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High degree of heterogeneity of PD-L1 and PD-1 from primary to metastatic endometrial cancer

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HIGHLIGHTS

- PD-L1 and PD-1 are frequently expressed in endometrial cancer, both across MSS and MSI.
- PD-L1 and PD-1 are not associated with prognosis in endometrial cancer.
- PD-L1 and PD-1 expression in primary tumors and corresponding metastases are discordant and expression is intra-variable.

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ABSTRACT

Objective. PD-L1 and PD-1 are predictive markers for immunotherapy and increasingly relevant in endometrial cancer. The reported fraction of positive primary tumors has been inconsistent. We investigated the expression of PD-L1 and PD-1 in primary tumors, also stratified by MSI. As immunotherapy is foremost relevant for metastatic disease, PD-L1 and PD-1 expression was also assessed in corresponding metastatic lesions.

Methods. PD-L1 and PD-1 was investigated in a prospective, population based endometrial cancer cohort of 700 patients with corresponding metastatic lesions from 68 and 74 patients respectively. Fresh tissue was used for gene expression analysis.

Results. In primary tumors, PD-L1 and PD-1 are expressed in 59% and 63%, respectively, but with no impact on survival, nor when stratified for MSS and MSI. Expression patterns of PD-L1 and PD-1 are similar in MSI and MSS tumors. Available metastatic lesions show heterogeneous expression of PD-L1 and PD-1. In gene expression analysis several genes related to immunological activity, including *CD274* (encoding for PD-L1), were upregulated in PD-1 positive tumors.

Conclusion. PD-L1 and PD-1 are frequently expressed in endometrial cancer and expression patterns are similar across MSS and MSI tumors. Expression in corresponding metastatic lesions is discordant compared to primary tumors. These findings are in particular relevant for treatment decisions in advanced and recurrent disease.

1. Background

Endometrial cancer is the most common gynecological malignancy in the Western world, and the incidence is increasing due to the higher prevalence of obesity and the longer life expectancy [1,2]. Although the prognosis is generally good, about 15–20% of patients experience recurrence and little improvement in survival has been achieved the last decades for advanced, recurrent and metastatic disease. Chemotherapy has been the standard of care with modest response rates [3]. In recent years, treatment with immune checkpoint inhibitors has emerged as a major therapeutic modality in oncology [4]. After the FDA approved pembrolizumab (PD-1-inhibitor) for treatment of microsatellite instable recurrent and metastatic endometrial cancer, treatment with immune checkpoint inhibitors has become an option also for endometrial cancer patients [5]. The KEYNOTE-028 study with pembrolizumab given to PD-L1 positive, advanced MSI-high endometrial cancer has demonstrated promising results [6]. However, the expression patterns of PD-L1 and PD-1 in primary tumors and metastases in particular have not been fully explored in endometrial cancer.

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PD-L1 is expressed on tumor cells and macrophages and binds to the receptor PD-1 on tumor-infiltrating CD4 and CD8 T-cells. Binding of PD-L1 to PD-1 suppresses the T-cell, a negative feedback system that represses the immune system [5]. Blockade of this pathway with antibodies to PD-L1 or PD-1, which re-activates the immune system has become an increasingly used treatment modality with promising response rates in the recurrent and metastatic setting in solid tumors. Results have demonstrated less toxicity than chemotherapeutic regimens and often more durable responses [7–9]. In endometrial cancer, immune checkpoint inhibitors to PD-L1 and PD-1 are increasingly studied and they are an attractive option for treatment [10]. TCGA (The Cancer Genome Atlas) classified endometrial cancer into four distinct molecular subtypes and gave momentum to further research on targeted therapy [11]. One of the four identified molecular subtypes, the microsatellite instable (MSI) tumors has demonstrated increased mutational burden that creates numerous neo-antigens responsible for the immune response. MSI has become an established predictive marker for response to immunotherapy in solid tumors [12–14], and PD-1 blockade has demonstrated better response rates in tumors with mismatch-repair deficiency compared with mismatch repair-proficient cancers [15]. However, only 20–30% of endometrial cancer patients are MSI-high, making only a fraction eligible for treatment with immune checkpoint inhibitors using this stratification [11]. Anti-tumor activity was demonstrated in a study with combination therapy with pembrolizumab and lenvatinib (VEGF-inhibitors) to biomarker unselected advanced endometrial cancer, supporting that treatment with immune checkpoint inhibitors may not only be reserved for MSI-high patients [16]. Also, promising results with combination therapy with PD-1 blockade and cytotoxic T-lymphocyte-associated protein-4 (CTLA-4) to MSS-tumors have been reported [17].

The expression of PD-L1 and PD-1 in endometrial cancer has previously been described in primary tumors in smaller cohorts, however, the previously described rates of expression in primary tumors have been inconsistent [18–20]. Moreover, the expression of PD-L1 and PD-1 in corresponding metastatic lesions has to our knowledge not been investigated in endometrial cancer. Expression patterns in metastatic lesions are of particular importance as treatment with immune checkpoint inhibitors has been of foremost relevance in the recurrent and metastatic setting. We aimed to investigate the expression of PD-L1 and PD-1 in primary tumors and corresponding metastatic lesions in both microsatellite stable and instable cancers, in relation to clinicopathological characteristics and follow-up.

2. Materials and methods

2.1. Patient samples

Patients included in the study were all diagnosed and treated at Haukeland University Hospital, Bergen, Norway and diagnosed with endometrial cancer from 2001 to 2015. All samples included in the study were retrieved from the Bergen Biobank for Gynecological Cancer (REK number 2014/1907). The biobank was collected prospectively after patients had given informed written consent. All parts of the study has been approved according to Norwegian legislation and Western Regional Committee for Medical and Health Research Ethics (REK 2009/2315 and 2014/1907). All patients were staged according to FIGO 2009 criteria and clinical data and follow-up were obtained from clinical records as previously described [21]. Formalin-fixed paraffin embedded (FFPE) tissue from 689 and 737 was used for immunohistochemistry for expression of PD-L1 and PD-1, respectively. Additionally, 275 corresponding metastases from 68 patients were assessed for PD-L1 expression, and 273 corresponding metastases from 74 patients were assessed for PD-1 expression. Number of corresponding metastases from each patient ranged from one to eight. Transcriptional alterations related to PD-L1 and PD-1 protein expression were investigated by mRNA microarray analysis for 260 freshly frozen samples.

2.2. Immunohistochemistry

Tissue microarrays (TMAs) from FFPE were constructed as previously described [22]. Briefly, the area with highest tumor grade was identified on hematoxylin and eosin stained slides. Tissue cylinders of 0.6 mm were punched out and mounted in a paraffin block using a custom-made precision instrument (Beecher Instruments, Silver Spring, MD, USA). Three cylinders were punched for primary tumors and one cylinder for metastatic lesions. TMA slides (5 μm) were cut and dewaxed with xylene and rehydrated in ethanol before microwave antigen retrieval in target retrieval solution, pH 6 for PD-L1 and pH 9 for PD-1, MSH6 and PMS2. Following peroxidase block, the TMAs were incubated for 1 h at room temperature with rabbit monoclonal antibody to PD-L1 (1:100; no. E1L3N, Cell Signaling, Danvers, MA, USA) or rabbit monoclonal antibody to PD-1 (1:300; no. D4W2J, Cell Signaling, Danvers, MA, USA) followed by 30 min of incubation with secondary HRP-conjugated anti-rabbit antibody and 8 min with DAB-chromogen (EnVision detection system, Dako, Glostrup, Denmark). Mouse monoclonal antibody to PMS2 (1:25; no PMS2-L-CE; Leica Biosystems, Wetzlar, Germany) and mouse monoclonal antibody to MSH6 (1:25; no MSH6-L-CE; Leica Biosystems, Wetzlar, Germany) were incubated for 1 h before incubation with secondary HRP-conjugated anti-mouse antibody for 30 min in room temperature and finally 3 min with DAB-chromogen (EnVision detection system, Dako, Glostrup, Denmark). Sections were counterstained in hematoxylin before dehydration and mounting. The immunostained sections were reviewed by light microscopy and scored visually by a semiquantitative and subjective method.

2.3. Evaluation of staining

Evaluation of staining was performed blinded for the clinical characteristics and outcome. For PD-L1, a staining index was calculated as a product of staining intensity (0–3) and area of positive tumor cells ($1 < 10\%$, $2 = 10\%–50\%$ and $3 > 50\%$). No expression was seen in stroma, and subsequently only glandular expression was evaluated. Expression was mainly cytoplasmic, however some membranous localization was seen and a score was given irrespectively of cellular localization of PD-L1. In subsequent statistical analyses, indexes were grouped in quartiles, considering the size of the subgroups and the number of events in each category. Quartile division was selected according to similarity in survival in each quartile. The lower quartile corresponded to negative (staining index = 0) expression only, quartile 2 to 4 were merged together and subsequently cut off was negative/positive. For PD-1, expression was evaluated as positive when >5% of stromal staining was detected. Two independent observers evaluated 88 cases and the κ-value was 0.74 for PD-L1 and 0.72 for PD-1. MSI tumors were identified by loss of one of the two mismatch-repair proteins, MSH6 and PMS2 by immunohistochemical staining according the published Promise classifier [23,24]. Positive stromal staining was used as internal control. For MSH6 and PMS2, staining was defined as negative when <10% glandular staining was observed. For negative cases with no stromal staining (lack of positive control), full sections were stained to determine status [23,24]. If either MSH6 or PMS2 was negative, cases were defined as negative and thus MSI.

2.4. Gene expression analysis

Gene expression alterations in relation to PD-L1 and PD-1 expression were investigated in previously generated microarray gene expression data from 260 primary endometrial cancers. RNA was extracted from fresh frozen tissue using the RNeasy Mini Kit (Qiagen, Hilden, Germany) and hybridised to Agilent Whole Human Genome Microarrays 44k (Cat.no. G4112F), according to the manufacturer's

instructions [25]. Overlapping data on both gene expression and IHC was available from 221 patients and were used in subsequent analysis. The expression data were normalised using quantile normalisation. Median spot signal was used as intensity measure. Differentially expressed genes in tumors expressing PD-1 were identified using SAM (significance analyses of microarray) (False Discovery Rate < 0.001, Fold Change >1.5). GSEA (gene set enrichment analyses) was performed applying gene sets from Molecular Signatures Database (MSigDb, version 6.2). Analyses were performed using the J-Express software (Molmine, Bergen, Norway).

2.5. Statistical analysis

Statistical analyses were performed using IBM SPSS Statistics software version 25 (IBM, Armonk, NY, USA). *p*-Values < 0.05 were considered statistically significant and all *p*-values were two-sided. Pearson's Chi-Square test or Fisher's exact test, when appropriate, were used for comparison between groups of categorical variables. The log-rank test, Kaplan-Meier method was used for univariate survival analysis of time to death due to endometrial cancer (disease-specific survival). The date of primary treatment is defined as entry

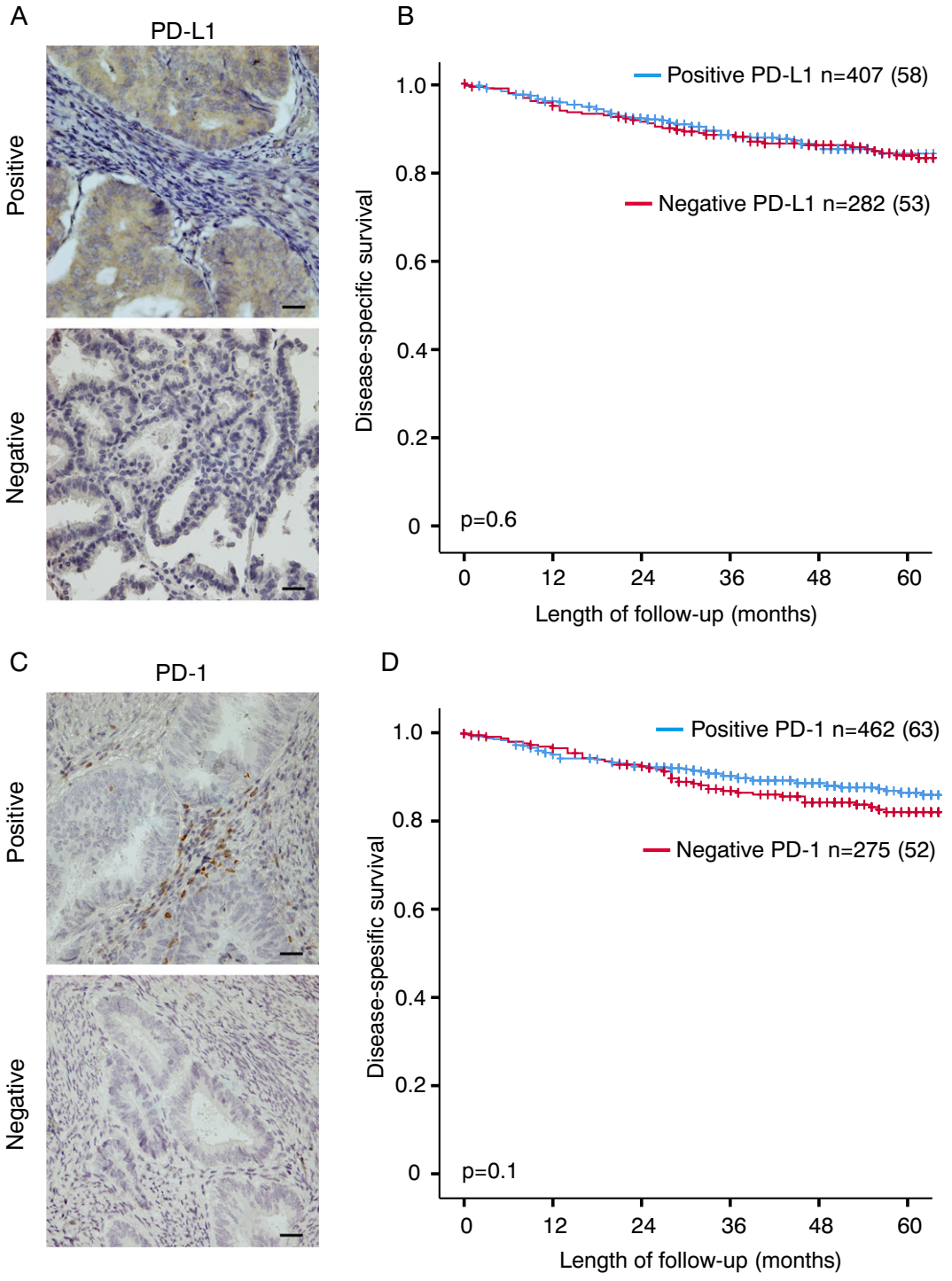

Fig. 1. Expression of PD-L1 and PD-1 demonstrates no difference in survival. A, Expression of PD-L1 is mainly glandular and cytoplasmic, 1 bar = 10 μm. A, Kaplan-Meier survival plot showing PD-L1 expression. Number of cases in each group is given and number of deaths in parenthesis. C, Expression of PD-1 is mainly stromal, 1 bar = 10 μm. D, Kaplan-Meier survival plot showing PD-1 expression. Number of cases in each group is given and number of deaths in parenthesis.

date. Patients who died from other causes were censored at the date of death.

3. Results

3.1. PD-L1 expression is independent of endometrial cancer type and grade, and has no impact on survival

Expression patterns of PD-L1 were evaluated by IHC in 689 endometrial cancer samples. PD-L1 was expressed in 59% of primary tumors and the staining was mainly glandular (Fig. 1A). PD-L1 expression was not associated with any specific patient subgroup, as positive expression of PD-L1 did not associate with age, FIGO stage, histologic type, deep myometrial infiltration or recurrence (Table 1). No significant difference in disease-specific survival was seen between patients with PD-L1 expression and patients with no expression of PD-L1 (Fig. 1B). No significant impact of PD-L1 expression on disease-specific survival was seen within the subgroup of endometrioid (5-year disease-specific survival, positive: 95% vs. negative: 91%, $p = 0.21$) or non-endometrioid histology (5-year disease-specific survival, positive: 51% vs. negative: 32%, $p = 0.74$).

3.2. Positive PD-1 expression associates with non-endometrioid histology, but not with prognosis

PD-1 expression was evaluated in 737 endometrial cancer samples by IHC. PD-1 was expressed in 63% of primary tumors and the staining pattern was mainly stromal (Fig. 1C). Positive PD-1 was significantly associated with low age (younger than 66; $p = 0.03$) and non-endometrioid histology ($p = 0.01$, Table 2), but not with FIGO stage, grade or myometrial infiltration. No difference in disease-specific survival between PD-1 positive and PD-1 negative patients was observed (Fig. 1D). When stratifying for patients with endometrioid histology, positive PD-1 was associated with better disease-specific survival compared to negative PD-1 (5-year disease-specific survival, positive: 96% vs. negative: 89%, $p = 0.02$). In patients with non-endometrioid histology PD-1 did not predict disease-specific survival (5-year disease-specific survival, positive: 60% vs. negative: 41%, $p = 0.23$).

In patients with microsatellite instable tumors, PD-L1 and PD-1 associate with favorable clinical characteristics but are not related to survival.

MSI tumors were identified by loss of one of the two mismatch-repair proteins, MSH6 and PMS2 by IHC. Patients with MSI-tumors have been considered better responders to checkpoint inhibitors [13,14] and establishing PD-L1/PD-1 expression level in patients with MSI-tumors is therefore important. In MSI-tumors PD-L1 expression associated with grade 1 tumors ($p = 0.005$, Supplementary Table 3), while PD-1 expression was not associated with any clinical characteristics (Supplementary Table 3). Neither PD-L1, nor PD-1 had impact on disease-specific survival (Fig. 2A and B).

In detailed analysis comparing MSS and MSI-tumors, no difference in expression patterns of PD-L1 and PD-1 was demonstrated (Supplementary Table 4). Overall, this supports that potential responders to PD-L1/PD-1 directed drugs are evenly distributed in the cohort.

3.3. Patients with PD-1 positive tumors show upregulation of genes associated with increased immunological activity

Patients were grouped according to PD-L1 or PD-1 status defined by IHC, and gene expression analyses were performed. Stratifying patients according to PD-L1 status did not result in any differentially expressed genes between the two groups, while several genes related to immunological activity, including *CD274* (encoding for PD-L1), were upregulated in PD-1 positive tumors. This might suggest that expression of PD-1 is linked to immune activation (false discovery rate; FDR < 0.001, Fold Change >1.5 in SAM analysis, Supplementary Table 5). To further

Table 1
PD-L1 expression related to clinicopathological variables in 689 patients with endometrial cancer.

	Negative, n (%)	Positive, n (%)	*p*-Value*
Age, y			0.56
<66	147 (42)	203 (58)	
≥66	135 (40)	204 (60)	
FIGO			0.90
I/II	238 (41)	342 (59)	
III/IV	44 (40)	65 (60)	
Histologic type			0.17
Endometrioid	235 (42)	322 (58)	
Non-endometrioid	47 (36)	85 (64)	
Non-endometrioid types			0.43#
Clear cell	8 (33)	16 (67)	
Serous	24 (37)	41 (63)	
Carcinosarcomas	13 (42)	18 (58)	
Undifferentiated	2 (17)	10 (83)	
Histologic grade**			0.02
Grade 1	105 (40)	159 (60)	
Grade 2	94 (50)	94 (50)	
Grade 3	32 (33)	64 (67)	
Myometrial infiltration			0.83
<50%	174 (41)	254 (59)	
≥50%	107 (42)	151 (58)	
MSI-status			0.24
Microsatellite stable	156 (42)	217 (58)	
Microsatellite instable	30 (50)	30 (50)	
Recurrence			0.5
Yes	63 (54)	53 (46)	
No	318 (60)	213 (40)	
Metastatic at primary	26 (62)	16 (38)	

FIGO: International Federation of Gynecology and Obstetrics. MSI: microsatellite instable.
* *p*-Values are calculated with Chi-Square test.
\# *p*-Values are calculated with Fisher exact test.
** Endometrioid included only.

Table 2
PD-1 expression related to clinicopathological variables in 737 patients with endometrial cancer.

	Negative, n (%)	Positive, n (%)	*p*-Value*
Age, y			0.03
<66	130 (34)	256 (66)	
≥66	145 (41)	206 (59)	
FIGO			0.65
I/II	235 (38)	389 (62)	
III/IV	40 (35)	73 (65)	
Histologic type			0.01
Endometrioid	233 (38)	369 (61)	
Non-endometrioid	42 (31)	93 (69)	
Non-endometrioid types			0.01#
Clear cell	3 (12)	23 (88)	
Serous	23 (36)	41 (64)	
Carcinosarcomas	14 (44)	18 (56)	
Undifferentiated	2 (15)	11 (85)	
Histologic grade**			0.11
Grade 1	120 (43)	159 (57)	
Grade 2	74 (36)	133 (64)	
Grade 3	33 (32)	71 (68)	
Myometrial infiltration			0.64
<50%	169 (37)	290 (63)	
≥50%	106 (39)	169 (61)	
MSI-status			0.04
Microsatellite stable	159 (40)	241 (60)	
Microsatellite instable	17 (27)	47 (73)	
Recurrence			0.02
Yes	62 (51)	59 (49)	
No	373 (65)	199 (35)	
Metastatic at primary	27 (61)	17 (38)	

FIGO: International Federation of Gynecology and Obstetrics. MSI: microsatellite instable.
* *p*-Values are calculated with Chi-Square test.
\# *p*-Values are calculated with Fisher exact test.
** Endometrioid included only.

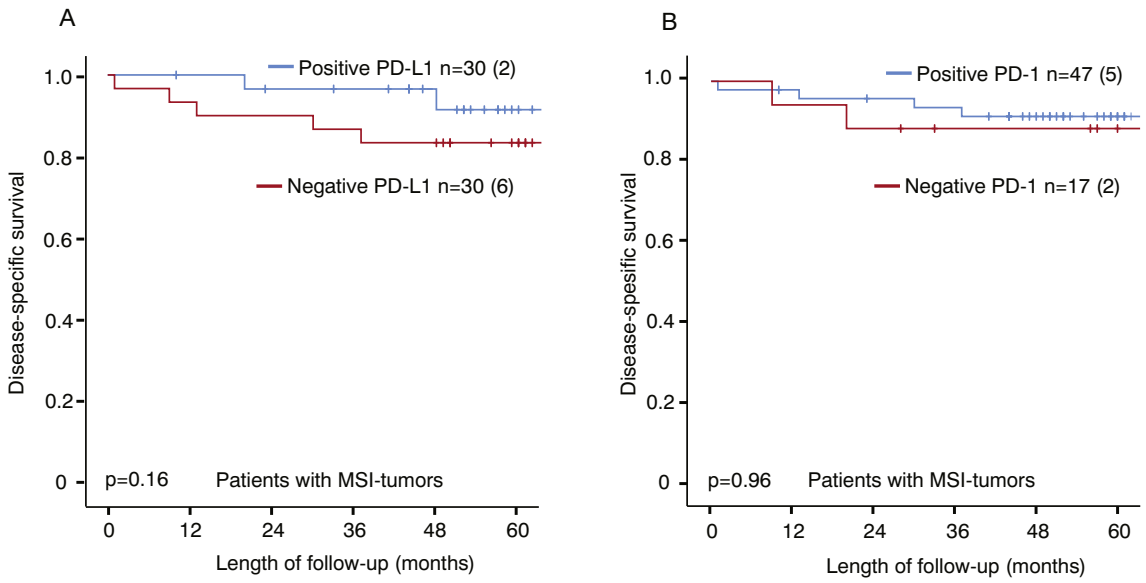

Fig. 2. Expression of PD-L1 and PD-1 in MSI-tumors demonstrates no impact on survival. Kaplan-Meier survival plot for A, PD-L1 expression and B, PD-1 expression. Number of cases in each group is given and number of deaths in parenthesis.

explore the molecular signaling pathways altered in tumors with PD-1 expression, gene set enrichment analysis (GSEA) was performed applying gene sets from Molecular Signatures Database (MSigDb, version 6.2). Tumors with expression of PD-1 show increased immunological activity (Supplementary Table 6) and further suggest that PD-1 is linked to immune activation.

3.4. Heterogeneous expression of PD-L1 and PD-1 from primary tumors to metastatic lesions

Protein expression was investigated in corresponding primary and metastatic lesions by IHC. A detailed overview of expression in individual patients and individual metastases is given in Fig. 3G. For PD-L1, 275 metastases from 68 patients were investigated (Fig. 3A–C). In primary tumors, 77% of these patients expressed PD-L1 (Fig. 3A). Only 40% (n = 21) expressed PD-L1 in all investigated metastatic lesions, while 21% (n = 11) of patients had lesions that were both positive and negative for PD-L1 (Fig. 3B). However, 39% (n = 20) of the patients did not express PD-L1 in any metastatic lesion, in spite of being positive in the primary setting (Fig. 3B). In patients with PD-L1 negative primary tumors (Fig. 3C), 38% (n = 6) of the patients remained negative also in the metastatic setting. However, 31% (n = 5) of the patients had PD-L1 positive metastatic lesions and 31% (n = 5) of the patients had both negative and positive metastatic lesions (Fig. 3C).

273 metastases from 74 patients were investigated for protein expression of PD-1 (Fig. 3D–F). PD-1 was expressed in 70% of primary tumors (Fig. 3D). Among patients with PD-1 positive primary tumors, only 27% (n = 14) remained positive in all metastatic lesions while 21% (n = 11) had both positive and negative metastasis (Fig. 3E). In 52% (n = 27) PD-1 expression was lost from primary to metastatic lesions (Fig. 3E). In patients with primary tumors with no expression of PD-1 (37%) (Fig. 3F), no patients showed expression of PD-1 in the metastatic lesions, as all metastatic lesions investigated were negative for PD-1. When examining all patients investigated in this study, 68% (n = 49) of all patients were negative for PD-1 in the metastatic lesions.

The high degree of discordant expression between biopsies from the primary tumor and corresponding metastatic lesions, and between multiple metastases from the same patient (Fig. 3G), depicts potential response in so far unidentified patient groups, and suggests investigation of protein expression in metastases when assigning patients for PD-L1 and PD-1 inhibitors.

4. Discussion

In the present study we investigated a large, population based and prospectively collected cohort of endometrial cancer. We depict expression of PD-L1 in 59% of patients and PD-1 in 63% of patients. We find that neither PD-L1 nor PD-1 is associated with prognosis, neither in the overall patient group, nor in subgroups of endometrioid or non-endometrioid cancers. PD-L1 was not associated with any clinical characteristics. Expression of PD-L1 has been reported to range from 14% to 36% [18–20], and has been associated with non-endometrioid histology and lymphovascular space invasion, but found no association with survival [18–20]. Results from recent studies investigating the expression patterns of PD-1 in endometrial cancer are also conflicting, with results ranging from 16% to 60% PD-1 positive patients [18,19]. In our study, expression of PD-1 in the overall cohort was significantly associated with low age ($p = 0.01$) and non-endometrioid histology ($p = 0.03$). Other studies report that negative PD-1 is associated with poor differentiation and non-endometrioid histology [18,19]. In regards to survival, no association with survival has previously been reported, which is in line with our findings [19]. Our results concur with previous findings from smaller cohorts which state that PD-L1 and PD-1 have no impact on survival, however results are inconsistent regarding association with clinical characteristics.

Inconsistent reports on fraction of positive tumor cells [26], as well as studies demonstrating differences when comparing biomarker assays for PD-L1 have raised concerns [27]. In the clinical context, the main concern is to ascertain that patients who could benefit from immune checkpoint inhibitors are identified and to keep the number of false-negative cases as low as possible, at the same time avoid false-positive tests and over-treatment with potentially severe side effects. In our study, we used validated antibodies that have previously been used in several publications [28,29]. It is also evident that expression of PD-L1 determined in small tissue biopsies might not be representative of the full tumor specimen. The use of TMAs for evaluating expression of PD-L1 and PD-1 may be a potential bias, and as for all biomarkers clinical implementation relies on validation on full sections [30]. However, previous studies have demonstrated a good sensitivity for detection of PD-L1 in TMAs when using our method of three tissue cylinders [31]. Further studies using large patient samples and preferably biomarker inclusion in clinical trials are vital to establish the robustness of these markers.

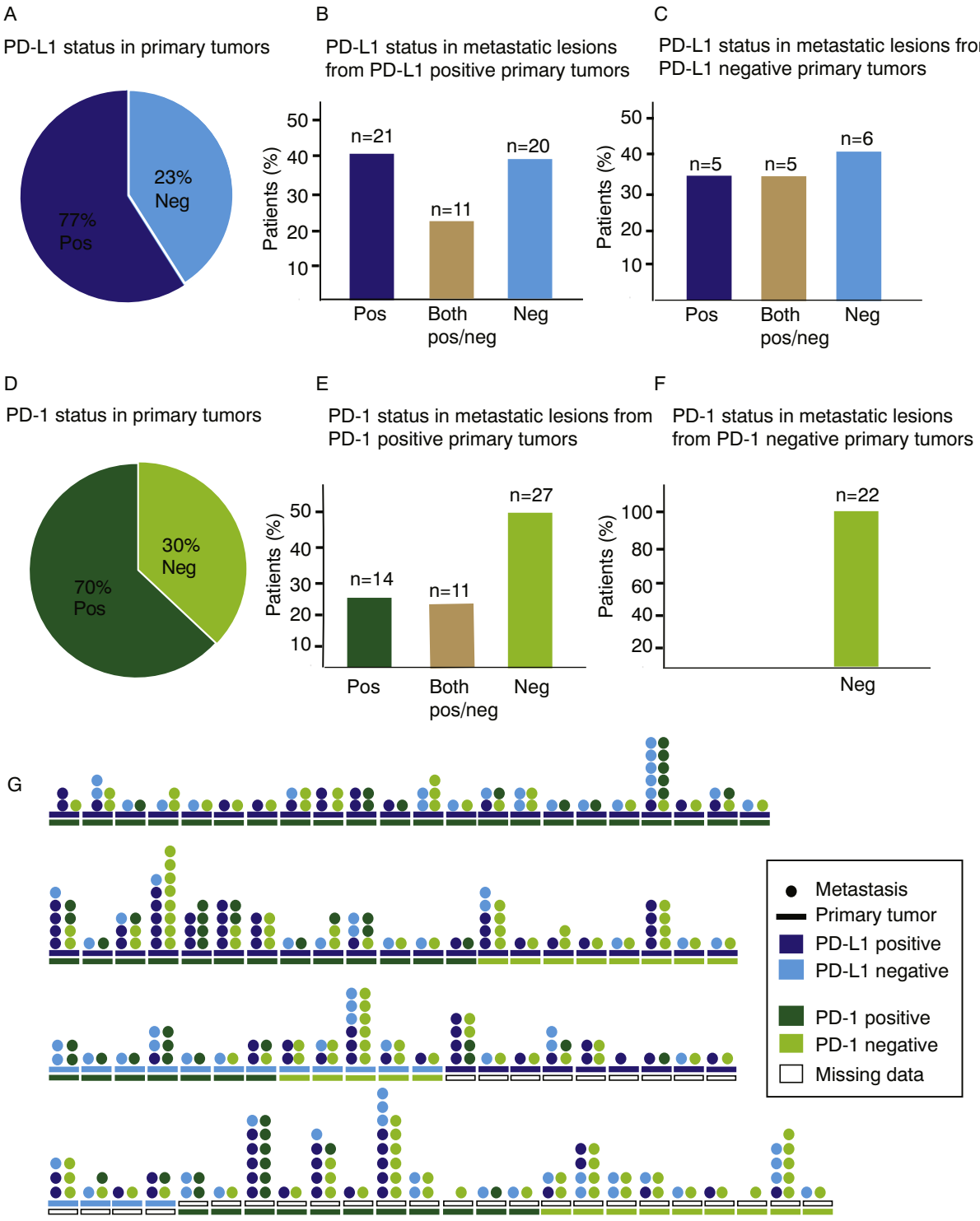

Fig. 3. Expression of PD-L1 and PD-1 in metastatic lesions. A, 77% of lesions were PD-L1 positive in primary lesions. B, Histogram showing PD-L1 status from PD-L1 positive primary tumors and C, PD-L1 status in PD-L1 negative primary tumors. D, 70% of patients were PD-1 positive in primary lesions. E, Histogram showing PD-1 status in PD-1 positive tumors and F, PD-1 negative primary tumors. G, Expression patterns in individual metastases from patients with PD-L1 positive (dark blue), PD-L1 negative (light blue), PD-1 positive (dark green) and PD-1 negative (light green). The primary tumor is illustrated with a bar. One circle represents one metastasis.

Immune checkpoint inhibitors have been suggested for treatment of patients with advanced or recurrent MSI-high, but the fraction of PD-L1 and PD-1 positive patients within this subgroup is still not clearly defined. We here validate the previous observation that neither PD-L1, nor PD-1 have prognostic value in the MSI subgroup. However, previous studies have shown more frequent expression of PD-L1 in MSI tumors compared to MSS tumors [20,32]. Expression of PD-L1 and PD-1 was similar in MSI and MSS tumors in our cohort and no significant differences were found in expression patterns. Recent clinical studies indicate that treatment with immune checkpoint inhibitors may not be exclusively for patients with MSI tumors [16,17]. The robustness of PD-L1 and PD-1 as predictive markers for response to immune checkpoint inhibitors are debated as studies have shown a variable predictive value of PD-L1 [6,33]. Interestingly, gene expression analyses of all patients with

expression of PD-1 identified upregulated genes related to immune activity, including the gene *CD274* (encoding for PD-L1). This might suggest that patients with expression of PD-1 may be responders to immune checkpoint inhibitors, regardless of MSI/MSS status. Clinical trials are needed to further elucidate the role of PD-L1 and PD-1 as predictive markers in endometrial cancer. In a recent biomarker-unselected trial combining pembrolizumab and lenvatinib multikinase inhibitor of VEGFR1–3 in metastatic endometrial cancer, an objective response was recorded in 16/45 patients with MSS-tumors compared to two out of four MSI-tumors [16]. Also, a study testing combination therapy with dostarlimab (PD-1-inhibitor) and chemotherapy (carboplatin and paclitaxel), regardless of MSI-status (NCT03981796) is in the pipeline and the results from this trial will hopefully suggest if also MSS patients are responders.

Immune checkpoint inhibitors to PD-1 in endometrial cancer has so far been approved in the recurrent and advanced setting only [5], however, expression patterns in metastases of endometrial cancers have not previously been investigated. This study finds discordant expression of PD-L1 and PD-1 in primary tumors compared to corresponding metastatic lesions, and a substantial number of the metastases have an intra-variable expression of PD-L1 or PD-1 (see Fig. 3G). Previous studies have found frequent expression of PD-L1 in metastatic colorectal cancer compared to primary tumors and an increase of PD-L1 during disease progression [34]. Also, discordant expression between primary tumors and corresponding metastatic lesions has previously been demonstrated in breast cancer, malignant melanoma and head and neck cancers [35–37]. It is interesting to note that among patients that were positive for PD-L1 and/or PD-1 in the primary tumor, we find that expression was lost in 39% ($n = 20$) of patients with PD-L1 positive primary tumors and in 52% ($n = 27$) of patients with PD-1 positive tumors. Treatment with pembrolizumab to PD-L1 positive, advanced endometrial cancer patients has previously demonstrated durable antitumor activity [6]; however, a variation in response was noted and heterogeneity was deemed as a possible explanation for the lack of response. The observed variation in PD-L1 and PD-1 expression in the metastases might thus be relevant for the response to treatment, also in endometrial cancer. PD-L1/PD-1 points to the need for evaluation of response markers in metastases when treating recurrent or advanced disease, as expression is heterogeneous and may be discordant for primary to metastatic lesions. Future studies exploring PD-L1 and PD-1 expression in metastatic lesions prior to treatment would be interesting to further pinpoint if these biomarkers really can predict response to checkpoint inhibitors.

In the present study, we demonstrate frequent expression of PD-L1 and PD-1 in endometrial cancer, and expression patterns are similar across MSS and MSI tumors. We identify a high number of positive PD-L1 tumors in our series, with 59% of all patients being positive for PD-L1 and 63% positive for PD-1. This inconsistency in the literature regarding frequency of PD-L1 and PD-1 expression may be explained by different antibodies and/or cut-offs for expression, and points to the importance of robust biomarkers and specific guidelines for expression evaluation. In corresponding metastatic lesions, expression is inconsistent and intra-variable compared to primary tumors and this should be considered when treatment strategies are decided. More research is needed to identify patients who may respond from immune checkpoint inhibitors to in endometrial cancer.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ygyno.2020.01.020.

Ethics approval and consent to participate

All parts of the study has been approved according to Norwegian legislation and Western Regional Committee for Medical and Health Research Ethics (REK 2009/2315 and 2014/1907). All patients gave informed written consent.

Consent for publication

Authors give full consent for publication.

Data availability

All data are stated in the manuscript.

Conflict of interest

The authors declare no conflict of interest.

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Author's contributions

H.E. and C.K. conceived and planned the experiments. H.E., H.F.B. and M.M. carried out the experiments. M.K.H., I.M.S., I.S.H., J.T., E.H and L.B. contributed to collection of samples and clinical data. H.E. and C.K. contributed to the interpretation of the results. H.E. and C.K. took the lead in writing the manuscript. C.K. supervised the project. All authors provided critical feedback and helped shape the research, analysis and manuscript.

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