ORIGINAL ARTICLE

Tumor immune microenvironment in brain metastases from gynecologic malignancies

Corey M. Gill[1] · Megan R. D'Andrea[1] · Shannon Tomita[2] · Jessa Suhner[2] · Melissa Umphlett[3] · Konstantin Zakashansky[2] · Stephanie V. Blank[2] · Nadejda Tsankova[3] · Raj K. Shrivastava[1] · Mary Fowkes[3] · Valentin Kolev[2]

Abstract

Introduction The density and distribution of the tumor immune microenvironment associated with brain metastases (BM) from gynecologic malignancies are unknown and have not been previously reported. We sought to describe the clinical features of a cohort of patients with BM from gynecologic malignancies and to characterize the tumor immune microenvironment from available archival surgical specimens.

Methods We performed a retrospective review of electronic medical records from 2002 to 2018 for patients with BM from gynecologic malignancies. Data on patient characteristics, treatment regimens, and clinical outcomes were procured. CD4, CD8, CD45RO, CD68, CD163, and FOXP3 immunohistochemistry were evaluated from available archival surgical specimens from primary disease site and neurosurgical resection.

Results A cohort of 44 patients with BM from gynecologic malignancies was identified, 21 (47.7%) endometrial primaries and 23 (52.3%) ovarian primaries. Tumor-infiltrating lymphocytes (TILs) and tumor-associated macrophages (TAMs) were evaluated in 13 primary cases and 15 BM cases. For the 13 primary cases, CD4$^+$ TILs were evident in 76.9% of cases, CD8$^+$ in 92.3%, CD45RO$^+$ in 92.3%, and FOXP3$^+$ in 46.2%, as well as CD68$^+$ TAMs in 100% and CD163$^+$ in 100%. For the 15 BM cases, CD4$^+$ TILs were evident in 60.0% of cases, CD8$^+$ in 93.3%, CD45RO$^+$ in 73.3%, and FOXP3$^+$ in 35.7%, as well as CD68$^+$ TAMs in 86.7% and CD163$^+$ in 100%.

Conclusion An active tumor immune microenvironment is present with similar distribution in the primary disease site and BM from patients with gynecologic malignancies.

Keywords Brain metastases · Endometrial · Gynecology · Ovarian · Tumor-associated macrophage · Tumor-infiltrating lymphocyte

Introduction

Brain metastases (BM) from gynecologic malignancies are rare. Among 1481 ovarian patients and 69,027 endometrial cancer patients in the SEER database, a total of 1.2% (18/1481) of ovarian cancer patients [1] and 0.2% of endometrial cancer patients (105/69027) [2] were reported to develop BM.

Survival for patients with BM is dismal. In patients with ovarian cancer BM, median survival from time of BM diagnosis is 0.5 months without treatment, but may improve to 20 months with multi-modal therapy that includes surgery and radiation with or without chemotherapy [3]. Similarly in endometrial cancer, median survival after BM diagnosis ranges from 2.0 to 4.0 months with improvement in survival

✉ Corey M. Gill
corey.gill@icahn.mssm.edu

1 Department of Neurosurgery, Icahn School of Medicine at Mount Sinai, One Gustave L. Levy Place, New York, NY 10029, USA

2 Obstetrics, Gynecology, and Reproductive Science, Icahn School of Medicine at Mount Sinai, New York, NY 10029, USA

3 Department of Pathology, Icahn School of Medicine at Mount Sinai, New York, NY 10029, USA

seen with multi-modal therapy [4–6]. On multivariable analysis, the presence of a single BM is associated with improved survival compared to multiple metastatic brain lesions [7]. Up to 20.8% of patients with BM from gynecologic malignancies may subsequently develop leptomeningeal disease [8].

Immunotherapy and the use of checkpoint inhibitors through the activation of T-cells [9] have demonstrated remarkable success in various malignancies. Biomarkers of favorable responses to immunotherapy include PD-L1 expression, tumor-infiltrating lymphocytes (TILs), and tumor-associated macrophages (TAMs), which help to characterize the activity of the tumor immune microenvironment [10–12].

Recent works have characterized the tumor immune microenvironment within BM from several malignancies to improve the understanding of the potential role of immunotherapy in the treatment of BM [13, 14]. Such characterization in BM has occurred in melanoma [15–18], NSCLC [19–25], breast cancer [26–32], small cell lung cancer [33], and colorectal cancer [34].

However, the density and distribution of the tumor immune microenvironment associated with BM from gynecologic malignancies are unknown and have not been previously reported. Given this unmet need, we sought to characterize TILs and TAMs associated with BM from gynecologic malignances, ovarian and endometrial cancer. Such insight ought to aid the understanding of how immunotherapy may play a role in the treatment of gynecologic BM beyond traditional neurosurgical resection and radiotherapy.

Materials and methods

Patient cohort

We performed a retrospective review of electronic medical records for patients with BM from gynecologic malignancies who were managed from 2002 to 2018 at a single institution. Inclusion criteria included the presence of a primary gynecologic malignancy confirmed by a board-certified pathologist and imaging evidence of at least one BM. A total of 44 patients met the inclusion criteria. The study was reviewed and approved by the Institutional Review Board and was in compliance with Health Insurance Portability and Accountability Act guidelines. Written informed consent was waived.

Immunohistochemistry

Archival formalin-fixed, paraffin-embedded samples (FFPE) were obtained from surgical specimens from primary disease site and BM for all patients when available. A board-certified pathologist reviewed Hematoxylin and Eosin (H&E) slides. Unstained slides were obtained from the best representative sample for each surgical case.

All immunohistochemistry (IHC) was performed on the Leica Bond III automated staining platform. Antibody FoxP3 from Biolegend, catalogue # 320102, clone 206D, was run at 1:50 dilution using the Leica Biosystems Refine Detection Kit with EDTA antigen retrieval. Antibody CD163 from Vector, catalogue # VP-C374, clone 10D6, was run at 1:250 dilution using the Leica Biosystems Refine Detection Kit with EDTA antigen retrieval. Antibody CD68 from Dako, catalogue # M0876, clone PG-M1, was run at 1:200 dilution using the Leica Biosystems Refine Detection Kit with citrate antigen retrieval. Antibody CD45RO from Dako, catalogue # M0742, clone UCHL1, was run at 1:500 dilution using the Leica Biosystems Refine Detection Kit without antigen retrieval. Antibody CD8 from Dako, catalogue # M7103, clone C8/144B, was run at 1:100 dilution using the Leica Biosystems Refine Detection Kit with EDTA antigen retrieval. Antibody CD4 from Dako, catalogue # M7310, clone 4B12, was run at 1:80 dilution using the Leica Biosystems Refine Detection Kit with EDTA antigen retrieval.

Tumor immune microenvironment analysis

We evaluated CD4, CD8, CD45RO, and FOXP3 TILs, as well as CD68 and CD163 TAMs, from 13 primary cases and 15 BM cases. The density of each marker was evaluated as previously reported [35], in an ordinal score of 1 "none or sporadic," 2 "moderate," 3 "abundant," and 4 "highly abundant" (Fig. 1). The density was reported for each case as "overall." The "solid tumor," "perivascular," and "border" compartments were individually evaluated. The border region represented the interface between tumor and surrounding parenchyma.

Statistical analysis

Statistical analysis was performed using JMP Pro 14.2 (SAS Institute Inc., Cary, NC). Variables were assessed for normality using the Shapiro–Wilk test. Chi-square and Fisher's exact test was used to detect statistical differences in categorical variables; t test and Wilcoxon test were used for continuous variables, as appropriate. TILs and TAMs were evaluated with an ordinal score and also as a binary variable, present (2, 3, or 4) and not present (1). Survival analysis utilized Kaplan–Meier and Cox proportional hazard methods. Overall survival was defined as time from date of BM diagnosis with imaging to date of death from any cause or date of last follow-up. $P \leq 0.05$ was used for statistical significance. Data presented as mean $\pm$ SD, unless otherwise indicated.
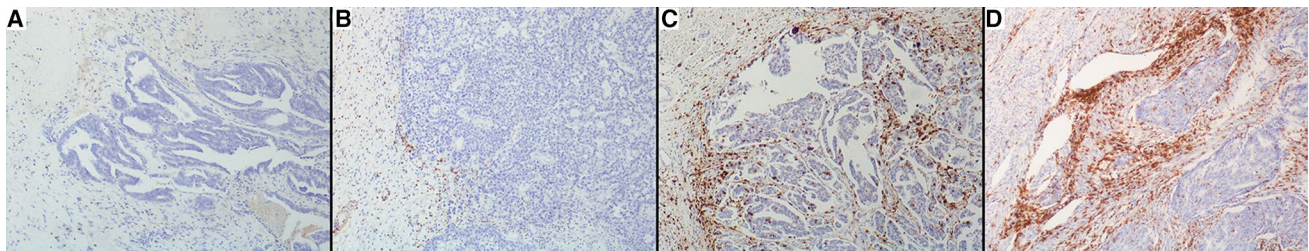
Fig. 1 Representative immunohistochemistry images (×10) depicting increasing density of the tumor immune microenvironment in the border compartment across four samples. **a** "none or sporadic," **b** "moderate," **c** "abundant," **d** "highly abundant"

Results

Clinical characteristics

A total of 44 patients with BM from gynecologic malignancies were identified. The cohort included 21 (47.7%) endometrial primaries and 23 (52.3%) ovarian primaries. The most common metastatic sites prior to BM diagnosis included lung ($n = 11$), liver ($n = 8$), and bone ($n = 7$). A total of 32 patients received systemic chemotherapy and 11 patients received radiation to the primary disease site. Median time to BM diagnosis from primary diagnosis was 2.1 years (95%CI 1.2, 3.5 years) (range 0–15.0 years), which did not significantly differ based on primary disease site (1.5 years, endometrial compared to 2.3 years, ovarian) ($P = 0.5$, log-rank).

Median age at BM diagnosis was 68.0 years (IQR: 59.1, 72.2 years) and range 37.2 to 86.2 years. The most common presenting symptoms included headache ($n = 12$), dizziness ($n = 9$), altered mental status ($n = 9$), cerebellar dysfunction ($n = 8$), seizure ($n = 6$), and cranial nerve neuropathy ($n = 2$). The number of BM identified on initial imaging included one BM in 18 (40.9%), two BM in 10 (22.7%), three BM in 4 (9.1%), and more than three BM in 12 (27.3%). The most common locations included frontal lobe in 24 (54.5%) and cerebellum in 21 (47.8%), followed by parietal lobe in 20 (45.5%), occipital lobe in 14 (31.8%), and temporal lobe in 9 (20.5%). Neurosurgical resection was performed in 17 (38.6%) patients and BM radiotherapy was performed in 22 (50.0%) patients.

Median overall survival from BM diagnosis was 6.8 months (95%CI 2.6, 14.7 months) (Fig. 2A). Compared to patients with one BM, there is a 3.94-fold increased risk of death for two BM ($P = 0.007$), 5.18-fold for three BM ($P = 0.02$), and 5.87-fold for more than three BM ($P = 0.002$). Median overall survival for one BM is 23.2 months (95%CI 4.3, 32.3 months), for two BM is 5.9 months (95%CI 0.4, 8.2 months), for three BM is 3 months (95%CI 1.3, 14.3 months), and for more than three BM is 2.2 months (95%CI 0.9, 14.1 months) ($P = 0.005$, log-rank) (Fig. 2B). Neurosurgical resection was associated with improved

survival: 15.4 months (95%CI 4.3, 21.3 months) compared to 2.4 months (95%CI 1.0, 6.6 months) ($P = 0.008$, log-rank) (Fig. 2C). There was no significant association with risk of death in regards to age at BM diagnosis, primary disease site (ovarian vs endometrial), or BM radiotherapy ($P > 0.05$, all variables).

Tumor immune microenvironment

From the 44 patients, the tumor immune microenvironment was evaluated in 13 primary cases and 15 BM cases (Fig. 3). CD4, CD8, CD45RO, CD68, CD163, and FOXP3 were reported for the overall case, as well as in the solid tumor, perivascular, and border compartments. For the 13 primary cases, CD4+ TILs were evident in 76.9% of cases, CD8+ in 92.3%, CD45RO+ in 92.3%, and FOXP3+ in 46.2%, as well as CD68+ TAMs in 100% and CD163+ in 100%. For the 15 BM cases, CD4+ TILs were evident in 60.0% of cases, CD8+ in 93.3%, CD45RO+ in 73.3%, and FOXP3+ in 35.7%, as well as CD68+ TAMs in 86.7% and CD163+ in 100%. There was no significant difference in the presence of TILs and TAMs between primary and BM cases, $P > 0.05$.

Among the 13 primary cases (Table 1 and Fig. 4), there was no significant difference in density of CD8, CD45RO, or FOXP3 between the solid tumor, perivascular, and border compartments. Highly abundant TILs and TAMs were only present in the border compartment and were seen for CD8, CD45RO, and CD163. There was significantly increased density of CD4+ TILs, as well as CD68+ and CD163+ TAMs, in the border compartment, $P = 0.04, 0.002$, and 0.01, respectively. All primary cases had CD68+ and CD163+ TAMs present. The overall presence of TILs in the primary cases did not significantly affect median time to BM diagnosis: CD4 ($P = 0.9$), CD8 ($P = 0.6$), CD45RO ($P = 0.9$), and FOXP3 ($P = 0.2$).

Among the 15 BM cases (Table 2 and Fig. 5), there was no significant difference in density of CD8, CD45RO, CD68, or FOXP3 between the solid tumor, perivascular, and border compartments. Similar to the primary cases, there was significantly increased density of CD4+ TILs and CD163+ TAMs in the border compartment, $P = 0.01$ and $P = 0.009$,
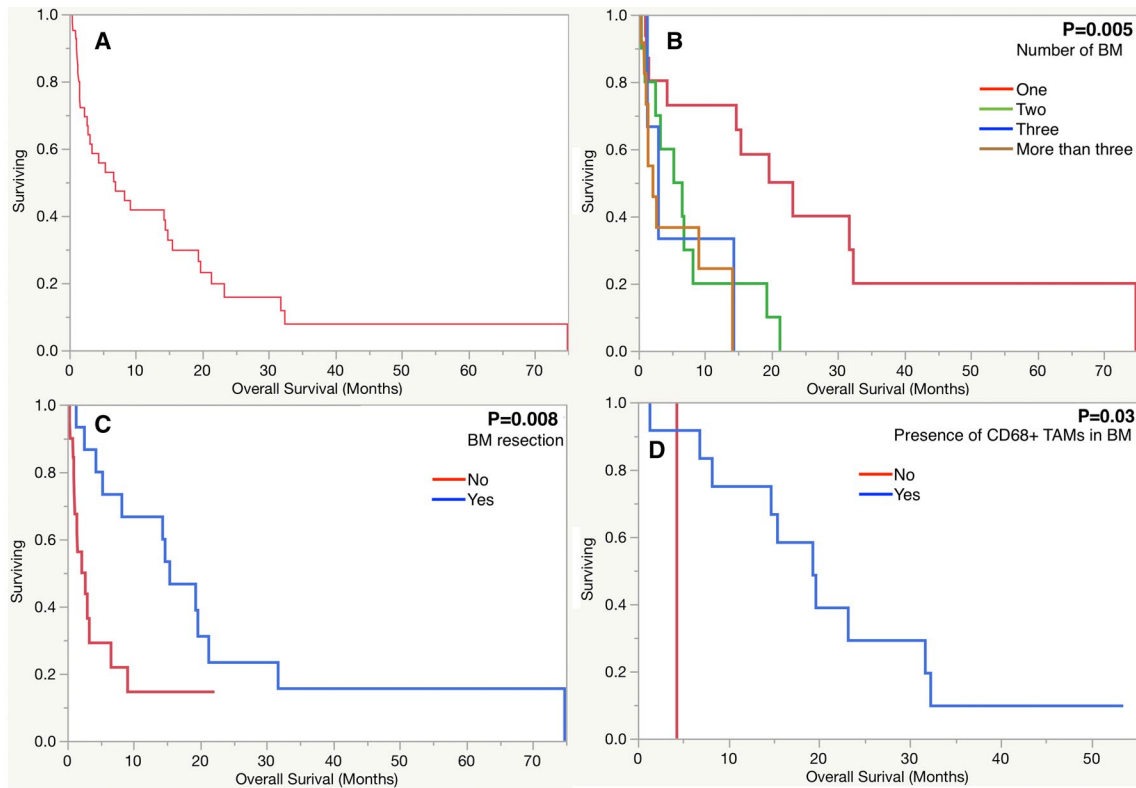
Fig. 2 Kaplan–Meier survival analysis of overall survival **a** for the whole cohort, **b** binned by number of BM, **c** binned by BM resection, and **d** binned by CD68 + TAMs in BM
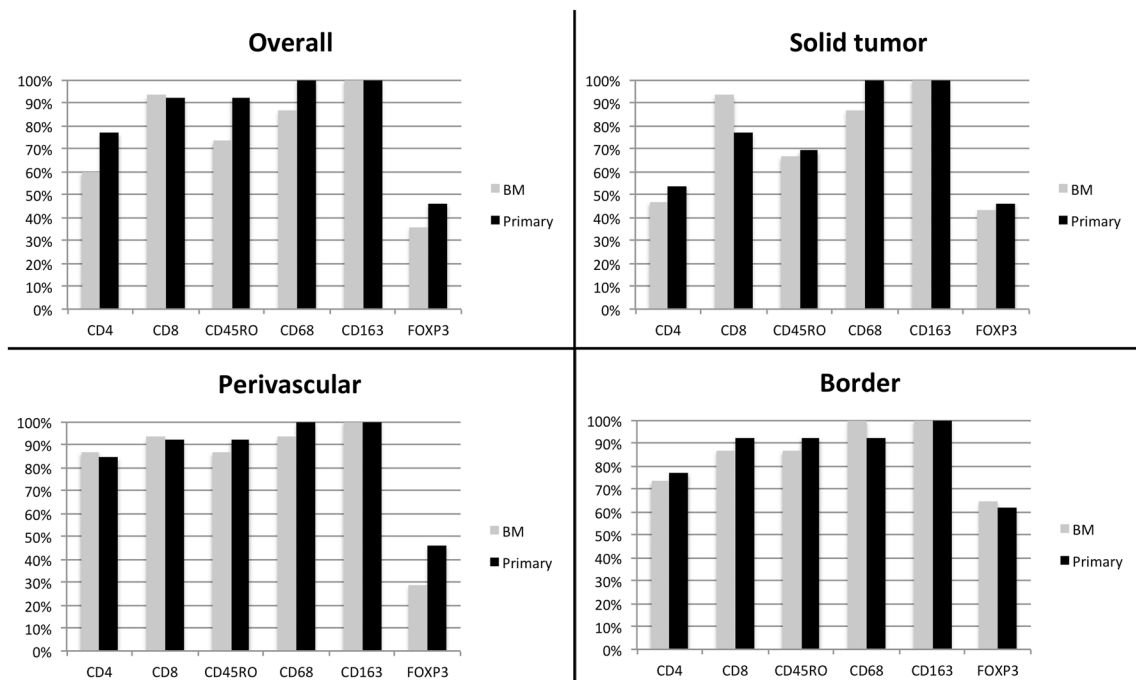

Fig. 3 Distribution of tumor-infiltrating lymphocytes and tumor-associated macrophages stratified by primary and BM. Y-axis reports binned percent of present (2, 3, or 4). No significant difference between primary and BM for all measurements, $P > 0.05$

Table 1 Distribution of tumor-infiltrating lymphocytes and tumor-associated macrophages from 13 primary cases

	CD4					CD8					CD45RO				
	1	2	3	4	P value	1	2	3	4	P value	1	2	3	4	P value
Overall	3 (23.1)	9 (69.2)	1 (7.7)	0 (0)		1 (7.7)	6 (46.2)	6 (46.2)	0 (0)		1 (7.7)	9 (69.2)	3 (23.1)	0 (0)	
Solid tumor	6 (46.2)	6 (46.2)	1 (7.7)	0 (0)	0.04	3 (23.1)	7 (53.8)	3 (23.1)	0 (0)	0.2	4 (30.8)	7 (53.8)	2 (15.4)	0 (0)	0.3
Perivascular	2 (15.4)	10 (76.9)	1 (7.7)	0 (0)		1 (7.7)	7 (53.8)	5 (38.5)	0 (0)		1 (7.7)	7 (53.8)	5 (38.5)	0 (0)	
Border	3 (23.1)	4 (30.8)	6 (46.2)	0 (0)		1 (7.7)	3 (23.1)	7 (53.8)	2 (15.4)		1 (7.7)	5 (38.5)	6 (46.2)	1 (7.7)	

	CD68					CD163					FOXP3				
	1	2	3	4	P value	1	2	3	4	P value	1	2	3	4	P value
Overall	0 (0)	8 (61.5)	5 (38.5)	0 (0)		0 (0)	2 (15.4)	11 (84.6)	0 (0)		7 (53.8)	6 (46.2)	0 (0)	0 (0)	
Solid tumor	0 (0)	8 (61.5)	5 (38.5)	0 (0)	0.002	0 (0)	4 (30.8)	9 (69.2)	0 (0)	0.01	7 (53.8)	6 (46.2)	0 (0)	0 (0)	0.8
Perivascular	0 (0)	12 (92.3)	1 (7.7)	0 (0)		0 (0)	5 (38.5)	8 (61.5)	0 (0)		7 (53.8)	6 (46.2)	0 (0)	0 (0)	
Border	1 (7.7)	3 (23.1)	9 (69.2)	0 (0)		0 (0)	0 (0)	9 (69.2)	4 (30.8)		5 (38.5)	8 (61.5)	0 (0)	0 (0)	

For each immune marker, ordinal data presented, 1: None or sporadic, 2: moderate, 3: abundant, 4: highly abundant. Numbers in parenthesis represent percentage within a row for each immune marker. Numbers may not add to 100 due to rounding. P value for Fisher's exact test comparing each marker's density between solid tumor, perivascular, and border compartments

respectively. All BM cases had CD163^{+} TAMs present. Overall presence of CD68^{+} TAMs among BM neurosurgical resections was significantly associated with improved survival (19.3 months vs 4.3 months) (P = 0.03, log-rank) (Fig. 2D). There was no significant association with CD4 (P = 0.4), CD8 (P = 0.8), CD45RO (P = 0.8), and FOXP3 (P = 0.7) and overall survival.

Discussion

We identified a large cohort of 44 patients with BM from gynecologic malignancies from endometrial and ovarian cancer. Time to BM diagnosis was 2.1 years (range 0–15.0 years), which is similar to a median of 24 months (range 0–133 months) reported in a systematic review of 591 ovarian cancer patients with BM [36]. Therefore, the treating gynecologic oncologist should be mindful of new-onset neurological symptomology, even over a decade from primary disease diagnosis. Initial neurosurgical resection was associated with improved overall survival (15.4 months compared to 2.4 months). Pothuri et al. similarly reported in a cohort of 14 ovarian BM patients that neurosurgical resection had improved survival of 18 months with a 2-year survival rate of 39% [37].

From available surgical specimens, we performed the first evaluation to our knowledge of the tumor immune microenvironment in BM from gynecologic malignances. Our data demonstrate an active tumor immune microenvironment within the central nervous system that is similar to the primary disease site.

Increased activity within the tumor immune microenvironment in BM patients correlates with improved prognosis [38]. In our cohort, the presence of CD68^{+} TAMs in the BM was associated with improved survival. FOXP3^{+} TILs, which support an immunosuppressive microenvironment [39], were least frequently observed in both primary and BM sites. In primary high-grade serous ovarian carcinoma, PD-L1^{+} CD68^{+} TAMs are associated with improved survival [40], see [41] for a comprehensive review of the immune microenvironment in primary disease sites in gynecologic malignancies. Evaluation of the immune microenvironment in a cohort of 84 breast cancer BM similarly identified that CD68^{+} TAMs were protective for overall survival in multivariable analysis (Hazard Ratio = 0.2) [26]. Such evidence adds encouragement for the potential role of checkpoint inhibitors in BM from gynecologic malignancies.

Compared to the solid tumor and perivascular compartments, we observed an increase in border region activity of TILs and TAMs within both primary and BM sites. Sobottka et al. similarly identified this pattern in a cohort of 87 breast cancer patients with tissue from primary site, extra-cranial metastases, and BM [31]. Evaluation of mononuclear cells,
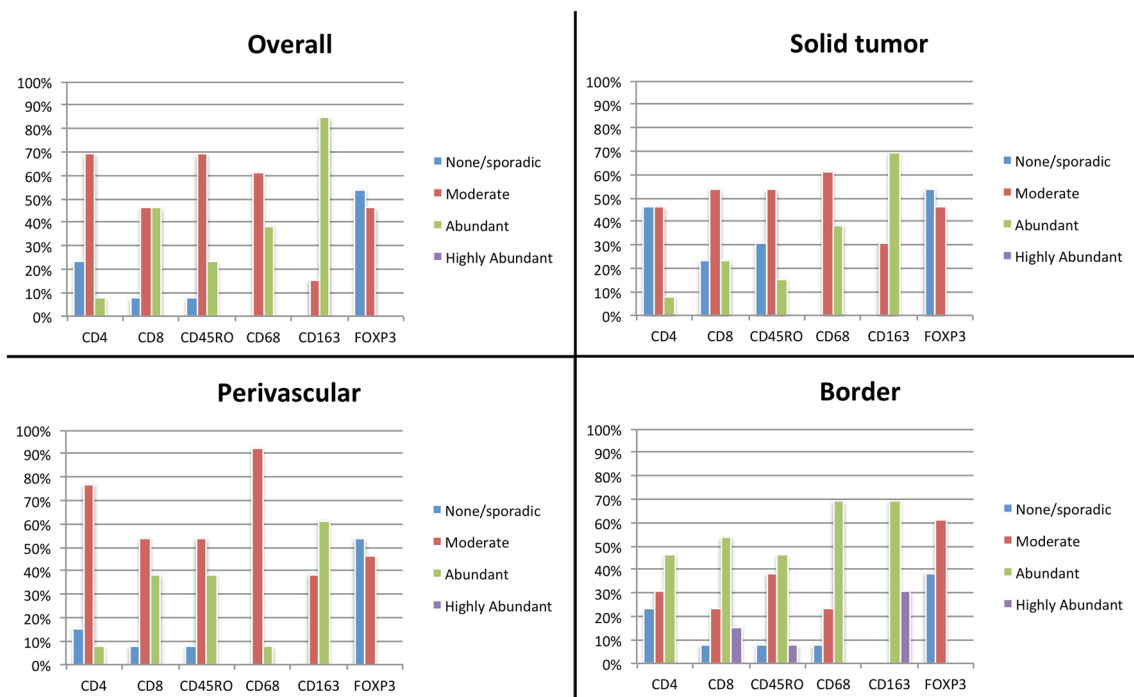
Fig. 4 Distribution of tumor-infiltrating lymphocytes and tumor-associated macrophages from 13 primary cases. Y-axis reports percent of ordinal data: none or sporadic, moderate, abundant, and highly abundant

which included lymphocytes, histiocytes, and plasma cells, in a cohort of 208 BM from lung adenocarcinoma found that increased border region activity, denoted as a "mononuclear ring," was associated with improved survival after BM surgery [24]. Increased activity within the border region between BM and surrounding brain parenchyma may represent a novel biomarker of cross-communication with an active tumor immune microenvironment.

Beyond TILs and TAMs, emerging evidence supports that underlying tumor oncogenes affect response to immunotherapy and play an integral role in immune escape through tumor microenvironment remodeling [42]. Thus, there is a need to understand the genomic complexity of BM beyond primary disease site. Indeed, there is growing evidence of divergent genomic evolution in metastatic brain lesions from primary disease site [43], which is similar to divergent PD-L1 expression seen in BM [44]. However, genomic BM data within gynecologic malignancies are limited to ovarian cancer. Notably, no study has evaluated underlying genomic alterations in a cohort of BM from endometrial cancer.

BRCA mutations may be associated with increased likelihood of BM for women with ovarian cancer. In a recent study of 4515 patients with ovarian cancer, a total of 46 developed BM [45]. From the 10% with a known *BRCA* mutation, 5.7% of patients at 5 years developed BM compared to 1.4% in patients who were *BRCA* wild type. Stasenko et al. evaluated 3649 patients with epithelial ovarian cancer, among which 91 patients developed BM. Overall survival from time of BM diagnosis was significantly reduced in patients with a *BRCA* mutation (29 months vs 9 months) [46]. Next-generation sequencing of 8 BM samples from ovarian cancer revealed a *BRCA1/2* mutation in 87.5% of 8 BM cases [47]. A second study that evaluated *BRCA1* mutation status in a cohort of 7 BM samples identified loss of heterozygosity of *BRCA1* in 57.1% of BM cases [48]. These data demonstrate that *BRCA* mutation status plays an important role in the risk of developing a BM in ovarian cancer.

The understanding of how the genomic complexity of BM differs from primary disease site may help to guide

Table 2 Distribution of tumor-infiltrating lymphocytes and tumor-associated macrophages from 15 BM cases

	CD4					CD8					CD45RO				
	1	2	3	4	P value	1	2	3	4	P value	1	2	3	4	P value
Overall	6 (40.0)	8 (53.3)	1 (6.7)	0 (0)		1 (6.7)	12 (80.0)	2 (13.3)	0 (0)		4 (26.7)	8 (53.3)	3 (20.0)	0 (0)	
Solid tumor	8 (53.3)	7 (46.7)	0 (0)	0 (0)	0.01	1 (6.7)	12 (80.0)	2 (13.3)	0 (0)	0.8	5 (33.3)	8 (53.3)	2 (13.3)	0 (0)	0.4
Perivascular	2 (13.3)	8 (53.3)	5 (33.3)	0 (0)		1 (6.7)	12 (80.0)	2 (13.3)	0 (0)		2 (13.3)	7 (46.7)	6 (40.0)	0 (0)	
Border	4 (26.7)	4 (26.7)	7 (46.7)	0 (0)		2 (13.3)	9 (60.0)	4 (26.7)	0 (0)		2 (13.3)	6 (40.0)	6 (40.0)	1 (6.7)	

	CD68					CD163					FOXP3				
	1	2	3	4	P value	1	2	3	4	P value	1	2	3	4	P value
Overall	2 (13.3)	5 (33.3)	8 (53.3)	0 (0)		0 (0)	2 (13.3)	12 (80.0)	0 (0)		9 (64.3)	5 (35.7)	0 (0)	0 (0)	
Solid tumor	2 (13.3)	8 (53.3)	5 (33.3)	0 (0)	0.3	0 (0)	5 (33.3)	9 (60.0)	0 (0)	0.009	8 (57.1)	6 (42.9)	0 (0)	0 (0)	0.2
Perivascular	1 (6.7)	9 (60.0)	5 (33.3)	0 (0)		0 (0)	1 (6.7)	13 (86.7)	0 (0)		10 (71.4)	4 (28.6)	0 (0)	0 (0)	
Border	0 (0)	5 (33.3)	9 (60.0)	1 (6.7)		0 (0)	1 (6.7)	7 (46.7)	1 (6.7)		5 (35.7)	9 (64.3)	0 (0)	0 (0)	

For each immune marker, ordinal data presented, 1: None or sporadic, 2: moderate, 3: abundant, 4: highly abundant. Numbers in parenthesis represent percentage within a row for each immune marker. Numbers may not add to 100 due to rounding. P value for Fisher's exact test comparing each marker's density between solid tumor, perivascular, and border compartments. Data for FOXP3 are available in only 14/15 BM cases

targeted therapy treatment regimens. In a patient with wide-spread metastatic low-grade serous ovarian cancer who underwent targeted therapy with binimetinib, a MEK inhibitor, genomic sequencing of the BM revealed copy number gain in Chr 13, Chr 20, and Chr 21 that were not present in the primary disease site [49]. Moreover, the BM had focal amplification of *ERBB3* at Chr 12q13.11-q14.1, which represents an acquired MEK inhibitor resistance alteration. A second patient with a known *BRCA2* mutation from ovarian cancer developed leptomeningeal disease in the setting of a previously treated solitary parietal BM [50]. Targeted therapy with olaparib, a PARP inhibitor, resulted in a clinical and radiological response for 1 year. PARP inhibitor targeted therapy with niraparib resulted in 17 months without disease progression in a third patient with BM from high-grade serous ovarian cancer [51].

Our study had several limitations. Given the study's retrospective nature spanning over 15 years, the availability of archival surgical specimens was limited, especially from primary disease site. Not all patients underwent neurosurgical resection of metastatic brain disease; tissue was not available for analysis for patients who did not undergo neurosurgery. Although we studied representative surgical specimens, it is not known how characterization of TILs and TAMs from the surgical specimen represents the entirety of the tumor. The degree of heterogeneity that may exist is unknown. *BRCA* status for the majority of our cases is unknown and therefore it was not included in the analysis. Given that BM from gynecologic malignancies is a rare occurrence, we were not adequately powered to perform substantial clinical correlates with TIL and TAM density and distribution. Inter-institutional and collaborative group efforts will be necessary to perform such definitive analyses.

Consortium-based guidelines, such as Response Assessment in Neuro-Oncology Brain Metastases (RANO-BM), may help to guide these advancements when developing clinical trials [52]. When safe, neurosurgical resection of BM should be considered, given the association with improved survival in our cohort and other reports in the literature. Furthermore, obtaining neurosurgical tissue from BM is critical to advancing the understanding of disease progression and mechanisms of resistance in gynecologic malignancies. Future work ought to couple genomic and immune evaluation of metastatic lesions to inform our understanding of how underlying tumor biology impacts immune response.
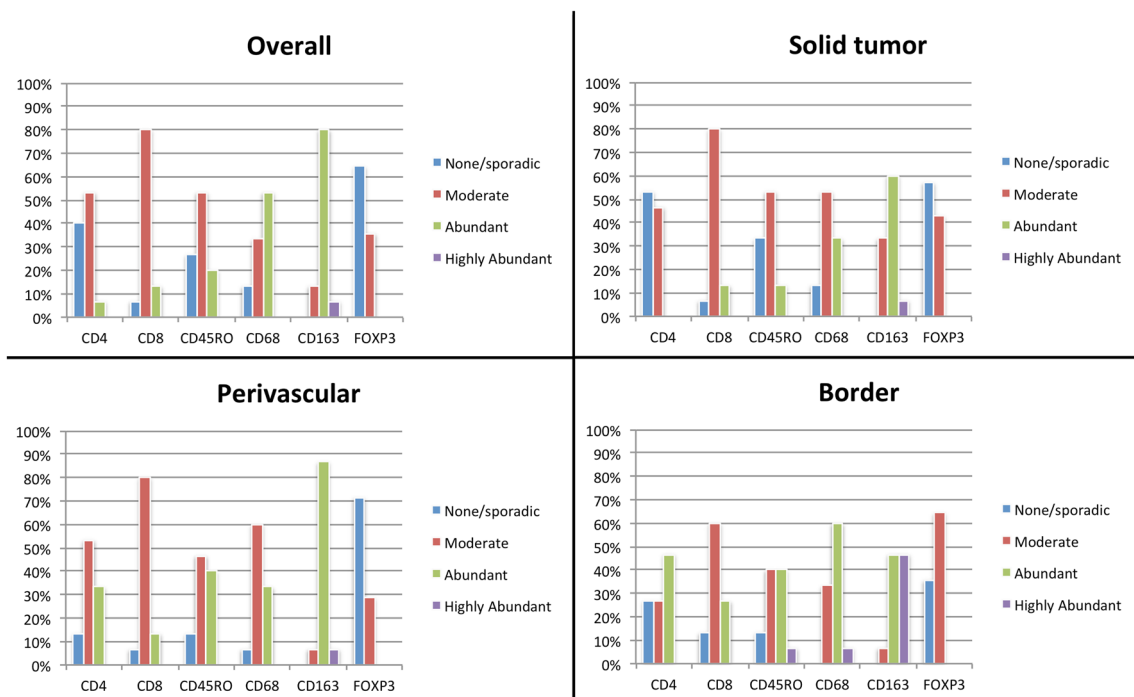
Fig. 5 Distribution of tumor-infiltrating lymphocytes and tumor-associated macrophages from 15 BM cases. Y-axis reports percent of ordinal data: none or sporadic, moderate, abundant, and highly abundant

Declarations

Ethics approval Ethics approval was obtained from Icahn School of Medicine at Mount Sinai. Written informed consent was waived.

Conflict of interest The authors declare that there are no conflicts of interest or competing interests.

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