




Pembrolizumab for patients with leptomeningeal metastasis from solid tumors: efficacy, safety, and cerebrospinal fluid biomarkers

Jarushka Naidoo ^{1,2,3}, Karisa C Schreck,^{1,4,5} Wei Fu,⁶ Chen Hu,⁶ Alexander Carvajal-Gonzalez,⁴ Roisin M Connolly,^{1,7} Cesar A Santa-Maria,^{2,8} Evan J Lipson ^{1,2}, Matthias Holdhoff ^{1,5}, Patrick M Forde,^{1,2} Christopher Douville,^{1,2,9,10} Joanne Riemer,^{1,2} Amanda Barnes,^{1,2} Kristin J Redmond,¹¹ Lawrence Kleinberg,¹¹ Brandi Page,¹¹ Nafi Aygun,¹² Kenneth W Kinzler,^{1,2,9,10} Nickolas Papadopoulos,^{1,2,9,10} Chetan Bettegowda,^{1,2,5,9,10} Arun Venkatesan,⁴ Julie R Brahmer,^{1,2} Stuart A Grossman¹

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For numbered affiliations see end of article.

Correspondence to

Dr Jarushka Naidoo; jnaidoo1@jhmi.edu

ABSTRACT

Background The benefit of immune checkpoint inhibitors (ICIs) in patients with leptomeningeal metastases (LMM) is unknown.

Methods We undertook a phase II trial of pembrolizumab in patients with LMM from solid tumors. Eligible patients had radiologic/cytologic LMM and Eastern Cooperative Oncology Group performance status 0–1. Pembrolizumab was administered intravenously at 200 mg q3W until disease progression/unacceptable toxicity. The primary endpoint was central nervous system (CNS) response after four cycles, defined radiologically/cytologically/clinically. Serial cerebrospinal fluid (CSF) was assessed for tumor-derived DNA (t-DNA) aneuploidy and cytokines.

Results Thirteen of a planned 16 patients were treated between April 2017 and December 2019. The study closed early for poor accrual. Median age was 57 years (range: 22–79). Sixty-two percent of patients had tumors not traditionally ICI-responsive (hormone-receptor (HR)-positive breast carcinoma=39%; high-grade glioma=23%), while 38% had ICI-responsive tumors (non-small cell lung cancer (NSCLC)=23%, head and neck carcinoma=8%, cutaneous squamous carcinoma (CSC)=8%). CNS response was observed in 38% of patients at 12 weeks (95% CI 13.9% to 68.4%) by pre-defined criteria and LM-RANO, and 2 achieved durable complete responses (CSC=1, overall survival (OS) 3+ years; NSCLC=1, OS 9 months). Median CNS progression-free survival and OS was 2.9 months (95% CI 1.3 to NR) and 4.9 months (95% CI 3.7 to NR), respectively. Grade 3+ treatment-related adverse events occurred in 15% of patients. Sensitivity for LMM detection by t-DNA and cytopathology was 84.6% (95% CI 54.6% to 98.1%) and 53.9% (95% CI 25.1% to 80.8%), respectively. Pre-therapy and on-therapy CSF cytokine analysis demonstrated complete responders clustered together.

Conclusions Pembrolizumab conferred a 38% CNS response rate in patients with LMM, a tolerable safety profile, and deep responses in selected patients with ICI-responsive tumors. CSF t-DNA may be sensitive for LMM

detection, and immunologic subsets of CNS response warrant further study.

Trial registration number NCT03091478

INTRODUCTION

Immune checkpoint inhibitors (ICIs) benefit selected patients with brain metastases (BM).^{1,2} In patients with metastatic melanoma, treatment with ipilimumab³ or the combination of ipilimumab and nivolumab resulted in improvements in central nervous system (CNS) response and survival.⁴ In patients with BM from either melanoma or PD-L1+ (>1%) non-small cell lung cancer (NSCLC),⁵ pembrolizumab led to CNS responses in a phase II trial. While patients with BM have been included in studies of ICIs, patients with leptomeningeal metastasis (LMM) have largely been excluded from these trials owing to poor patient prognosis, except for one study in melanoma.^{4,6} There remains a paucity of treatment options for patients with LMM from solid tumors. In patients with LMM from breast cancer, local radiation therapy (RT) for symptomatic control±high-dose systemic or intrathecal chemotherapy may be used.^{7,8} In patients with LMM from oncogene-addicted tumors with targeted treatment options (eg, osimertinib in EGFR-mutant NSCLC⁹), CNS responses and improved survival outcomes occur. However, most patients with LMM have poor outcomes due to a lack of treatments that are effective both intracranially and extracranially. While there has been one published study demonstrating a higher 3-month

survival rate from pembrolizumab for patients with LMM from solid tumors mainly in those with breast cancer,¹⁰ it is unknown whether anti-PD(L)1 ICIs may lead to CNS responses in those with LMM from solid tumors. In addition, biomarkers of ICI response in this subset of patients have not been elucidated.

Thus, we proposed a phase II trial of pembrolizumab in patients with LMM from any solid tumor. Our primary objective was to determine if pembrolizumab led to CNS response in LMM. Secondary objectives were to determine if pembrolizumab led to improvements in CNS progression-free survival (PFS), overall survival (OS), and acceptable safety, compared with historic data. We also sought to identify whether tumor-derived DNA (t-DNA) shed into the cerebrospinal fluid (CSF) and CSF immunologic features would be detectable in patients with LMM, and correlate with CNS response or survival.

PATIENTS AND METHODS

Patients

Eligible patients were ≥ 18 years old with LMM from a pathologically confirmed solid tumor malignancy. In this study, LMM was defined as either radiologically measurable LMM on contrast-enhanced MRI (>3 mm lesion) or cytologically-detectable LMM by CSF sampling. Patients with CNS metastases but without LMM were not eligible. Measurable disease outside the CNS was not a requirement for study enrollment. All potentially eligible patients were discussed at a multidisciplinary neuro-oncology conference comprising ≥ 2 medical oncologists, a radiation oncologist, neurologist, and neuro-radiologist to confirm the diagnosis of LMM and suitability for enrollment. All enrolled patients had an Eastern Cooperative Oncology Group (ECOG) performance status (PS) of 0 or 1, and adequate organ function (absolute neutrophil count $\geq 1500/\mu\text{L}$; platelets $\geq 100,000/\mu\text{L}$; Hb ≥ 9 g/dL; serum creatinine ≤ 1.5 times upper limit of normal, serum total bilirubin ≤ 1.5 times upper limit of normal, AST and ALT ≤ 2.5 times upper limit of normal, albumin ≥ 2.5 mg/dL, international normalized ratio or prothrombin time or international normalized thromboplastin time ≤ 1.5 times upper limit of normal). Premenopausal women were required to have a negative pregnancy test, and all participants of childbearing potential were required to use contraception. Patients may have received any number of prior lines of therapy, but could not have received prior therapy with anti-PD-(L)1 ICIs. Patients with oncogene-addicted or hormone-responsive tumors who were receiving either targeted therapy or hormonal therapy prior to study enrollment were permitted to continue this therapy concurrently with pembrolizumab, determined on a case-by-case basis, if there was published and acceptable safety data of the combination in question. Patients were permitted to enroll if they were receiving corticosteroids, as long as the steroid requirement was non-escalating. Patients who underwent major surgery to the brain within 3 weeks, had concurrent brain metastasis

with mass effect, or a history of steroid-requiring pneumonitis were not eligible. Prior radiation therapy (RT) to LMM was allowed, if administered more than 3 months before study start or to non-target areas of LMM. An area of LMM that had received RT at any time could not be used as a target lesion for the assessment of CNS response.

Study design

This was an investigator-initiated, open-label single-institution phase II trial of pembrolizumab monotherapy in patients with LMM from any solid tumor. Pembrolizumab was administered intravenously at a flat dose of 200 mg every 3 weeks until disease progression or unacceptable toxicity. Screening investigations were completed within 2 weeks of study start and included standard laboratory investigations, CSF sampling for cytology, and a pre-treatment gadolinium-enhanced MRI scan of the brain and total spine. During the trial, patients were monitored with a clinical visit, adverse event (AE) assessment, and routine laboratory tests at the time of each dose of pembrolizumab. Patients had blood draws, CT imaging of the body, and a contrast-enhanced MRI scan of the brain and total spine after 2 and 4 cycles of pembrolizumab, and every 6–9 weeks thereafter until disease progression or coming off study. Patients were followed for 90 days after the end of therapy for AE monitoring or 30 days after the end of therapy if a new anti-cancer therapy was initiated. Serial CSF samples were obtained by lumbar puncture and assessed for cytopathology, genomic and immunologic assays pre-treatment, and after 2 and 4 cycles of pembrolizumab. We assayed CSF for the presence of t-DNA by chromosomal copy number changes (aneuploidy) using a PCR-based approach (RealSeqS). We assessed CSF for immunologic features by 16-color flow cytometry, immunophenotyping, and multiplex cytokine panels, as outlined later.

Study endpoints

The primary endpoint of the study was CNS response assessed after 12 weeks of therapy using prospectively defined criteria.⁷ CNS response was defined either as radiologic, cytologic, or clinical response to therapy assessed by the treating investigator, and outlined in the protocol (online supplemental table 1). Radiologic response was defined as a reduction in size of LMM on gadolinium-enhanced MRI of the brain and total spine including T1-weighted, T2-weighted, fluid-attenuated inversion recovery, and post-gadolinium sequences. All radiologic responses were confirmed by a dedicated study neuro-radiologist. Cytologic response was defined as conversion of positive to negative CSF cytology on two consecutive CSF samples taken at the time of study treatment. Clinical response was defined as an improvement in objective neurologic signs or symptoms attributable to LMM by the treating investigator. Retrospective analysis using LM-RANO criteria¹¹ was also performed for all patients who were assessed for response by the study radiologist (NA). Secondary endpoints included CNS-PFS, OS and

safety. Adverse events were graded according to Common Terminology Criteria for Adverse Events (CTCAE version 4.0). OS was defined as time from pembrolizumab start until death from any cause. CNS-PFS was defined as time from pembrolizumab start until radiologic, cytologic or clinical progression in LMM, whichever came first.

Correlative analysis

RealSeqS can sensitively detect aneuploidy using a single PCR primer pair. In RealSeqS, for each sample a single primer pair was used to amplify ~350,000 loci spread throughout the genome.¹² One of the primers included a unique identifier sequence (UID) as a molecular barcode of 16 degenerate bases to reduce error rates associated with PCR and sequencing. Massively parallel sequencing was performed on an Illumina HiSeq 4000. Sequencing data were processed to identify single chromosomal arm gains or losses using the Within-Sample Aneuploidy Detection (WALDO), incorporated into the RealSeqS work flow.¹³ WALDO used a set of normal non-cancer healthy plasma controls as references (that were excluded from all other analyses), and based on the closest reference generated a Z score, for likelihood of alterations on each acrocentric chromosomal arm. WALDO further incorporated a support vector machine (SVM) to discriminate between aneuploid and euploid samples and to provide a summary Genome Aneuploid Score statistic. The SVM classifier was trained using 1334 normal euploid plasma samples and 2651 aneuploid samples. The software and all datasets used to generate the scores for this manuscript have been posted and are available online (<https://doi.org/10.5281/zenodo.3656943>). A cut-off of 0.333 was selected for a positive call; this cut-off was selected based on specificity of 98% reported in the manuscript on 1348 normal samples.¹³

The immunologic features of collected CSF samples were analyzed by flow cytometry following immunostaining for CD3 to identify T cells (anti-CD3-Percp Cy5.5, 1:50, clone: OKT3, Cat#45-0037-42; Invitrogen) and CD19 to identify B cells (anti-CD19-FITC, 1:50, clone: HIB19, Cat#11-0199-42; Invitrogen). Cells were incubated with antibodies on ice for 30 minutes in the dark, followed by washing, re-suspension, and analysis via MACSQuant Analyzer 10 (Miltenyi Biotec). Expression profiles of each cell type was analyzed via FlowJo_V10 (Tree Star Inc., Ashland, OR) software. T cells and B cells were enumerated for all baseline and on-treatment samples based on flow cytometry panels. The V-PLEX Plus Human Cytokine 30-Plex assay was used per manufacturer's instructions (Meso Scale Diagnostics, CA) to characterize cytokine profiles. Cytokines and chemokines were annotated on baseline and on-treatment samples and represented by heatmaps, using absolute and rank order values, excluding a coefficient of variation in excess of 10%.

Statistical analysis

All enrolled patients who received at least one dose of study therapy were included in this analysis. CNS response proportion and its associated CI was calculated using Clopper-Pearson exact method, and the study size

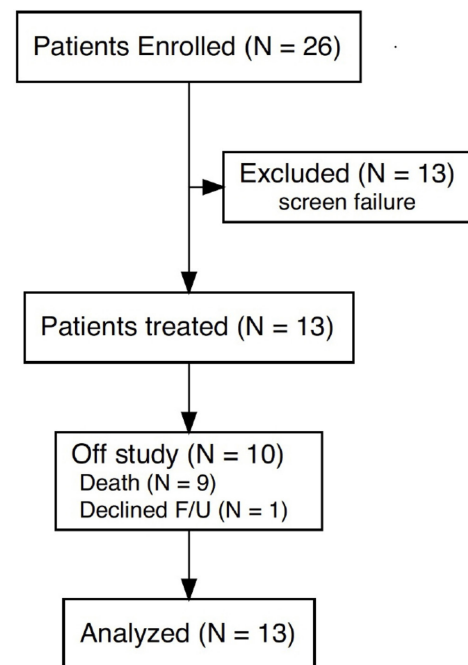


Figure 1 Consort diagram.

was determined as 16 analyzable patients to control the estimation precision prospectively (appendix I). Kaplan-Meier method was used to estimate PFS and OS and the log-rank test was used to compare differences in CNS-PFS and OS rates by baseline cytologic LMM. Treatment-related adverse events (TRAEs) were reported for all patients. Baseline and on-treatment genomic and immunologic data from CSF samples were presented descriptively, and correlated with CNS response and OS.

RESULTS

Between February 15, 2017 and December 9, 2019, 26 patients were screened for study entry. The study was stopped early for poor accrual. Thirteen patients received at least one dose of pembrolizumab, and were included in this analysis (figure 1).

Baseline characteristics

Patient and disease characteristics at baseline are listed in table 1. The median age was 57 years (range: 22–79). The majority of patients (n=8, 62%) had tumors not traditionally responsive to ICI monotherapy (“ICI non-responsive”: breast carcinoma=5, 39%; high-grade glioma=3, 23%), while 38% had tumors that traditionally respond to ICI monotherapy (ICI-responsive=5, 38%), consisting of NSCLC (n=3, 23%), head and neck carcinoma (HNSCC) (n=1, 8%), and cutaneous squamous carcinoma of the skin (CSC) (n=1, 8%). Of the patients with breast carcinoma, three had HR-positive/HER2-positive breast cancers, and two had HR-positive/HER2-negative tumors. One patient with HR-positive/HER2-positive breast cancer continued trastuzumab and tamoxifen during the study concurrently with pembrolizumab, based on safety data of this combination that


Table 1 Baseline patient demographics and disease characteristics

Patient demographics	Total (n=13, %)
Median age, years (range)	57 (22–79)
Sex	
Female	7 (53.8)
Male	6 (46.2)
Race	
Caucasian	9 (69.2)
Asian	2 (15.4)
Unknown	2 (15.4)
ECOG Performance Status	
0	12 (92.3)
1	1 (7.7)
Primary Tumor Diagnosis	
Non-small cell Lung Cancer*	3 (23.1)
Head and Neck Squamous Carcinoma	1 (7.6)
Cutaneous Squamous Carcinoma of Skin	1 (7.6)
Breast carcinoma†	5 (38.4)
High-grade Glioma	3 (23.1)
Presence of Extracranial Disease	
Yes	4 (30.7)
No	9 (69.3)
Neurologic Symptoms of LMM	
Yes	13 (100)
No	0 (0)
Baseline CSF cytology	
Positive	6 (46.2)
Negative	7 (53.8)
Median CSF White Cell Count (range)	6 (1–131)
Median Serum Absolute Lymphocyte count (range)	0.91 (0.3–2.4)
Disease Characteristics	
Prior Chemotherapy	
Yes	10 (76.9)
No	3 (23.1)
Prior Brain/Spine Radiotherapy	
Yes‡	10 (76.9)
No	3 (23.1)
No. of Prior Lines of Systemic Therapy	
0	3 (23.1)
1	1 (7.7)
2+	9 (69.2)
Any corticosteroids at the time of study enrollment	
Yes	5 (38.4)
No	8 (61.5)

*Two NSCLCs were PD-L1 <1%, 1 harbored a MET-exon14 skipping alteration; 1 had no oncogenic driver mutation; 1 NSCLC harbored an NRAS mutation and was PD-L1 30%.

†3 patients had HER2-positive breast cancers; and 2 patients had ER-positive/PR-negative/HER-2-negative breast cancers.

‡Whole-Brain Radiation Therapy= 4; Stereotactic Radiosurgery= 1; Focal RT to LMM= 5; all received > 3 months before study enrollment. ECOG, Eastern Cooperative Oncology Group.

had been presented prior to enrollment.¹⁴ No patients received targeted therapy concurrently with protocol-directed pembrolizumab in this study. Of the patients with NSCLC (n=3), two patient's tumors were PD-L1-negative (<1%), one of which harbored a MET-exon 14 skipping alteration, while the other did not harbor an oncogenic-driver alteration. The third NSCLC tumor had a PD-L1 score of 30% (22C3) and harbored an *NRAS* Q61K mutation. The majority of patients in this study had LMM only without disease in the body (n=11, 84.6%). Interestingly, in slightly majority of enrolled patients, cytologic LMM was not detectable using CSF cytopathology (n=7, 53.8%). Overall, most of the study population was heavily pre-treated (2+ prior lines of systemic therapy=9, 69.2%) and had received prior radiation to the brain >3 months prior to study enrollment (n=10, 76.9%). A minority of patients received non-escalating corticosteroid therapy at the time of enrollment (n=5, 38%), the dose at enrollment was equivalent to prednisone 25 mg/day in one patient, while all others were equivalent to prednisone ≤10 mg/day for symptomatic management of LMM.

Efficacy

Overall CNS response among the 13 patients treated with pembrolizumab was assessed (table 2). Six patients received more than four doses of pembrolizumab (n=6/13, 46%). CNS response was assessed using pre-defined study criteria as outlined in the protocol or retrospectively using LM-RANO criteria, as outlined in table 2. Since LM-RANO criteria do not differentiate between partial and complete responses to treatment and in this manuscript CNS response included stable disease, the CNS response rate from both assessments of response were identical. CNS response was observed in 38% (n=5/13, 95% CI 13.9% to 68.4%), while most had progressive disease (PD) in the CNS (n=8, 61.5%). Two patients achieved complete responses (CR) in the CNS after pembrolizumab, one had a partial response (7.7%), and two (15.4%) had stable disease. Both patients with CRs had ICI-responsive tumors (CSC; MET-exon14+NSCLC) (figure 2). The patient with NSCLC had received prior crizotinib and sustained prolonged stable disease from this therapy, as well as a short-lived response to second-line chemotherapy. This patient received five doses of study therapy and had complete radiographic resolution of LMM, but developed grade 2 pneumonitis and grade 2 inflammatory arthritis, necessitating treatment hold. During this period, the patient had PD in the body and subsequently died, while CNS disease did not recur (OS 9 months). Interestingly, this patient commenced study therapy while receiving corticosteroids at a dose of prednisone ≤10 mg/day. The other patient with a CNS CR had newly diagnosed CSC and received 17 doses of study therapy as first-line treatment, with ongoing CNS CR and OS 3+ years from ICI start. Both patients who sustained CRs to study therapy had not received prior radiation therapy to LMM. The duration of response to pembrolizumab by CNS radiologic response

Table 2 Response to treatment

	CNS response assessment methods						
	CNS response in LMM	CNS Radiologic Response (RECIST 1.1)	CNS Radiologic Response (irRC)	CNS Cytologic Response	CNS Clinical Response	Extracranial Radiologic Response (RECIST 1.1)	LM-RANO Response*
No. evaluable patients	13†	11	11	7	13	4	13
Complete Response	2 (15)	0 (0)	1 (8)	N/A	2 (15)	0 (0)	Response2 (15)
Partial Response	1 (8)	1 (8)	1 (8)	N/A	0 (0)	0 (0)	
Stable Disease	2 (15)	7 (54)	5 (38)	N/A	3 (23)	1 (8)	3 (23)
Progressive Disease	8 (62)	3 (23)	4 (31)	N/A	8 (62)	3 (23)	8 (62)
Not evaluable	0 (0)	2 (15)	2 (15)	6	0 (0)	9 (69)	0 (0)
Present				4 (31)			
Absent				3 (23)			

*LM-RANO: Leptomeningeal Metastasis Response Assessment in Neuro-Oncology.

†Primary Response assessment for Overall CNS response as radiologic in 10 cases, and clinical in 3 cases.

CNS, central nervous system; irRC, immune-related response criteria; LMM, leptomeningeal metastasis; N/A, not applicable; RECIST, response evaluation criteria for solid tumors.

and tumor type is depicted in [figure 3](#). The median CNS-PFS was 2.9 months (95% CI 1.3 to NR) and the median OS was 4.9 months (95% CI 3.7 to NR) ([figure 4](#)). While not statistically significant, the presence of cytologic LMM was associated with numerically poorer OS versus those without cytologically detectable LMM (3.7 months vs 10.3 months, log-rank $p=0.29$) (online supplemental figure 1).

Adverse events

TRAEs occurred in 38.5% of patients ($n=5/13$), and the majority were low grade (23.1%, 3/13) (online supplemental table 2). There were no high-grade immune-related adverse events (irAEs). Drug therapy was withheld for TRAEs that were also irAEs in both patients with CNS CR (NSCLC: pneumonitis; CSC: pain in extremity, joint reduced range of motion).

CSF analysis

CSF was obtained for cytopathology, t-DNA, and immunologic analysis (flow cytometry, immunophenotyping, cytokine analysis) at baseline in all patients ($n=13$), and in patients with available samples after two ($n=10$) and four cycles ($n=4$) of pembrolizumab.

Seven patients had positive CSF cytopathology (53.8%) at baseline. No CSF samples had significant contamination from peripheral blood (red blood cells $<500/\mu\text{L}$) for all patients. Positive CSF cytopathology did not correlate with CSF white blood cell count, protein, glucose, or baseline radiographic LMM. There were no significant differences in CNS response or OS ($p=0.278$) in patients with positive versus negative baseline CSF cytopathology (online supplemental figure 1). Given the limitations of traditional CSF

cytopathology, we evaluated the sensitivity of CSF t-DNA by aneuploidy assay, for the diagnosis of LMM and associations with CNS response and OS. The sensitivity of CSF for the detection of LMM by t-DNA aneuploidy was 84.6% (95% CI 54.6% to 98.1%) and 53.9% (95% CI 25.1% to 80.8%) by cytopathology (online supplemental table 3). We were unable to assess specificity as all patients in the cohort had LMM. There were no significant associations between CSF t-DNA by aneuploidy assay and either CNS response ($p=0.65$) or OS (0.79).

Flow cytometric analysis demonstrated a median of 35 024 live cells (IQR 14,043, 117,736) per sample. T cells (CD3⁺ expression) comprised 44% (SD 36%) of the total, while B cells (CD19⁺ expression) comprised a smaller proportion (mean: 3.9%; SD 7.7%). Baseline CSF cytokine analysis demonstrated that both patients who sustained CNS CRs (#3, #11) as well as two patients with short-lived responses (#8, #24) clustered together, while patients with clear CNS PD (#1, #4, #10, #16) clustered differently (online supplemental figure 2). Lower levels of baseline pro-inflammatory cytokines were seen in patients 3, 11, 8, and 24. When examining the log-fold change of cytokines comparing baseline with the post cycle 2 visit, once again both CRs clustered together (online supplemental figure 2), exhibiting a further reduction in the levels of pro-inflammatory cytokines.

DISCUSSION

In this prospective clinical trial, we identified that 38% of patients with LMM from solid tumors had CNS responses to pembrolizumab. The median CNS-PFS and OS was

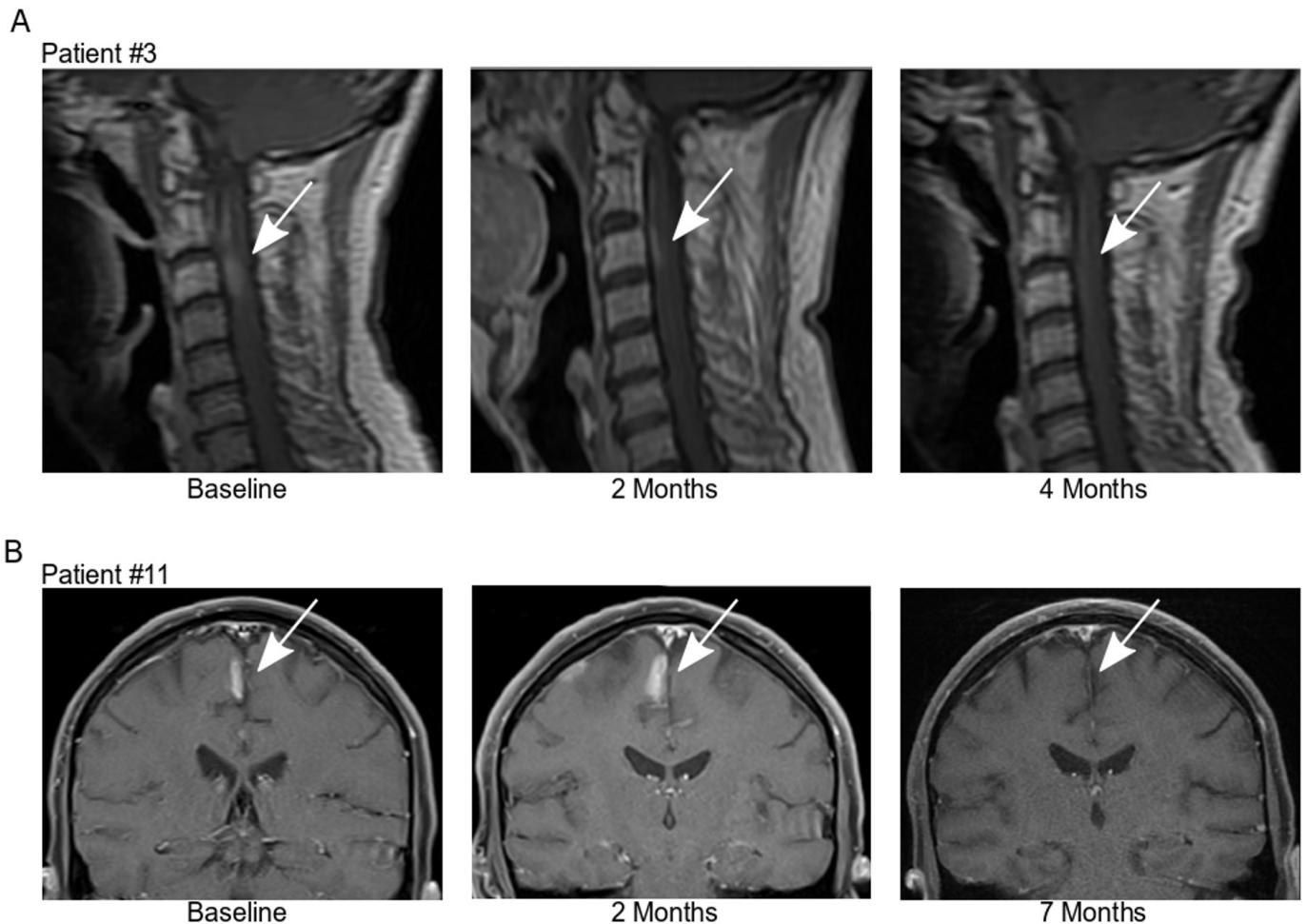


Figure 2 Radiologic imaging in complete central nervous system responders to pembrolizumab.

2.9 months and 4.9 months, respectively, in keeping with published prospective studies in LMM.^{7 8 15–17} Pembrolizumab was also safe to administer, with a low incidence of high-grade TRAEs. Interestingly, two patients with ICI-responsive tumors (NSCLC, CSC) demonstrated complete responses in the CNS, clinically and radiographically.

The main goal of this investigator-initiated trial was to identify whether anti-PD-1 could lead to CNS responses. We adopted stringent criteria for inclusion and assessment of LMM, only enrolling patients with clearly measurable radiographic disease or cytologic proof of LMM, followed by multidisciplinary consensus. While this led to a high rate of screen failure, it also ensured reliable assessment of CNS response. OS has been used as a primary endpoint for several studies in LMM, but may not account for prolonged OS seen in subsets of patients with oncogene-addicted cancers and LMM. OS was assessed as secondary endpoint, together with CNS-PFS and safety. We included patients in our study who had received prior RT to the CNS, but restricted this to 3+ months before ICI start, to limit the effect on CNS response assessment. Patients receiving non-escalating doses of corticosteroids were also allowed to enroll despite the potential deleterious effects on ICI response,¹⁸ and only one patient received a dose

higher than prednisone 10 mg/day, which has historically been deemed acceptable in ICI studies.

The study population in this trial was heavily pre-treated, comprised mainly of patients with tumors that are not typically responsive to ICI monotherapy, and with CNS-only disease. This was unsurprising since anti-PD-1 was already available for many cancers, and our study did not require measurable disease in the body. Despite this, 38% of enrolled patients exhibited CNS responses from pembrolizumab. Re-analysis of responses using the recently published consensus LM-RANO criteria matched our protocol-defined criteria for assessing response based on neurological examination, radiographic features, and CSF cytopathology. Objective responses occurred in patients with tumor types that typically respond to anti-PD-1 (CR: NSCLC, CSC; PR: NSCLC). Interestingly, two patients sustained CRs in their LMM, substantiating that certain patients may derive prolonged benefit. A potential explanation for these CNS responses is that activated T cells were able to reach the CNS through a less effective blood–brain barrier, owing to the presence of LMM. One other prospective study has explored the use of anti-PD-1 in patients with LMM. This study enrolled 18 patients with breast cancer, with the goal of assessing OS rate from

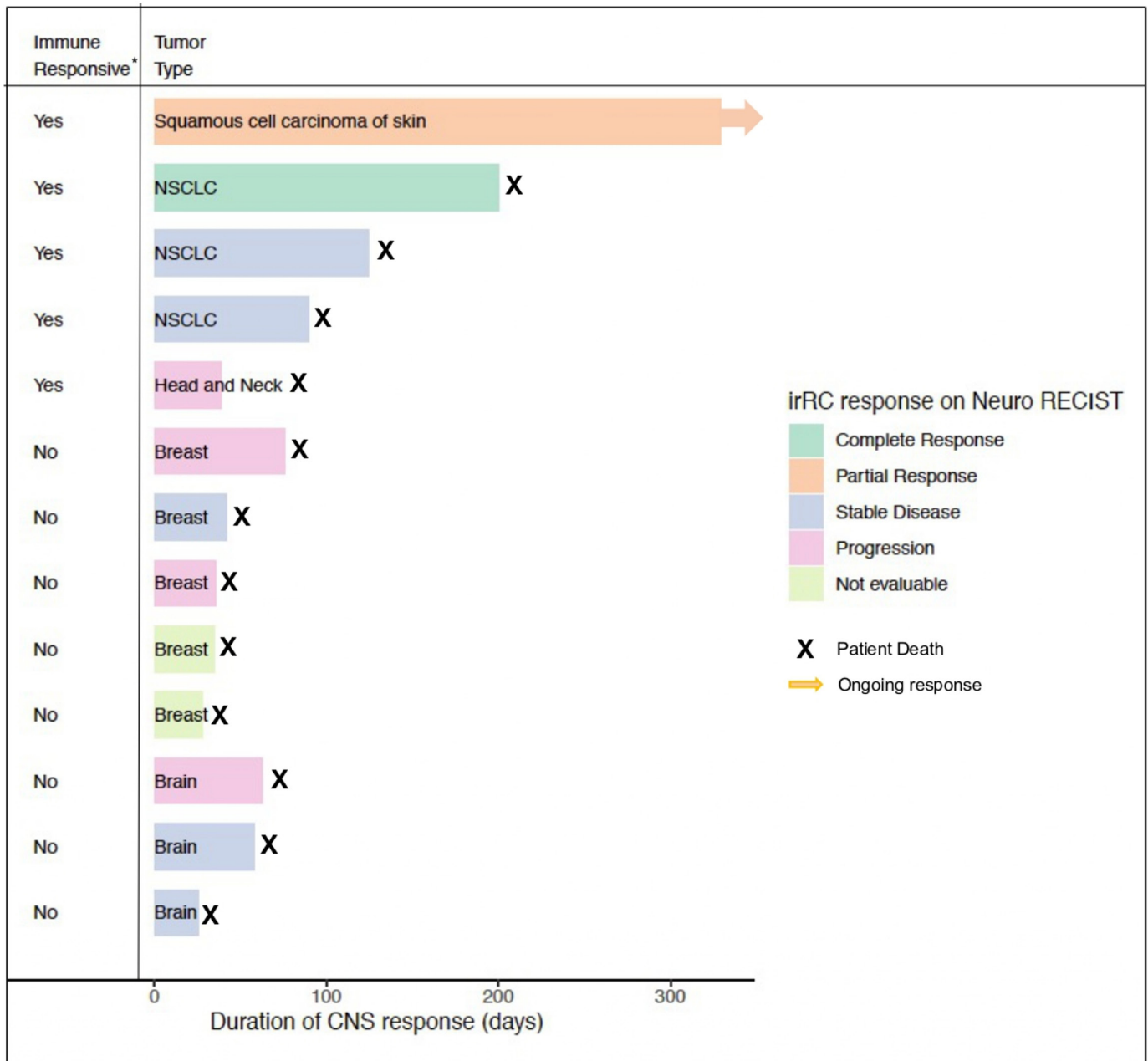


Figure 3 Swimmer plot of radiologic response duration for patients with leptomeningeal metastasis treated with pembrolizumab. CNS, central nervous system; NSCLC, non-small cell lung cancer.

pembrolizumab at 3 months. Consistent with our survival outcomes, this study and others in similar cohorts¹⁹⁻²¹ demonstrated an OS rate of 60% at 3 months.

Uniquely, in our study we aimed to elucidate the biologic features of CNS response to pembrolizumab. We undertook a comprehensive genomic and immunologic assessment of the CSF from enrolled patients at baseline and serially on-treatment. We observed that CSF t-DNA by aneuploidy assay may be sensitive for detection of LMM, and that there may be important immunologic subsets of patients relative to ICI response based on multiplex cytokine assays. Lower pro-inflammatory cytokine levels have been correlated in prior studies to lower tumor burden in the CNS.^{22,23} Thus, this may account for lower

CSF cytokine levels in selected responders in our cohort and further reductions in those with a declining burden of disease (response to therapy). Given the small sample size, our findings are exploratory in nature. Based on these interesting results, studies examining CSF t-DNA in larger numbers of patients with suspected LMM are underway to assess the potential diagnostic utility of this assay, as well as further immunologic studies on those with differing ICI responses.

Lastly, we identified that pembrolizumab was well tolerated in this population of patients, who are often deemed too unwell to pursue systemic therapy. These data have important implications for treatment choices in patients with LMM since other treatments for LMM are

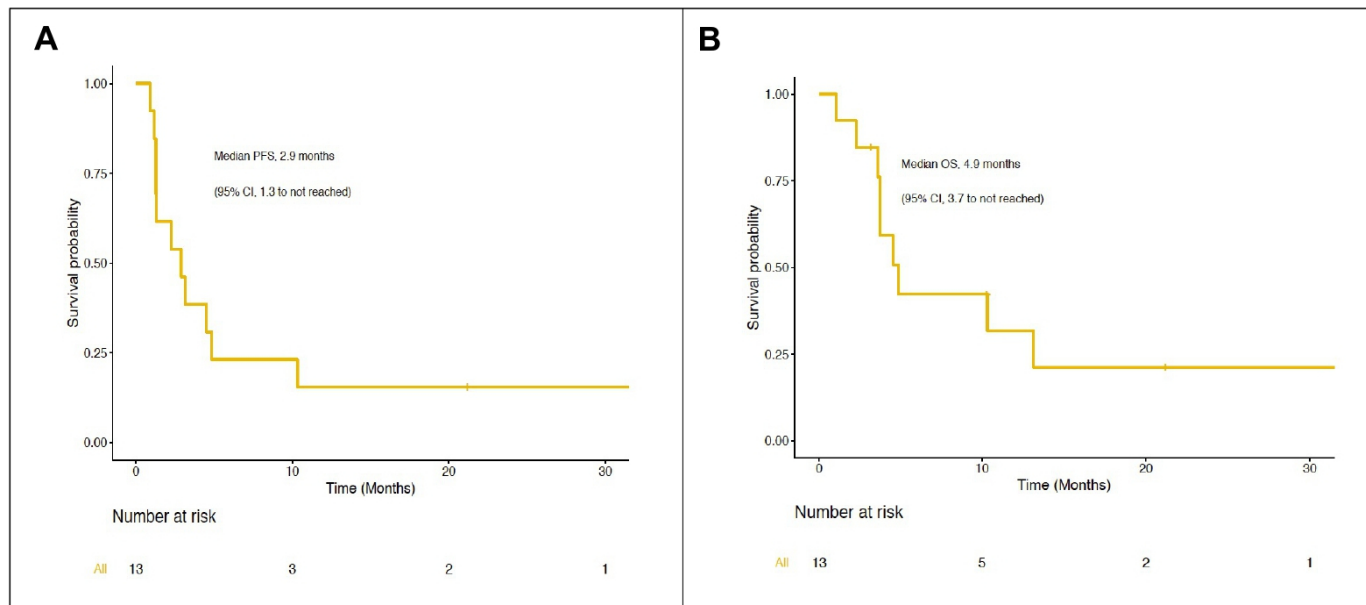


Figure 4 Central nervous system progression-free survival and overall survival for patients with leptomeningeal metastasis treated with pembrolizumab.

associated with much higher rates of toxicity, and in-patient administration.

While our study demonstrated several important findings, there were also limitations. We only enrolled physically robust patients who could tolerate clinical trial treatment. Thus, our study may not represent the entire population of patients with LMM, who tend to have a poor ECOG PS. Given the rarity of LMM and strict eligibility criteria, our study closed early for poor accrual. We were thus unable to estimate CNS response with the same planned precision. Despite this, we identified compelling CNS response, survival, and safety findings. In order to expand the reach of this study, we chose to enroll patients with any solid tumor. While this may have broadened the applicability of our findings, including patients with tumors not traditionally responsive to ICIs may have resulted in a lower CNS response rate. In terms of assessment of CNS response, when this study was developed there was no consensus regarding optimal radiographic response assessment of LMM. We thus used published but older CNS response criteria for LMM that incorporated radiographic, cytologic, and clinical response, which may be less applicable using modern MRI techniques, and should be considered in the interpretation of CNS response in this study.⁷ Subsequently published LM-RANO criteria assess response using similar criteria (MRI, CSF cytology, neurologic examination)¹¹ to our study; however, there is controversy over its reproducibility.²⁴ While findings were not statistically significant, we also note relatively disparate outcomes for patients in this study based on the presence or absence of detectable CSF cytology. In addition, while CNS response is a clinically relevant endpoint for patients with LMM, given the challenges in assessing LMM response, OS (or 3-month landmark OS) is now more commonly used as an endpoint

for trials of novel agents in LMM. Lastly, while we pursued investigation of potential CSF biomarkers, the number of immune cells present in the CSF and sample size of our study limited the ability to reach statistically significant conclusions or in analyzing serial samples over time. Future CSF work using single-cell approaches^{25, 26} and multicenter collaborations should be sought.

In conclusion, in this phase II trial of pembrolizumab for patients with LMM from solid tumors, 38% of patients demonstrated CNS responses by predefined study criteria. Pembrolizumab was well tolerated, and survival outcomes were consistent with published studies. Novel CSF biomarker studies in enrolled patients demonstrated that CSF aneuploidy of t-DNA may be sensitive for the detection of LMM, and that immunologic subsets of ICI-response based on cytokine profiles warrant further study. These data support further study of ICIs in patients with LMM in ICI-responsive tumor types and an adequate performance status.

Author affiliations

¹Department of Oncology, Sidney Kimmel Comprehensive Cancer Center, Johns Hopkins University, Baltimore, Maryland, USA

²Department of Immunology, The Bloomberg–Kimmel Institute for Cancer Immunotherapy, Johns Hopkins University, Baltimore, Maryland, USA

³Department of Oncology, Beaumont Hospital and RCSI University of Health Sciences, Dublin, Ireland

⁴Department of Neurology, John Hopkins Medicine, Johns Hopkins University, Baltimore, Maryland, USA

⁵Department of Neurosurgery, Johns Hopkins University, Baltimore, Maryland, USA

⁶Department of Biostatistics, Sidney Kimmel Comprehensive Cancer Center, Johns Hopkins University, Baltimore, Maryland, USA

⁷Cancer Research@UCC, College of Medicine and Health, University College Cork, Cork, Ireland

⁸Department of Immunology, Johns Hopkins Medicine Sidney Kimmel Comprehensive Cancer Center, Baltimore, Maryland, USA

⁹Ludwig Center for Cancer Genetics and Therapeutics, Johns Hopkins University, Baltimore, Maryland, USA

¹⁰Sol Goldman Pancreatic Cancer Research Center, Johns Hopkins University, Baltimore, Maryland, USA

¹¹Department of Radiation Oncology, Sidney Kimmel Comprehensive Cancer. John Hopkins University, Baltimore, Maryland, USA

¹²Division of Radiology, Johns Hopkins Hospital, Baltimore, Maryland, USA

Twitter Jarushka Naidoo @DrJNaidoo and Joanne Riemer @jriemer3

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ORCID iDs

Jarushka Naidoo <http://orcid.org/0000-0002-3470-8686>

Evan J Lipson <http://orcid.org/0000-0003-2976-0911>

Matthias Holdhoff <http://orcid.org/0000-0002-3285-3484>

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