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Composition of the immune microenvironment differs between carcinomas metastatic to the lungs and primary lung carcinomas

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ABSTRACT

Lungs are among the most common sites for development of both primary and metastatic carcinomas. Tumor cells expression (TC) of PD-L1 is an important predictor of the of response to immune check-point inhibition in NSCLC, while the composition of the immune cells (IC) in the tumor microenvironment including PD-L1 + cells is believed to predict responses in tumors of some other primary sites. Total mutational load (TML) and microsatellite instability (MSI) also play a role in response to the immune checkpoint blockade. We investigated immune microenvironment characteristics (PD-1, PD-L1, CD8) of 257 lung biopsies including 81 primary (NSCLC) and 176 metastatic tumors to the lungs. TML and MSI were calculated from massively parallel sequencing (592-gene panel). TC expression of PD-L1 was more common in NSCLC than in metastatic carcinomas (28% vs. 10%, $p = 0.009$), while PD-L1-positive IC were present at relevant percentages (1–5%) exclusively in metastatic carcinomas (31% IC positive vs. 0%, $p < 0.001$). Metastatic carcinomas carried significantly lower TML in comparison with the NSCLCs (6.6 mutations on average vs. 10, $p = 0.01$). All primary NSCLC were microsatellite stable, and only 2 metastatic carcinomas exhibited MSI-H status. The number of PD-1 + and CD8 + tumor infiltrating lymphocytes did not differ significantly between the primary and metastatic carcinomas. Our study revealed significant differences in tumor immune microenvironment (PD-L1 in IC and TC), and its relationship to TML between NSCLC and metastatic cancers. These differences could determine the choice of a predictive biomarker test and subsequently effect(s) of the immune therapy treatments in various advanced cancers.

1. Introduction

Immune checkpoint inhibitors have improved cancer treatment in the recent years, with significant survival benefits in advanced malignancies of diverse lineages (e.g. melanoma, non-small cell lung cancer [NSCLC], renal cell carcinoma, bladder carcinoma, classical Hodgkin lymphoma). Tumor expression of CD274 (programmed cell death 1 ligand 1 or PD-L1) is the most commonly used predictive biomarker for selection of patients for immune check point inhibition, but it is still in need of refinement, particularly differentiating its expression on cancer cells and in the immune cells of the tumor environment [1].

Suppression of the programmed cell death 1 (PD1 encoded by *PDCD1*gene), expressed on activated T-lymphocytes by its ligands PD-L1 and PD-L2 (CD273, *PDCD1LG2*) represent a major immunosuppressive mechanism in the tumor microenvironment [2]. Blockade of that inhibition may reactivate T-cell function and induce their antineoplastic activity [2,3]. PD-L1 expression, measured by immunohistochemistry, can be found in both tumor (cancer) cells (TC) and inflammatory/reactive “immune” cells (IC), and TC of PD-L1has been successfully used to select patients for immune check point inhibitors [4-6]. However, a subset of PD-L1 TC-negative tumors may still respond to the PD-1/PD-L1 blockade while failure to the therapy has been observed in some PD-L1 TC-positive cancers [1]. Therefore, substantial efforts have been invested in refining existing and identifying additional biomarkers that would predict patients' responses to the immune checkpoint inhibition. Consequently, in recurrent and metastatic bladder (urothelial) carcinomas, expression of PD-L1 on immune cells (IC) had been described as a better predictive biomarker to atezolizumab [3,7]. Among other potential predictive biomarkers, increased CD8 + T-cell density and PD-1 overexpression on T-cells, have been investigated [1,2,8-10]. Most recently, an increased expression of the cancer neoantigens and measurement of tumor mutational load and microsatellite instability have emerged as the potent predictors of the response to the immune check point blockade therapies [11,12].

While PD-L1 expression in cancer cells (TC) of the NSCLC has been particularly well characterized, PD-L1 in cancers metastatic to lungs

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(the most common site of dissemination for numerous malignancies) was not. We comparatively analyzed distribution of PD-L1 along with PD-1 and CD8 in neoplastic (TC) and immune cells (IC) of the tumor microenvironment between primary (NSCLC) and metastatic tumors to the lung (carcinomas, sarcomas, melanomas) in order to gain insight in their differences which could lead to improved selection and treatment outcomes for both primary lung carcinomas and for a wide variety of disseminated malignancies.

2. Materials and methods

2.1. Samples

Two-hundred fifty seven formalin-fixed paraffin-embedded tissue samples (81 NSCLC and 176 metastatic tumors to the lung) were profiled at the CLIA/CAP/ISO-certified laboratory (Caris Life Sciences, Phoenix, AZ). Histologic diagnosis for all cases was confirmed by a board certified pathologist (Z.G.) and appropriate slides were used for molecular profiling.

Caris Life Sciences maintains a de-identified database that houses commercial laboratory results stripped of identifiers. The tumor profiling data for this study was obtained from this de-identified database. This analysis was retrospective and only consisted of results that were already stored in the database. This research was compliant with 45 CFR 46.101(b). Therefore, the project was deemed exempt from IRB oversight and consent requirements were waived.

2.2. Immunohistochemistry

The samples were evaluated for PD-L1 (SP142 antibody), PD-1 (NAT105 antibody), and CD8 expression (SP57 antibody) using automated immunohistochemical (IHC) staining methods. Expression of 4 mismatch repair proteins (MMRP) was tested in selected cases (equivocal microsatellite result in NGS analysis) by IHC (MLH1, M1 antibody; MSH2, G2191129 antibody; MSH6, 44 antibody; PMS2, EPR3947 antibody).

PD-L1 positivity was defined as expression of membranous staining at ≥ 5% cells in TCs or ICs as suggested earlier [13-16]. Due to the observed low PD-L1 expression in IC (none of the tumors had PD-L1 positivity in ICs exceeding 5%), when IC was statistically analyzed alone we dichotomized PD-L1 IC variable into two categories (< 1% = negative and ≥ 1% = positive).

PD-1 and CD8 expressions were investigated in the IC (T-lymphocytes, histiocytes and dendritic cells) component. Whenever possible, ten consecutive tumor fields were microscopically reviewed under 40 × objective (high-power field, hpf) and the total number of PD-1 + and CD8 + cells was recorded. In case of small biopsies, the whole slides were evaluated for both markers. Mean cohort values for both variables were used for dichotomization in the statistical analysis.

All cases were further stratified into 4 categories based on the presence or absence of PD-L1 expression on TC or ICs (tumor microenvironment, TME, Table 4) [17].

All cases were evaluated by 2 investigators (W.S. and board-certified pathologist Z.G.); discordances in interpretations were resolved at the double headed microscope evaluation.

2.3. Next-generation sequencing (NGS)

Tumor mutational load (TML) was calculated using the massively parallel (next-generation) sequencing (Illumina NextSeq platform). Only missense mutations that were not previously reported as germline variants were used for TML estimation. NGS panel included 592 genes (list of the genes is available here: http://www.carismolecularintelligence.com/solid_tumors_international).

The TML variable was categorized as follows: Low TML (≤ 6); intermediate (7-16) and high TML (≥ 17). This categorization was previously validated, based on the microsatellite instability (MSI) and NGS data comparisons (available here: http://www.carislifesciences.com/platforms/cmi-overview/total-mutational-load-tml/).
Microsatellite instability (MSI) status was determined by sequence analysis of microsatellite repeat tracts in 7317 target loci in the 592-gene panel.

2.4. Statistical methods

The two-tailed Fisher exact test and χ^2 test were applied for the correlation between the variables ($p \leq 0.05$).

3. Results

3.1. Patients and tumor sample characteristics

The study included the samples from 120 male and 137 female patients (mean age: 62.4 for male and 62.6 for female patients; ranges: 12–90 years for male and 7–95 years for female patients).

The histologic subtypes of primary NSCLC included 15 squamous cell carcinomas, 61 adenocarcinomas and 5 other NSCLCs (two adenosquamous, 2 large cell carcinomas and one NSCLC not further specified). Metastatic tumors to the lung, most commonly included carcinomas (n = 126), including colon (n = 51), gynecologic (n = 22), breast (n = 21), head and neck (n = 15), pancreas (n = 10) and kidney (n = 7) primary sites; the remaining 50 metastatic tumors included 15 soft tissue sarcomas, 11 malignant melanomas and 24 cases of miscellaneous histologic types of solid cancers.

Types of specimens submitted for evaluation included 169 small (needle) biopsies (51 NSCLC and 118 metastatic tumors) and 88 surgically resected samples (30 NSCLC and 58 metastatic tumors) (p = 0.57).

3.2. PD-L1 expression in primary (NSCLC) and metastatic tumors to the lung

The results of PD-L1 expression in TC and IC are summarized in Tables 1–3 and illustrative cases of primary NSCLC and metastatic colorectal carcinoma are shown on Figs. 1–2. Specimen type (small vs. surgical biopsy) had no influence on frequency of PD-L1 expression in TCs and ICs (p = 0.23 and 0.86, respectively).

Overall, TC PD-L1 positivity in primary NSCLC was observed in 23 of 81 cases (28%) and in 24 of 176 metastatic tumors (14%) (p = 0.009, Fisher's exact test). Among the 24 positive metastatic tumors, 13 were carcinomas (Table 1). Interestingly, all three PD-L1 + breast carcinomas were triple-negative (ER-/PR-/Her2-) carcinomas while 5 out of six head and neck carcinomas were squamous cell carcinomas. In non-carcinomatous metastases, PD-L1 expression was also observed in 4/11 (36%) metastatic melanomas and 3/12 (20%) soft tissue sarcomas (Table 1).

In adjacent normal lung tissue, PD-L1 expression was observed in alveolar macrophages (positive internal control cell type). However, PD-L1 expression in intratumoral IC was generally low in both cohorts (none of the tumors had IC PD-L1 above 5%).

However, when > 1% IC threshold for positivity was applied, a significantly higher proportion of IC PD-L1staining was observed in metastatic carcinomas than in primary NSCLCs (31% vs. 0%) (p < 0.001) (Tables 1). Notably, other histologic types of metastatic tumors (e.g. melanomas and sarcomas) also showed significantly higher IC PD-L1 expression (13–36% of cases) than NSCLCs. Consequently, tumor immune microenvironment (TME) categories differed significantly between the primary NSCLC and metastatic carcinomas to the lung (Table 3) (see Fig. 3).

No significant difference in TC PD-L1 expression was observed within the two major primary NSCLC subgroups (adenocarcinoma vs. squamous cell carcinoma, p = 0.19, Table 1) whereas significant

Table 1
PD-L1 expression in tumor cells was significantly higher in NSCLC compared with the metastatic carcinomas (p = 0.003) while IC within metastatic tumors exhibited significantly higher PD-L1 expression (p < 0.001).

Histotype	PD-L1 expression in tumor cells		Total
	[< 5%]	[≥ 5%]	
NSCLC	58 (72%)	23 (28%)	81
- Adenocarcinoma	44 (72%)	17 (28%)	61
- Squamous cell carcinoma	11 (73%)	4 (27%)	15
- Other NSCLC	3 (60%)	2 (40%)	5
Metastatic carcinomas	113 (90%)	13 (10%)	126
- Colorectal carcinoma	49 (96%)	2 (4%)	51
- Gynecologic carcinomas	21 (95%)	1 (5%)	22
- Breast carcinoma	18 (86%)	3 (14%)	21
- Head and neck carcinomas	9 (60%)	6 (40%)	15
- Pancreatic carcinoma	10 (100%)	0 (0%)	10
- Renal cell carcinoma	6 (86%)	1 (14%)	7
Other metastatic tumors	39 (78%)	11 (22%)	50
- Soft tissue tumors	12 (80%)	3 (20%)	15
- Malignant melanoma	7 (64%)	4 (36%)	11
- Other cancers	20 (83%)	4 (17%)	24
Total	210	47	257

Histotype	PD-L1 expression in inflammatory cells		Total
	[< 1%]	[≥ 1%]	
NSCLC	81 (100%)	0 (0%)	81
- Adenocarcinoma	61 (100%)	0 (0%)	61
- Squamous cell carcinoma	15 (100%)	0 (0%)	15
- Other NSCLC	5 (100%)	0 (0%)	5
Metastatic carcinomas	87 (69%)	39 (31%)	126
- Colorectal carcinoma	32 (63%)	19 (37%)	51
- Gynecologic carcinomas	16 (73%)	6 (27%)	22
- Breast carcinomas	13 (62%)	8 (38%)	21
- Head and neck carcinomas	12 (80%)	3 (20%)	15
- Pancreatic carcinoma	7 (70%)	3 (30%)	10
- Renal cell carcinoma	7 (100%)	0 (0%)	7
Other metastatic tumors	40 (81%)	10 (19%)	50
- Soft tissue tumors	13 (87%)	2 (13%)	15
- Malignant melanoma	7 (64%)	4 (36%)	11
- Other cancers	20 (80%)	4 (20%)	24
Total	208	49	257

differences in TC PD-L1 expression were seen among the metastatic carcinomas based on their lineages (from 0% positivity in pancreatic to 40% positivity in head and neck carcinomas, Table 1).

3.3. Relationship between the PD-L1 expression and tumor mutational load (TML)

Tumor mutational load (TML) was analyzed in 229 samples (76 NSCLC and 153 metastatic tumors). The NSCLC cases exhibited significantly higher TML in comparison with the metastatic carcinomas (10 mutations on average vs. 6.6, p < 0.001, Table 4). Also, PD-L1 TC positive NSCLCs had higher TML compared with the PD-L1 negative primary tumors (p = 0.05).

MSI status was evaluated in 256 cases total; only two metastatic tumors (1.3%) to the lung (one endometrial carcinoma and one adenocarcinoma of presumably intestinal origin) exhibited MSI-H status. None of the NSCLC (0%) had MSI-H (three equivocal cases by NGS had intact MMRP expression by IHC).

No significant differences in TML were observed between the various histologic types in the metastatic carcinomas group. However, metastatic melanomas (n = 10) had particularly high TML with an average of 32.7 mutations (range, 1–130 mutations) (Table 4).

Table 2
PD-1 and CD8 expression in inflammatory (T-cell) population [T-cell density] (mean values were used to dichotomize both variables). The number of PD-1 + and CD8 + cells did not differ significantly between the primary and metastatic tumors to the lung (p = 1.0 and 0.13, respectively).

Histotype	PD-1 expression		Total
	Low (< 34)	High (≥ 34)	
NSCLC	55 (68%)	26 (32%)	81
- Adenocarcinoma	45 (74%)	16 (26%)	61
- Squamous cell carcinoma	8 (53%)	7 (47%)	15
- Other NSCLC	2 (40%)	3 (60%)	5
Metastatic carcinomas	40 (70%)	17 (30%)	57
- Colorectal carcinoma	17 (59%)	12 (41%)	29
- Gynecologic carcinomas	6 (67%)	3 (33%)	9
- Breast carcinoma	5 (71%)	2 (29%)	7
- Head and neck carcinomas	8 (100%)	0 (0%)	8
- Pancreatic carcinoma	2 (100%)	0 (0%)	2
- Renal cell carcinoma	2 (100%)	0 (0%)	2
Other metastatic tumors	21 (65%)	11 (35%)	32
- Soft tissue tumors	10 (71%)	4 (29%)	14
- Malignant melanoma	1 (33%)	2 (67%)	3
- Other cancers	10 (67%)	5 (33%)	15
Total	116	54	170

Histotype	CD8 expression		Total
	Low (< 389)	High (≥ 389)	
NSCLC	53 (65%)	28 (35%)	81
- Adenocarcinoma	40 (65%)	21 (35%)	61
- Squamous cell carcinoma	9 (60%)	6 (40%)	15
- Other NSCLC	4 (80%)	1 (20%)	5
Metastatic carcinomas	44 (77%)	13 (23%)	57
- Colorectal carcinoma	19 (66%)	10 (34)	29
- Gynecologic carcinomas	9 (100%)	0 (0%)	9
- Breast carcinomas	5 (71%)	2 (29%)	7
- Head and neck carcinomas	8 (100%)	0 (0%)	8
- Pancreatic carcinoma	2 (100%)	0 (0%)	2
- Renal cell carcinoma	1 (50%)	1 (50%)	2
Other metastatic tumors	26 (81%)	6 (19%)	32
- Soft tissue tumors	13 (93%)	1 (7%)	14
- Malignant melanoma	1 (33%)	2 (67%)	3
- Other cancers	12 (80%)	3 (20%)	15
Total	123	47	170

3.4. PD-1 and CD8 expression in primary (NSCLC) and metastatic tumors to the lung

Table 3
Significantly different TME categories between NSCLC and metastatic carcinomas to the lung (p < 0.001).

Histotypes	TME categories (PD-L1 expression)				Total
	TC + /IC +	TC-/IC-	TC + /IC-	TC-/IC +	
NSCLC	0 (0%)	58 (72%)	23 (28%)	0 (0%)	81
Metastatic carcinomas	4 (3%)	78 (62%)	9 (7%)	35 (28%)	126
Total	4	136	32	35	207

TC = tumor cells; IC = inflammatory (immune cells); TME = tumor microenvironment; NSCLC = non-small cell lung cancer.

The mean number of CD8 + IC cells per 10 hpf/whole slides (small biopsies) was 388 (range, 3 to > 3000) while the mean number of PD-1 positive cells was 33.7 (range, 0 to 280). The average number of PD-1 + and CD8 + ICs did not differ significantly between the primary and metastatic tumors to the lung (p = 1.0 and 0.13, respectively) (Table 2). A good correlation between PD-1 + and CD8 + cells (T-

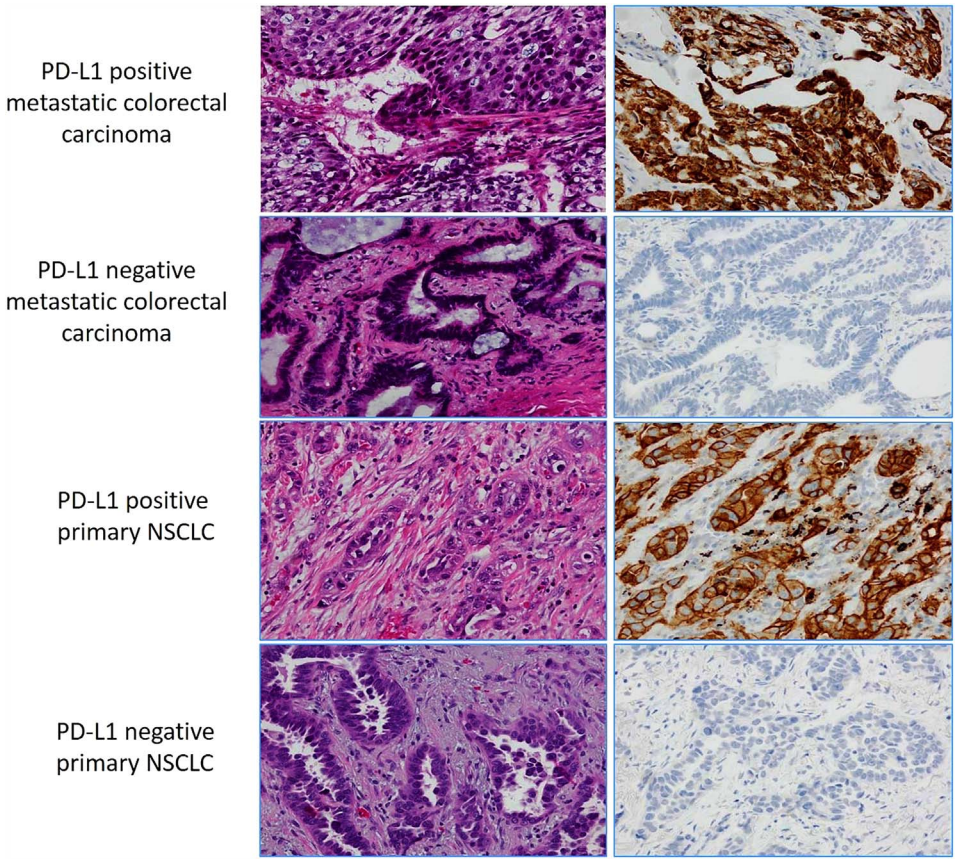

Fig. 1. Various PD-L1 expression in tumor cells in primary (NSCLC) and metastatic tumor to the lung (colorectal carcinoma).

lymphocytes) was also observed ($p < 0.001$, $r_s = 0.454$). No significant association between the number of CD8 + T-lymphocytes and TML among the NSCLC and metastatic carcinomas was observed ($p = 0.59$). No significant differences in presence of PD-1 + and CD8 + IC was observed between small (core) biopsies and resection samples ($p = 0.56$ and 0.38, respectively).

4. Discussion

Immunotherapy with immune PD1/PD-L1 checkpoint inhibitors has achieved remarkable therapeutic benefits in various solid and hematologic malignancies [18,19]. However, predictive biomarkers still need refinement [19]. A compelling body of evidence indicates that no single biomarker may be sufficient to identify the optimal "PD-1/PD-L1 immunotype" in predicting the successful immune checkpoint treatment strategy [2,9]. In the present study, we comparatively analyzed distribution of the two key targets of the immune checkpoint inhibitors in a cohort of primary NSCLC and tumors metastatic to the lungs. Our data support the previous results on the relatively common TC PD-L1 expression in NSCLC [20,21]. In contrast to the previous studies, we also explored PD-L1 in IC that exhibited rare or no PD-L1 expression. On the other hand, metastatic tumors were more commonly enriched by the PD-L1 + IC cells with uncommon PD-L1 TC positivity. We did not have a chance to perform the paired sample analysis comparing the metastatic tumors' TME to their primary sites' TME, to observe dynamics of the changes, if any. Couple of earlier studies indicated that some discordance in PD-L1 expression in TC of NSCLC can occur between the primary and metastatic sites [22,23], but we are not aware of any such studies outside NSCLC.

In NSCLC, PD-L1 expression level on tumor cells has been directly correlated with response to anti-PD1/PDL1 immune checkpoint inhibitors. Low expression and high expression of PD-L1 testing is now used in clinical practice to identify treatment-naïve and previously treated patients most likely to obtain benefit from an anti-PD-1 therapy, respectively [5,24]. On the other hand, the expression of PD-L1 on tumor infiltrating lymphocytes may be important in identifying responders to specific anti-PDL1 immune checkpoint inhibitors, and a combinatorial approach to evaluate PD-L1 expression on both tumor cells and tumor infiltrating lymphocytes can help to identify responders [3,25,26]. Even though the PD-L1 tumor expression is shown here to be much lower in tumors metastatic to the lung compared to NSCLC, a notably higher expression of PD-L1 expression on tumor infiltrating immune cells was observed and may provide additional treatment opportunities for anti-PDL1 immune checkpoint inhibitors [26,27]. In non-lung tumors, PD-L1 expression on tumor infiltrating immune cells has been shown to help identify patient response [3,25,27]. Therefore, our findings highlight the TME differences between primary and metastatic tumors and reveal new therapeutic options in metastatic tumors. Several recent studies showed a potential therapeutic benefit of PD-1/PD-L1 blockade in locally advanced and/or metastatic tumors enriched by the PD-L1 + ICs [3,7,13,28].

Regarding the different thresholds reported in literature for predictive value of PD-L1 in different tumor types, in our study we used the uniform 5% threshold for PD-L1 positivity, because of the heterogeneous nature of both primary and metastatic cancers. Several clinical trials and systematic reviews recommended 5% threshold [5,15,16,29,30]; a systematic review with meta-analysis conducted by Carbognin et al. revealed significant differences in therapeutic responses when 5% cutoff was used in the patients with NSCLC, genitourinary cancers and malignant melanoma. No differences were observed when 1% threshold was used [15].

Preliminary data indicate that the tumors with high levels of somatic mutations (TML) are more sensitive to PD-1/PD-L1 blockade [1,18,31]. Our TML study revealed significantly higher TML in NSCLC that in the metastatic carcinomas, which may predict their better response to the immune therapy; of the metastatic tumors, metastatic

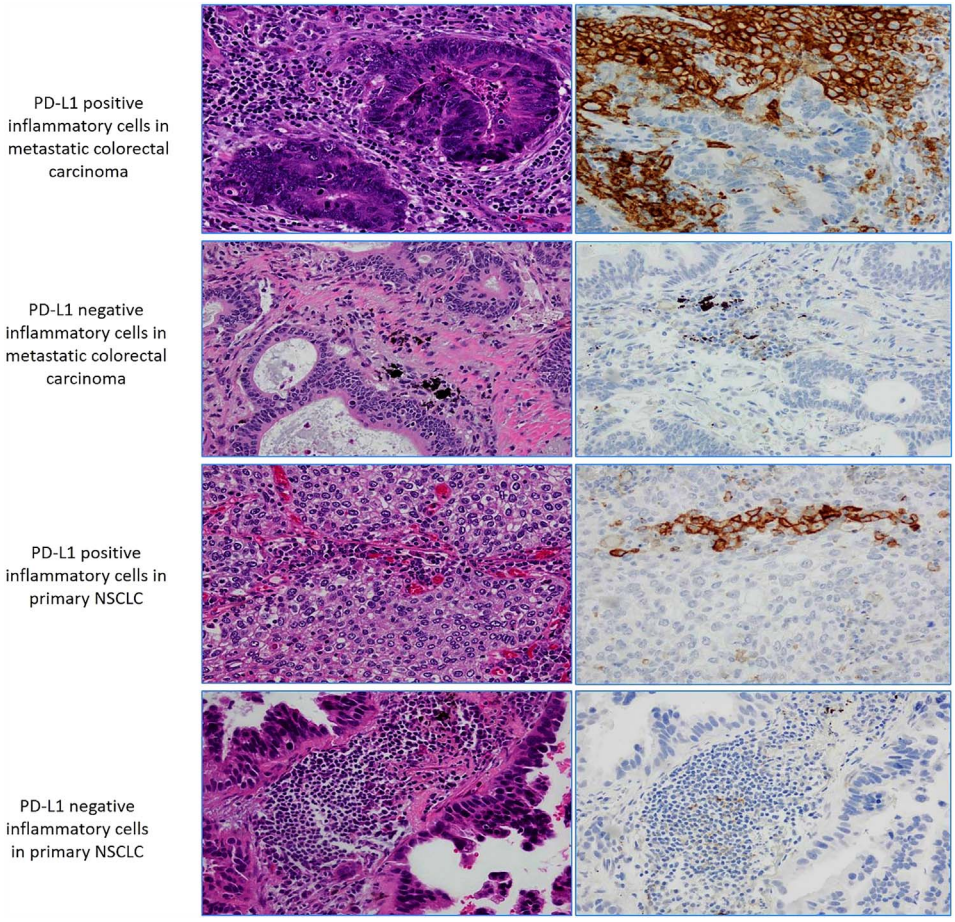

Fig. 2. Various PD-L1 expression in inflammatory (immune) cells in primary (NSCLC) and metastatic tumor to the lung (colorectal carcinoma).

melanomas exhibited particularly high TML, which, in part, elucidate their increased sensitivity to the PD-1/PD-L1 blockade. Interestingly, a heterogeneous group of metastatic carcinomas to the lung showed no significantly different TML.

Recently, tumor microsatellite instability has raised to the level of lineage-agnostic biomarker of the response to the immune check point inhibitors [12,32]. However, in our cohort, only a small proportion (1.3%) of metastatic cancers and none of the primary NSCLC exhibited MSI-H.

Beside its confirmed prognostic value, increased number of CD8 + T-lymphocytes within the tumor ("T-cell density") has been proposed as another biomarker associated with more favorable response to the immune check point inhibitors [33,34]. Our study revealed no differences in the CD8 + T-cell density between the primary NSCLC and

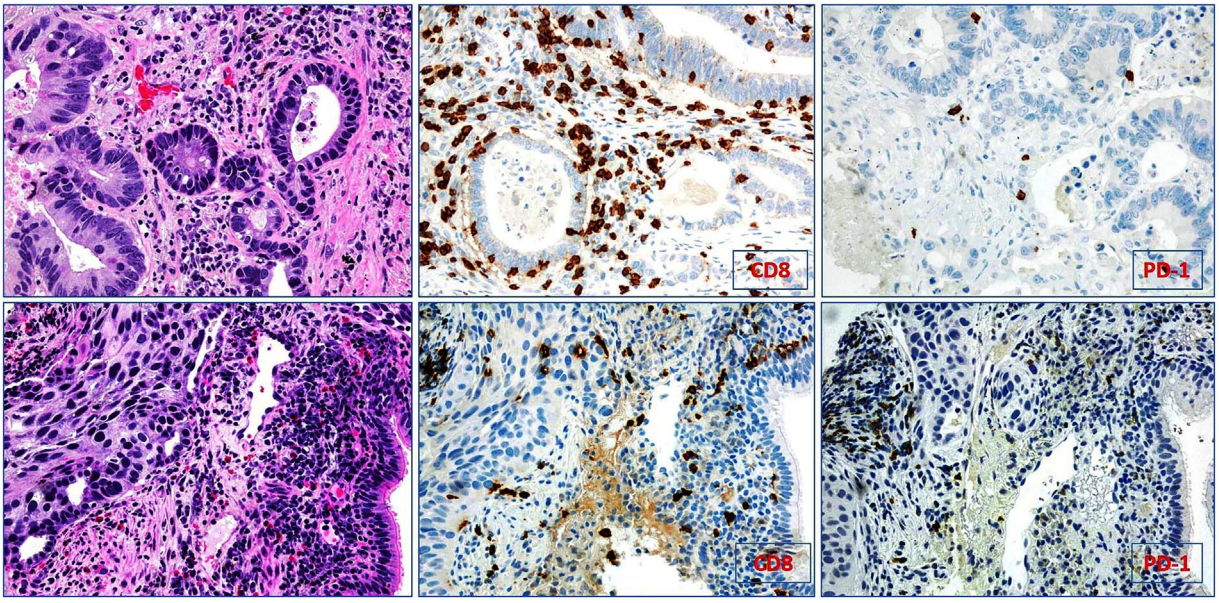

Fig. 3. PD-1 and CD8 distributions (T-lymphocytes) in a case of metastatic colorectal carcinoma (upper figures) and primary (NSCLC) (lower figures).

Table 4
Tumor mutational load differences between the primary (NSCLC) and metastatic tumors to the lung ($p < 0.001$); Metastatic melanomas exhibited particularly high TML (mean 32.70, SD: 43.721).

Histotype	TML category			Total
	Low (≤ 6)	Intermediate (7-16)	High (≥ 17)	
NSCLC (mean: 10.07, SD 5.608)	22 (29%)	49 (64%)	5 (7%)	76
Metastatic carcinomas (mean: 6.60, SD 2.785)	64 (60%)	41 (39%)	1 (1%)	106
-Colorectal carcinoma	22 (50%)	22 (50%)	0 (0%)	44
-Gynecologic carcinomas	11 (65%)	5 (29%)	1 (6%)	17
-Breast carcinoma	11 (56%)	8 (42%)	0 (0%)	19
-Head and neck carcinomas	10 (72%)	4 (28%)	0 (0%)	14
-Pancreatic carcinoma	4 (86%)	1 (14%)	0 (0%)	7
-Renal cell carcinoma	6 (84%)	1 (16%)	0 (0%)	5
Other metastatic tumors (mean 11.76, SD 21.405)	27 (57%)	12 (26%)	8 (17%)	47
Total	113	102	14	229

NSCLC = Non-small cell lung carcinoma; TML = tumor mutational load; SD = standard deviation.

metastatic tumors including metastatic carcinomas. Although a study of Brown et al. indicated that immunogenic mutations in several solid malignancies (lung, ovary, breast, colorectal, brain, and kidney cancer in combined analysis) correlated with T-cell density, our study could not confirm these observations [35]. Of note, our study included a relatively small number of metastatic breast, gynecologic and kidney carcinomas, which may be a limiting factor for the statistical analysis and comparisons.

Our study has several limitations. Firstly, we used a single monoclonal antibody (SP142 clone) to assess the status of PD-L1 in a wide range of tumors and cell types. Although it was recently reported that the sensitivity of the SP142 antibody was somewhat lower in detection of IC in NSCLC [36], in our own laboratory utilizing a modified, validated laboratory developed test, SP142 performs comparably to 3 other antibodies (SP263, 28-8, 22c3 antibodies) when all tumor types are evaluated together [37] (Gatalica Z, manuscript in preparation). Furthermore, SP142 is a widely used antibody clone with numerous studies showing its utility not only to detect PD-L1 expression in TC but also to predict a response to atezolizumab when measured in the immune cells (IC) of the recurrent and metastatic urothelial carcinomas [3]. In NSCLC, SP142 antibody has been shown to measure TC and IC. While response was seen regardless of PD-L1 expression, extension of overall survival was significantly longer in patients with higher PD-L1 expression level [6]. Secondly, we used a uniform threshold for the assessment of PD-L1 positivity, as recently 2 different thresholds (using 22c3 antibody) were introduced for the NSCLC treatment decision based on the previous treatments status [38]. Since we used a wide variety of cancer lineages (both primary and metastatic cancers), we believe that a single antibody (SP142) and single threshold (5%) is best suited for such comparative study, when no outcome data were measured. However, comparisons between the same histotypes (e.g. primary squamous vs. metastatic or primary adenocarcinoma vs. metastatic) was limited by a small number of each histotype. Thirdly, a significant proportion of the assays was performed on small biopsies. Although biopsy type (small vs. surgical) in our study had no a significant impact on PD-1, PD-L1 and CD8 expressions, this issue should be taken into account and results cautiously interpreted, as shown in one previous study [39].

In conclusion, our study revealed significant differences in the presence of PD-L1 expressing cells in the microenvironments of primary NSCLC and carcinomas metastatic to lungs. Additionally, it also confirmed potential value in measuring the tumor mutational load, which may provide for immune checkpoint blockade in selected cases.

Conflict of interest

Wijendra Senarthne, Joanne Xiu, Inga Rose, Peggy Gates and Zoran Gatalica are all employees of Caris Life Sciences. Semir Vranic had received honoraria from Caris Life Sciences.

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