Systemic Anti–PD-1 Immunotherapy Results in PD-1 Blockade on T Cells in the Cerebrospinal Fluid

Jana Portnow, MD; Dongrui Wang, PhD; M. Suzette Blanchard, PhD; Vivi Tran, MS; Darya Alizadeh, PhD; Renate Starr, MS; Ramsinh Dodia, MS; Vivian Chiu, BS; Alfonso Brito, MS; Julie Kilpatrick, MSN; Paige McNamara, PA-C; Stephen J. Forman, MD; Behnam Badie, MD; Timothy W. Synold, PharmD; Christine E. Brown, PhD

IMPORTANCE Little is known about the penetration and bioactivity of systemically administered programmed cell death 1 (PD-1) antibodies in the central nervous system. Such information is critical for advancing checkpoint antibody therapies for treatment of brain tumors.

OBJECTIVE To evaluate pembrolizumab concentrations and PD-1 blockade on T cells in the cerebrospinal fluid (CSF) after intravenous administration.

DESIGN, SETTING, AND PARTICIPANTS Cerebrospinal fluid and blood samples were collected from 10 adult patients with high-grade gliomas who were participating in clinical trials of intracranially administered chimeric antigen receptor (CAR) T cells and intravenous pembrolizumab at City of Hope in Duarte, California, from 2017 through 2019. Neuropharmacokinetic and immunologic correlative studies were performed on CSF and serum samples.

INTERVENTIONS OR EXPOSURES Pembrolizumab, 200 mg, was given intravenously every 3 weeks with a median of 2 cycles (range, 1-8). CAR T cells were administered intracranially every 1 to 4 weeks. Cerebrospinal fluid and blood samples were collected on the day of CAR T-cell administration and then 24 hours later for a total of 100 paired samples.

MAIN OUTCOMES AND MEASURES Pembrolizumab concentrations were measured by enzyme-linked immunosorbent assay, PD-1 blocking on T cells by flow cytometry, and results of PD-1 blockade on CAR T-cell function by in vitro tumor rechallenge assays.

RESULTS Of the 10 patients included in this study, the mean (SD) age was 45.7 (11.0) years, and 6 (60%) were women. Steady-state pembrolizumab concentrations in the CSF were achieved by 24 hours after initial intravenous administration, with a mean CSF:serum ratio of 0.009 (95% CI, 0.004-0.014). The CSF concentrations of pembrolizumab effectively blocked PD-1 on both endogenous T cells and intracranially administered CAR T cells in the CSF, with flow cytometric detection of surface PD-1 on the T cells decreasing from a mean (SD) of 39.3% (20.2%) before pembrolizumab to a mean (SD) of 3.8% (5.8%) 24 hours after pembrolizumab infusion. Steady-state concentrations in the CSF were maintained throughout the 21-day cycle of pembrolizumab, as was the PD-1 blocking effect, evidenced by no increase in detectable surface PD-1 on T cells in the CSF during that time period. Incubation of PD-1-expressing T cells with CSF samples from patients treated with pembrolizumab also resulted in PD-1 blockade.

CONCLUSIONS AND RELEVANCE Results of this study demonstrate steady-state concentrations of pembrolizumab in CSF after intravenous administration as well as CSF concentrations that are sufficient for blocking PD-1 on endogenous and adoptively transferred T cells. This provides mechanistic insight regarding the ability of systemically administered PD-1 blocking antibodies to modulate T-cell activity in the brain.

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Author Affiliations: Author affiliations are listed at the end of this article.

Corresponding Author: Christine E. Brown, PhD, Department of Hematology and Hematopoietic Cell Transplantation, T Cell Therapeutics Research Laboratories, City of Hope Beckman Research Institute and Comprehensive Cancer Center, 1500 E Duarte Rd, Duarte, CA 91010 (cbrown@coh.org).
Programmed cell death 1 (PD-1) blocking antibodies are effective against many types of cancer because of their ability to reinvigorate antitumor T-cell responses. Not only do they improve survival in patients with cancer who have systemic disease, they have also shown promising activity against brain metastases from melanoma and non-small cell lung cancer.\(^1,2\) Responses to anti-PD-1 therapy for primary brain tumors, such as glioblastoma, have been disappointing,\(^3,4\) although recent small studies have suggested clinical activity in the neoadjuvant setting.\(^5,6\) Improving responses to immunotherapy for patients with glioblastoma or other brain tumors requires a better understanding of the neuropharmacokinetics and neuropharmacodynamics of systemically administered PD-1 antibodies.

Chimeric antigen receptor (CAR) T cells are also being investigated as a treatment for primary and metastatic brain tumors.\(^7,10\) Our author group has been studying locoregional delivery of interleukin-13 receptor α2–targeted and ERBB2–targeted CAR T cells in patients with recurrent high-grade gliomas, and we previously reported that CAR T cells delivered intrathecally mediated complete tumor regression in a patient with multifocal glioblastoma.\(^8\) However, CAR T cells can be vulnerable to functional exhaustion mediated by PD-1. The addition of PD-1 blockade might enhance the efficacy of CAR T cells against brain tumors, yet it is currently unknown whether systemically administered PD-1 antibodies can achieve sufficient concentrations in the central nervous system to potentiate locoregionally delivered T-cell therapies.

**Methods**

**Patients and Sample Collections**

Cerebrospinal fluid (CSF) and blood samples were collected from 10 patients with high-grade gliomas (eTable 1 in the Supplement) who were participating in CAR T-cell clinical trials with cells given either intrathecally or both intrathecally and intracavitary (eFigure 1 in the Supplement). All patients also received pembrolizumab, 200 mg, intravenously every 21 days (eTable 2 in the Supplement). This study was conducted in accordance with the Declaration of Helsinki and approved by the City of Hope Institutional Review Board. All patients provided written informed consent. See eMethods in the Supplement for more details.

**Sample Analyses**

Concentrations of pembrolizumab in serum and cell-free CSF samples were determined using a PD-1 ligand–based enzyme-linked immunosorbent assay.\(^12\) Immune cells in the CSF were analyzed by flow cytometry. See eMethods in the Supplement for more details, including methods for statistical analysis.

**Statistical Analysis**

Statistical analyses are described in Figures 1 and 2, and in eMethods in the Supplement.
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Figure 1. Detection of Intravenously Administered Pembrolizumab in Cerebrospinal Fluid

A, Steady-state concentrations of pembrolizumab in serum and cerebrospinal fluid (CSF) from 10 patients with high-grade gliomas who received pembrolizumab, 200 mg, intravenously. All samples collected 1 day or more after the first pembrolizumab infusion are plotted. The median number of pembrolizumab cycles that patients received was 2 (range, 1-8), and the median number of paired samples collected at steady state from each patient was 6 (range, 1-25). Error bars indicate the mean 95% CIs for each patient, and the dashed line indicates the antilog of the average across all patients’ means of either serum or CSF. B, Concentrations of pembrolizumab in the serum and CSF from the 7 patients for whom 1-hour and 24-hour time points were collected after the first pembrolizumab infusion (ie, patients 213, 215, 226, 234, 239, 266, and 292). The patients received was 2 (range, 1-8), and the median number of paired samples collected at steady state from each patient was 6 (range, 1-25). Error bars indicate the mean 95% CIs for each patient, and the dashed line indicates the antilog of the average across all patients’ means of either serum or CSF. B, Concentrations of pembrolizumab in the serum and CSF from the 7 patients for whom 1-hour and 24-hour time points were collected after the first pembrolizumab infusion (ie, patients 213, 215, 226, 234, 239, 266, and 292). The P value is based on a 1-sided paired t test. C, Concentrations of pembrolizumab in the serum and CSF of a representative patient (patient 239) during administration of multiple cycles of pembrolizumab. Dotted lines indicate intravenous pembrolizumab infusions. D, Evaluation of programmed cell death 1 (PD-1) expression on CSF T cells. Percentages of PD-1 staining on T cells in patient CSF samples (patients 213, 215, 226, 234, 239, 268, 275, and 292) collected before (n = 8), 24 hours after the administration of intravenous pembrolizumab (n = 8), and toward the end of a pembrolizumab cycle (n = 6). The P value is based on a 1-sided paired t test.

We also analyzed PD-1 blockade on CAR T cells that were administered directly into the CSF. Despite initial negligible PD-1 expression on the CAR T-cell product (eFigure 6 in the Supplement), analysis of a representative CSF sample obtained prior to pembrolizumab treatment showed similar PD-1 expression on both locoregionally delivered CAR-positive T cells (administered intracavitary and/or intraventricularly) and endogenous CAR-negative T cells (Figure 2B). In CSF obtained after pembrolizumab administration, blockade of PD-1 (Figure 2C) and detection of bound pembrolizumab (eFigure 6 in the Supplement) was seen on both CAR-positive and CAR-negative T cells, demonstrating that CSF pembrolizumab concentrations were sufficient to block PD-1 on the T cells.

To confirm that CSF pembrolizumab concentrations were sufficient to block PD-1, healthy donor T cells were stimulated with CD3/CD28 Dynabeads (Thermo Fisher Scientific) to induce PD-1 expression and then incubated with CSF obtained before and after treatment with pembrolizumab. Blockade of PD-1 (Figure 2A) and detection of bound pembrolizumab (eFigure 5A in the Supplement) was only observed in CSF samples obtained after pembrolizumab administration. A similar blocking effect was seen after incubating T cells with either pembrolizumab or nivolumab, another anti-PD-1 monoclonal antibody, at concentrations similar to that measured in patient CSF (Figure 2A) and as low as 1 ng/mL (eFigure 5B in the Supplement).
Discussion

Recent studies have documented that intravenously administered anti–PD-1 antibodies can enhance endogenous antitumor immune responses in the brain; however, these studies do not demonstrate whether PD-1 blockade can occur on T cells residing within the central nervous system. To our knowledge, this study is the first to report CSF concentrations of PD-1 above isotype controls (light gray histogram) are depicted. B-C, Flow cytometric analysis of positive chimeric antigen receptor (CAR)–gated and negative CAR–gated T cells in CSF samples of a representative patient (patient 275) collected before (B) and 21 days after the second pembrolizumab infusion (C). Percentages of CD3-gated cells staining for surface PD-1 above isotype controls (gray histograms) are depicted. D, Healthy donor-derived CAR T cells were cocultured with primary brain tumor (PBT) cells, or PBT cells overexpressing programmed cell death ligand 1 (PD-L1) with or without the indicated amount of pembrolizumab in a rechallenge assay where additional target cells (with and without pembrolizumab) were added every 48 hours (arrowheads). Viable target cell numbers over time are depicted. The day 7 values were compared using a 1-sided 2-sample t test. The P values were corrected to achieve a familywise error rate of .05 based on a Hochberg procedure.

Limitations

This study is limited by the small sample size. It remains possible that the observed PD-1 blockade of endogenous T cells in the CSF occurred in the systemic circulation before the cells crossed into the CSF. However, both in vitro functional assays and PD-1 T-cell blocking data establish that concentrations of pembrolizumab in the CSF are effective for blocking PD-1 on T cells.

Conclusions

This case series study has demonstrated that CSF concentrations of systemically administered pembrolizumab can functionally block PD-1 on T cells. These results provide rationale for combining PD-1 checkpoint inhibitors with locoregionally delivered CAR T cells and other cellular therapies for the treatment of brain tumors.
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Author Affiliations: Department of Medical Oncology and Therapeutics Research, City of Hope Beckman Research Institute and Comprehensive Cancer Center, Duarte, California (Portnow); Department of Hematology and Hematopoietic Cell Transplantation, T Cell Therapeutics Research Laboratories, City of Hope Beckman Research Institute and Comprehensive Cancer Center, Duarte, California (Wang, Alizadeh, Starr, Dodia, Chiu, Brito, Forman, Brown); Department of Computational and Quantitative Medicine, City of Hope Beckman Research Institute and Comprehensive Cancer Center, Duarte, California (Blanchard); Department of Cancer Biology, City of Hope Beckman Research Institute and Comprehensive Cancer Center, Duarte, California (Tran, Synold); Department of Clinical Research, City of Hope Beckman Research Institute and Comprehensive Cancer Center, Duarte, California (kilpatrick); Division of Neurosurgery, Department of Surgery, City of Hope Beckman Research Institute and Comprehensive Cancer Center, Duarte, California (McNamara, Badie).

Author Contributions: Drs Synold and Brown had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. Drs Portnow and Wang contributed equally to the study.

Concept and design: Portnow, Wang, Badie, Synold, Brown.

Acquisition, analysis, or interpretation of data: All authors.

Drafting of the manuscript: Portnow, Wang, Blanchard, Synold, Brown.

Critical revision of the manuscript for important intellectual content: Portnow, Wang, Blanchard, Synold, Brown.

Statistical analysis: Wang, Blanchard, Synold.

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REFERENCES


