Supplementary Methods

Microarray and Database Analysis

The Cancer Genome Atlas (TCGA) data for grade II and grade IV glioma and molecular subtypes were downloaded from the UCSC Xena browser (http://xena.ucsc.edu/). mRNA expression data, normalized using log2 (RPKM+1) method was represented by RNASeq (Illumina Hi-seq platform). Data for gene expression from specific anatomical and spatial regions (identified by H&E staining) in glioblastoma was downloaded from *Ivy Glioblastoma Atlas Project (*http://glioblastoma.alleninstitute.org/) as previously described (1). Total RNA from tumor tissue was isolated using Qiagen (Hilden, Germany) all prep kit (cat # 80204) and quantified using a Nano drop. Integrity of RNA preparation was confirmed with the Agilent RNA 6000 Nano Kit (cat # 5067-1511). 500 ng of total RNA was used for the expression analysis using Affymetrix Clariom D human arrays (# 902922; Thermo-Fisher, Grand Island, NY). Manufacturer's protocol was followed for all procedures. Raw data was processed using the Affymetrix expression console and further data analyses were performed using the Affymetrix transcriptome analysis console. Raw data were normalized using gene-level RMA Sketch, and bi-weight average signal (log2) values were used for analysis.

Human PBMCs

Leukoepharesis pack was obtained from healthy donors by Research Blood Components, LLC (Boston, MA). PBMCs were isolated form leukoepharesis pack using Ficoll-Paque PLUS (GE Healthcare Bio-Sciences, Pittsburgh,PA).

Immune competent tumor models

C57BL/6 (H-2^b, CD45.2) mice were purchased from Charles River Laboratories (Wilmington, MA). Mice were exposed to 4 Gy total body irradiation (TBI) one-day prior to implant to help tumor

engraftment. To develop intracranial tumors, 6-7-week old mice were anesthetized and immobilized on a stereotactic frame, a midline scalp incision was made and tumor cells ($2x10^5$ suspended in 2 µl PBS) were injected using a Hamilton syringe localized to specific coordinates 2 mm posterior to bregma and 2 mm mediolateral from the midline and at a depth of 3 mm. Mice were imaged 6-8 days after implant using Multihance (Bracco Diagnostics, Cranbury, NJ, USA) diluted in sterile saline delivered via tail vein injection under anesthesia. Images were acquired from anesthetized mice using on a 3.0 T benchtop MRI (MR Solutions, Guildford, UK). Fast spin-echo T2 and T1 weighted images were acquired pre and post contrast injection. Tumor volumes were determined by delineating areas of contrast enhancement on the T1 weighted fast spin echo sequence using the image processing PBAS tool in PMOD software (PMOD Technologies, Zurich, Switzerland). To develop subcutaneous tumors, tumor cells ($2x10^6$) suspended in 100 µL of sterile PBS were injected into the right flank of C57BL/6 mice. Tumors were measured using digital calipers weekly and tumor volume was calculated using formula (πab^2)/6 (a=largest diameter, b=smallest diameter) (2). After verification of tumors, mice were randomized into described treatment arms. Radiation was delivered using the Faxitron model CP160 (Faxitron X-Ray Corp., Wheeling, IL, USA) in custom jigs designed to shield the remaining body at a dose rate of 0.69 Gy/min, tube voltage of 160 kVp and current of 4 mA, to a total of 6Gy GDC-0919 was given as oral gavage twice (starting from day of first dose of RT) a day (200 mg/kg) for two weeks (6 days a week). 1-L-MT was given in drinking water (4 mg/ml a*d libitum*) for two weeks.

Kynurenine estimation

Mouse cells were cultured in the presence or absence of murine recombinant IFN-γ (100 ng/ml) and human cells were cultured in the presence of human recombinant IFN-γ (50 ng/ml) from Peprotech (Rocky Hill, NJ) for three days. Cell culture supernatants were collected for kynurenine estimation using Ehrlich's reagent (*p*-dimethylaminobenzaldehyde dissolved in glacial acetic

acid). Measurements were obtained using X-Mark Bio-Rad spectrometer (Hercules, CA) at 492 nM (3,4).

Western Blot

Western blot was performed using methods previously described (5). Human IDO1 (#86630) and tubulin (#2144) antibodies were obtained from Cell Signaling Technology (Danvers, MA). Mouse IDO1 (clone 2E2/IDO1) antibody was obtained from Biolegend (San Diego, CA) and TDO2 (clone G-18) antibody was obtained from Santa Cruz Biotechnology, Inc. (Dallas, TX). PD-1 (#AF1021) antibody was obtained from R&D Systems (Minneapolis, MN). IRDye 800CW secondary antibodies were obtained from Li-Cor (Lincoln, NE). Blots were imaged using Odyssey infrared imaging system by Li-Cor (Lincoln, NE).

Metabolite Extraction from Tumors and Plasma

Tumor tissue was snap frozen in liquid nitrogen, lyophilized, and homogenized manually to a fine powder and 10 mg was added to 300 µl of methanol in a sterile 2 ml Eppendorf tube. Samples were mixed for 15 minutes, centrifuged at 3,000 *g* for 5 minutes at 4°C and the supernatants collected. 300 µl of methanol was added to 100 µl of plasma in a sterile 2 ml Eppendorf tube and the samples were mixed and centrifuged as previously described for tumor tissue. All the supernatants were subsequently filtered through 0.22 µm Costar Spin-X Centrifuge Tube Filters (10,000 *g* at 4°C for 5 min; Corning Incorporated, Corning, NY 14831, USA). All solvents used (formic acid, methanol) were purchased from Fisher Scientific (Pittsburg, USA) and were LC-MS grade or equivalent.

Targeted Liquid Chromatography Mass Spectrometry Analysis

Chromatography was performed on a Waters Acquity UPLC I-Class system, equipped with column oven, coupled to a Waters TQS mass spectrometer (Milford, MA, USA) equipped with an

electrospray ionization source operating in positive mode. 3 µl of tumor and plasma extracts were injected onto an Acquity UPLC® BEH column (2.1 mm × 50 mm; 1.7 m) column (Waters, Wexford, Ireland). The column oven was set to 40°C and the sample manager temperature set to 10°C. The gradient elution buffers were 0.1 % formic acid in water (A) and 0.1 %acetonitrile (B) and the flow rate was 0.6 ml.min^{-1}. The elution gradient (A:B, v/v) was as follows (time minutes, %B): (0, 1), (4, 50), (5, 95), (6, 95), (6.1, 1), (7,1). To accurately measure tryptophan, kynurenine and GDC-0919, the mass range window of 134.936 to 317.311 was monitored. Standard calibration curves (8 points) were generated for the 3 compounds of interest (GDC-0919, tryptophan and kynurenine) to determine their tissue and plasma concentrations.

Flow cytometry and magnetic bead sorting and IFN-γ ELISA

Blood, spleen, and tumors collected from mice were dissociated and suspended as single cell solutions using DNase I and Collagenase IV (Sigma Aldrich, St. Louis, MO). Blood was treated with ACK lysing buffer to lyse RBCs before staining. Subsequently, cells were stained with fluorochrome conjugated antibodies, which included T regulatory cells ($CD45^+CD4^+FoxP3^+CD25^+$), MDSCs ($CD45^+CD11b^+Gr1^+$), or TAMs ($CD45^+CD68^+CD11b^+F4/80^+$). Cells were also stained for CD8, granzyme B, perforin, CD69. Fluorochrome conjugated antibodies CD45 (clone I3/2.3), CD4 (clone GK1.5), CD8a (clone 53-6.7), CD25 (clone PC61), CD11b (clone M1/70), Gr1 (clone RB6-8C5), CD69 (clone H1.2F3), F4/80 (clone BM8), CD68 (clone FA-11) were purchased from Biolegend (San Diego, CA). FoxP3 staining was performed using a Foxp3 staining buffer set and FoxP3 antibody (clone-150D/E4) (eBioscience/Thermo Fisher; Grand Island, NY). Granzyme B (eBioscience, clone NG2B) staining was performed using a fixation and permeabilization kit (BD Biosciences; San Jose, CA). All samples were analyzed on a FACS Canto II flow cytometer (Becton Dickinson; Mountain View, CA). Analysis of flow cytometry data was performed using FlowJo V10 software (FlowJo, LLC; Ashland, OR). $CD4^+CD25^+$ (Tregs) were sorted from splenocytes using FlowComp Dynabeads

(Invitrogen/Thermo Fisher; Grand Island, NY). CD8 T cells were isolated from splenocytes using a MojoSort™ Mouse CD8 T Cell Isolation Kit (Biolegend; San Diego, CA). Plasma was isolated from mouse blood and use for analyzing IFN-γ using ELISA-MAX kit (BioLegend; San Diego, CA). Measurements were obtained using X-Mark spectrometer (Bio-Rad; Hercules, CA).

Cell suppression assay

$CD8^+$ sorted cells were stained with CFSE CellTrace (GIBCO/Thermo; Grand Island, NY) and activated with plate bound anti-CD3 (1µg/ml; clone- 145-2C11) and antiCD28 (5µg/ml; clone- 37.51) antibodies (Biolegend; San Diego, CA). For suppressing proliferation, sorted Tregs ($CD4^+CD25^+$) were added in a 3:1 ratio ($CD8^+$: Tregs) with and without 50µM of kynurenine. CFSE dilution on $CD8^+$ T cells was analyzed three days after activation using a FACS Canto II flow cytometer (Becton Dickinson, Mountain View, CA).

References

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