PD-L1 Expression of Lung Cancer Cells, Unlike Infiltrating Immune Cells, Is Stable and Unaffected by Therapy During Brain Metastasis

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Abstract

Patient selection criteria for immune checkpoint inhibitor therapy is still debated. We compared the immune cell infiltration and programmed cell death 1 (PD-1)/programmed death ligand 1 (PD-L1) expression of primary lung adenocarcinoma with their corresponding brain metastasis and found a strong correlation of PD-L1–positive tumor cells not influenced by oncotherapies. PD-L1 positivity in the primary tumor could serve as a therapeutic criterion even for brain metastases.

Background: Approximately 50% of brain metastases originate from non–small-cell lung cancer. The median survival of patients with brain metastases is 1 month without treatment. Novel immunotherapeutic strategies, such as those targeting the programmed death ligand 1 (PD-L1)/programmed cell death 1 (PD-1) axis, are promising in patients with advanced systemic disease but are often preferentially administered to patients with tumors showing PD-L1 positivity.

Patients and Methods: Surgically resected paired primary lung adenocarcinoma and brain metastasis samples of 61 patients were analyzed. We compared the paired samples regarding the amount of peritumoral and stromal mononuclear infiltration, PD-L1 expression of tumor and immune cells, and PD-1 expression of immune cells. We investigated the effect of radiotherapy, chemotherapy, and steroid therapy on PD-L1 expression in brain metastases.

Results: There was significant positive correlation regarding the PD-L1 expression of tumor cells between the paired primary lung adenocarcinoma and brain metastatic samples with the use of different cutoff levels (1%, 5%, 50%). We found no impact of chemotherapy or steroid therapy on the changes of PD-L1 expression of tumor cells between the 2 sites. There is no or only limited concordance of the proportion of PD-1– or PD-L1–positive tumor-associated immune cells between the paired tumor samples, which suggests that brain metastases develop their own immune environment. **Conclusion:** We observed a strong correlation of PD-L1 positive tumor cells between primary lung adenocarcinoma cases and their corresponding brain metastases, which is not significantly influenced by chemotherapy or steroid therapy.

Clinical Lung Cancer, Vol. 20, No. 5, 363-9

Keywords: Immune checkpoint, Immune environment, Lung adenocarcinoma, Programmed death ligand 1

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Submitted: Jan 27, 2019; Revised: Mar 25, 2019; Accepted: May 2, 2019; Epub: May 15, 2019

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1525-7304/$ - see frontmatter
https://doi.org/10.1016/j.cllc.2019.05.008

Introduction

Metastatic brain tumors are the most common type of central nervous system malignancies in adults.[1] Approximately 50% of brain metastases originate from non–small-cell lung cancer (NSCLC).[2] In addition to surgical resection, whole-brain radiotherapy, and stereotactic radiosurgery, small-molecule *EGFR* or *ALK* inhibitors have also shown some efficacy in controlling brain metastases of NSCLC.[1,3-6] Novel immunotherapeutic strategies are also promising in patients with advanced systemic disease.[7-9] However, little is known about their effect on brain metastases; indeed, most clinical trials exclude patients with central nervous system involvement.[10,11]

Patient selection criteria for immune checkpoint inhibitor therapy are still under debate, but higher programmed death ligand 1 (PD-L1) expression of tumor cells (TC) has been shown to be associated with favorable outcome in clinical studies of programmed cell death 1 (PD-1) or PD-L1 checkpoint blockades in advanced NSCLC.[12-16] At the same time, the presence and PD-L1 expression of tumor-infiltrating lymphocytes may be more relevant to immune checkpoint inhibitor response, as PD-1 inhibitors may have no effect on a PD-L1–expressing tumor deficiency of a substantial immune cell (IC) infiltrate.[7] On the basis of these facts, patients with tumors showing both the presence of tumor-infiltrating lymphocytes and PD-L1 expression will likely benefit the most from a PD-1/PD-L1 inhibitor therapy.[17]

Expression of PD-L1 has been recognized as heterogeneous within the tumor,[18] as well as inducible and dynamic subject to changes taking place in the tumor microenvironment.[19,20] Some studies have also detected changes of PD-L1 expression in response to different chemotherapeutic agents,[21,22] suggesting that multiple tumor biopsies may be necessary to find the optimal therapy during the clinical course.

We investigated whether the amount of tumor-associated IC and/or PD-1/PD-L1 expression is concordant between primary lung adenocarcinomas (ADC) and their corresponding brain metastases, and whether this concordance is affected by therapy. Because many patients are not eligible to undergo both primary and metastatic tumor biopsies, a strong concordance would suggest that characterizing the IC infiltration/PD-1/PD-L1 expression in one site may serve as a surrogate measure of the same immunobiological status of the other site.

Patients and Methods

Surgically resected paired primary lung ADC and brain metastasis samples of 61 patients were analyzed in this study. The diagnosis and management of the primary tumor of all patients were carried out in the National Korányi Institute of Pulmonology, Budapest, Hungary. Tumors were classified according to the latest World Health Organization classification.[23] We used formalin-fixed, paraffin-embedded (FFPE) lung ADC samples from our institution's archives. Brain metastasis surgeries were carried out in the National Institute of Clinical Neurosciences, Budapest, Hungary. We used the FFPE brain metastases samples from the archive of the 1st Department of Pathology and Experimental Cancer Research, Semmelweis University, Budapest, Hungary. Permission to use the archived tissue was obtained from the local ethics committee of Semmelweis University (TUKEB-1552012, -5102013, and -862015), and the study was conducted in accordance with the Declaration of Helsinki.

We compared the paired primary lung ADC and brain metastasis samples of each patient from several aspects, including the amount of peritumoral and stromal mononuclear infiltration, PD-L1 expression of TC and IC, and PD-1 expression of IC. We also investigated the possible effects of radiotherapy, chemotherapy, and steroid therapy.

The amount of mononuclear IC, including lymphocytes, histiocytes, and plasma cells associated with lung ADC and brain metastasis, was determined on hematoxylin and eosin–stained sections by 2 independent pathologists. We investigated the presence of peritumoral mononuclear IC and created 2 groups, present and absent. Peritumoral mononuclear IC were considered present if a thick or thin layer of mononuclear cell infiltrate was detected at least focally within the lung or brain parenchyma surrounding the tumor. In contrast, peritumoral mononuclear IC was considered absent when only very few scattered or no mononuclear cells were present around the tumor. The amount of intratumoral stromal mononuclear IC infiltration was also recorded by a semiquantitative method when $< 20\%$ or $\geq 20\%$ of the tumor stroma contained IC.[24,25]

Immunohistochemistry was performed on 3 μm thick sections of tissue microarray blocks. The primary antibody for PD-L1 was the US Food and Drug Administration (FDA)-approved clone SP142 (dilution 1:100; Spring Bioscience; Ventana, Tucson, AZ) and for PD-1 the commonly used ab52587 (dilution 1:100; Abcam, Cambridge, UK)[26-30] because there is no FDA-approved assay for PD-1 detection. Each case was represented by 3 cores (2 mm in diameter) taken from representative areas of the viable tumor from the original FFPE tissue blocks. Staining was performed on one of each tissue microarray slides according to standard laboratory practice on a Leica Bond-Max automated immunostaining system (Leica Biosystems, Danvers, MA). Placenta and tonsillar tissues were used as positive controls for PD-L1 and PD-1, respectively. All the visible cells were evaluated in each case, including at least 100 viable TC and any amount of IC present. The amounts of positive TC and IC were determined by a semiquantitative method by 2 independent pathologists as the percentage of positive cells in each core. The final score was given as the mean values of the 3 representative cores. The cells were considered to be PD-L1 or PD-1 positive if the cell membrane was partially or completely stained. For TC 1%, 5%, and 50%, and for IC 1%, 5%, and 10% cutoff levels were recorded, which are the various thresholds used in related research studies.[31-34] The overall agreement between the 2 pathologists was $> 90\%$ both for TC and IC. When the readers gave discrepant scores to a core, the final score was determined after discussing findings viewed under a multihead microscope.

Spearman correlation between different variables were calculated by Python 3.5.3 with the help of the scipy.stats statistical package.

Because true associations between quantitative variables are best detected without applying artificial cutoff levels, we aimed to establish the significance of correlations using the original semiquantitative scale of the histology parameters described above. However, because all the values of this scale are commonly used as cutoff levels to separate patients into 2 distinct groups both in previous studies[31,32] and clinical practice,[34-37] preliminary investigations were carried out to determine significant correlations when patients were stratified on the basis of a given threshold level. Thus, for all investigated parameters, the cutoff level with the most significant result was chosen, and

Table 1 Characteristics of 61 Patients

Characteristic	Value
Age (Y)	
Mean ± SD	59.81 ± 7.07
Range	42-72.5
Sex	
Male	30 (49.2)
Female	31 (50.8)
Smoking Status	
Nonsmoker	5 (8.2)
Former smoker	15 (24.6)
Current smoker	39 (63.9)
Unknown	2 (3.3)
COPD	
Yes	21 (34.4)
No	37 (60.6)
Unknown	3 (5.0)
Clinical Stage at Diagnosis	
IA	4 (6.6)
IB	18 (29.5)
IIA	9 (14.8)
IIB	6 (9.8)
IIIA	15 (24.6)
IIIB	1 (1.6)
IV (brain)	6 (9.8)
Unknown	2 (3.3)
Chemotherapy Before Lung Surgery	
Yes	4 (6.6)
No	57 (93.4)
Radiotherapy Before Lung Surgery	
Yes	2 (3.3)
No	59 (96.7)
Steroid Treatment Before Brain Metastasis Surgery	
Yes	37 (60.6)
No	15 (24.6)
Unknown	9 (14.8)
Chemotherapy Before Brain Metastasis Surgery (Any Time)	
Yes	32 (52.5)
No	29 (47.5)
Chemotherapy Before Brain Metastasis Surgery (Within 1 Year)	
Yes	14 (22.95)
No	47 (77.05)
Radiotherapy Before Brain Metastasis Surgery	
Yes	5 (8.2)
No	56 (91.8)
***EGFR/KRAS* Status**	
EGFR mutant[a]	2 (3.3)
KRAS mutant	9 (14.8)
Double wild type	4 (6.5)
Unknown	46 (75.4)

Data are presented as n (%) unless otherwise indicated.
Abbreviations: COPD = chronic obstructive pulmonary disease; SD = standard deviation.
[a]Only classical sensitizing mutations were included.

Bonferroni corrections were applied to this filtered set of *P* values using .05 as the significance level.

Changes in the different investigated parameters between primary lung ADC and the corresponding brain metastasis were defined as either positive (+1), negative (−1), or neutral (0), according to whether the value of the given parameter increased, decreased, or did not change in the brain metastasis compared to the primary tumor. The mean of the direction of changes in differently treated groups was compared by unequal variance *t* tests using the scipy.stats Python package. Whenever multiple cutoff levels were available, the one with the lowest *P* value for the given parameter was chosen for further investigation. This filtered set of *P* values was corrected for multiple testing with the Holm-Šidák method, given the nonindependent nature of the tests.

Results

Patient Characteristics

Sixty-one patients with primary lung ADC with corresponding brain metastasis were selected for this study. The clinical data for all cases, including smoking status, chronic obstructive pulmonary disease, clinical stage at the time of lung cancer diagnosis, *EGFR/KRAS* status, and therapies applied to treat the primary and metastatic tumor, are summarized in Table 1. None of the patients had received immune checkpoint inhibitor therapy.

Correlation of Intratumoral Stromal and Peritumoral IC Between Primary Lung ADC and Corresponding Brain Metastasis Sample

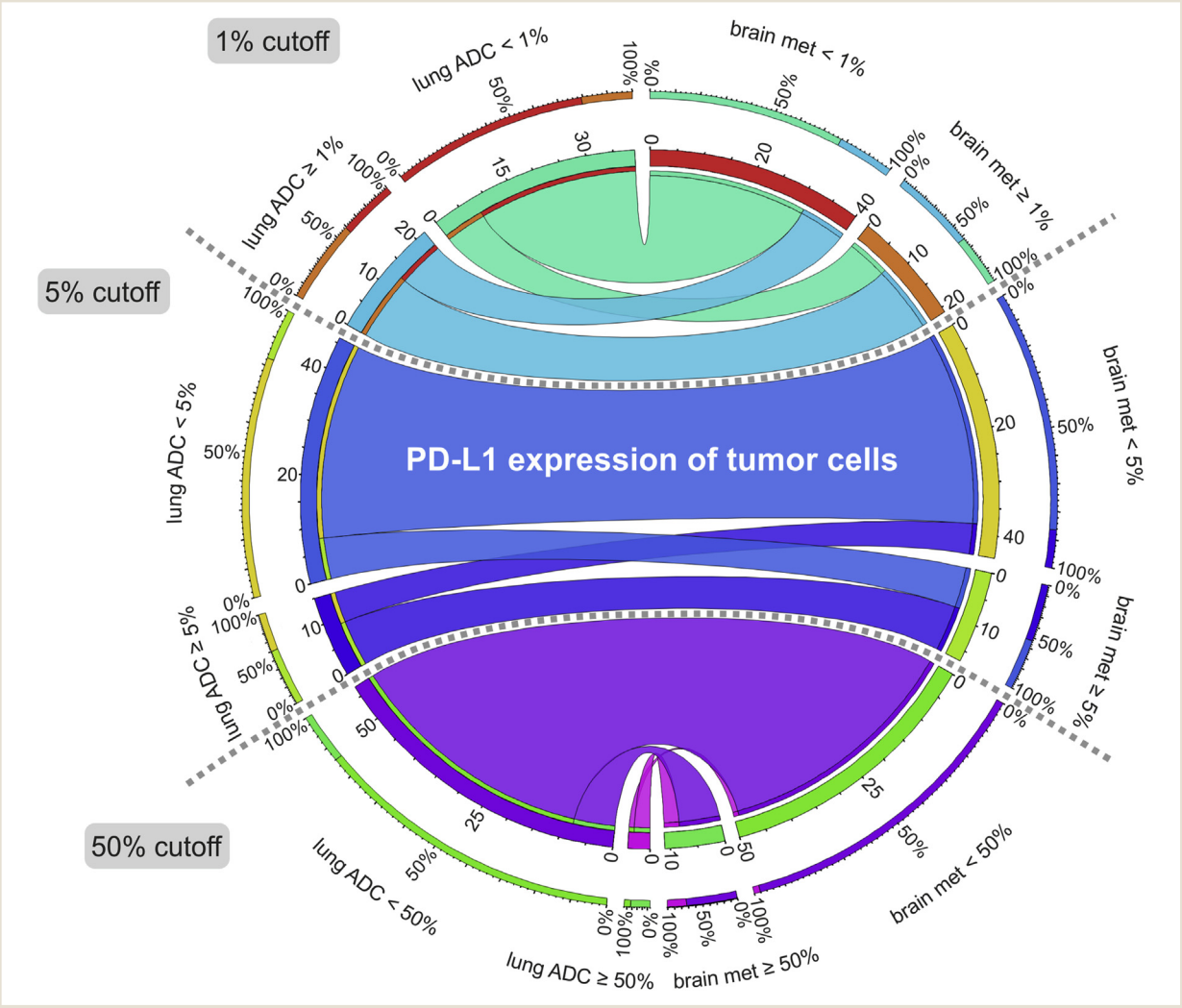
In patients with primary lung ADC, 83.60% had < 20% and 16.40% had ≥ 20% intratumoral stromal IC; 77.59% of patients presented with peritumoral mononuclear IC and 22.41% without. Among the brain metastases, 68.97% of patients had < 20% and 31.03% ≥ 20% intratumoral stromal IC, and 62.75% presented with peritumoral mononuclear IC and 37.25% without.

There was no correlation regarding the amount of intratumoral stromal ($P = .353$) or peritumoral IC ($P = .818$) between the paired primary lung ADC and brain metastatic samples (Supplemental Figure 1 in the online version). In addition, no significant correlation was detected between the peritumoral and stromal IC within the tumor samples (primary lung ADC: $P = .063$; brain metastases: $P = .158$) (Supplemental Figure 1 in the online version).

Correlation of PD-1/PD-L1 Expression Between Primary Lung ADC and Brain Metastatic Pairs

There was a significant positive correlation regarding the PD-L1 expression of TC between the paired primary lung ADC and brain

Figure 1 Circos Diagram Showing PD-L1 Expression of Tumor Cells Between Paired Primary Lung ADC and Brain Metastases for Different Cutoff Levels. For Each Cutoff and Each Tumor Site, Patients Were Categorized Into 2 Groups according to whether Measured Level of PD-L1 TC Expression Fell Below or Above Given Cutoff Level. Thus, 3 Horizontal Parts of figure should be Considered Separately. Ribbons Between Different Categories of Tumor Sites Indicate How Patients Might Have Changed Groups Due to Sufficient Difference Between Expression Levels in Lung ADC (Left Side) and Brain Metastasis (Right Side). Outer Ring Represents Distribution of Patients of Given Group Among Groups of Other Tumor site. Numbers on Inner Ring Display Actual Patient Counts. Most Ribbons Run Horizontally and Connect Same Groups Between Tumor Sites, Thus Demonstrating Positive Correlation of PD-L1 TC Expression Levels Between Primary Lung ADC and Brain Metastasis



Abbreviations: ADC = adenocarcinoma; CNS = central nervous system; FFPE = formalin fixed, paraffin embedded; IC = immune cell; met = metastasis; NSCLC = non–small-cell lung cancer; PD-1 = programmed cell death 1; PD-L1 = programmed cell death ligand 1; TC = tumor cell; TIL = tumor-infiltrating lymphocyte.

metastatic samples with all cutoff levels (Figure 1). The most prominent correlation was observed when using no cutoff levels; however, correlations with all cutoff levels remained significant when corrected for multiple comparisons (Table 2).

A similar, albeit much weaker, trend of positive correlation could be observed for the PD-L1 expression of IC in lung ADC samples and brain metastases for the 10% cutoff level, but this tendency did not remain significant when correcting for multiple comparisons (Table 2). Further, there was no correlation regarding the PD-1 expression of IC between the paired samples (Table 2).

The number of lung ADC and brain metastasis cases with different expression of PD-L1 and PD-1 according to the various cutoff values is summarized in Supplemental Table 1 in the online version.

Effects of Various Treatments on Direction of Changes in Amount of Intratumoral Stromal and Peritumoral IC From Primary Lung ADC to Corresponding Brain Metastasis

The direction of changes (increase, decrease, or no change) in the amount of intratumoral stromal and peritumoral IC from primary

Table 2 Correlation of PD-L1 and PD-1 Expression Between Primary Lung ADC and Corresponding Brain Metastasis

Characteristic	Percentage of Positive Cells	*P*	Pearson *R*
PD-L1 tumor cells in primary lung ADC vs. brain metastasis	No cutoff	<001[a]	0.464
	< 1% vs. ≥ 1%	.002[a]	0.390
	< 5% vs. ≥ 5%	.001[a]	0.409
	< 50% vs. ≥ 50%	.002[a]	0.393
PD-L1 immune cells in primary lung ADC vs. brain metastasis	No cutoff	NS	—
	< 1% vs. ≥ 1%	NS	—
	< 5% vs. ≥ 5%	NS	—
	< 10% vs. ≥ 10%	.013	0.322
PD-1 immune cells in primary lung ADC vs. brain metastasis	No cutoff	NS	—
	< 1% vs. ≥ 1%	NS	—
	< 5% vs. ≥ 5%	NS	—
	< 10% vs. ≥ 10%	NS	—

Abbreviations: ADC = adenocarcinoma; NS = not significant; PD-1 = programmed cell death 1; PD-L1 = programmed cell death ligand 1.
[a]Statistically significant after Bonferroni correction.

lung ADC to the corresponding brain metastasis was similar in groups of patients who did or did not receive radiotherapy, chemotherapy, or steroid therapy before the surgical resection of lung ADC or brain metastasis (Supplemental Table 2 in the online version).

Effect of Various Treatments on Direction of Changes in PD-1/PD-L1 Expression of TC and IC From Primary Lung ADC to Corresponding Brain Metastasis

There was no significant difference in the direction of changes of PD-L1 expression of TC/IC and PD-1 expression of IC from primary lung ADC to the corresponding brain metastasis in virtually all of the various treatment groups of patients when corrected for multiple testing (Supplemental Table 2 in the online version). Of note, patients who received radiotherapy before surgical resection of lung ADC showed a significant increase in PD-1 expression of IC in their brain metastasis compared to the primary lung ADC ($P < .001$). However, this correlation could only be observed at 1% cutoff value, and the group with radiotherapy comprised only 2 cases, versus 59 cases without (Supplemental Table 2 in the online version).

Discussion

We performed a comprehensive analysis of the amount of tumor-associated IC and PD-1/PD-L1 expression between primary lung ADC and their corresponding brain metastases. This is to our knowledge the first study to investigate PD-1 expression of tumor-associated IC between paired lung ADC and brain metastases samples, and more importantly the potential influence of chemotherapy, radiotherapy, and steroid therapy on the studied parameters.

Immune checkpoint inhibitor therapy is playing an emerging role in lung cancer treatment.[16,37] Some clinical trials have started to recruit brain metastatic patients with melanoma and lung origin,[8,11,38,39] although most clinical trials still exclude such patients.[10,11] Patient selection criteria for PD-1/PD-L1 inhibitor therapy is still under debate, and little is known about the predictive markers for patients with brain metastasis. The presence of tumor-associated IC and their PD-1/PD-L1 expression as well as PD-L1 expression of TC in brain metastases may all affect the response to PD-1/PD-L1 inhibitor therapy. This was recently recognized with the introduction of a combined positive score instead of the tumor proportional score as a predictive marker for the efficacy of checkpoint inhibitor therapies.[40] At present, 4 FDA-approved antibodies are used for PD-L1 immunohistochemistry: SP142, SP263, 22C3, and 28-8. In several comparison studies, the SP142 assay was found to be an outlier, as it stained fewer TC and did not correlate well with the other 3 assays. IC staining also showed variability among the 4 assays.[41-43] According to the literature, the concordance between the readers who assess PD-L1 expression of TC is high.[43-47] However, a good concordance is equivocal when assessing PD-L1 expression of IC. On the one hand, Scheel et al[43] showed that scoring of IC yielded low concordance rates with all 4 FDA-approved PD-L1 antibodies. On the other hand, Vennapusa et al[47] using the SP142 antibody and Rebelatto et al[45] using the SP263 antibody detected > 90% inter- and intraobserver agreement. On the basis of these discrepant results, the scoring of IC may require specific standardization in the near future.

We found a significant correlation of the amount of PD-L1–positive TC between primary lung ADC cases and their corresponding brain metastases. Our result is comparable to previous studies, in which the correlation of PD-L1 expression was studied between primary NSCLC and corresponding brain metastases with the use of a 5% cutoff level.[35,36] Kim et al[34] demonstrated that the concordance of PD-L1 expression of TC between primary and metastatic lung ADC is high with the use of 1% and 50% cutoff levels. In clinical practice and clinical trials, different cutoff levels of PD-L1 expression have been used according to the various assay antibodies and the corresponding drugs.[48,49] For example, in case of disease progression after platinum-based doublet chemotherapy in NSCLC, anti–PD-L1/PD-1 therapy is recommended in second-line therapy in patients with ≥ 1% PD-L1 expression of TC.

Moreover, the anti–PD-1 drug pembrolizumab received approval for first-line NSCLC treatment in patients with ≥ 50% PD-L1 expression of TC.[37] In several CheckMate studies, the 5% cutoff level was used.[50] Rizvi et al[51] noticed that the reduction of the targeted tumor by nivolumab was more common in patients with PD-L1–positive than in PD-L1–negative tumors with the use of 5% cutoff levels. Therefore, we tested all 3 different cutoff levels that are commonly used in clinical and research practice.

Because brain metastases are believed to arise from only a few cells,[52,53] we have to consider 2 hypotheses to explain this remarkable and robust concordance. The first assumes that brain metastases are developing from a clump of primary cells that, by random chance selection, reflects the proportion of PD-L1–positive cells. The second hypothesis assumes that the proportion of PD-L1–positive cells in the brain metastases are recreated from one or few metastasizing primary cells independent of their initial PD-L1 expression status. This hypothesis is supported by experimental evidence that cell populations exist in equilibria in various transcriptomic states, and when those subpopulations are isolated, the same equilibria are again reached from each subpopulation.[54]

Primary lung cancer is often treated by various systemic chemotherapy regimens or irradiation; therefore, it was important to determine whether the proportion of PD-L1–positive TCs of brain metastases significantly changes upon therapy, thereby potentially leading to altered immune checkpoint inhibitor eligibility. We found no impact of chemotherapy or steroid therapy before brain metastasis surgery on the changes of PD-L1 expression of TC between the 2 sites.

Conclusion

On the basis of this study, there is no or only limited concordance of the proportion of PD-1–or PD-L1–positive tumor-associated IC between the primary lung ADC and corresponding brain metastases. This suggests that brain metastases develop their own immune environment irrespective of that of the primary tumor. This is in stark contrast with the high concordance of the proportion of PD-L1–positive TCs across those sites. Furthermore, there was no correlation of PD-1/PD-L1 expression of tumor-associated IC between the 2 sites in the presence or absence of chemotherapy, radiotherapy, or steroid therapy.

We observed a strong correlation of PD-L1–positive TC between primary lung ADC cases and their corresponding brain metastases, which is not significantly influenced by chemotherapy or steroid therapy. If PD-L1 positivity of TCs remains to be a therapeutic decision point, then our observations, in accordance with other similar results reported, will provide a strong rationale to use presystemic therapy PD-L1 positivity of TC in the primary tumor as a therapeutic criterion, even if such data are not obtainable in the brain metastases. However, in case of introducing the combined positive score as predictors for checkpoint inhibitors, metastatic disease would also require analysis of the metastatic tissue.

Clinical Practice Points

- Despite the increasing use of novel immune checkpoint inhibitors, patient selection criteria for eligibility, especially for patients with brain metastasis, are still under debate.
- Little is known about the correlation of the amount of tumor-associated IC infiltration and PD-L1/PD-1 expression between primary lung ADC and paired brain metastases.
- We found a strong correlation of PD-L1–positive TCs between the 2 sites not influenced by chemotherapy or steroid therapy.
- There is no or only limited concordance of the proportion of PD-1– or PD-L1–positive tumor-associated IC, suggesting that brain metastases develop their own immune environment irrespective of that of the primary tumor.
- PD-L1 positivity in the primary tumor could serve as a therapeutic criterion even for brain metastases. However, in case of introducing the combined positive score as predictors for checkpoint inhibitors, metastatic disease would require analysis of the metastatic tissue as well.

Acknowledgments

Supported in part by the Hungarian Brain Research Program (grant 2017-1.2.1-NKP-2017-00002), Breast Cancer Research Foundation (BCRF-17-156), Novo Nordisk Foundation Interdisciplinary Synergy Programme (grant NNF15OC0016584), Hungarian National Research,Development and Innovation Office (grant NVKP_16-1-2016-0004), Hungarian Science Foundation (grant OTKA-K116151), Hungarian National Research, Development and Innovation (NRDI) Office (grants K109626 and SNN114490), Austrian Science Fund (grant FWF I 3977), Hungarian NRDI Office (grant K129065), and Hungarian Brain Research Program (grant 2017-1.2.1-NKP-2017-00002). We thank Anna Tamási, Mónika Szilágyiné Paulusz, and Zsuzsanna Kaminszky for excellent technical assistance.

Disclosure

The authors have stated that they have no conflict of interest.

Supplemental Data

A supplemental figure and tables accompanying this article can be found in the online version at https://doi.org/10.1016/j.cllc.2019.05.008.

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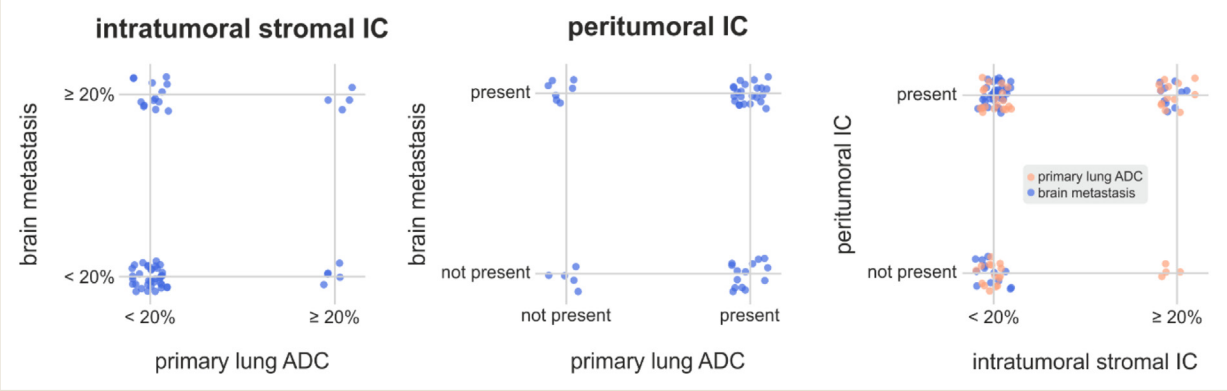
Supplemental Data

Supplemental Figure 1 Correlations of Amount of Intratumoral and Peritumoral Immune Cells. There Was No Correlation Regarding Amount of Intratumoral Stromal ($P = .353$) or Peritumoral ICs ($P = .818$) Between Paired Primary Lung ADC and Brain Metastatic Samples. Moreover, No Significant Correlation Was Detected Between Peritumoral and Stromal IC Within Tumor Samples (Primary Lung ADC: $P = .063$; Brain Metastases: $P = .158$)



Abbreviations: ADC = adenocarcinoma; IC = immune cell.

Supplemental Table 1 Ratio of Cases With PD-L1 and PD-1 Expression in Primary Lung ADC and Brain Metastasis According to Different Cutoff Levels

Characteristic	Primary Lung ADC						Brain Metastasis					
	< 1% vs. ≥ 1% (N)		< 5% vs. ≥ 5% (N)		< 50% vs. ≥ 50% (N)		< 1% vs. ≥ 1% (N)		< 5% vs. ≥ 5% (N)		< 50% vs. ≥ 50% (N)	
PD-L1 TC	63.93% (39)	36.07% (22)	75.41% (46)	24.59% (15)	93.44% (57)	6.56% (4)	65.57% (40)	34.43% (21)	72.13% (44)	27.87% (17)	81.97% (50)	18.03% (11)
	< 1% vs. ≥ 1% (N)		< 5% vs. ≥ 5% (N)		< 10% vs. ≥ 10% (N)		< 1% vs. ≥ 1% (N)		< 5% vs. ≥ 5% (N)		< 10% vs. ≥ 10% (N)	
PD-L1 IC	55.74% (34)	44.26% (27)	90.16% (55)	9.84% (6)	96.72% (59)	3.28% (2)	64.41% (38)	35.59% (21)	93.22% (55)	6.78% (4)	93.22% (55)	6.78% (4)
PD-1 IC	16.39% (10)	83.61% (51)	54.10% (33)	45.90% (28)	78.69% (48)	21.31% (13)	36.07% (22)	63.93% (39)	75.41% (46)	24.59% (15)	88.52% (54)	11.48% (7)

Abbreviations: ADC = adenocarcinoma; IC = immune cells; PD-1 = programmed cell death 1; PD-L1 = programmed cell death ligand 1;TC = tumor cell.

Supplemental Table 2 Effect of Different Treatments on Changes in Amount of Tumor-Associated IC and PD-1/PD-L1 Expression of IC and TC Between Primary Lung ADC and Corresponding Brain Metastasis

Characteristic	Changes in Amount of Intratumoral IC	Changes in Amount of Peritumoral IC	Changes in PD-1 Expression of IC				Changes in PD-L1 Expression of TC				Changes in PD-L1 Expression of IC			
	< 20%/≥ 20%	Present/Not Present	No Cutoff	< 1%/≥ 1%	< 5%/≥ 5%	< 10%/≥ 10%	No Cutoff	< 1%/≥ 1%	< 5%/≥ 5%	< 50%/≥ 50%	No Cutoff	< 1%/≥ 1%	< 5%/≥ 5%	< 10%/≥ 10%
RT before lung ADC surgery	.073[a]	.862[a]	.217	<001[b,c]	.374	.430	.640	.476[a]	.978	.571	.436	.260[a]	.468	.510
CT before lung ADC surgery	.654[a]	.620[a]	.602	.645	.619	.234[a]	.165[a]	.342	.426	.249	.080[a]	.109	.484	.321
RT before BM surgery	.852[a]	.356[a]	.399	.433	.466	.134[a]	.400	.389[a]	.597	.714	.426	.440	.484	.321[a]
CT before BM surgery (any time)	.412[a]	.946[a]	.401	.614	.442	.291[a]	.038[c]	.096	.103	.251	.199[a]	.752	.508	.297
CT before BM surgery (<1 year)	.196[a]	.670[a]	.056	.403	.183	.016[c]	.962	.638	.796	.313[a]	.691	.264[a]	.720	.540
CT before BM surgery (>1 year)	.629[a]	.805[a]	.859	.895	.774	.620[a]	.007[c]	.167	.117	.052	.181	.738	.313	.056[a]
Steroid therapy before BM surgery	.086[a]	.416[a]	.189	.734	.027[c]	.135	.691	.774	.306[a]	.986	.511	.514	.059[a]	.192

Data are *P* values.
Abbreviations: ADC = adenocarcinoma; BM = brain metastasis; CT = chemotherapy; IC = immune cells; PD-1 = programmed cell death 1; PD-L1 = programmed cell death ligand 1; RT = radiotherapy; TC = tumor cell.
[a]Result with lowest *P* value among different cutoff levels of same parameter.
[b]Significant with correction for multiple testing.
[c]Statistically significant.