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Cerebrospinal fluid immunological cytokines predict intracranial tumor response to immunotherapy in non-small cell lung cancer patients with brain metastases

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ABSTRACT

Background: Immunotherapy has shown intracranial efficacy in non-small cell lung cancer (NSCLC) patients with brain metastases. However, predictive biomarkers for intracranial response to immunotherapy are lacking. This post-hoc analysis aimed to explore the potential of immunological cytokines in cerebrospinal fluid (CSF) to predict intracranial tumor response to immunotherapy in patients with brain metastases.

Methods: Treatment-naive NSCLC patients with brain metastases who received camrelizumab plus chemotherapy were enrolled. Paired plasma and CSF samples were prospectively collected at baseline and the first treatment assessment. All samples were analyzed for 92 immuno-oncology cytokines using Olink's panels.

Results: A total of 28 patients were included in this analysis. At baseline, most immunological cytokines were significantly lower in CSF than in plasma, whereas a subset comprising CD83, PTN, TNFRSF21, TWEAK, ICOSLG, DCN, IL-8, and MCP-1, was increased in CSF. Baseline CSF levels of LAMP3 were significantly higher in patients with intracranial tumor response, while the levels of CXCL10, IL-12, CXCL11, IL-18, TIE2, HGF, and PDCD1 were significantly lower. Furthermore, the CXCL10, CXCL11, TIE2, PDCD1, IL-18, HGF, and LAMP3 in CSF were also significantly associated with intracranial progression-free survival for immunotherapy. The identified cytokines in CSF were decreased at the first treatment evaluation in patients with intracranial tumor response. The logistic CSF immuno-cytokine model yielded an AUC of 0.91, as compared to PD-L1 expression (AUC of 0.72).

Conclusions: Immunological cytokines in CSF could predict intracranial tumor response to immunotherapy in NSCLC patients with brain metastases, and the findings warrant validation in a larger prospective cohort study.

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Introduction

Brain metastases, the most common type of central nervous system (CNS) tumors, are often associated with poor prognosis and unsatisfactory therapeutic outcomes.[1] Lung cancer is the leading malignancy associated with brain metastases, and approximately 20–40% of lung cancer patients would develop brain metastases during the course of their treatment.[2] Immune checkpoint inhibitors (ICIs) have been the standard treatment for advanced non-small cell lung cancer (NSCLC), either as monotherapy or in combination with chemotherapy.[3,4] In NSCLC patients with brain metatases, pembrolizumab showed CNS efficacy in a prospective phase 2 study.[5] Recently, our multicenter, prospective clinical trial explored the efficacy and safety of camrelizumab combined with chemotherapy as a first-line therapy for NSCLC patients with brain metastases, revealing favorable active with manageable toxicity.[6] However, only a subset of patients responds to immunotherapy. Therefore, finding appropriate predictive biomarkers for immunotherapy is urgently needed for patients with brain metastases.

The brain is regarded as an immune-privileged organ, and brain metastatic tumors exhibit unique genomic characteristics and tumor immune microenvironment compared to their primary tumors.[7,8] Recent studies have demonstrated temporal and spatial discordance in PD-L1 expression and tumor-infiltrating lymphocytes between paired primary lung tumors and brain metastases.[9] However, obtaining intracranial tumor samples is challenging due to the invasive nature of the surgical procedures. Additionally, the inherent tumor heterogeneity limits the representativeness utility of extracranial tumors for predicting intracranial tumor efficacy. Our previous study, along with others, has shown the potential of circulating tumor DNA in cerebrospinal fluid (CSF) to reflect the genetic profiles of brain metastases and predict intracranial responses.[10,11] Moreover, a recent study revealed that immune cell profiling within CSF could represent the characteristics of the brain metastases microenvironment.[12] However, it remains unknown whether CSF can predict the intracranial response to immunotherapy.

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We prospectively collected paired CSF and plasma samples at baseline and during treatment from a prospective clinical trial of camrelizumab plus pemetrexed and carboplatin chemotherapy as first-line treatment for NSCLC with brain metastases. We aimed to explore immunological cytokines in the CSF to predict intracranial tumor responses to immunotherapy in NSCLC patients with brain metastases in this post hoc analysis.

Methods

Study design and participants

A multi-center, single-arm, prospective clinical trial (NCT04211090), was completed and has been reported (PMID:36738928). Eligible participants met the following criteria: pathological or cytologically confirmed non-squamous NSCLC without *EGFR* mutations or *ALK* rearrangement; brain metastases confirmed by enhanced brain magnetic resonance imaging (MRI) at primary diagnosis; at least one measurable brain lesion with a longest diameter of >5 mm; treatment-naïve (no previous systemic therapy, surgery, or radiotherapy); brain metastases were asymptomatic or responding to corticosteroid treatment; and had no contraindication for lumbar puncture. All enrolled patients received camrelizumab (200 mg) plus pemetrexed (500 mg/m^2) and carboplatin (area under the curve [AUC] of 5) chemotherapy every 21 days for four cycles, followed by camrelizumab (200 mg) plus pemetrexed (500 mg/m^2) maintenance until disease progression, unacceptable toxicity, death, or 24 months of camrelizumab.

For this analysis, we included patients from Sun Yat-sen University Cancer Center (Guangzhou, China) who provided paired CSF and plasma samples before treatment (baseline) and at the first treatment assessment. The CSF samples were centrifuged at 500 g for 10 min, plasma samples were centrifuged at 2000 rpm for 10 min, and then stored at −80°C until assayed. All samples were processed within two hours. All patients provided written informed consent to participate in the study and to provide samples for the analysis.

Immunological cytokines assay

The Target 92 Immune-Oncology panel from Olink's (Uppsala, Sweden) was used to quantify 92 cytokines in the CSF and plasma at Sequanta Technologies Co., Ltd. To ensure objectivity, samples were distributed randomly across assay plates, and laboratory technicians remained unaware of the corresponding clinical data.

A multiplex proximity extension assay panel was used to quantify each protein as previously described.[13] In this assay, a dual-recognition immunoassay mechanism was utilized, wherein two paired antibodies, each tagged with unique DNA oligonucleotides, concurrently bound to the target protein in the liquid medium. This brings the two antibodies into proximity, allowing hybridization and serving as a template for the DNA polymerase-dependent extension step. Double-stranded DNA is unique to a specific antigen and is amplified using a primer, which is quantitatively proportional to the sample concentration of the target protein. The amplified targets were then quantified by RT-PCR using a Fluidigm BioMarkTM Microfluidic qPCR instrument. Protein abundance was reported as normalized protein expression (NPX), Olink's arbitrary relative unit, which is on the Log2 scale. Four internal and three negative controls were used to calculate the lower limit of detection (LOD) for each protein. Proteins with levels below the LOD in more than 50% of the samples were excluded from further analysis.

Immunohistochemistry

To evaluate PD-L1 expression, formalin-fixed paraffin-embedded samples of primary lung tumors were employed. Immunohistochemical staining was conducted utilizing the PD-L1 Clone 22C3 pharmDx Kit and Dako Automated Link 48 platform. The tumor proportion score (TPS) was defined as the percentage of cells with positive membrane staining after inspecting 100 viable cancer cells, which were categorized into three groups based on TPS values (TPS of 0%, 1–49% and ≥50%). The combined proportion score (CPS) was defined as expression of PD-L1 in both tumor and inflammatory cells, CPS was evaluated by the following formula: (PD-L1 positive tumor cells + PD-L1 positive inflammatory cells)/(total tumor cells) * 100, the optimal cut-off of PD-L1 CPS was unclear, we also categorized CPS into three groups (CPS of 0, 1–49, and ≥50) according to recent studies.[14,15]

Clinical outcomes

Baseline radiographic evaluations were conducted, followed by assessments every two cycles according to the modified RECIST 1.1, until disease progression (i.e., enhanced computerized tomography scans for extracranial lesions and enhanced magnetic resonance imaging for intracranial lesions). Intracranial progression-free survival (PFS) (time from the start of treatment to intracranial disease progression or death), extracranial PFS (time from the start of treatment to extracranial disease progression or death), intracranial objective response rate (ORR) (proportion of patients with complete or partial response of intracranial lesions), extracranial ORR (proportion of patients with complete or partial response of extracranial lesions), and overall survival (OS: time from the start of treatment to death from any cause) were assessed.

Statistical analysis

The Mann-Whitney U test was performed to compare immunological cytokines between the different clinical response groups. Paired plasma-CSF analyses were performed using the Wilcoxon matched-pairs signed-rank test ("ggpubr"). Kaplan-Meier curves were used to estimate the survival and determine the optimal cutoff levels of cytokines with significant differences observed in the Mann-Whitney U test, and differences between the groups were evaluated using the log-rank test ("survival" and "survminer" package). Considered a covariant, significant cytokines were further assessed using logistic LASSO regression models to predict clinical response ("glmnet" package). Independent variants were subsequently integrated into multivariate logistic regression analysis. The

model performance was further assessed by two internal cross validation methods: i) ten-fold cross validation for 100 times. ii) leave-one-out cross validation method (R package "caret"). The predictive performance was evaluated based on the area under the ROC curve (AUC), accuracy, sensitivity, and specificity ("pROC" package). Fisher's exact test was using for the comparison of categorical variables. All statistical analyses were performed using the R software (version 3.5.3). Two-tailed *p*-values of less than 0.05 were considered statistically significant.

Results

Patients' characteristics and clinical outcomes

Between April 2020 and May 2022, a cohort of 28 untreated NSCLC patients with brain metastases was enrolled at the Sun Yat-sen University Cancer Center. The baseline clinical characteristics of patients are shown in Table 1. The cohort patients had a median age of 57 years (rang: 43–72 years), with 27 male patients, 22 smokers, and 20 patients with an Eastern Cooperative Oncology Group Performance Status (ECOG PS) score of 0–1. Ten patients had symptomatic brain metastases, which could be alleviated with dehydration therapy using dexamethasone and/or mannitol. All the patients received camrelizumab combined with pemetrexed and carboplatin chemotherapy as their first-line treatment. Evaluation of PD-L1 expression was performed on primary lung tumor tissues, with 14.3% (4/28) demonstrating PD-L1 TPS of 0%, 32.1% (9/28) with PD-L1 TPS of 1–49%, and 53.6% (15/28) with PD-L1 TPS ≥ 50%. Representative immunohistochemistry results illustrating distinct PD-L1 expressions are provided in Supplementary Figure 1.

At the data cutoff, the median follow-up time was 28.0 months (IQR, 9.30–21.03 months). The intracranial objective response rate was 39.3% (comprising two patients with complete response and nine patients with partial response), and the extracranial objective response rate was 53.6% (15 patients with partial response) in the entire study cohort.

The median intracranial PFS was 8.0 months (95%CI 3.0–17.7), the median extracranial PFS was 8.5 months (95%CI 3.0–14.0), and the median OS was 29.2 months (95%CI 13.1–45.3). In total, 28 CSF and 25 plasma samples were collected from 28 patients at baseline (before treatment), and 11 CSF and 9

Table 1. Baseline characteristics of this study cohort.

Characteristics	No. of patients (%)
Age, median (range)	57 (43–72)
Sex	
Male	27 (96.4)
Female	1 (3.6)
Smoking status	
Smokers	22 (78.6)
Non-smokers	6 (21.4)
ECOG PS	
0–1	20 (71.4)
≥2	8 (28.6)
CNS symptom	
Yes	10 (35.7)
No	18 (64.3)
CSF cytology	
Positive	1 (3.6)
Negative	25 (89.3)
Unknown	2 (7.1)
Lung-molGPA[a]	
0–1	3 (10.7)
1.5–2	15 (53.6)
2.5–3	10 (35.7)
extracranial metastases	
Yes	18 (64.3)
No	10 (35.7)
PD-L1 TPS[b]	
<1%	4 (14.3)
1–49%	9 (32.1)
≥50%	15 (53.6)
Intracranial tumor size[c]	
>20mm	15 (53.6)
≤20mm	13 (46.4)
Intracranial tumor number[c]	
1–3	15 (53.6)
≥4	13 (46.4)

[a]An update of the diagnosis-specific Graded Prognostic Assessment (DS-GPA) using molecular markers.
[b]PD-L1 expression was evaluated by immunohistochemistry in primary lung tumor tissues.
[c]Intracranial tumor characteristics were evaluated according to baseline enhanced brain magnetic resonance imaging.
Abbreviations: ECOG PS, Eastern Cooperative Oncology Group performance status; CNS, central nervous system; CSF, cerebrospinal fluid.

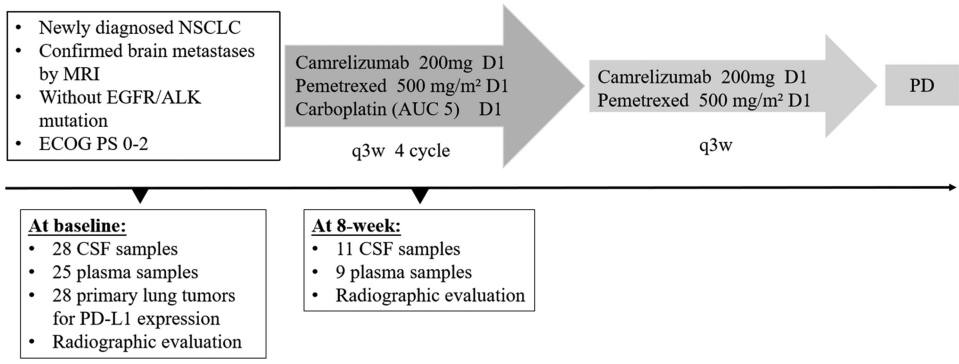

Figure 1. Flow chart of this study.

plasma samples were collected from 11 patients at the first treatment evaluation (Figure 1). Supplementary Table S1 provided detailed patient information.

Baseline immunological cytokines profiles between paired CSF and plasma

Among the 92 immunological cytokines assayed in this study, 23 cytokines in CSF and 7 cytokines in plasma were excluded due to low detection levels falling below the LOD in more than 50% of the samples. We finally focused on 69 cytokines in paired CSF and plasma samples for further analyses (Supplementary Table S2). Among the 25 patients with paired CSF and plasma samples at baseline, we compared 69 immunological cytokines profiles between two sample types. At baseline, with the exceptions of CAIX, IL-15, IL-6, MIC-A/B, PGF, and CXCL10 cytokines, all other cytokines exhibited significantly different expression levels between the CSF and plasma samples. Notably, the majority of immunological cytokines were significantly lower in paired CSF compared to plasma. However, a subset including CD83, PTN, TNFRSF21, TWEAK, ICOSLG, DCN, IL-8, and MCP-1 exhibited elevated levels in CSF samples (Figure 2).

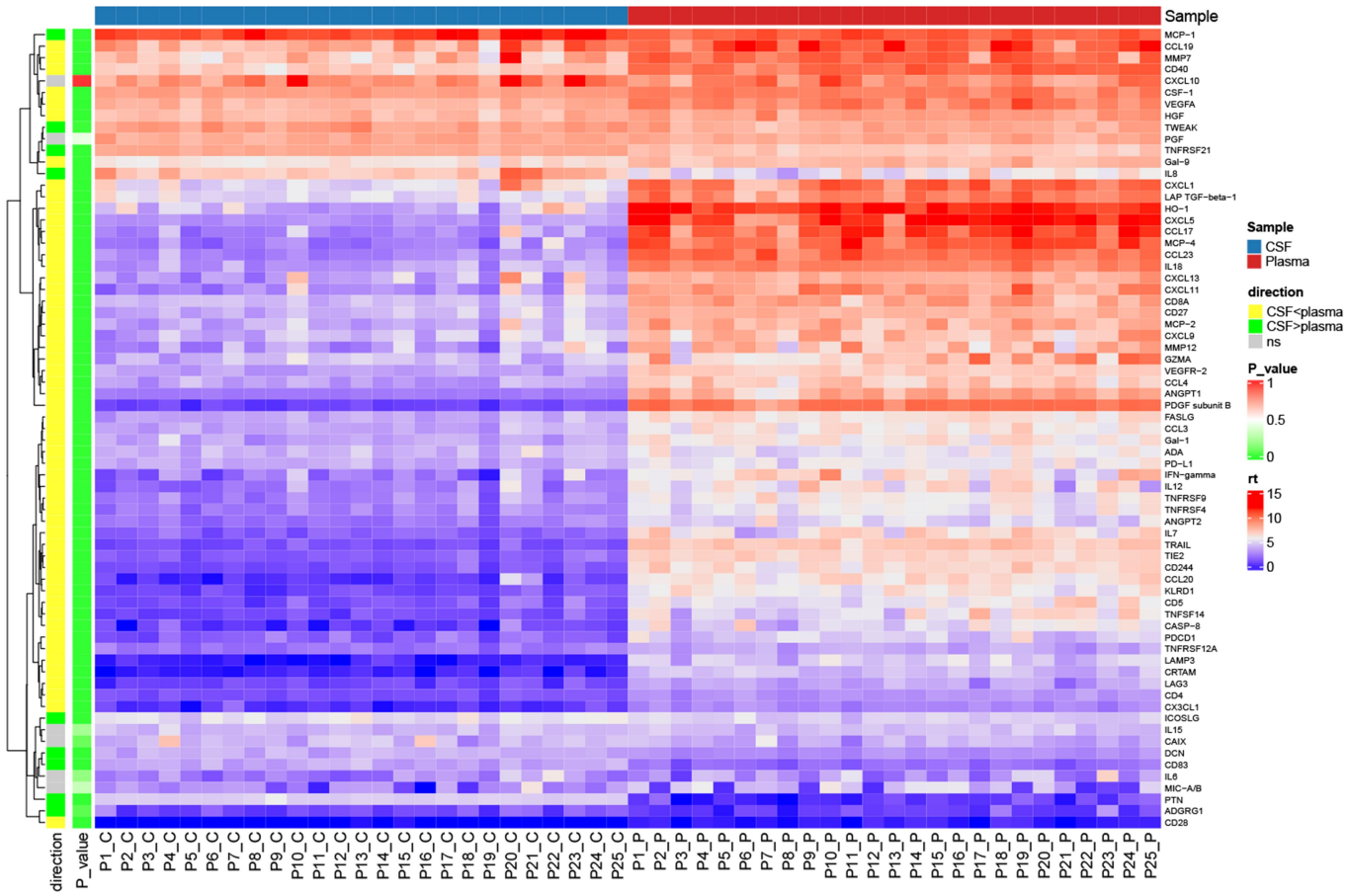

Figure 2. Heatmap of baseline immunological cytokines in paired CSF and plasma samples. *P* value was evaluated using Wilcoxon matched-pairs signed rank test. Abbreviations, CSF, cerebrospinal fluid.

We then compared the levels of immunological cytokines between different PD-L1 expression groups, but found no cytokine in CSF or plasma was significantly different expressed across PD-L1 expression groups (PD-L1 TPS: 0% vs. 1–49% vs. ≥50%). Furthermore, the tumor PD-L1 expression also exhibited poor correlations with the cytokines levels in either CSF or plasma (Supplementary Figure S2–4). We then explored the correlation between OS and cytokines in baseline CSF and plasma samples. However, no cytokine demonstrated significant association with OS using Cox regression analysis (data not shown).

Immunological cytokines in CSF predict intracranial tumor response to immunotherapy

Within this study cohort, 11 patients achieved intracranial tumor response (2 patients with complete response and 9 patients with partial response) following camrelizumab plus chemotherapy treatment, and 17 patients achieved stable or progressive disease of intracranial tumors. The baseline clinical characteristics were comparable between the two groups (Supplementary Table S3). We investigated the association between immunological cytokines in CSF and intracranial tumor response. We found that LAMP3 levels in CSF were significantly higher in the intracranial response group compared to the non-response group. Conversely, CXCL10, IL-12, CXCL11, IL-18, TIE2, HGF, and PDCD1 levels were significantly lower in the intracranial response group (Figure 3(a)). For further analysis, Kaplan-Meier curves were employed to estimate the intracranial PFS and identify the optimal cytokines cutoff values. Patients with lower levels of CXCL10, CXCL11, TIE2, PDCD1, IL-18, and HGF in baseline CSF had significantly longer intracranial PFS than those with higher levels. While patients with higher levels of LAMP3 had significantly longer intracranial PFS (Supplementary Figure S5). Furthermore, logistic LASSO regression analysis identified TIE2, IL-18, LAMP3, PDCD1, and IL-12 as significant cytokines associated with intracranial tumor response (Figure 3(b)).

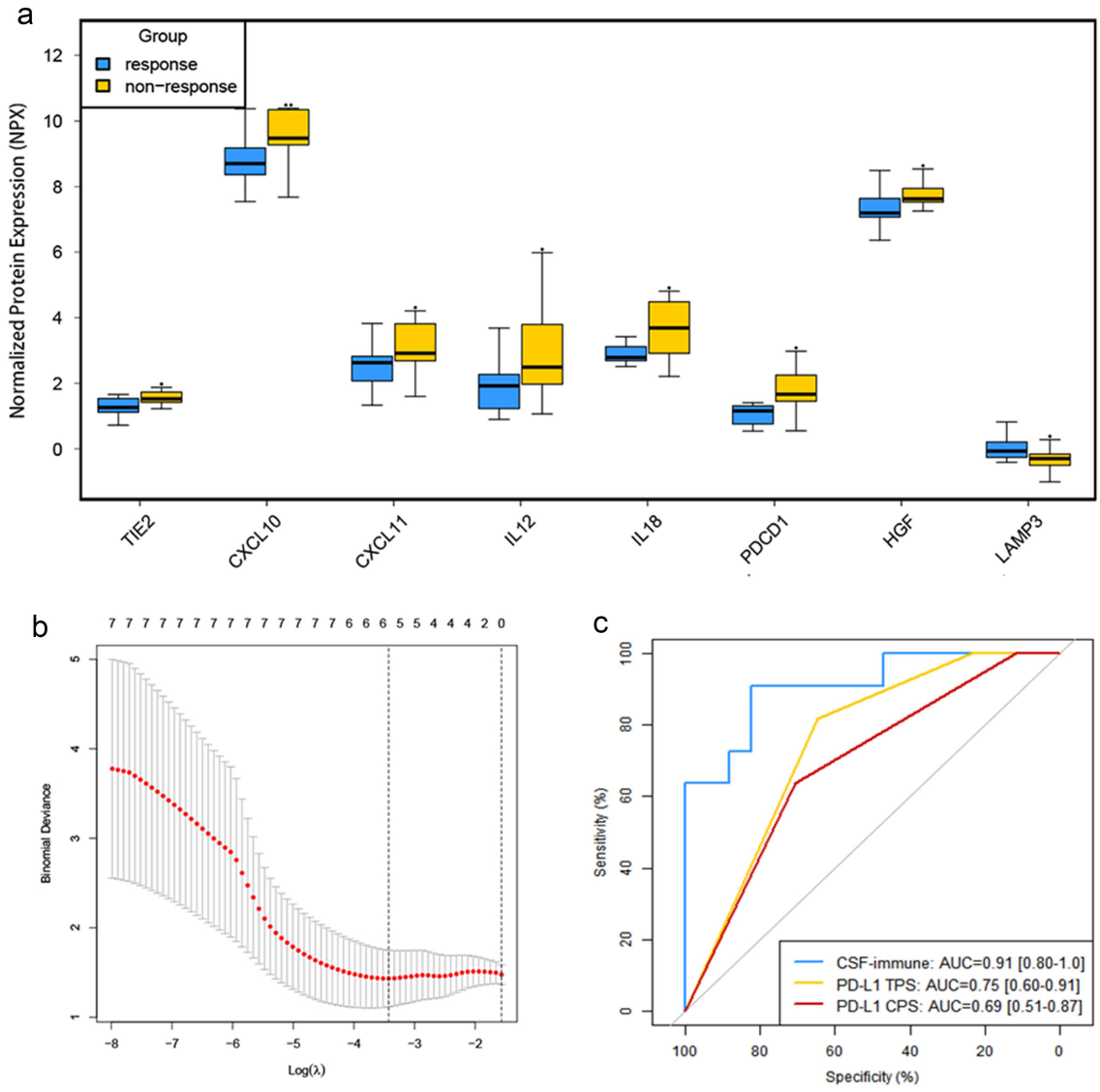

Figure 3. Comparison of immunological cytokines in CSF between intracranial tumors response and non-response groups. (a) Boxplot of significantly different cytokines in CSF between intracranial tumor response and non-response groups. *P* value was evaluated by Mann-Whitney U test. (b) LASSO logistic regression model. (c) Receiver operating characteristic (ROC) curve of the CSF immuno-cytokines and PD-L1 expression for intracranial tumors response.

We conducted ROC analyses to compare the predictive abilities of immunological cytokines in the CSF with PD-L1 expression in primary lung tumors. Our logistic CSF immuno-cytokines model yielded an AUC of 0.91 (95%CI 0.80–1.0) for predicting intracranial tumor response. While the PD-L1 expression of primary tumors achieved an AUC of 0.75 (95% CI 0.60–0.91) with TPS, and an AUC of 0.69 (95%CI 0.51–0.87) with CPS (Figure 3(c)). Model performance was further verified through two internal cross-validation methods. The 10-fold cross-validation, repeated 100 times, demonstrated an AUC of 0.76, sensitivity of 0.55, and specificity of 0.71. And the leave-one-out cross-validation yielded an AUC of 0.76, sensitivity of 0.55, and specificity of 0.71 (Supplementary Table S4).

In further multivariate analysis, we identified LAMP3 in baseline CSF as an independent predictive biomarker for intracranial tumor response to immunotherapy in NSCLC patients with brain metastases (Table 2). Interestingly, among the 11 patients with paired dynamic CSF samples at the first treatment evaluation, we observed these CSF immunological cytokine levels significantly decreased following immunotherapy in patients with intracranial tumor response. While these changes were not significant in patients with non-response (Figure 4). However, no significant cytokine in the baseline plasma was associated with intracranial tumor response to immunotherapy in patients with brain metastases (data not shown).

We also evaluated the association between baseline plasma cytokines levels and extracranial tumor response to immunotherapy. Fourteen plasma cytokines levels were significantly higher in patients with extracranial tumor responses compared to those without response (Supplementary Figure S6). Logistic LASSO regression analysis identified CD40, Gal-9, VEGFA, and ADA levels were significantly associated with extracranial tumor response. In further multivariate analysis, Gal-9 in baseline plasma was an independent predictive biomarker of extracranial tumor response to immunotherapy (Supplementary Table S5).

Immunological cytokines in CSF predict durable clinical benefit to immunotherapy

In our cohort, we observed six patients with PFS >18 months (both intracranial and extracranial PFS) to camrelizumab plus chemotherapy. This group was considered as durable clinical benefit (DCB). And we observed 14 patients with less than 6 months of PFS, considered as non-DCB. We compared the baseline immunological cytokines in the CSF and plasma between patients with DCB and non-DCB. While no plasma cytokine was associated with DCB to immunotherapy, we found significantly lower levels of ADGRG1, CXCL1, CCL17, CCL20, IL-18, CCL23, CXCL5, and CXCL13 in the CSF of patients with DCB than in those with non-DCB, and levels of PTN were significantly higher in patients with DCB (Figure 5(a)).

Through LASSO regression model analysis, we identified ADGRG1, IL-18, CXCL1, PTN, and CCL20 in CSF as significant cytokines associated with durable clinical benefit to immunotherapy (Figure 5(b)). Our CSF immuno-cytokines model achieved an AUC of 1.0 in predicting durable clinical benefit to immunotherapy, as compared to the PD-L1 TPS with an AUC of 0.79, and PD-L1 CPS with an AUC of 0.71 (Figure 5(c)). Model performance was also evaluated by two internal cross-validation methods. The 10-fold cross validation, repeated 100 times, yielded an AUC of 0.88, sensitivity of 0.67, and specificity of 0.93. And the leave-one-out cross-validation yielded an AUC of 0.85, sensitivity of 0.67, and specificity of 0.93, respectively (Supplementary Table S4). Moreover, when we extend the CSF immuno-cytokines model to the entire study cohort, it consistently demonstrated superior predictive performance (AUC of 0.88) for DCB compared to PD-L1 expression (AUC of 0.71 with TPS, and AUC of 0.67 with CPS) within the entire cohort (Supplementary Figure S7). Internal cross-validations for CSF immuno-cytokines model in whole cohort demonstrated the AUC of 0.73 (10-fold cross validation, repeated 100 times) and the AUC of 0.70 (leave-one-out cross-validation).

Further multivariate analysis found IL-18 and PTN in CSF were independent predictive markers for the durable clinical benefit to immunotherapy in NSCLC patients with brain metastases (Table 2). The baseline clinical characteristics were comparable between patients with DCB and non-DCB (Supplemental Table S6).

Discussion

In this prospective study, we performed immunological cytokines profiling in the CSF, and found that immunological cytokines in the CSF could better predict intracranial tumor response to immunotherapy in NSCLC patients with brain metastases. To the best of our knowledge, this is the first study to report CSF predictive biomarkers for immunotherapy, providing a relatively noninvasive tool for predicting intracranial response to immunotherapy in patients with brain metastases.

Traditionally, the brain is considered as an immune-privileged organ, and sheltered from immune surveillance by the blood-brain barrier (BBB)[16]. Brain metastases, exhibit different molecular and microenvironmental characteristics compared to their primary tumors. Our previous study also revealed that the immune microenvironment of brain metastases is further immunosuppressed compared with primary lung tumors, and the expression of PD-L1 in brain metastases is poorly correlated with paired primary lung tumors[17]. However, the anatomical location of brain metastases limits its availability and thus the characterization of the microenvironment of brain metastases. A recent study compared immune cell profiles in brain tumors with those in paired CSF samples using single-cell RNA sequencing. The study found immune microenvironment in brain metastatic lesions can be characterized by CSF immune cell profiles,[12] thus it may be a noninvasive tool to characterize and predict tumor responses to immunotherapy.

In our study, we found that most immunological cytokines were expressed at significantly lower levels in the CSF than in paired plasma. This finding was consistent with the observations from a previous study, which found reductions of chemokine CCL22, as well as cytokines IL1a, IL4, and IL5 in CSF samples, while CXCL10, CCL4, CCL17, and IL8 were increased in CSF samples from patients with melanoma brain metastases

Table 2. The immunological cytokines in baseline CSF between different clinical group.

Cytokines	Response (NPX)	Non-response (NPX)	Univariate analysis (*p* value)	Multivariate analysis (*p* value)[a]
Intracranial tumor response				
TIE2	1.26	1.52	0.022	0.289
IL-12	1.92	2.49	0.024	0.118
IL-18	2.79	3.69	0.038	0.211
PDCD1	1.16	1.66	0.038	0.392
HGF	7.19	7.62	0.048	–
CXCL10	8.70	9.47	0.011	–
CXCL11	2.63	2.91	0.043	–
LAMP3	−0.067	−0.30	0.030	0.002 *
Durable clinical benefit				
Cytokines	DCB (NPX)	non-DCB (NPX)	Univariate analysis (*p* value)	Multivariate analysis (*p* value)
ADGRG1	0.44	1.05	0.043	1.0
CXCL1	4.69	5.43	0.029	1.0
CCL17	2.28	2.60	0.035	–
CCL20	−0.01	0.65	0.035	1.0
CXCL5	2.79	3.17	0.043	–
CXCL13	3.22	3.71	0.035	–
IL-18	2.78	3.51	0.023	0.009 *
CCL23	2.34	2.82	0.043	–
PTN	4.81	4.73	0.035	0.001 *

[a]Cytokines selected in the LASSO logistic models were further evaluated using multivariate analysis.
P-values in the univariate analysis were evaluated using the Mann-Whitney U test.
Abbreviations: CSF, cerebrospinal fluid; DCB, durable clinical benefit; NPX, normalized protein expression.

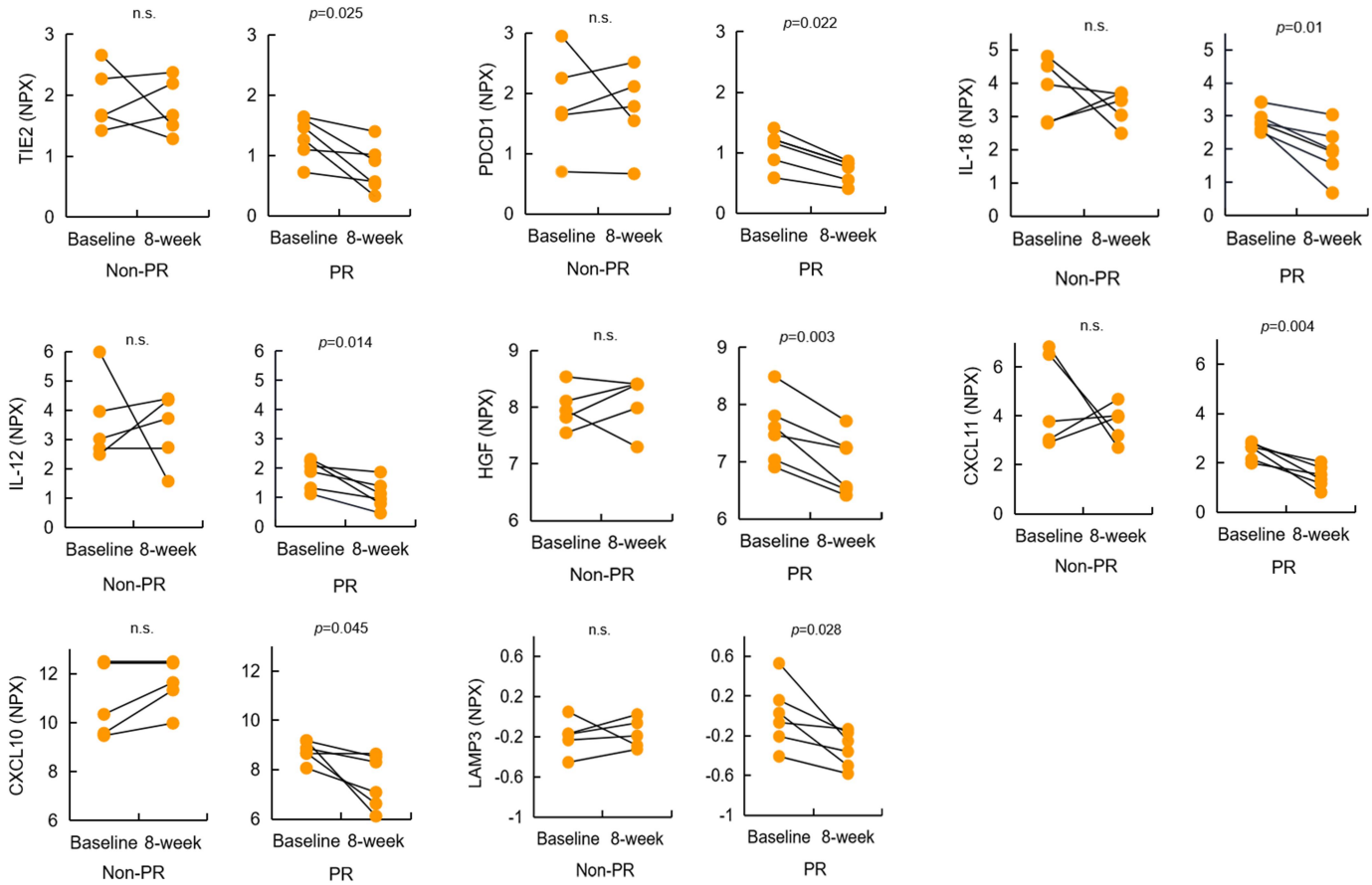

Figure 4. Changes of significant cytokines at baseline and at the first treatment evaluation in CSF between different groups. *P* value was evaluated by Mann-Whitney U test.

compared to non-disease controls.[18] Together, these data showed immune suppression within the CNS compared with peripheral tumors in patients with brain metastases. On the other hand, we observed a subset cytokines were increased in CSF compared to paired plasma samples, such as CD83, PTN, TNFRSF21, TWEAK, ICOSLG, DCN, IL-8, and MCP-1. Two of these, MCP-1 and IL-8 are known to be secreted not only by tumor cells but also by microglia within the brain,[19] and could

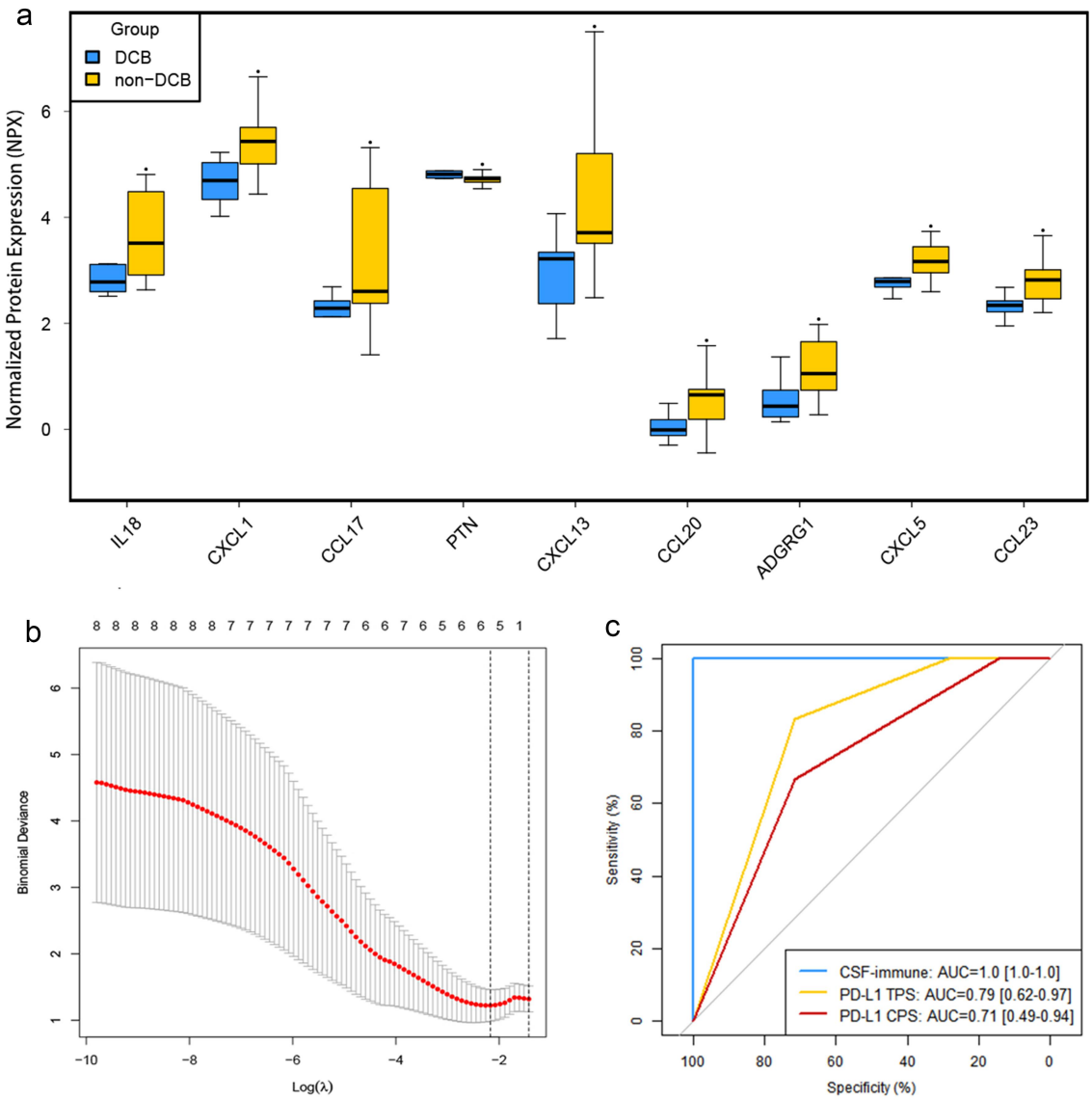

Figure 5. Comparison of immunological cytokines in CSF between DCB and non-DCB groups. (a) boxplot of significantly different cytokines in CSF between DCB and non-DCB groups. *P* value was evaluated by Mann-Whitney U test. (b) LASSO logistic regression model. (c) Receiver operating characteristic (ROC) curve of the CSF immuno-cytokines and PD-L1 expression for durable clinical benefit.

recruit monocytes and neutrophils into the CNS, which may contribute to suppressed immune responses, facilitate tumor progression, and promote the development of brain metastases.

TWEAK and TNFRSF21 are members of the tumor necrosis factor (TNF) superfamily, and participate in cell proliferation and death, angiogenesis, carcinogenesis and inflammation.[20–22] Meanwhile, ICOSLG, belonging to the B7 family of ligands, emerges as a central player in immune regulation mediated by regulatory T cells.[23,24] These cytokines regulated the immune microenvironment through complex interactions and participated in the process of CNS disease.

Immune checkpoint inhibitors, alone or in combination with chemotherapy, have been the standard first-line treatment strategies for patients with advanced NSCLC without *EGFR* mutations or *ALK* rearrangements. For patients with brain metastases, subgroup analyses from previous studies have reported that ICIs combined with chemotherapy achieved an overall response rate of approximately 40% in NSCLC patients with brain metastases.[25,26] Recently, several prospective studies have reported that immunotherapy has a certain intracranial tumor response in NSCLC patients with untreated brain metastases.[5,6] Although PD-L1 expression is a confirmed biomarker for immunotherapy in NSCLC, its ability to predict intracranial efficacy in patients with brain metastasis remains unclear. A phase 2 study reported that PD-L1 expression in both tumor and stromal cells was associated with prolonged OS, however, no statistically significant association between PD-L1 expression and response or PFS was observed.[5] Importantly, most patients only had tissues from systemic sites of disease, which may have different microenvironments from those of brain metastases. Therefore, identifying appropriate predictive biomarkers for brain metastases is worthwhile.

In our study, we found LAMP3 in CSF was significantly associated with intracranial response in multivariate analysis.

LAMPs (lysosome associated membrane proteins) represent a family of glycosylated proteins present predominantly on the membrane of lysosomes, is considered as a marker of mature dendritic cells in humans.[27] Recent study found LAMP3 positive dendritic cells in tumor microenvironment, and this dendritic cells expressed multiple immune ligand to interact with T lymphocytes, thus may be a therapeutic target in the future.[28,29] Furthermore, we established a CSF immunocytokines model combined significant immunological cytokines, and this model had better predictive value for intracranial tumor response to immunotherapy than PD-L1 expression of primary lung tumor tissues. It seemed that higher proinflammatory cytokines were associated with poor intracranial efficacy of immunotherapy, and their levels decreased after tumor response, which correlated with lower tumor burden in the CNS according to previous studies.[30,31] A prospective study also explored the efficacy of pembrolizumab in patients with leptomeningeal metastasis and found that lower proinflammatory cytokines levels in the CSF were associated with CNS response and further reduced in those with a response to pembrolizumab.[32] However, given the small sample size, our findings are exploratory and require further validation in larger cohorts.

On the other hand, the cytokines in plasma could not predict intracranial tumor response, but was associated with extracranial tumor response. In multivariate analysis, the galectin-9 in plasma was an independent predictive cytokine for extracranial response to immunotherapy. Galectin-9 regulates immune homeostasis and tumor cell survival through its interaction with its receptor Tim-3.[33,34] In lung cancer, recent study showed that increased galectin-9 expression in tumor cells may inhibit lung metastasis, which may be related to the suppressive effect of galectin-9 on adhesion and invasion of tumor cells.[35] Galectin-9 induced macrophages to differentiate into plasmacytoid DC-like macrophages, which may enhance the activation of NK cells that prolonged the survival of lung cancer-bearing mice.[36] The interaction between Gal-9 and Tim-3 plays a key role in tumor immunity, and is an emerging immunotherapy combination following PD-1/PD-L1 treatment.

One of the specificities of immunotherapy is that some patients can achieve a durable tumor response in long-term survival. While studies assessing biomarkers of long-term response were scarce, owing to lack of long-term clinical follow-up and the low proportion of long-term responders. The most reported predictive biomarker of a durable response to immunotherapy is PD-L1 expression in tissues,[37–39] however, its reliability in patients with brain metastases is uncertain. Our findings indicate that CSF cytokines have a better predictive value than PD-L1 expression for durable response in patients with brain metastases. We found that lower IL-18 and PTN levels in CSF were associated with durable clinical benefit to immunotherapy in multivariate analysis, whereas no significant association was observed with plasma cytokines. Pleiotrophin (PTN), a heparin-binding glycoprotein could promote cell growth, migration and cellular activities through binding its receptor PTPRZ1 and increasing phosphorylation of the downstream effectors.[40] Previous study showed that tumor associated macrophages secreted abundant PTN to stimulate glioma stem cells, and thus promoting glioblastoma growth and predicting poor prognosis.[41] IL-18 is a member of IL-1 superfamily of cytokines and plays a key regulatory role in tumor immunity.[42] The analysis of serum IL-18 in human cancer patients shows that high levels of IL-18 strongly correlate with shorter survival.[43,44] This cytokine promotes cancer cells to escape from the immune system by suppressing CD70.[45] In tumor microenvironment, IL-18 could mediate macrophage M2 polarization, and induce suppressed immune-microenvironment, as well as enhance angiogenesis to promote tumor progression and metastases.[46] In central nervous system, IL-18 were mainly secreted by microglia, and induced microglia to produce matrix metalloproteinases and other proinflammatory cytokines, thus participating in the development of central system diseases.[47,48] The treatment targeted for IL-18 and PTN in brain metastases need further investigation in future.

A major limitation of our study was the small sample size from a single center, and our findings warrant further validation in larger cohort studies and the predictive value of cytokines for other immunotherapy regimes need further validated. Besides, majority female patients had driver mutations, that could received targeted therapies, the most patients we enrolled were male patients in this study, which may induce gender bias. Moreover, further exploratory CSF studies using multi-omics analysis to identify more potential predictive biomarkers are necessary for patients with brain metastases.

In conclusion, this prospective study demonstrated the immunological cytokines profiles of CSF and found that immunological cytokines in CSF can predict intracranial tumor response to immunotherapy in NSCLC patients with brain metastases, which requires validation in larger prospective cohorts.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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List of Abbreviations

CSF	cerebrospinal fluid
ICIs	immune checkpoint inhibitors
ORR	objective response rate
PFS	progression-free survival
OS	overall survival
CNS	central nervous system
NSCLC	non-small cell lung cancer
DCB	durable clinical benefit

MRI	magnetic resonance imaging
ROC	receiving operating curve
AUC	area under curve
ECOG PS	Eastern Cooperative Oncology Group performance status
LOD	limit of detection

Ethics approval and consent to participate

This study was approved by the Ethics Committee of the Guangdong Association Study of Thoracic Oncology (GASTO ID:1060, Approval No. A2020–004). Written consent was obtained from all patients.

Author contributions

Dr. Likun Chen had full access to all the data in the study and took responsibility for the integrity of the data and the accuracy of the data analysis.

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Patient recruitment and enrollment: Meichen Li, Xue Hou, Jing Chen, Baishen Zhang, Honghua Jiang, and Likun Chen.

Data acquisition and curation: Meichen Li.

Data analysis and interpretation: All authors.

Manuscript draft writing: Meichen Li.

Critical revision: Meichen Li, Dan Xie and Likun Chen.

Study supervision: Dan Xie and Likun Chen.

Availability of data and material

Dr. Chen had full access to all the data in the study and took responsibility for the integrity of the data and the accuracy of the data analysis.

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