-L1 was associated with a reduced risk of death, albeit the CIs overlapped 1.0, (age-adjusted HR, 0.66 CI: 0.35–1.27). As shown in Table 5, after adjustment for confounders, compared with MSLN+/PD-L1+ patients, including tumour histology, all other biomarker subgroups had an increased risk of death; a four-fold increased risk for MSLN-/PD-L1+ patients, a three-fold increased for MSLN-/PD-L1- patients, and a two-fold increased risk for MSLN+/PD-L1- patients.

4. DISCUSSION

Despite growing interest in the therapeutic effect of personalised cancer treatment that targets tumours overexpressing MSLN and PD-L1 in patients with MM, few studies have evaluated the co-expression patterns of these two biomarkers and its joint prognostic value. Our study showed that patients with mesothelioma tumours overexpressing MSLN have a better prognosis compared with patients without MSLN tumour overexpression in crude and adjusted survival models. Likewise, overexpression of PD-L1 was not associated with survival in crude models, but we found an association with longer survival after adjusting for potential confounders. Interestingly, we found an apparent interaction between PD-L1 and MSLN overexpression and their association with death, with the risk of death being four times higher among MSLN-/PD-L1+ patients than in MSLN+/PD-L1+ patients. Among MSLN+ patients, the difference in survival between those whose tumours did or did not overexpress PD-L1 was less pronounced. Although a two-fold higher risk of death was seen among MSLN+ patients without PD-L1 overexpression (vs. with PD-L1 overexpression), our findings suggest that MSLN overexpression may be a stronger predictor of prognosis than PD-L1 overexpression. We also found that overexpression of the two biomarkers is inversely correlated, and previous studies suggest that this may be driven by tumour histology [27, 40].

Our finding that MSLN tumour expression was associated with better survival is consistent with findings from previous studies in other cancer types such as gastric and ovarian cancer [41, 42]. A biological plausible mechanism through which this association could be mediated may involve the triggering of an anti-MSLN immune response involving both T cells and B cells [35]. This is supported by studies that have evaluated the suitability of MSLN as a target for immunotherapy [40]. An immune response alongside surgical removal of the tumour and chemotherapy may increase survival [41]. A similar conclusion and interpretation was elicited by Yen and colleagues in their analysis of around 200 samples of ovarian serous carcinomas [42]. However, another study conducted in 25 cholangiocarcinoma samples found contradicting results [43], and this is supported by data from an in vitro study, which found that MSLN expression increased cell proliferation and migration, and resulted in worse prognosis [44]. Interestingly, a meta-analysis of eight studies (579 patients) reported that high levels of soluble MSLN in serum were consistently associated with a poorer prognosis of malignant pleural mesothelioma. However, only one of the studies included in the meta-analysis used pleural effusion rather than blood serum as a source of soluble MSLN and reported no association with cancer prognosis [20]. With respect to PD-L1 membrane expression, some studies have shown an association with poor prognosis in mesothelioma patients [27], but there is still controversy around its prognostic value [28].

Owing to the limited research on this topic, our study provides valuable findings into the effects of MSLN and PD-L1 co-expression in patients with MM. Another strength of our study is that we evaluated co-

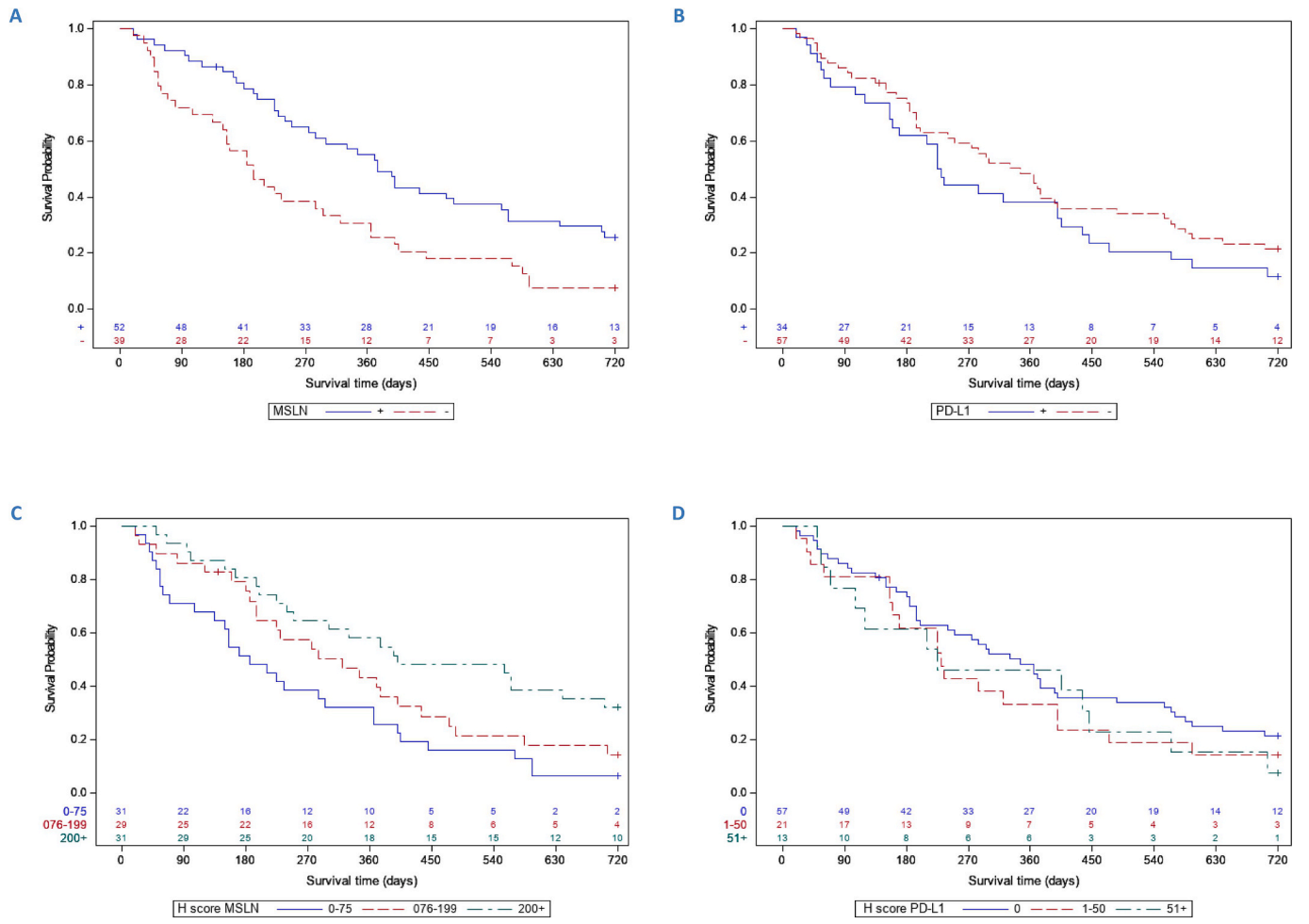

Fig.. 1. Two-year Kaplan–Meyer survival curves of mesothelioma patients at diagnosis: A) By MSLN over-expression (expression at moderate or strong membrane intensity (2+ or 3+) on ≥30% of tumour cells), B) By PD-L1 over expression (expression showing a tumour Proportion Score (TPS) ≥ 1%), C) By tertile categories of the MSLN H-score, D) By tertile categories of the PD-L1 H-score.

expression of the two biomarkers using the same tumour sample for each patient. Also, our analyses used actual survival time for nearly all patients. Only three patients were still alive at the end of follow-up for whom survival time will have been underestimated. However, our study has limitations that must be considered when interpreting its results. Firstly, our study population was derived from a specific region of Finland and was slightly younger compared with patients with mesothelioma from Turku in Finland's national cancer registry [45]. Although, this potentially raises doubts about its external validity, we believe that our findings have good generalisability to broader populations. We would not expect the biological properties of the biomarkers or the aetiology of the disease to differ across geographic regions, the treatment guidelines are similar across developed countries, and healthcare patterns are broadly similar across Scandinavian countries and more widely across Europe. Secondly, our sample size was small, and this limited the precision of the estimates in our study. Although we used all the available MM tumour samples in the Biobank, MM is a rare cancer (approximately only 25–30 cases arise every year in the Biobank's catchment area). While cohorts in other molecular epidemiology studies of MM have been larger, these have not evaluated PDL1 expression, and our study is the largest to investigate co-expression of both MSLN and PDL1. Notwithstanding this, it would be interesting to compare our results with those from future studies that evaluate co-expression of both these biomarkers in larger cohorts, and our results have the potential to be included in future pooled/meta-analyses on the topic. Other factors contributing to our small sample size included the need to obtain patient consent to store biological samples, and the need for a large enough sample to provide sufficient tumour content for analysis. If the tumour content of samples was associated with clinical factors such as severity of disease or the likelihood of undergoing certain clinical procedures, this may have introduced some selection bias into the study. Thirdly, we did not use methodologies to homogenise the compared subgroups at baseline such as 1:1 matching or propensity scores. This was due to the very limited sample size of our MM cohort. We controlled for confounding bias adjusting for co-variables that proved to be confounders in the regression models, although it must be noted that residual confounding, from unknown or unmeasured variables, may have occurred. Fourthly, we were unable to assess the two biomarkers over time as only two individuals from our cohort had two tumour samples that were obtained at different points in time. Finally, our study design depended on secondary data collection for all variables except for biomarker expression measurements, therefore we could not control for data collection quality and relied on the information in the EHRs and administrative databases.

Only 15% of patients in the study had a tumour overexpressing both markers and 20% had a tumour that expressed neither. Hence, 65% of mesothelioma patients overexpressed only one of the two biomarkers reducing in theory the population that could potentially benefit from a combined therapy targeting both markers. The prognostic value of MSLN and PD-L1 was evident through evaluation of their co-expression. Both markers measure in sections of the same sample demonstrated good value in evaluating survival probability irrespective of histology,

Table. 3
Age-adjusted hazard ratios (95% CI) for death associated with various factors according to overexpression to MSLN and PDL1.

	MSNL- HR (95% CI)	MSNL+ HR (95% CI)	PD-L1- HR (95% CI)	PD-L1+ HR (95% CI)
PD-L1+	1.88 (0.96–3.71)	0.66 (0.35–1.27)	1.10 (0.70–1.72)	–
MSLN+	0.52 (0.34–0.81)	–	0.77 (0.43–1.35)	0.26 (0.11–0.58)
Age at diagnosis, years				
<65	1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)
65–74	(1.67 (0.77–3.61)	1.07 (0.56–2.05)	1.34 (0.72–2.49)	1.06 (0.45–2.49)
≥75	2.34 (0.95–5.73)	2.65 (1.19–5.89)	2.50 (1.17–5.32)	2.14 (0.82–5.61)
Histology				
Epithelioid	1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)
Mixed	5.53 (1.79–17.06)	1.52 (0.45–5.14)	8.31 (1.81–38.21)	2.86 (1.03–7.94)
Sarcomatoid	1.78 (0.82–3.87)	–	1.87 (0.65–5.40)	2.85 (1.14–7.13)
Exposure to asbestos	1.00 (0.52–1.89)	1.75 (0.98–3.15)	1.39 (0.80–2.41)	1.78 (0.80–3.99)
Radiotherapy	0.48 (0.24–0.96)	0.89 (0.50–1.58)	0.64 (0.37–1.13)	0.61 (0.27–1.36)
Chemotherapy				
None	1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)
Maintained pemetrexed & platinum-based therapy	0.21 (0.08–0.56)	2.27 (0.77–6.70)	0.23 (0.09–0.60)	1.56 (0.53–4.55)
Pemetrexed & platinum-based therapy followed by other therapies	0.10 (0.03–0.30)	0.83 (0.29–2.40)	0.10 (0.04–0.28)	0.69 (0.21–2.30)
Sole pemetrexed & platinum-based therapy monotherapy	–	0.56 (0.15–2.19)	0.09 (0.02–0.38)	0.54 (0.10–2.81)
Comorbidities				
II. Neoplasms	0.45 (0.16–1.29)	1.41 (0.74–2.70)	0.85 (0.44–1.64)	1.34 (0.52–3.42)
IV. Endocrine	3.60 (0.81–15.98)	1.90 (0.83–4.34)	2.16 (0.84–5.55)	1.28 (0.42–3.87
IX. Circulatory	0.96 (0.40–2.31)	0.64 (0.19–2.13)	1.14) (0.40–3.21	1.05 (0.44–2.53)
X. Respiratory	2.30 (0.96–5.50)	1.16 (0.60–2.22)	1.07 (0.55–2.07)	2.09 (0.92–4.72)
XVIII. Symptoms & signs	1.03 (0.47–2.27)	0.67 (0.26–1.70)	0.94 (0.44–2.01)	0.78 (0.30–2.05)
Charlson comorbidity score				
0–3	1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)
4–5	2.31 (1.04–5.10)	1.43 (0.77–2.68)	1.68 (0.91–3.10)	1.14 (0.51–2.56)
≥6	1.77 (0.76–4.10)	4.35 (1.67–11.32)	2.80 (1.20–6.52)	2.11 (0.80–5.54)

CI, confidence interval; HR, hazard ratio; MSLN, mesothelin; PD-L1, programmed cell death 1 ligand 1.

type, age at diagnosis, sex or chemotherapy. However, the fact that our results indicated that the association between MSLN and survival was evidently less confounded by other factors such as histology than PD-L1, makes it potentially more useful as a prognostic marker. The current and future paradigm of personalized, proactive and preventive healthcare administration makes it very important to understand mechanisms of drug action at the populational level. This, in turn, would help in optimizing combination treatments. Our study approach to investigate clinical and molecular epidemiology questions in MM – use of secondary data sources for clinical and demographic information linked to biological samples from a well-established academic biobank – proved to be feasible and cost-efficient. In addition, it showed a strong potential for further use in the area of oncology and other therapeutic areas such as cardiovascular disease. The results of this manuscript are of particular interest considering the recent FDA approval in October 2020 of the immune checkpoint inhibitor drug combination nivolumab and ipilimumab as first-line treatment for unresectable malignant pleural mesothelioma, based on the prespecified interim analysis of the phase III CheckMate-743 study [46]. In CheckMate-743 [47] patients were not required to express PD-L1 and, in light of our data, it would be very interesting to retrospectively analyse the tumour tissues from that study for expression of PD-L1 and MSLN.

Our results indicate that PD-L1 and MSLN expression in mesothelioma tumors is often not concomitant, and this may be driven by the histological type. In addition, MSLN and PD-L1 overexpression in the tumour cells may indicate a better prognosis independent of other factors such as histology. Further studies are needed to confirm these results in different mesothelioma patient populations, as well as in

Table. 4
Hazard ratios (95% CI) for death by MSLN/PD-L1 tumour overexpression status.

MSLN & PD-L1 overexpression status	Crude HR (95% CI)	Adjusted* HR (95% CI)
MSLN+ / PD-L1+	1.00	1.00
MSLN+ / PD-L1-	1.47 (0.78–2.79)	2.13 (0.97–4.67)
MSLN- / PD-L1-	1.92 (0.94–3.90)	3.03 (1.35–6.77)
MSLN- / PD-L1+	3.83 (1.85–7.94)	4.18 (1.71–10.23)

* Adjusted for gender, overexpression of MSLN and PD-L1, histology, exposure to asbestos, radiotherapy, chemotherapy, and Charlson comorbidity score. CI, confidence interval; HR, hazard ratio; MSLN, mesothelin; PD-L1, programmed cell death 1 ligand 1.

Table. 5
Hazard ratios (95% CI) of death associated with various factors and overexpression of both MSLN and PD-L1.

	Age & sex-adjusted HR (95% CI)	Multivariable-adjusted*HR (95% CI)
Overexpression of MSLN	0.56 (0.36–0.88)	0.53 (0.31–0.91)
Overexpression of PD-L1	1.15 (0.73–1.80)	0.56 (0.32–0.99)
Histology		
Epithelioid	1.00 (reference)	1.00 (reference)
Mixed	2.84 (1.30–6.22)	4.62 (1.89–11.36)
Sarcomatoid	2.33 (1.19–4.55)	1.82 (0.83–3.98)
Exposure to asbestos	1.18 (0.74–1.89)	1.00 (0.60–1.65)
Radiotherapy	0.61 (0.39–0.96)	0.63 (0.39–1.04)
Chemotherapy sequence		
None	1.00 (reference)	1.00 (reference)
P & P maintained	0.57 (0.28–1.16)	0.50 (0.23–1.12)
P & P followed by other therapy	0.24 (0.11–1.20)	0.16 (0.06–0.39)
Monotherapy	0.40 (0.14–1.20)	0.57 (0.18–1.85)
Comorbidities		
II. Neoplasms	0.83 (0.48–1.42)	–
IV. Endocrine	1.41 (0.69–2.87)	–
IX. Circulatory	0.95 (0.50–1.79)	–
X. Respiratory	1.33 (0.79–2.45)	–
XVIII. Symptoms & signs	0.86 (0.48–1.55)	–
Charlson comorbidity score		
0–3	1.00 (reference)	1.00 (reference)
4–5	1.71 (1.03–2.83)	1.29 (0.75–2.22)
≥6	2.57 (1.38–4.80)	1.77 (0.92–3.41)

* Adjusted for gender, overexpression of MSLN and PD-L1, histology, exposure to asbestos, radiotherapy, chemotherapy, and Charlson comorbidity score.

different tumour types such as ovarian cancer that commonly express MSLN and PD-L1.

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6. Role of the funding source

David Vizcaya, Bahman Farahmand, Annette O. Walter, Christoph Kneip and Arndt A. Schmitz (all employees of Bayer, the funder of the study) were involved in the study design (DV, BF, and AS), collection, analysis and interpretation of data (all), writing the manuscript draft (DV), reviewing the manuscript (all) and the decision to submit the article for publication (all).

CRediT authorship contribution statement

David Vizcaya: Conceptualization, Methodology, Writing - original draft, Writing - review & editing, Visualization, Supervision, Project administration, Funding acquisition. **Bahman Farahmand:** Conceptualization, Methodology, Software, Data curation, Formal analysis, Investigation, Writing - review & editing, Visualization. **Annette O. Walter:** Conceptualization, Writing - review & editing, Visualization. **Christoph Kneip:** Conceptualization, Methodology, Writing - review & editing, Visualization. **Korinna Jöhrens:** Methodology, Resources, Investigation, Writing - review & editing. **Mikko Tukiainen:** Methodology, Resources, Investigation, Writing - review & editing. **Arndt A. Schmitz:** Conceptualization, Methodology, Writing - review & editing, Visualization.

Declaration of Competing Interest

DV, BF, AOW, CK and AAS are full-time employees of Bayer. AS also holds stocks of Bayer AG. KJ and MT have no interests to declare.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ctarc.2020.100260.

References

[1] AJ Moore, RJ Parker, J Wiggins, Malignant mesothelioma, Orphanet. J. Rare Dis. 3 (2008) 34.
[2] C Bianchi, L Giarelli, G Grandi, et al., Latency periods in asbestos-related mesothelioma of the pleura, Eur. J. Cancer Prev. 6 (1997) 162–166.
[3] L Stayner, LS Welch, R Lemen, The worldwide pandemic of asbestos-related diseases, Annu. Rev. Public Health 34 (2013) 205–216.
[4] A Marinaccio, A Binazzi, G Cauzillo, et al., Analysis of latency time and its determinants in asbestos related malignant mesothelioma cases of the Italian register, Eur. J. Cancer 43 (2007) 2722–2728.
[5] GLOBOCAN. Cancer Today. Available at https://gco.iarc.fr/today/online-analysis-table.
[6] SI Ferlay J, M Ervik, R Dikshit, S Eser, C Mathers, et al., GLOBOCAN 2012 v1.0, Cancer Incidence and Mortality Worldwide: IARC CancerBase No. 11, International Agency for Research on Cancer, Lyon, France, 2013 [cited 2016]. Available from, http://globocan.iarc.fr.
[7] EK Park, K Takahashi, T Hoshuyama, et al., Global magnitude of reported and unreported mesothelioma, Environ. Health Perspect. 119 (2011) 514–518.
[8] D Vizcaya, K Rough, L Stayner, et al., Estimation of country-specific mesothelioma deaths: a multivariate predictive model based on asbestos use, Occup. Environ. Med. 73 (2016).
[9] W Amin, F Linkov, DP Landsittel, et al., Factors influencing malignant mesothelioma survival: a retrospective review of the National Mesothelioma Virtual Bank cohort, F1000Res 7 (2018) 1184.
[10] V Verma, CA Ahern, CG Berlind, et al., Survival by histologic subtype of malignant pleural mesothelioma and the impact of surgical resection on overall survival, Clin. Lung. Cancer 19 (2018) e901–e912.
[11] P Baas, D Fennell, KM Kerr, et al., Malignant pleural mesothelioma: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up, Ann. Oncol. 26 (5) (2015) v31–v39. Suppl.
[12] B Addis, H. Roche, Problems in mesothelioma diagnosis, Histopathology 54 (2009) 55–68.
[13] P Krasuski, A Poniecka, E Gal, The diagnostic challenge of peritoneal mesothelioma, Arch. Gynecol. Obstet. 266 (2002) 130–132.
[14] J Creaney, IM Dick, TM Meniawy, et al., Comparison of fibulin-3 and mesothelin as markers in malignant mesothelioma, Thorax 69 (2014) 895–902.
[15] A Morello, M Sadelain, PS Adusumilli, Mesothelin-targeted CARs: driving T cells to solid tumors, Cancer Discov. 6 (2016) 133–146.
[16] R Hassan, A Thomas, C Alewine, et al., Mesothelin immunotherapy for cancer: ready for prime time? J. Clin. Oncol. 34 (2016) 4171–4179.
[17] A Scherpereel, F Wallyn, SM Albelda, et al., Novel therapies for malignant pleural mesothelioma, Lancet Oncol. 19 (2018) e161–e172.
[18] UB Hagemann, C Ellingsen, J Schuhmacher, et al., Mesothelin-targeted thorium-227 conjugate (MSLN-TTC): preclinical evaluation of a new targeted alpha therapy for mesothelin-positive cancers, Clin. Cancer Res. 25 (2019) 4723–4734.
[19] R Cornelissen, Aerts J.Abstract 2249, Checkpoint inhibitor therapy after dendritic cell vaccination elicits tumor response in mesothelioma patients, AACR (2019).
[20] J Creaney, D Yeoman, LK Naumoff, et al., Soluble mesothelin in effusions: a useful tool for the diagnosis of malignant mesothelioma, Thorax 62 (2007) 569–576.
[21] BWS Robinson, RA. Lake, Advances in malignant mesothelioma, New Engl. J. Med. 353 (2005) 1591–1603.
[22] K Hollevoet, JB Reitsma, J Creaney, et al., Serum mesothelin for diagnosing malignant pleural mesothelioma: an individual patient data meta-analysis, J. Clin. Oncol. 30 (2012) 1541–1549.
[23] M Linch, S Gennatas, S Kazikin, et al., A serum mesothelin level is a prognostic indicator for patients with malignant mesothelioma in routine clinical practice, BMC Cancer 14 (2014) 674.
[24] J Creaney, RJ Francis, IM Dick, et al., Serum soluble mesothelin concentrations in malignant pleural mesothelioma: relationship to tumor volume, clinical stage and changes in tumor burden, Clin. Cancer Res. 17 (2011) 1181–1189.
[25] P Wheatley-Price, B Yang, D Patsios, et al., Soluble mesothelin-related Peptide and osteopontin as markers of response in malignant mesothelioma, J. Clin. Oncol. 28 (2010) 3316–3322.
[26] BD Grigoriu, A Scherpereel, P Devos, et al., Utility of osteopontin and serum mesothelin in malignant pleural mesothelioma diagnosis and prognosis assessment, Clin. Cancer Res. 13 (2007) 2928–2935.
[27] S Cedres, S Ponce-Aix, J Zugazagoitia, et al., Analysis of expression of programmed cell death 1 ligand 1 (PD-L1) in malignant pleural mesothelioma (MPM), PLoS ONE 10 (2015), e0121071.
[28] S Valmary-Degano, P Colpart, L Villeneuve, et al., Immunohistochemical evaluation of two antibodies against PD-L1 and prognostic significance of PD-L1 expression in epithelioid peritoneal malignant mesothelioma: a RENAPE study, Eur. J. Surg. Oncol. 43 (2017) 1915–1923.
[29] S Khanna, A Thomas, D Abate-Daga, et al., Malignant mesothelioma effusions are infiltrated by CD3(+) T cells highly expressing PD-L1 and the PD-L1(+) tumor cells within these effusions are susceptible to ADCC by the anti-PD-L1 antibody Avelumab, J. Thorac. Oncol. 11 (2016) 1993–2005.
[30] C Combaz-Lair, F Galateau-Salle, A McLeer-Florin, et al., Immune biomarkers PD-1/PD-L1 and TLR3 in malignant pleural mesotheliomas, Hum. Pathol. 52 (2016) 9–18.
[31] S Brosseau, C Danel, A Scherpereel, et al., Shorter survival in malignant pleural mesothelioma patients with high pd-l1 expression associated with sarcomatoid or biphasic histology subtype: a series of 214 cases from the bio-MAPS cohort, Clin. Lung Cancer 20 (2019) e564–e575.
[32] S Cedres, S Ponce-Aix, N Pardo-Aranda, et al., Analysis of expression of PTEN/PI3K pathway and programmed cell death ligand 1 (PD-L1) in malignant pleural mesothelioma (MPM), Lung Cancer 96 (2016) 1–6.
[33] T Watanabe, K Okuda, T Murase, et al., Four immunohistochemical assays to measure the PD-L1 expression in malignant pleural mesothelioma, Oncotarget 9 (2018) 20769–20780.
[34] M Quanz, UB Hagemann, S Zitzmann-Kolbe, et al., Anetumab ravtansine inhibits tumor growth and shows additive effect in combination with targeted agents and chemotherapy in mesothelin-expressing human ovarian cancer models, Oncotarget 9 (2018) 34103–34121.
[35] FR Hirsch, M Varella-Garcia, PA, Jr. Bunn, et al., Epidermal growth factor receptor in non-small-cell lung carcinomas: correlation between gene copy number and protein expression and impact on prognosis, J. Clin. Oncol. 21 (2003) 3798–3807.
[36] T John, G Liu, MS Tsao, Overview of molecular testing in non-small-cell lung cancer: mutational analysis, gene copy number, protein expression and other biomarkers of EGFR for the prediction of response to tyrosine kinase inhibitors, Oncogene 28 (2009) S14–S23. Suppl 1.
[37] R Hassan, R Jennens, JPV Meerbeeck, et al., A pivotal randomized phase II study of anetumab ravtansine or vinorelbine in patients with advanced or metastatic pleural mesothelioma after progression on platinum/pemetrexed-based chemotherapy (NCT02610140), J. Clin. Oncol. (2016) 34. TPS8576-TPS8576.

[38] HL Kindler, S Novello, D Fennell, et al., OA 02.01 Randomized Phase II study of anetumab ravtansine or vinorelbine in patients with metastatic pleural mesothelioma, J. Thoracic Oncol. 12 (2017). S1746.
[39] G Hutarew, PD-L1 testing, fit for routine evaluation? From a pathologist's point of view, Memo 9 (2016) 201–206.
[40] R Hassan, M Ho, Mesothelin targeted cancer immunotherapy, Eur. J. Cancer 44 (2008) 46–53.
[41] K Baba, S Ishigami, T Arigami, et al., Mesothelin expression correlates with prolonged patient survival in gastric cancer, J. Surg. Oncol. 105 (2012) 195–199.
[42] MJ Yen, CY Hsu, TL Mao, et al., Diffuse mesothelin expression correlates with prolonged patient survival in ovarian serous carcinoma, Clin. Cancer. Res. 12 (2006) 827–831.
[43] R Nomura, H Fujii, M Abe, et al., Mesothelin expression is a prognostic factor in cholangiocellular carcinoma, Int. Surg. 98 (2013) 164–169.
[44] M Li, U Bharadwaj, R Zhang, et al., Mesothelin is a malignant factor and therapeutic vaccine target for pancreatic cancer, Mol. Cancer Ther. 7 (2008) 286–296.
[45] L Teppo, E Pukkala, M Lehtonen, Data quality and quality control of a population-based cancer registry: experience in Finland, Acta Oncol. (Madr.) 33 (1994) 365–369.
[46] P Baas, A Scherpereel, A Nowak, et al., ID:2908 first-line nivolumab + ipilimumab vs chemotherapy in unresectable malignant pleural mesothelioma: checkMate 743, J. Thoracic Oncol. 15 (2020) e42.
[47] JM Mankor, MJ Disselhorst, M Poncin, et al., Efficacy of nivolumab and ipilimumab in patients with malignant pleural mesothelioma is related to a subtype of effector memory cytotoxic T cells: translational evidence from two clinical trials, EBioMedicine 62 (2020), 103040.