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Concurrent Dexamethasone Limits the Clinical Benefit of Immune Checkpoint Blockade in Glioblastoma

J. Bryan Iorgulescu[1,2], Prafulla C. Gokhale[3], Maria C. Speranza[1], Benjamin K. Eschle[3], Michael J. Poitras[3], Margaret K. Wilkens[3], Kara M. Soroko[3], Chhayheng Chhoeu[3], Aine Knott[3], Yan Gao[1], Mary Jane Lim-Fat[4], Gregory J. Baker[5], Dennis M. Bonal[6], Quang-Dé Nguyen[6], Gareth R. L. Grant[7], Keith L. Ligon[2,8,9], Peter K. Sorger[5], E. Antonio Chiocca[10], Ana C. Anderson[11,12], Paul T. Kirschmeier[3], Arlene H. Sharpe[12], Gordon J. Freeman[1], and David A. Reardon[1,4]

ABSTRACT

Purpose: Dexamethasone, a uniquely potent corticosteroid, is frequently administered to patients with brain tumors to decrease tumor-associated edema, but limited data exist describing how dexamethasone affects the immune system systemically and intratumorally in patients with glioblastoma (GBM), particularly in the context of immunotherapy.

Experimental Design: We evaluated the dose-dependent effects of dexamethasone when administered with programmed cell death 1 (PD-1) blockade and/or radiotherapy in immunocompetent C57BL/6 mice with syngeneic GL261 and CT-2A GBM tumors. Clinically, the effect of dexamethasone on survival was evaluated in 181 patients with isocitrate dehydrogenase (IDH) wild-type GBM treated with PD-(L)1 blockade, with adjustment for relevant prognostic factors.

Results: Despite the inherent responsiveness of GL261 to immune checkpoint blockade, concurrent dexamethasone administration with anti–PD-1 therapy reduced survival in a dose-dependent manner. Concurrent dexamethasone also abrogated survival following anti–PD-1 therapy with or without radiotherapy in immune-resistant CT-2A models. Dexamethasone decreased T-lymphocyte numbers by increasing apoptosis, in addition to decreasing lymphocyte functional capacity. Myeloid and natural killer cell populations were also generally reduced by dexamethasone. Thus, dexamethasone appears to negatively affect both adaptive and innate immune responses. As a clinical correlate, a retrospective analysis of 181 consecutive patients with IDH wild-type GBM treated with PD-(L)1 blockade revealed poorer survival among those on baseline dexamethasone. Upon multivariable adjustment with relevant prognostic factors, baseline dexamethasone administration was the strongest predictor of poor survival [reference, no dexamethasone; <2 mg HR, 2.16; 95% confidence interval (CI), 1.30–3.68; $P = 0.003$ and ≥2 mg HR, 1.97; 95% CI, 1.23–3.16; $P = 0.005$].

Conclusions: Our preclinical and clinical data indicate that concurrent dexamethasone therapy may be detrimental to immunotherapeutic approaches for patients with GBM.

Introduction

Although inhibition of immune checkpoints, such as programmed cell death 1 (PD-1), has transformed the treatment of many cancers, some tumors, such as glioblastoma (GBM), have responded poorly as exemplified by recently reported negative phase III trials among patients with recurrent (CheckMate-143) and newly diagnosed GBM (CheckMate-498; press release, Bristol Myers Squibb, May 9, 2019; ref. 1). These disappointing results likely reflect multifaceted mechanisms of immunosuppression exploited by GBM tumors, which are particularly pronounced in older patients (2–4). However, increasing data suggest that exogenous corticosteroid exposure also can limit the therapeutic benefits of immunotherapeutics (e.g., PD-1 and PD-L1 inhibitors) for patients with cancer, including those with GBM (5–7). Subgroup analyses of the CheckMate-143 study revealed that baseline dexamethasone use was associated with worse survival among nivolumab recipients than those treated with bevacizumab (1). In a recent clinical trial of immunogene therapy, dexamethasone dose was associated with decreased survival among patients with recurrent malignant glioma (8). Likewise, patients with newly diagnosed GBM on dexamethasone failed to generate immune responses following neoantigen vaccination, whereas those not on dexamethasone generated responses to multiple vaccinated neoepitope peptides (9).

Many patients with brain cancer receive dexamethasone to treat symptomatic cerebral edema generated by the tumor as well as by standard therapies, like external beam radiotherapy (10). Dexamethasone, which is five to 10 times more potent than other corticosteroids (e.g., prednisone and methylprednisolone), is the steroid of choice for

[1]Department of Medical Oncology, Dana-Farber Cancer Institute, Boston, Massachusetts. [2]Department of Pathology, Brigham and Women's Hospital, Boston, Massachusetts. [3]Experimental Therapeutics Core and Belfer Center for Applied Cancer Science, Dana-Farber Cancer Institute, Boston, Massachusetts. [4]Center for Neuro-Oncology, Dana-Farber Cancer Institute, Boston, Massachusetts. [5]Department of Systems Biology, Harvard Medical School, Boston, Massachusetts. [6]Lurie Family Imaging Center, Dana-Farber Cancer Institute, Boston, Massachusetts. [7]University of Glasgow Medical School, Glasgow, Scotland, United Kingdom. [8]Department of Oncologic Pathology, Dana-Farber Cancer Institute, Boston, Massachusetts. [9]Broad Institute of Harvard and MIT, Cambridge, Massachusetts. [10]Department of Neurosurgery, Brigham and Women's Hospital, Boston, Massachusetts [11]Department of Neurology, Brigham and Women's Hospital, Boston, Massachusetts. [12]Department of Immunology, Blavatnik Institute, Harvard Medical School and Evergrande Center for Immunologic Diseases, Harvard Medical School, Boston, Massachusetts.

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J.B. Iorgulescu, P.C. Gokhale, and M.C. Speranza contributed equally to this article.

Corresponding Author: David A. Reardon, Clinical Director, Center for Neuro-Oncology; Dana-Farber Cancer Institute; 450 Brookline Avenue, Dana 2134, Boston, MA 02215-5450. Phone: 617-632-2166; Fax: 617-632-4773; E-mail: david_reardon@dfci.harvard.edu

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Translational Relevance

Increasing data indicate that corticosteroids can exert a detrimental effect on immunotherapy for oncology patients. Dexamethasone, a uniquely potent corticosteroid, is frequently administered to patients with glioblastoma (GBM) to decrease tumor-associated edema, but limited data exist describing how it affects systemic and intratumoral immune activity, particularly in the context of immunotherapy. We demonstrate that concurrent dexamethasone administration, even at a low dose, limits the therapeutic benefit of anti–PD-1 therapy both in mouse GBM models and in a retrospective cohort of 181 patients with isocitrate dehydrogenase wild-type GBM. Mechanistically, dexamethasone decreased intratumoral T cells and systemic levels of T cells, natural killer cells, and myeloid cells, while qualitatively impairing lymphocyte function. The mechanism of T-cell depletion included induction of apoptosis. These findings indicate that dexamethasone hinders both adaptive and innate immune responses and its administration should be carefully assessed among patients with GBM undergoing immunotherapy clinical trials.

patients with brain cancer based on its potency, long half-life, and high brain penetrance (11). Although well known to induce myriad potentially severe, systemic side effects including proximal myopathy, truncal weight gain, hypertension, and glucose intolerance, the specific effects of dexamethasone on immune function and response to PD-1 immune checkpoint therapy for GBM tumors have not been well described (12).

Herein, we evaluated the impact of dexamethasone administration on response to anti–PD-1 immune checkpoint blockade in the syngeneic immunosensitive GL261 and immune-resistant CT-2A murine GBM models and how dexamethasone affects intratumoral and systemic immune cell populations and functionality. In addition, we assessed how concurrent dexamethasone administration affected the survival outcomes of 181 patients with isocitrate dehydrogenase (IDH) wild-type GBM treated with PD-1 or PD-L1 inhibitors [anti–PD-(L)1] in analyses adjusted for patients' key prognostic factors.

Methods and Materials

Cell Lines, antibodies, and reagents

Luciferase-transduced GL261 cells (GL261-luc2; PerkinElmer, Inc.) were expanded and frozen at the same generation. CT-2A cells (obtained from Thomas Seyfried, Boston College, Newton, MA) were transduced using firefly luciferase lentiviral particles (CT-2A-luc; Kerafast Inc.). Thawed cells were cultured for up to three passages in DMEM supplemented with 10% heat-inactivated FCS and 100 μg/mL G418 (for GL261-luc2) or 2 μg/mL puromycin (for CT-2A-luc) at 37°C in a humidified incubator maintained at 5% CO_2 prior to intracranial implantation, with periodic testing for *Mycoplasma*. Cells were maintained in logarithmic growth phase for all experiments. The 332.8H3 mouse anti-mouse PD-1 mAb (IgG1) was generated in the laboratory of G.J. Freeman and MOPC21 (IgG1; BioXCell) was used for isotype control (13). The mAbs contained less than 2 EU/mg endotoxin protein. Dexamethasone sodium phosphate (4 mg/mL, USP; Fresenius Kabi USA, LLC) was diluted with normal saline and injected intraperitoneally at doses described in the information to follow.

Intracranial tumor cell inoculation

A total of 1×10^5 GL261-luc2 cells or 0.25×10^5 CT-2A-luc cells, which are syngeneic in C57BL/6 mice, were resuspended in PBS and injected stereotactically into the right striatum of anesthetized, 7- to 10-week-old, female, albino C57BL/6 mice (The Jackson Laboratory) using a Hamilton syringe and stereotactic frame (14). Mice were euthanized for either signs of morbidity due to tumor burden or after at least 100 days to terminate the study, if mice appeared healthy. All animal experiments were approved by the Dana-Farber Cancer Institute (DFCI, Boston, MA) Animal Care and Use Committee.

In vivo treatment and tumor assessment

For all studies, mice with enlarging tumor burden, defined by increasing bioluminescence signal between days 3 and 6 after tumor implantation, were randomized into control and treatment cohorts. Tumor response assessment was done by quantifying bioluminescence signal in all animals, as well as MRI in a subset, as performed previously (13). Therapeutic anti–PD-1 and isotype controls were administered via intraperitoneal injection beginning on day 6 after tumor implantation, using two dosing regimens. A dose-intensive regimen, consisting of a loading dose (500 μg) with repeat injections every 3 days (250 μg/dose) for a total of six to eight injections, was employed to evaluate the effect of dexamethasone in the setting of maximal therapeutic benefit from anti–PD-1 therapy. An abbreviated regimen, comprising of only four doses (250 μg/dose every 3 days) without a loading dose, was also used to examine the effects of dexamethasone when the therapeutic effect of anti–PD-1 was reduced. Control animals received equivalent doses of isotype murine IgG according to the same dosing schedule. Dexamethasone was administered as a single agent at 10 mg/kg/day intraperitoneally and in combination with PD-1 mAbs at either low (1 and 2.5 mg/kg/day) or high (10 mg/kg/day) doses on days 6–27. No treatment was administered after day 27 following tumor implantation. Using this treatment schedule, we systematically evaluated antitumor activity as measured by bioluminescence imaging (BLI), MRI, and overall survival (OS).

Next, we evaluated whether the timing of dexamethasone administration impacted the therapeutic efficacy of inhibitory immune checkpoint blockade. In these experiments, anti–PD-1 was initiated on day 6 and administered every 3 days for eight doses over 27 days and dexamethasone (10 mg/kg/day i.p.) was administered on days 1–5. We then evaluated the effect of concurrent dexamethasone when added to anti–PD-1 therapy plus fractionated radiotherapy in both the GL261 and CT-2A models. Fractionated radiotherapy was administered using a X-Rad 225Cx Image Guided Biological Irradiator System (Precision X-Ray). Each mouse received a total dose of 10 Gy, delivered as 5×2 Gy in 5 consecutive days, using two parallel-opposed fields, including an anterior–posterior collimated field and posterior–anterior collimated field (Supplementary Materials and Methods). Dexamethasone (10 mg/kg) treatment in the combination studies was administered on days 6–16 in GL261-luc2 studies and days 6–27 in CT-2A-luc studies.

For rechallenge experiments that assessed immunologic memory responses to tumor, 1×10^5 GL261 nonluciferase-transduced cells were injected intracranially into the contralateral hemisphere in a cohort of mice that were previously treated and survived for more than 100 days. A similar tumor cell inoculum was administered to a cohort of treatment-naïve mice as a control. Rechallenged mice were followed for a minimum of 128 additional days and received no additional therapy.

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Flow cytometry characterization of immune responses

Immune response assessment studies were performed on material obtained from euthanized, tumor-bearing animals on day 16 following a 500-μg anti–PD-1 loading dose on day 6 and 250-μg doses on days 9, 12, and 15 and/or dexamethasone (administered at 10 mg/kg/day i.p. on days 6–16). For comprehensive profiling of the immune microenvironment by flow cytometry analysis, whole tumor-bearing brain, superficial cervical lymph nodes (cLNs), spleen, and thymus were homogenized using enzymatic (1.5 mg/mL collagenase IV, 200 U/mL DNaseI, and Hank's Balanced Salt Solution with calcium and magnesium) and/or mechanical tissue disaggregation. Red blood cells were removed using a Ficoll Gradient (GE Life Sciences). Brain homogenates were resuspended in 25% Percoll Plus (Sigma) and centrifuged (1,500 rpm for 20 minutes, with minimum acceleration and no brake) to remove myelin and isolate leukocytes (13). Samples were split for staining with antibody panels and completely enumerated by flow cytometry. The following antibodies (from BioLegend, unless otherwise indicated) were used for flow cytometric analysis: anti-CD45 (30-F11), anti-CD3 (17A2), anti-CD4 (RM4-5), anti-CD8 (53-6.7), anti-CD11b (M1/70), anti-CD80 (16-10A1), anti-CD86 (GL-1), anti-NK1.1 (PK136), anti-Ly6G (1A8), anti-Ly6C (HK1.4), anti–PD-1 (RMP1-30, noncompeting epitope to PD-1 treatment mAb, eBioscience), anti–PD-L1 (10F.9G2), and anti-CD69 (H1.2F3) Dead cells were excluded using the Zombie NIR Fixable Viability Kit (BioLegend). Following surface staining, cells were processed with the FOXP3 Fixation/Permeabilization Kit (eBioscience). The following antibodies were used for intracellular staining: anti-FOXP3 (FJK-16s, eBioscience) and anti-Ki67 (16A8, BioLegend), and anti-IFNγ (XMG1.2). To assess IFNγ expression, splenocytes were stimulated for intracellular cytokine staining as per the manufacturer's instructions (Cell Stimulation Cocktail, Invitrogen eBioscience). In addition, late apoptosis of splenic lymphoid populations was measured (via annexin-V and 7-AAD staining) in nontumor-bearing mice euthanized at either 1 hour after the first dexamethasone dose or 1 hour after the sixth dexamethasone dose (121 hours after initiation of dexamethasone), including both low (1 mg/kg) or high (10 mg/kg) daily intraperitoneal dexamethasone dosing and IgG-treated controls for comparison. Acquisition was performed on an LSR Fortessa Flow Cytometer (BD Biosciences) and analyzed using FlowJo software, with the gating strategies displayed in Supplementary Fig. S1.

Clinical cohort

To assess the clinical effect of dexamethasone, we retrospectively identified consecutive patients with GBM diagnosed before April 1, 2019 who were evaluated at DFCI (Boston, MA) and received anti–PD-(L)1 therapy on either a formal clinical trial or a compassionate use basis. Among these patients, 181 had ≥1 month of follow-up after the start of PD-(L)1 blockade, as well as: (i) tumor available at DFCI (Boston, MA) for an integrated histomolecular diagnosis of IDH wild-type GBM, World Health Organization grade 4; (ii) annotated clinical data; and (iii) survival outcome. Pharmacy data and clinic notes were reviewed to identify whether patients were receiving dexamethasone at the time of PD-(L)1 treatment initiation and, if so, the dexamethasone dose (none, <2 mg, and ≥2 mg; Supplementary Table S1). Tumor volumes of interest were manually selected and measured from patients' contrast enhancing T1 MRI sequences taken prior to PD-(L)1 treatment. These data were collected under DFCI Institutional Review Board (Boston, MA) protocol 10-417. OS was estimated using the Kaplan–Meier method from time of PD-(L)1 treatment start to date of death, with censorship at the date of last clinical assessment, and comparisons by log-rank test. The cutoff for survival data was 17 April 2020. Multivariable Cox regression analysis was performed to adjust OS among the 163 patients with complete annotated data for relevant prognostic factors including age at GBM diagnosis and *MGMT* promoter methylation status, as well as the following at the time of PD-(L)1 therapy initiation: disease status (newly diagnosed vs. recurrent), Karnofsky performance scale (KPS; ≤70 vs. 80 vs. ≥90), tumor volume (on MRI T1 sequence, postcontrast), and whether a pre-anti–PD-(L)1 treatment gross total resection (GTR) was performed.

Statistical analysis

For flow cytometry characterization of immune responses, dexamethasone versus IgG control and concurrent dexamethasone with anti–PD-1 versus anti–PD-1 alone were prospectively determined to be the comparisons of interests. Complete absolute cell counts from flow cytometry experiments were evaluated using multiple linear regression, including adjustment for data derived from multiple experiments (i.e., batch effect). Data are displayed as mean ± SE. For visualization, cell counts were normalized to that experiment's IgG group's average counts. Apoptosis was evaluated within and between timepoints by two-way ANOVA with Sidák correction for multiple comparisons. Murine OS estimates were determined using Kaplan–Meier methods and were compared using log-rank test and Cox regression. Two-sided $P < 0.05$ was considered statistically significant. Statistical analysis was performed with GraphPad Prism 8 (GraphPad Software, Inc) and STATA (v15.1, IBM).

Results

Concurrent dexamethasone limits the survival benefit of anti–PD-1 monotherapy and combination with radiotherapy in preclinical models

We first evaluated the effect of dose and timing of dexamethasone on the antitumor activity of PD-1 blockade. OS was assessed when dexamethasone was concurrently administered at either low (1 or 2.5 mg/kg) or high (10 mg/kg) daily dosing with a dose-intensive anti–PD-1 schedule (**Fig. 1A**). As published previously (13), a majority of mice [71.4%; 95% confidence interval (95% CI), 55.2–82.7] with growing intracranial GL261-luc2 tumors were effectively cured with anti–PD-1 monotherapy (**Fig. 1B**). In contrast, in anti–PD-1–treated mice who received concurrent dexamethasone, OS decreased in a dose-dependent fashion that was most pronounced at higher dexamethasone doses, although lower doses also decreased OS. OS rates at 100 days were 47.1% (95% CI, 29.8–62.5; $P = 0.04$), 31.3% (95% CI, 11.4–53.7; $P = 0.008$), and 26.5% (95% CI, 13.2–41.8; $P < 0.001$) as concurrent dexamethasone dosage increased from 1 to 2.5 mg/kg and to 10 mg/kg, respectively (**Fig. 1B**). Mice treated with dexamethasone alone had similarly poor OS compared with control mice treated with IgG ($P = 0.31$). Changes in tumor burden were confirmed by bioluminescence (**Fig. 1C**; Supplementary Fig. S2A) and MRI (**Fig. 1D**). Animals surviving long term (i.e., ≥100 days) were rechallenged by implantation of 1×10^5 GL261 nonluciferase-transduced cells in the contralateral hemisphere. Among those treated with anti–PD-1 alone or anti–PD-1 with concurrent dexamethasone at either 1 or 10 mg/kg, 85.7% (6/7), 100% (5/5), and 75.0% (3/4) successfully cleared the rechallenge tumors and survived for at least an additional 128 days ($P = 0.57$), respectively (Supplementary Fig. S2B). All challenged-naïve control mice died (median OS, 27 days).

We then evaluated dexamethasone administered on days 1–5 prior to the initiation of anti–PD-1 therapy on day 6, but not during anti–PD-1 therapy, in the GL261-luc2 GBM model. In contrast to the

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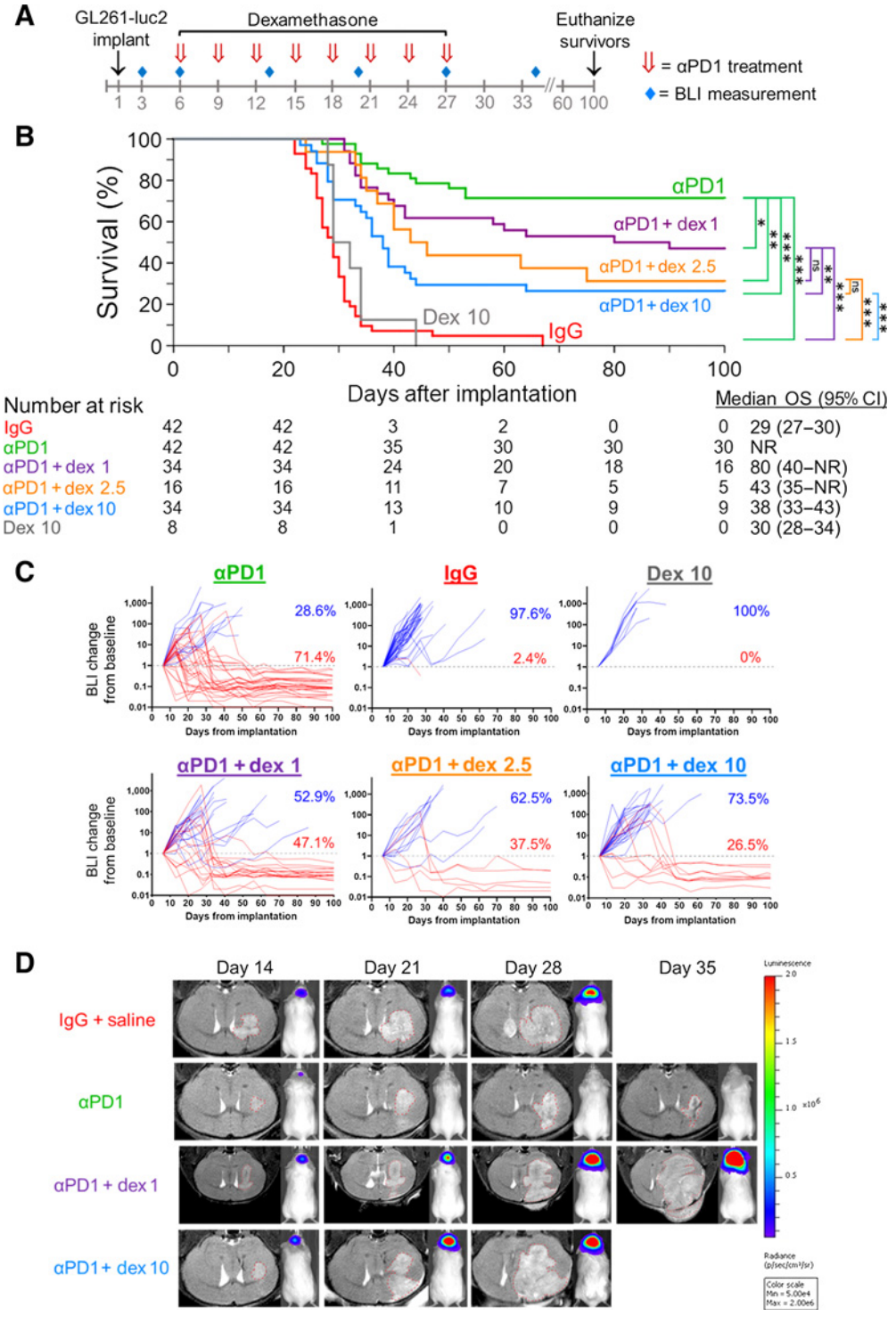

Figure 1.

Concurrent dexamethasone reduces the survival benefit of anti–PD-1 therapy in GL261-luc2 GBM mouse models in a dose-dependent manner. **A,** Experimental schema. Anti–PD-1 (αPD1, red arrows) was administered in a dose-intensive schedule, that is, intraperitoneally beginning on day 6 (500 μg) followed by seven additional doses (250 μg/dose) at 3-day intervals, with dexamethasone delivered intraperitoneally daily from day 6 to 27. **B,** Kaplan–Meier survival estimates of anti–PD-1 therapy without dexamethasone ($n = 42$, data derived from five experiments, with 8–10 mice each) and anti–PD-1 therapy with concurrent dexamethasone at 1 mg/kg ($n = 34$, data derived from four experiments, with 8–10 mice each), 2.5 mg/kg ($n = 16$, data derived from two experiments, with 8 mice each), or 10 mg/kg ($n = 34$, data derived from four experiments, with 8–10 mice each), compared with IgG ($n = 42$, data derived from five experiments, with 8–10 mice each) and 10 mg/kg dexamethasone only ($n = 8$, data derived from a single experiment) controls; with comparison by Cox regression. (*Continued on the following page.*)

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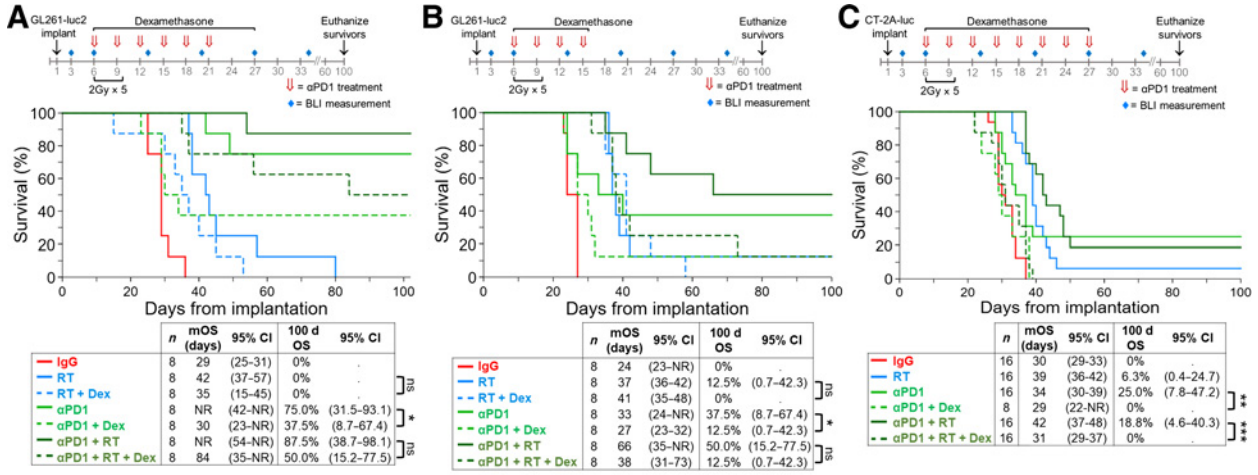

	n	mOS (days)	95% CI	100 d OS	95% CI	
IgG	8	29	(25–31)	0%	.	
RT	8	42	(37–57)	0%	.	ns
RT + Dex	8	35	(15–45)	0%	.	
αPD1	8	NR	(42–NR)	75.0%	(31.5–93.1)	*
αPD1 + Dex	8	30	(23–NR)	37.5%	(8.7–67.4)	
αPD1 + RT	8	NR	(54–NR)	87.5%	(38.7–98.1)	ns
αPD1 + RT + Dex	8	84	(35–NR)	50.0%	(15.2–77.5)	

	n	mOS (days)	95% CI	100 d OS	95% CI	
IgG	8	24	(23–NR)	0%	.	
RT	8	37	(36–42)	12.5%	(0.7–42.3)	ns
RT + Dex	8	41	(35–48)	0%	.	
αPD1	8	33	(24–NR)	37.5%	(8.7–67.4)	*
αPD1 + Dex	8	27	(23–32)	12.5%	(0.7–42.3)	
αPD1 + RT	8	66	(35–NR)	50.0%	(15.2–77.5)	ns
αPD1 + RT + Dex	8	38	(31–73)	12.5%	(0.7–42.3)	

	n	mOS (days)	95% CI	100 d OS	95% CI	
IgG	16	30	(29–33)	0%	.	
RT	16	39	(36–42)	6.3%	(0.4–24.7)	
αPD1	16	34	(30–39)	25.0%	(7.8–47.2)	**
αPD1 + Dex	8	29	(22–NR)	0%	.	
αPD1 + RT	16	42	(37–48)	18.8%	(4.6–40.3)	***
αPD1 + RT + Dex	16	31	(29–37)	0%	.	

Figure 2.

Concurrent dexamethasone administration decreases the OS benefit of anti–PD-1 plus radiotherapy in syngeneic GL261-luc2 and CT-2A-luc GBM mouse models. Kaplan–Meier OS estimates are depicted, with comparison by log-rank test and Cox regression. **A,** To assess concurrent dexamethasone's effect on a dose-intensive schedule of anti–PD-1 with or without radiotherapy in GL261-luc2 mice ($n = 8$/group from a single experiment), anti–PD-1 was administered intraperitoneally via a loading dose (500 μg) followed by five additional doses (250 μg/dose) at 3-day intervals. Radiotherapy was administered in 2 Gy fractions/day for 5 days beginning on day 6. Dexamethasone was delivered intraperitoneally daily from days 6 to 27 at 10 mg/kg. **B,** For GL261-luc2 mice ($n = 8$/group from a single experiment), anti–PD-1 (αPD1) was administered intraperitoneally via an abbreviated dosing schedule every 3 days beginning on day 6 for a total of four doses (250 μg/dose). **C,** For CT-2A-luc mice ($n = 8$–16/group, derived from two experiments), anti–PD-1 was administered intraperitoneally via a loading dose (500 μg) followed by seven additional doses (250 μg/dose) at 3-day intervals. Radiotherapy was administered in 2 Gy fractions/day for 5 days beginning on day 6. Dexamethasone was delivered intraperitoneally daily from days 6 to 27 at 10 mg/kg. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; dex, dexamethasone; NR, not reached.

decreased OS exhibited when dexamethasone was administered concurrently with anti–PD-1, dexamethasone administered prior to, but not during, anti–PD-1 therapy did not alter survival ($P = 0.64$; Supplementary Fig. S2C).

Next, given that radiotherapy is standard therapy for patients with GBM, we evaluated the effect of concurrent dexamethasone on OS when PD-1 therapy was administered with radiotherapy relative to either therapy alone. Initially anti–PD-1 was administered using the dose-intensive schedule to mice with growing intracranial GL261-luc2 tumors (**Fig. 2A**). With this schedule, 75.0% (95% CI, 31.5–93.0) of mice treated with anti–PD-1 monotherapy were long-term survivors (i.e., ≥100 days). As expected, the addition of dexamethasone markedly reduced the median OS benefit of anti–PD-1 therapy [from >100 days (95% CI, 42–not reached) to 30 days (95% CI, 23–not reached); $P = 0.049$] and decreased the long-term survivor rate by half. Radiotherapy, administered at 2 Gy × 5 daily fractions beginning 6 days after tumor implantation, modestly prolonged median OS compared with isotype controls (42 days, 95% CI, 37–57 vs. 29 days, 95% CI, 25–31; $P = 0.001$). Radiotherapy added to dose-intensive anti–PD-1 therapy had a nominal effect on survival. However, the addition of dexamethasone to anti–PD-1 plus radiation demonstrated a trend toward decreased median and long-term survival, but did not achieve statistical significance ($P = 0.15$).

Using the abbreviated anti–PD-1 dosing schedule (**Fig. 2B**), concurrent dexamethasone with anti–PD-1 monotherapy significantly reduced the median OS and the long-term OS rate, from 37.5% (95% CI, 8.7–67.4) to 12.5% (95% CI, 0.1–42.3; $P = 0.04$). Median OS increased from 37 days for radiotherapy alone to 66 days for radiotherapy plus anti–PD-1, although the net OS improvement for the combination did not achieve significance ($P = 0.25$). However, when dexamethasone was administered with anti–PD-1 plus radiotherapy, the survival decreased to the level of radiotherapy alone ($P = 0.78$), showing that dexamethasone abrogated the therapeutic benefit of anti–PD-1.

We then repeated this experiment using the CT-2A-luc model, which is known to be less responsive to anti–PD-1 than GL261 (15), using the dose-intensive anti–PD-1 schedule (**Fig. 2C**). Compared with isotype controls, anti–PD-1 exhibited a statistically significant OS benefit with a modest number of long-term survivors ($P < 0.001$). Radiotherapy also moderately improved OS [median of 39 days (95% CI, 36–42) vs. 30 days in isotype controls (95% CI, 29–33); $P < 0.001$], while the combination of anti–PD-1 plus radiotherapy achieved limited additive benefit compared with either modality alone. As in the GL261-luc2 model, the addition of dexamethasone to anti–PD-1 plus radiotherapy significantly decreased OS (median 31 days; 95% CI, 29–37) compared with either single-agent anti–PD-1 (median 34 days;

(*Continued.*) **C,** The corresponding longitudinal BLI, displayed as change from baseline (day 6 after implantation, dotted gray line), for mice treated with anti–PD-1 alone ($n = 42$, baseline BLI median 415,600 ph/sec/cm^2/sr; IQR, 201,500–981,050) or anti–PD-1 with concurrent 1 mg/kg ($n = 34$, baseline BLI median 434,700 ph/sec/cm^2/sr; IQR, 246,975–835,500), 2.5 mg/kg ($n = 16$, baseline BLI median 490,950 ph/sec/cm^2/sr; IQR, 268,800–1,081,875), or 10 mg/kg dexamethasone ($n = 34$, baseline BLI median 367,300 ph/sec/cm^2/sr; IQR, 227,400–636,675), as compared with IgG control ($n = 16$, baseline BLI median 463,650 ph/sec/cm^2/sr; IQR, 286,775–1,059,250) and dexamethasone 10 mg/kg only control ($n = 8$, baseline BLI median 159,550 ph/sec/cm^2/sr; IQR, 143,725–231,700). Tumor response visualized in red and lack of response in blue. **D,** Representative longitudinal MRI findings demonstrating increased tumor growth when low (1 mg/kg) or high (10 mg/kg) doses of dexamethasone were coadministered during PD-1 therapy, compared with anti–PD-1 without dexamethasone. Images are obtained serially from the same mice over time. Dotted red line outlines the tumor on coronal MRI plane. ns, not significant, $P \geq 0.05$; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; dex, dexamethasone; NR, not reached.

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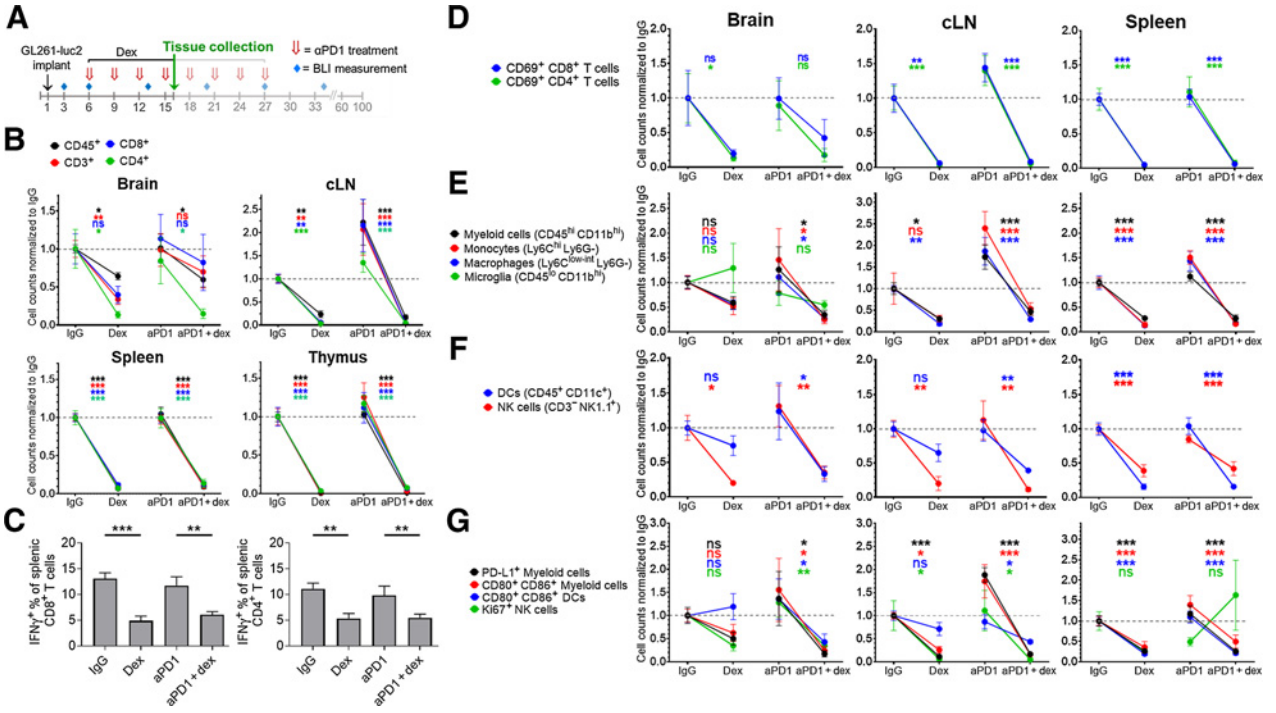

Figure 3.

Concurrent dexamethasone negatively affects intratumoral and systemic adaptive and innate immune cell populations in the GL261-luc2 GBM mouse model. **A,** Experimental schema. Tissue was collected at day 16 of a dose-intensive regimen of anti–PD-1, in which anti–PD-1 (αPD1) was administered intraperitoneally beginning on day 6 (500-μg loading dose) followed by three additional doses (250 μg) at 3-day intervals, with dexamethasone (10 mg/kg) administered intraperitoneally on days 6–16. Tissue ($n = 4$–8/group, derived from two experiments) was harvested on day 16 and analyzed by flow cytometry. Immune cell counts were evaluated by multiple linear regression, normalized to the corresponding IgG control group's mean count (displayed as dashed gray line), and displayed as mean ± SE. **B,** Differences in $CD45^+$ leukocytes and $CD45^+$ $CD3^+$ lymphocytes, including $CD4^+$ and $CD8^+$ T cells between treatment groups. **C,** Percentage of splenic IFNγ$^+$ $CD4^+$ and $CD8^+$ lymphocytes by treatment group. **D,** Change in the number of early activated $CD69^+$ T cells by site for each treatment group. In addition, differences between treatment groups in innate immune cells including myeloid cells ($CD45^{hi}$ $CD11b^{hi}$), macrophages ($Ly6C^{lo\text{-}int}$ Ly6G-), monocytes ($Ly6C^{hi}$ Ly6G-), and microglia (in the brain, $CD45^{lo}$ $CD11b^{hi}$; **E**), DCs ($CD45^+$ CD11c$^+$) and NK cells ($CD45^+$ $CD3^-$ NK1.1$^+$; **F**), as well as activated ($CD80^+$ $CD86^+$) myeloid cells and DCs, PD-L1$^+$ myeloid cells, and $Ki67^+$ NK cells (**G**) were analyzed. Dex, dexamethasone; ns, not significant, $P \geq 0.05$; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

95% CI, 30–39; $P = 0.003$) or the combination of anti–PD-1 with radiotherapy (median 42 days; 95% CI, 37–48; $P < 0.001$). The addition of dexamethasone was likewise associated with a complete abrogation of the benefits of anti–PD-1 monotherapy on OS (HR of adding dexamethasone: 4.34; 95% CI, 1.68–11.21; $P < 0.002$).

Concurrent dexamethasone administration decreases intratumoral and systemic immune effector cell populations

To investigate the mechanisms by which concurrent dexamethasone administration limits anti–PD-1 therapeutic benefit, we used flow cytometry to quantify the adaptive and innate immune cell populations isolated from intracranial tumor, cLNs, and spleen. Concurrent dexamethasone (10 mg/kg) administration significantly decreased the numbers of $CD45^+$, $CD3^+$, $CD4^+$, and $CD8^+$ cells from the cLN, spleen, and thymus independent of anti–PD-1 therapy (**Fig. 3A** and **B**). These patterns were also observed in some brain tumor-infiltrating lymphocyte (TIL) populations as well. Dexamethasone also reduced regulatory $CD4^+$ $FOXP3^+$ T cells among TILs, cLNs, and spleens, with a significant reduction of the CD8 to $CD4^+$ $FOXP3^+$ ratio in cLNs compared with IgG control (Supplementary Fig. S3A and S3B). Decreased $CD8^+$, $CD4^+$ $FOXP3^-$, and $CD4^+$ $FOXP3^+$ T cells were also observed in the tumor immune microenvironment by visualization with multiplexed immunofluorescence (CyCIF) staining (Supplementary Fig. S4; Supplementary Materials and Methods).

To evaluate the effect of dexamethasone on T-cell function, we assayed IFNγ cytokine expression in *ex vivo*–stimulated splenic T cells from tumor-bearing mice by intracellular cytokine staining. Concurrent dexamethasone significantly decreased the percentage of IFNγ-positive $CD4^+$ and $CD8^+$ T cells (**Fig. 3C**). We then evaluated the effect of dexamethasone on T-cell activation as assessed by expression of the early activation marker, CD69. Dexamethasone, either alone or concurrent with anti–PD-1, reduced the number of $CD69^+$ $CD4^+$ and $CD69^+$ $CD8^+$ cells, particularly in the cLN and spleen (**Fig. 3D**).

Regarding innate immunity, dexamethasone significantly decreased intratumoral natural killer (NK) cells ($CD45^+$ $CD3^-$ NK1.1$^+$), while other myeloid populations [e.g., tumor-associated macrophages, monocytes, and dendritic cells (DCs)] trended downward, particularly if dexamethasone was added to anti–PD-1 (**Fig. 3E** and **F**). Most innate immune cell populations were also consistently decreased in cLN and spleen following dexamethasone. Similarly, declines in PD-L1$^+$ myeloid cells ($CD45^{hi}$ $CD11b^{hi}$), activated myeloid cells ($CD45^{hi}$ $CD11b^{hi}$ $CD80^+$ $CD86^+$), and mature DCs ($CD45^+$ $CD11c^+$ $CD86^+$ $CD80^+$) were observed with dexamethasone administration (**Fig. 3G**).

Dexamethasone induces lymphocyte apoptosis

We then investigated how dexamethasone quantitatively decreases lymphocyte levels. Dexamethasone increased the percentage of splenic $CD4^+$ and $CD8^+$ T cells expressing late apoptosis markers (7-AAD$^+$

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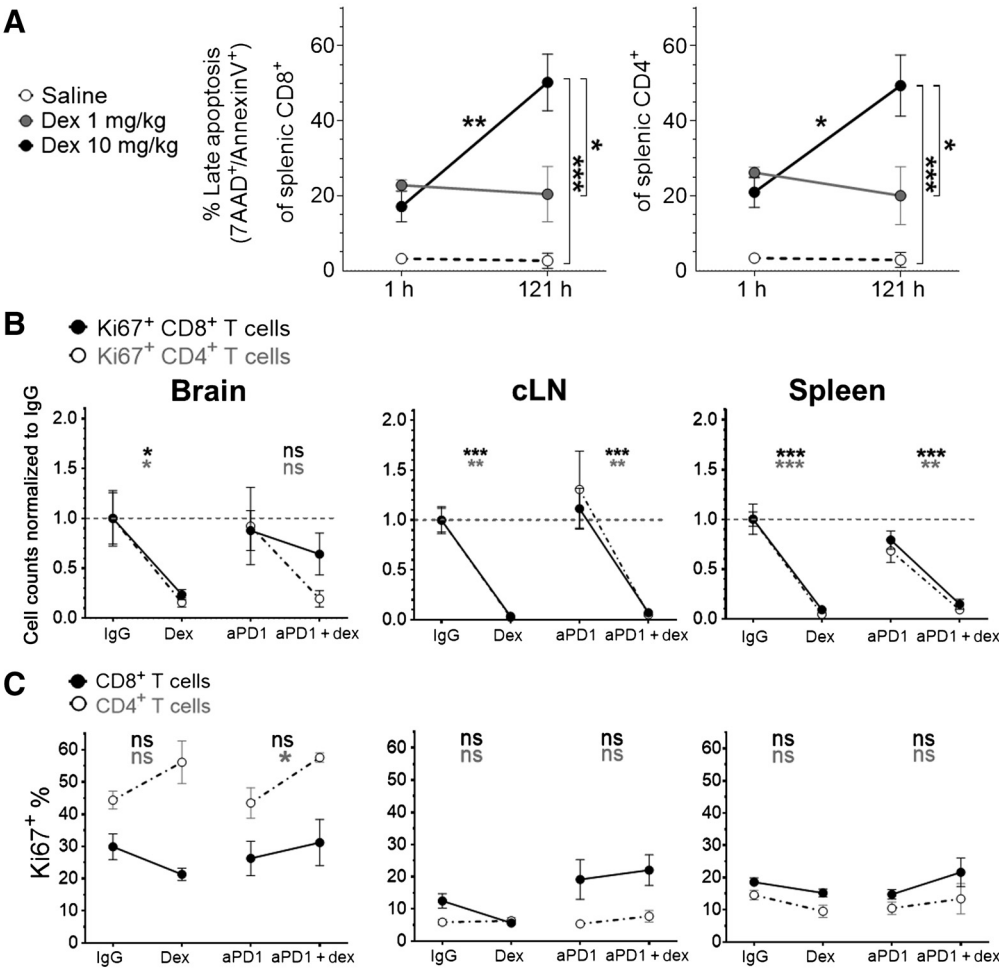

Figure 4.

Concurrent dexamethasone increases apoptosis of $CD4^+$ and $CD8^+$ T cells in mice. **A,** Late apoptosis was evaluated by 7-AAD^+ and Annexin-V^+ staining in nontumor-bearing mouse spleens (n = 3/group, from a single experiment) either 1 hour after the first dexamethasone dose or 1 hour after the sixth daily dexamethasone dose. Apoptosis differences were tested by two-way ANOVA with post-test correction. Cell counts normalized to the corresponding IgG control group's mean count (**B**) and percent of proliferating $CD4^+$ and $CD8^+$ T cells (**C**) was evaluated by Ki67 staining, using the same dosing schema and analyses as **Fig. 3** (n = 4–8/group, derived from two experiments). Dex, dexamethasone; ns, not significant, $P \geq 0.05$; ns, not significant, $P \geq 0.05$; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

Annexin-V^+) as early as 1 hour after either low (1 mg/kg) or high (10 mg/kg) doses (**Fig. 4A**). With continued daily dexamethasone dosing for 6 days, the percentage of late apoptotic $CD8^+$ and $CD4^+$ T cells remained stable following low dosing and significantly increased following high dosing ($P_{adj} = 0.005$ for $CD8^+$ T cells and $P_{adj} = 0.03$ for $CD4^+$ T cells). Dexamethasone significantly decreased the absolute counts of Ki67$^+$ $CD4^+$ and $CD8^+$ T cells from cLN and spleen, as well as Ki67$^+$ TILs in the absence of anti–PD-1 (**Fig. 4B**); however, dexamethasone did not reduce the proportion of $CD4^+$ and $CD8^+$ T cells that were proliferative (**Fig. 4C**). These data show that dexamethasone reduces T lymphocytes at least, in part, by inducing apoptosis.

Dexamethasone decreases the adjusted survival among patients with GBM undergoing anti–PD-(L)1 therapy

To examine the influence of dexamethasone on the clinical activity of anti–PD-(L)1 therapy among patients with GBM, we analyzed the OS of 181 consecutive patients with IDH wild-type GBM at our institution who were treated with anti–PD-(L)1 therapy, including 75.7% (n = 137) at recurrence and 24.3% (n = 44) in the newly diagnosed setting. The median follow-up from diagnosis of these patients was 22.1 months [interquartile range (IQR), 15.3–30.7 months) and 153 (84.5%) have died. Baseline dexamethasone, either at <2 mg daily (n = 29, 16.0%) or ≥2 mg daily (n = 35, 19.3%), significantly decreased unadjusted median OS to 8.1 months (95% CI, 5.5–9.5; $P < 0.001$) and 6.3 months (95% CI, 4.5–9.6; $P = 0.001$), respectively, from 13.1 months (95% CI, 11.3–14.6) for those not on baseline dexamethasone (n = 117, 64.6%; **Fig. 5A**). The detrimental effect of baseline dexamethasone persisted in multivariable analyses adjusted for disease setting (newly diagnosed vs. recurrent), patient age, *MGMT* promoter methylation status, KPS, tumor volume at anti–PD-(L)1 initiation, and extent of resection (n = 163; **Fig. 5B**; **Table 1**). Baseline dexamethasone use was the strongest identified negative risk factor for OS. Even after multivariable adjustment, baseline dexamethasone eliminated the survival benefit when administered at either lower (i.e., <2 mg daily; HR, 2.16; 95% CI, 1.30–3.60; $P = 0.003$) or higher doses (i.e., ≥2 mg daily; HR, 1.97; 95% CI, 1.23–3.16; $P = 0.005$) compared with no baseline dexamethasone. Similar results with

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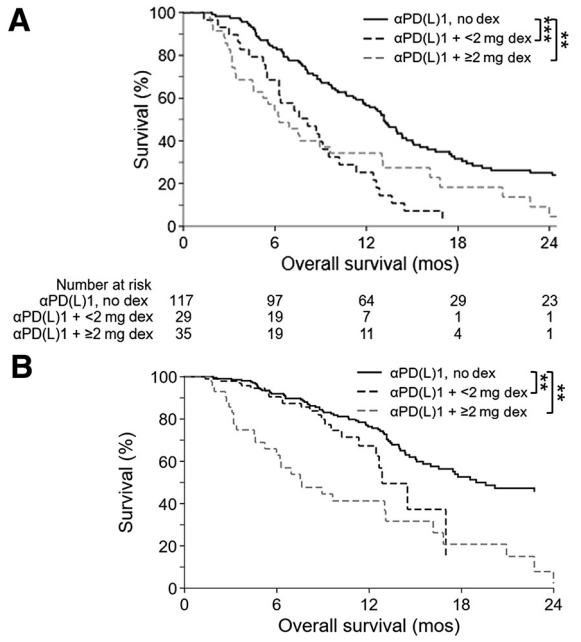

Figure 5.

Baseline dexamethasone is associated with decreased OS among patients with GBM receiving anti–PD-(L)1 therapy, regardless of dexamethasone dose. Kaplan–Meier OS estimates for 181 patients with IDH wild-type GBM treated with anti–PD-(L)1 therapy, who were either on ≥2 mg (dashed gray line), <2 mg (dashed black line), or no (solid black line) baseline dexamethasone, are depicted, including both unadjusted analyses ($n = 181$; **A**) and analyses adjusted (by a Cox regression model; $n = 163$; **B**) for relevant prognostic factors, including disease setting (newly diagnosed vs. recurrent), patient age, *MGMT* promoter methylation, KPS, tumor volume prior to anti–PD-(L)1 initiation, and extent of resection. **, $P < 0.01$; ***, $P < 0.001$; dex, dexamethasone; mos, months.

dexamethasone were observed in our preclinical GL261 (**Figs. 1** and **2**) and CT-2A (**Fig. 2**) murine models. As expected, multivariable analysis also identified newly diagnosed patients, younger patients, and *MGMT* promoter methylated tumors as having improved OS.

Discussion

Immune checkpoint blockade has transformed the treatment of many cancers. Immune-related side effects of immune checkpoint inhibitors are often treated with corticosteroids, such as prednisone and methylprednisolone. While some studies show that the use of corticosteroids does not compromise therapeutic benefit, other studies indicate that they may weaken efficacy (16, 17). Accumulating data also support the idea that baseline corticosteroid use or administration early in the course of immune checkpoint therapy may be detrimental. For example, baseline corticosteroid use portends poorer outcome among patients with advanced non–small cell lung cancer following immune checkpoint blockade, while patients with advanced melanoma who received corticosteroids within 7 weeks of initiating CTLA-4 blockade had worse outcomes compared with patients who received corticosteroids at a later timepoint, particularly among those with a low mutational burden tumor (5–7).

Patients with primary as well as metastatic secondary tumors of the central nervous system are frequently prescribed dexamethasone, a potent anti-inflammatory agent used to treat symptomatic cerebral edema induced by the tumor or its treatment, for prolonged periods. Recent multivariable analyses indicate that corticosteroid use portends a worse survival for patients with newly diagnosed GBM that is independent of established prognostic factors such as degree of resection and baseline performance status (18, 19). The mechanism underlying decreased survival among patients with corticosteroid-treated brain tumor remains to be clarified, but the suppressive effects of corticosteroids on immune function and antitumor immune responses are likely a contributing factor. Patients with GBM on dexamethasone exhibit notable lymphopenia, including lower levels of circulating $CD4^+$ and $CD8^+$ T cells compared with patients with GBM not on dexamethasone or age-matched normal donors (20, 21).

To better understand the effects of dexamethasone therapy on anti–PD-(L)1 therapy, we replicated the dexamethasone dosing and administration schedules administered to patients with GBM using the immunocompetent, syngeneic GBM GL261 and CT-2A murine models. Our group and others have previously demonstrated that the GL261 model responds favorably to anti–PD-1 therapy, most likely due to its inherent immunogenicity and high tumor mutational burden (13, 22, 23). The GL261 model has been appropriately criticized as being unrepresentative of human GBM tumors, which typically exhibit low immunogenicity and mutational burden, creating an immunologically "cold" tumor microenvironment (24, 25). Despite the heightened immunogenicity of the GL261 model, we find that concurrent dexamethasone limits the therapeutic benefit of anti–PD-1 immune checkpoint blockade even at low doses. We also noted that the timing of dexamethasone administration appears to be relevant. Dexamethasone administered concurrently with anti–PD-1 exerted a detrimental effect on survival in our murine GBM models, whereas dexamethasone administered prior to initiation of anti–PD-1 did not, although this difference may have reflected the short half-life of dexamethasone in mice and the reduced exposure associated with the pre-anti–PD-1 dexamethasone administration schedule. In addition, the effects of concurrent dexamethasone were dose dependent, with high dexamethasone dose levels (10 mg/kg) reducing the long-term survival rate by half as compared with low dose levels (1 mg/kg).

Because radiotherapy is an established cornerstone of GBM therapy, we evaluated the effect of concurrent dexamethasone on the survival associated with anti–PD-1 therapy when combined with fractionated radiotherapy using a schedule analogous to that used to treat human patients. We deliberately employed a radiation schedule that prolongs survival, but fails to cure most tumor-bearing mice, as this is the typical effect of radiation in patients with GBM. Using a dosing schedule of 2 Gy daily for 5 days, we observed a modest survival benefit for both the GL261-luc2 and CT-2A-luc syngeneic GBM models. When dexamethasone was concurrently administered, there was a trend toward decreased survival from PD-1 blockade combined with radiotherapy; although these analyses were not powered to detect an additive effect of PD-1 blockade with radiotherapy. Of note, concurrent dexamethasone did not appear to affect memory T-cell responses based on our demonstration that long-term survivors were capable of rejecting tumor rechallenges, regardless of whether the mice had received concurrent dexamethasone, a finding that recapitulates what has previously been reported in both intracranial and subcutaneous tumor models (7, 26). Although studies from both tumor and viral preclinical settings found that memory T cells are sensitive to glucocorticoid-induced apoptosis, recent work indicates that concurrent corticosteroids, through suppression of critical fatty acid metabolism pathways, selectively diminish and impair the low-affinity, but not the high-affinity, memory $CD8^+$ T cells (7, 27, 28). Together, these data suggest that for highly immunogenic tumors, high-affinity antitumor memory T-cell populations may persist despite corticosteroid-induced

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Table 1. Multivariable Cox regression analysis of the effect of baseline dexamethasone on OS in patients with GBM treated with anti–PD-(L)1.

	n	Multivariable Cox regression HR (95% CI)	*P*
Dexamethasone at αPD-(L)1 baseline			
None	105	Reference	
<2 mg dexamethasone	25	2.16 (1.30–3.60)	**0.003**
≥2 mg dexamethasone	33	1.97 (1.23–3.16)	**0.005**
Age at diagnosis (year)			
<45	27	Reference	
45–54	41	1.37 (0.75–2.52)	0.31
55–64	58	1.95 (1.10–3.45)	**0.02**
≥65	37	2.19 (1.16–4.14)	**0.02**
Disease setting			
Recurrent	120	Reference	
Newly diagnosed	43	0.45 (0.29–0.70)	<**0.001**
KPS at αPD-(L)1 baseline			
≤70	29	0.89 (0.52–1.53)	0.68
80	53	Reference	
≥90	81	0.93 (0.61–1.43)	0.75
MGMT promoter status			
Unmethylated	93	Reference	
Methylated	56	0.48 (0.32–0.72)	<**0.001**
Partially methylated	14	1.54 (0.80–2.95)	0.19
Tumor volume at αPD-(L)1 baseline			
Lowest tertile	40	0.71 (0.43–1.18)	0.18
Middle tertile	44	Reference	
Highest tertile	45	1.30 (0.81–2.09)	0.28
n/a	34	1.20 (0.68–2.11)	0.54
GTR prior to αPD-(L)1			
No	88	Reference	
Yes	75	0.82 (0.57–1.18)	0.29

Note: Bold terms are statistically significant.

lymphopenia at the time of immune checkpoint blockade therapy. Further study of the effects of corticosteroids on T-cell memory responses are warranted.

We then investigated the mechanisms underlying the attenuated survival associated with the addition of dexamethasone to anti–PD-1 therapy in our syngeneic GBM models. In our experiments, concurrent dexamethasone markedly decreased overall $CD3^+$ T-lymphocyte counts, including $CD4^+$ and $CD8^+$ T cells, isolated from the tumor, draining cLNs, spleen, and thymus. Our findings corroborate those of a recent study in which the number of peripheral blood $CD4^+$ and $CD8^+$ T cells was reduced when dexamethasone was administered to GL261-luc–bearing mice (26). Similar to other tumor models, we observed that the same dose of corticosteroids can exert differential effects on T cells depending on whether they reside in peripheral or intratumoral compartments, with greater lymphodepletion generally displayed by the peripheral compartments (29). We found that the mechanism of T-cell depletion associated with concurrent dexamethasone dosing involved, at least in part inducing apoptosis of $CD4^+$ and $CD8^+$ T cells beginning as early as 1 hour after dexamethasone initiation, even at relatively low dexamethasone dose levels. This effect persisted through 5 days after dexamethasone initiation and was increased with repeated higher dexamethasone doses. In addition, we investigated the effect of dexamethasone on lymphocyte proliferation and found that dexamethasone reduced the absolute numbers of proliferative T cells, although the proportion of surviving T cells that were proliferative did not decrease. Depending on their dosing and timing, corticosteroids have been found to have varied suppressive and supportive effects on lymphocyte proliferation (29–31). In addition, distinct immune cell types and states have been shown to exhibit differential sensitivity to dexamethasone, which may underlie the differences that we observed in $CD4^+$ and $CD8^+$ TILs and peripheral T cells. For example, in *in vitro* cultures of human T cells, dexamethasone impaired proliferation of naïve T cells, but not memory T cells, by upregulating expression of CTLA-4 in naïve T cells. Inhibition of CTLA-4, but not PD-1, was then able to partially restore proliferation (27).

In addition to quantitative effects on immune cell subsets, concurrent dexamethasone dosing also impacted functional capacity. We observed that dexamethasone decreased the ability of splenic $CD4^+$ and $CD8^+$ T cells to generate IFNγ responses and decreased the number of $CD4^+$ and $CD8^+$ cells expressing the early activation marker, CD69, that were isolated from cLN, spleen, and intracranial tumor. We also evaluated the effect of dexamethasone on innate immunity and noted decreases in most myeloid subsets and NK cells, as well as decreased levels of activated ($CD80^+$ $CD86^+$) myeloid cells and DCs. To comprehensively profile the immune microenvironment irrespective of response to anti–PD-1 therapy, entire tumor-bearing tissues were evaluated, therefore it is possible that endogenous extratumoral immune cells were included in our analyses. However, their contribution is expected to be marginal due to the brain's unique immunologic niche, which is characterized by a relative paucity of lymphocytes, nonmicroglial myeloid cells, and DCs (32).

In accordance with our study findings, a recent study utilizing the GL261-luc model also demonstrated that dexamethasone administered for 5 days concurrently with anti–PD-1 therapy was associated with decreased survival, including fewer long-term survivors, compared with mice who did not receive dexamethasone (26). However, protracted dexamethasone administration before, during, and after PD-1 dosing did not appear to impact survival in that study. In contrast to our study, a subset of tumor-bearing mice treated with dexamethasone alone remained long-term survivors, whereas we found that all mice treated with dexamethasone monotherapy succumbed to progressive tumor with comparable survival with that of untreated controls. The discordance between our results and the findings of the previous study may reflect the different anti–PD-1 antibodies and dosing schedules, different tumor cell inocula, and different sources of dexamethasone.

To evaluate whether our preclinical findings are clinically relevant, we retrospectively evaluated the survival outcome among 181 consecutive patients with IDH wild-type GBM treated at our institution with anti–PD-(L)1 therapy. We found that patients who were not on baseline dexamethasone had an improved OS compared with those on dexamethasone, independent of key prognostic factors like disease setting, age, *MGMT* promoter methylation status, KPS, tumor size, or extent of resection prior to anti–PD-(L)1 treatment. In addition, the detrimental effects of dexamethasone were independent of dexamethasone dose: both lower (<2 mg) and higher (≥2 mg) doses were associated with worse OS following anti–PD-(L)1 treatment. Our analysis, however, was not powered to assess OS differences between dose levels. Because of the retrospective and heterogenous nature of our data, our results require prospective validation in a randomized controlled trial, but they are consistent with the planned subgroup analyses of a recent randomized phase III study in which patients with recurrent GBM treated with nivolumab had poorer survival if they were on baseline dexamethasone compared with those who were not (1). This result supports our preclinical and clinical data indicating

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that dexamethasone contributes to limit the therapeutic benefit of immune checkpoint blockade among patients with GBM. Our findings have salient implications for ongoing and planned clinical trials that are evaluating combinations of immunotherapeutic agents, including checkpoint inhibitors, with other therapeutic agents for patients with GBM; as well as for patients with a spectrum of brain metastasis types, where immune checkpoint inhibitors are part of standard-of-care management and corticosteroids are often indicated (33–35). Further evaluation of the effect of dexamethasone and other corticosteroids on patients' outcomes for such immunotherapy treatments for GBM and oncology in general is warranted.

Conclusions

In conclusion, we demonstrate that the concurrent systemic administration of dexamethasone diminishes the survival benefits associated with PD-1 immune checkpoint blockade in both anti–PD-1–resistant (CT-2A) and anti–PD-1–responsive (GL261) immunocompetent, syngeneic GBM models in a dose-dependent manner. Although the GL261 model recapitulates some of the cell-of-origin and histopathologic features of human GBM tumors, its marked mutational load and intrinsic immunogenicity lead to an overestimation of therapeutic benefit from immune checkpoint blockade relative to what has been observed among patients with GBM (23, 24, 36). The heightened sensitivity of this model to immune checkpoint blockade makes it all the more striking that concurrent dexamethasone administration, even at relatively low doses, attenuated the therapeutic benefit of PD-1 inhibition. These findings were also replicated in our experiments with the more clinically relevant, immune-resistant CT-2A syngeneic GBM model; where concurrent dexamethasone abrogated the survival benefits associated with either PD-1 monotherapy or anti–PD-1 administered along with fractionated radiotherapy. Our findings are consistent with preclinical studies demonstrating that concurrent dexamethasone also diminished antitumor immune responses and therapeutic benefits associated with Delta24-RGD oncolytic virus therapy (37). We further demonstrated that concurrent dexamethasone led to quantitative and qualitative/functional decreases in both adaptive and innate immune effector cells. Our data attribute decreased lymphocyte levels to increased apoptosis associated with dexamethasone administration. Among patients with IDH wild-type GBM undergoing anti–PD-(L)1 treatment, we demonstrate that baseline dexamethasone use is associated with poorer survival even after adjustment for disease setting, age, tumor size, tumor resection, *MGMT* promoter methylation, and patient's performance status. Our data also support recent findings that older patients with GBM have worse survival than younger patients following immune checkpoint therapy (4).

Taken together, our results further support accumulating concerns that corticosteroids can be detrimental to immunotherapy for oncology patients: dexamethasone therapy, which is typically used to treat symptomatic cerebral edema in patients with GBM, limits the therapeutic benefit of immune checkpoint blockade. Careful evaluation of dexamethasone use is warranted for neuro-oncology patients undergoing immunotherapy clinical trials. Our preclinical analyses also indicate that the detrimental effect of dexamethasone appears to be dose dependent, suggesting that the lowest possible dose should be used for patients where the concurrent use of dexamethasone is unavoidable. Evaluation of alternative approaches to treat symptomatic cerebral edema, such as inhibition of vascular permeability induced by VEGF, merits further study as strategies to limit dexamethasone exposure among patients with GBM receiving immunotherapy.

Authors' Disclosures

M.C. Speranza reports being an investigator at GlaxoSmithKline. K.L. Ligon reports grants and personal fees from BMS outside the submitted work. P.K. Sorger reports grants and other from NCI during the conduct of the study, other from Glencoe Inc, personal fees and other from Applied Biomath LLC, and grants, personal fees, and other from RareCyte Inc outside the submitted work. A.C. Anderson reports personal fees from Tizona Therapeutics, Compass Therapeutics, Zumutor Biologics, and Astellas Global Pharma outside the submitted work. A.H. Sharpe reports personal fees from Surface Oncology, Sqz Biotech, Selecta, Elstar, and Elpiscience, other from Monopteros, grants from Novartis, Merck, Roche, Ipsen, and Quark outside the submitted work, as well as has a patent 7,432,059 with royalties paid from Roche, Merck, Bristol-Myers-Squibb, EMD-Serono, Boehringer-Ingelheim, AstraZeneca, Leica, Mayo Clinic, Dako, and Novartis, a patent 7,722,868 with royalties paid from Roche, Merck, Bristol-Myers-Squibb, EMD-Serono, Boehringer-Ingelheim, AstraZeneca, Leica, Mayo Clinic, Dako, and Novartis, a patent 8,652,465 licensed to Roche, a patent 9,457,080 licensed to Roche, a patent 9,683,048 licensed to Novartis, a patent 9,815,898 licensed to Novartis, a patent 9,845,356 licensed to Novartis, a patent 10,202,454 licensed to Novartis, a patent 10,457,733 licensed to Novartis, a patent 9,580,684 issued to none, a patent 9,988,452 issued to none, and a patent 10,370,446 issued to none, as well as reports being on the scientific advisory boards for the Massachusetts General Cancer Center, Program in Cellular and Molecular Medicine at Boston Children's Hospital and the Human Oncology and Pathogenesis Program at Memorial Sloan Kettering Cancer Center and scientific editor for the Journal of Experimental Medicine. G.J. Freeman reports grants from NCI during the conduct of the study, and personal fees from Roche, Bristol-Myers-Squibb, Xios, Origimed, Triursus, iTeos, NextPoint, IgM, and Jubilant outside the submitted work, as well as has a patent for PD-L1/PD-1 pathway with royalties paid from Roche, a patent for PD-L1/PD-1 pathway with royalties paid from Merck MSD, a patent for PD-L1/PD-1 pathway with royalties paid from Bristol-Myers-Squibb, a patent for PD-L1/PD-1 pathway with royalties paid from Merck KGA, a patent for PD-L1/PD-1 pathway with royalties paid from Boehringer-Ingelheim, a patent for PD-L1/PD-1 pathway with royalties paid from AstraZeneca, a patent for PD-L1/PD-1 pathway with royalties paid from Dako, a patent for PD-L1/PD-1 pathway with royalties paid from Leica, a patent for PD-L1/PD-1 pathway with royalties paid from Mayo Clinic, and a patent for PD-L1/PD-1 pathway with royalties paid from Novartis. D.A. Reardon reports grants from Acerta Pharmaceuticals, Incyte, Midatech, Omniox, and Tragara, grants and personal fees from Agenus, Celldex, EMD Serono, and Inovio, and personal fees from AbbVie, AdvantaGene, Amgen, Bayer, Bristol-Myers Squibb, Delmar, Genentech/Roche, Imvax, Medicenna Biopharma, Inc., Merck, Merck KGaA, Monteris, Novocure, Oncorus, OxiGene, Regeneron, Stemline, Sumitono Dainippon Pharma, and Taiho Oncology, Inc. outside the submitted work. No disclosures were reported by the other authors.

Authors' Contributions

J.B. Iorgulescu: Data curation, formal analysis, validation, investigation, visualization, methodology, writing-original draft, writing-review and editing. **P.C. Gokhale:** Conceptualization, resources, formal analysis, investigation, visualization, methodology, writing-original draft, writing-review and editing. **M.C. Speranza:** Formal analysis, investigation, visualization, writing-original draft, writing-review and editing. **B.K. Eschle:** Investigation, methodology, writing-review and editing. **M.J. Poitras:** Investigation, writing-review and editing. **M.K. Wilkens:** Investigation, writing-review and editing. **K.M. Soroko:** Investigation, writing-review and editing. **C. Chhoeu:** Investigation, writing-review and editing. **A. Knott:** Validation, writing-review and editing. **Y. Gao:** Formal analysis, investigation, writing-review and editing. **M.J. Lim-Fat:** Data curation, investigation, writing-review and editing. **G.J. Baker:** Formal analysis, investigation, visualization, methodology, writing-original draft, writing-review and editing. **D.M. Bonal:** Formal analysis, investigation, writing-review and editing. **Q.-D. Nguyen:** Formal analysis, investigation, writing-review and editing. **G.R.L. Grant:** Data curation, writing-review and editing. **K.L. Ligon:** Writing-original draft, writing-review and editing. **P.K. Sorger:** Methodology, writing-original draft, writing-review and editing. **E.A. Chiocca:** Writing-original draft, writing-review and editing. **A.C. Anderson:** Writing-original draft, writing-review and editing. **P.T. Kirschmeier:** Conceptualization, formal analysis, methodology, writing-original draft, writing-review and editing. **A.H. Sharpe:** Writing-original draft, writing-review and editing. **G.J. Freeman:** Conceptualization, investigation, methodology, writing-original draft, writing-review and editing. **D.A. Reardon:** Conceptualization, data curation, supervision, funding acquisition, validation, investigation, visualization, methodology, writing-original draft, project administration, writing-review and editing.

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