Supplementary Appendix

This appendix has been provided by the authors to give readers additional information about their work.

Supplement to: Brown CE, Alizadeh D, Starr R, et al. Regression of glioblastoma after chimeric antigen receptor T-cell therapy. N Engl J Med 2016;375:2561-9. DOI: 10.1056/NEJMoa1610497

SUPPLEMENTAL APPENDIX

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Supplemental Methods

Clinical vector and T cell manufacturing

The codon optimized CAR sequence (IL13BBζ) contains a membrane-tethered human IL-13 ligand mutated at a single site (E13Y) to reduce potential binding to IL13Rα1,[1,2] a human IgG4 Fc spacer containing two mutations (L235E; N297Q) that prevent Fc receptor-mediated recognition,[3] a human CD4 transmembrane domain, a human costimulatory 4-1BB cytoplasmic signaling domain, and a human CD3ζ cytoplasmic signaling domain. A T2A ribosome skip sequence[4] then separates this IL13BBζ CAR sequence from a truncated human CD19 sequence (CD19t), an inert, nonimmunogenic cell surface marker. The T2A linkage enables co-expression of both IL13BBζ and CD19t from a single transcript (**Fig. S6**).[4] Details for the generation of the lentiviral vector encoding the IL13BBζ CAR and the CD19t transgene are available upon request.

For IL13BBζ-CAR T-cell manufacturing, on the day of leukapheresis, peripheral blood mononuclear cells (PBMC) were isolated by density gradient centrifugation over Ficoll-Paque (GE Healthcare) followed by two washes in PBS/EDTA. PBMC were then washed once in PBS, resuspended in X Vivo15 media (Bio Whittaker) containing 10% fetal calf serum (FCS) (Hyclone), and stored on a 3-D rotator overnight at room temperature (RT). The following day, PBMC were incubated with clinical grade anti-CD14, anti-CD25 and anti-CD45RA microbeads (Miltenyi Biotec). $CD14^+$, $CD25^+$, and $CD45RA^+$ cells were then depleted using the CliniMACS™ depletion mode according to the manufacturer's instructions (Miltenyi Biotec). After centrifugation, the unlabeled negative fraction of cells was resuspended in CliniMACS™ PBS/EDTA buffer (Miltenyi Biotec) containing 0.5% human serum albumin (HSA) (CSL Behring) and then labeled with clinical grade biotinylated-DREG56 mAb (City of Hope Center for Biomedicine and Genetics, COH CBG). The cells were then washed and resuspended in CliniMACS™ PBS/EDTA containing 0.5% HSA and then incubation with anti-biotin microbeads (Miltenyi Biotec), the $CD62L^+$ fraction (central memory T cells, or T_{CM}) was purified with positive selection on CliniMACS™ according to the manufacturer's instructions, and resuspended in X Vivo15 containing 10% FCS.

Following enrichment, 26.9 x 10^6 (first product) or 64.5 x 10^6 (second product) T_{CM} were stimulated with GMP Dynabeads® Human T expander CD3/CD28 (Invitrogen) at a 1:3 ratio (T cell:bead), and transduced with clinical grade IL13BBζ-T2A-CD19t_epHIV7 at an MOI of 0.3 in X Vivo15 containing 10% FCS with 5μg/mL protamine sulfate (APP Pharmaceutical), 50 U/mL rhIL-2 and 0.5 ng/mL rhIL-15 . Cultures were then maintained at 37°C, 5% CO_2 with addition of X-Vivo15 10% FCS as required to keep cell density between 4x10^5 and 2x10^6 viable cells/mL, with cytokine supplementation (final concentration of 50 U/mL rhIL-2 and 0.5 ng/mL rhIL-15) every Monday, Wednesday and Friday of culture. Seven days after the lentiviral transduction, the CD3/CD28 Dynabeads were removed using the Dynal ClinEx Vivo Magnetic Particle Concentrator bag magnet. Cultures were propagated until approximately 4.53x10^8 (first product) or 6.96 x 10^8 (second product) cells were generated as determined by Guava PCA, at which time cultures were harvested, washed in Isolyte (Braun) with 2% HSA, then resuspended in Cryostor CS5 (BioLife Solutions) for cryopreservation. Overall, this manufacturing process was completed in 18 (first product) or 15 (second product) days. Quality control tests on freshly thawed cells included viability, potency (CD19t expression), identity (CD3 expression), transgene copy number (WPRE qPCR), replication competent virus testing (VSV-G qPCR and formal RCL testing at the University of Indiana), residual bead count, and sterility. Manufacturing methods and product release testing are approved as per our FDA-authorized clinical trial NCT02208362.

Patient Eligibility

Eligibility included prior histologically-confirmed diagnosis of an IL13Rα2+ recurrent malignant glioma, age > 18 years with a Karnofsky performance status > 60, adequate cardiopulmonary function, and a survival expectation > 4 weeks. The patient must have radiographic evidence of recurrence/progression of measurable disease more than 12 weeks

after the end of initial radiation therapy. The patient must not have any other active malignancies, infections or intercurrent illness, nor require more than 6 mg daily dexamethasone. Eligible patients provided written informed consent upon enrollment.

Infusion of IL13BBζ-CAR T Cells

Cryopreserved cell banks of quality control released autologous IL13BBζ-CAR T cells were thawed and reformulated for infusion by washing twice with phosphate buffered saline (PBS) with 2% HSA and resuspending in 0.5 mL pharmaceutical preservative-free normal saline with 2% HSA. While cells were not purified based on CD19t expression, dosing was calculated using the potency (CD19t expression) results from quality control testing. Delivery of the therapeutic CAR T cells into either the glioma resection cavity (intracavitary; ICT) or the lateral ventricle (intraventricular; ICV) was achieved within six hours of reformulation using a Holter™ Rickham Ventriculostomy Reservoir (Codman), with a ventricular catheter (Integra Pudenz), and a stylet. Cells were manually injected into the Rickham reservoir using a 21 gauge butterfly needle to deliver a 0.5 mL volume over 5-10 minutes, followed by up to 1 mL PFNS flush delivered over two hours.

For ICT delivery, the catheter device was inserted at the time of tumor resection, and the tip of the catheter was partially embedded into the resection wall in order to allow for cell delivery both into the cavity and into the peritumoral brain tissue. Post-operative imaging (CT and MRI) were obtained to confirm catheter position and extent of tumor resection.

Patient Sample Processing and Analysis

Tumor resection material was collected through the COH Department of Pathology according to the clinical protocol.

IL13Rα2 immunohistochemistry (IHC) was performed on 5 µm-sections of formalin-fixed paraffin-embedded (FFPE) specimens as previously described.[5] IL-13Rα2 immunoreactivity was scored by a clinical neuropathologist and quantified based on the percentage of tumor cells exhibiting weak (1+), moderate (2+), or strong (3+) intensity staining of cell surface, cytoplasmic and golgi-like staining. The H score is obtained by the formula: (3 x percentage of strongly staining cells) + (2 x percentage of moderately staining cells) + percentage of weakly staining cells, giving a range of 0 to 300 (Modified from [6]). The H score can be translated into the intensity scoring system described in the enrollment criteria as follows: 0 representing negative (H score 0), 1+ low (H score 1-100), 2+ moderate (H score 101-200) and 3+ high (H score 201-300). The criteria for inclusion was at least 20% of the cells scoring 1+ staining intensity (> 20%, 1+), representing an H score of 20. Appropriate positive (testicular) and negative (prostate) controls were employed for IL-13Rα2 IHC staining. This test has been performed at the COH Department of Pathology and is regarded as investigational for research. This laboratory is certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA) as qualified to perform high complexity clinical laboratory testing.

Peripheral blood samples were collected in vacutainer tubes ±EDTA. Samples with EDTA were ficolled immediately upon receipt and PBMC were frozen in Crystor CS5 at -80°C and then transferred to liquid nitrogen for long term storage. Samples without EDTA were allowed to coagulate for 2-3 hours at room temperature; serum was collected by centrifugation, aliquoted in single use 100-200 µl aliquots and stored at -80°C. Cerebral spinal fluid (CSF) was collected from the ICV reservoir in a 3cc syringe, spun down, and cell-free supernatants were aliquoted and stored at -80°C. The CSF cells were resuspended in HBSS-/- (Corning CellGro) with 2% FCS and sodium azide for immediate flow cytometric analysis, with the remaining cells resuspended and frozen in Cryostor CS5 at -80°C and then transferred to liquid nitrogen for long term storage.

Cell surface phenotyping of immune cells was performed by flow cytometry using fluorochrome conjugated antibodies specific for CD3, CD4, CD11b, CD14, CD19, CD27, CD28, CD62L, CD45RA, CD45RO, IL-13, TCR-α/β (BD Biosciences), CD15 (BioLegend), HLA-DR (eBiosciences), CD8 (Fisher Scientific),or CCR7 (R&D Systems), and their respective isotype controls.

Research participant serum and CSF samples were analyzed by cytokine bead array. Assays were performed by the COH Clinical Immunobiology Correlative Studies Laboratory (CICSL) using the Human Cytokine 30-Plex Panel kit (Invitrogen) and a FLEXMAP 3D® (Luminex).

The Oncoscan® assay array was performed by COH Cytogenetics Core Laboratory. For this assay tumor area identified on a sequential H&E slide was used as a guide to macrodissect the tumor region from unstained slides. DNA was isolated using the Maxwell® 16 FFPE Plus LEV DNA purification kit according to manufacturer's directions (Promega). DNA was quantified using the Quantus® fluorometer (Promega), and then processed and hybridized on the OncoScan® assay array according to manufacturer's directions (Affymetrix). The OncoScan® assay uses MIP (molecular inversion probe) technology to identify copy number changes, loss of heterozygosity and somatic mutations. The assay has over 220,000 SNPs across the genome with a 300kb genome wide resolution and 50-100kb resolution across ~900 cancer genes. The assay also detects 74 common somatic mutations in nine cancer genes (BRAF, KRAS, EGFR, IDH1, IDH2, PTEN, PIK3CA, NRAS and TP53).

Clinical Imaging

The post-gadolinium T1 weighted MRI sequences of the brain and spine were acquired on a Siemens Viro 3 Tesla scanner. Tumor foci were measured on axial T1 MPR weighted images obtained after the administration of Multihance. Imaging with 18-F-fluorodeoxyglucose (18-F-FDG) was performed using a GE Discovery DST HP60 PET-CT scanner (70 cm axial field of view, slice thickness 3.75mm). Maximal standardized uptake values were obtained utilizing Vital Images Vitrea version 6.7.2 software. Regions of contrast-enhancing tumor foci were outlined by a radiologist for measurements of largest tumor area (mm^2) and tumor volumes (cm^3) were computed on the Medtronic StealthStation™ with Stealth3D™ software (version 2.2.0).

RANO Criteria used to Define Complete Response

Imaging features:
- disappearance of all enhancing disease (measurable and non-measurable)
- sustained for at least 4 weeks
- stable or improved non enhancing FLAIR/T2 lesions
- no new lesions

Clinical features:
- no corticosteroids (physiological replacement doses allowed)
- clinically stable or improved

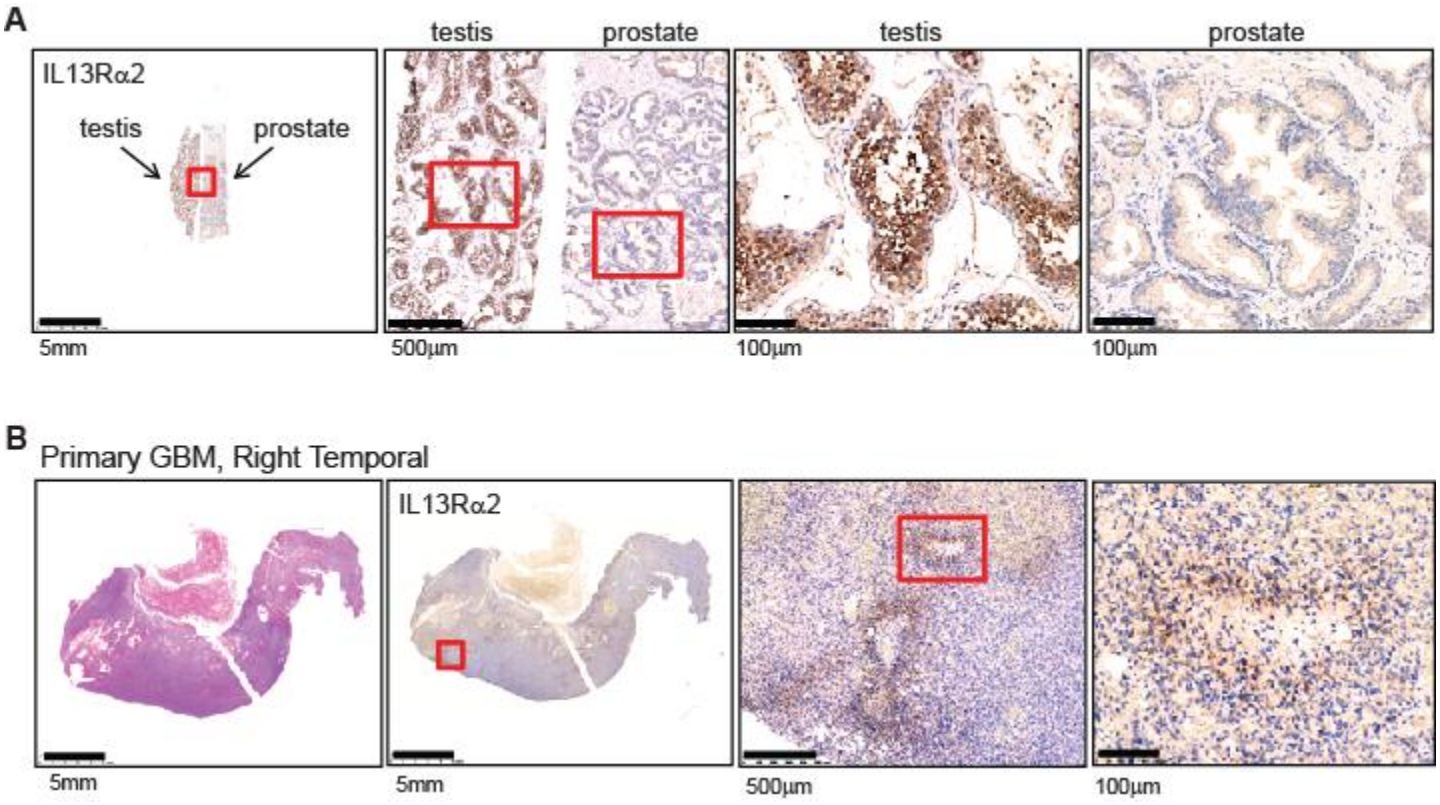
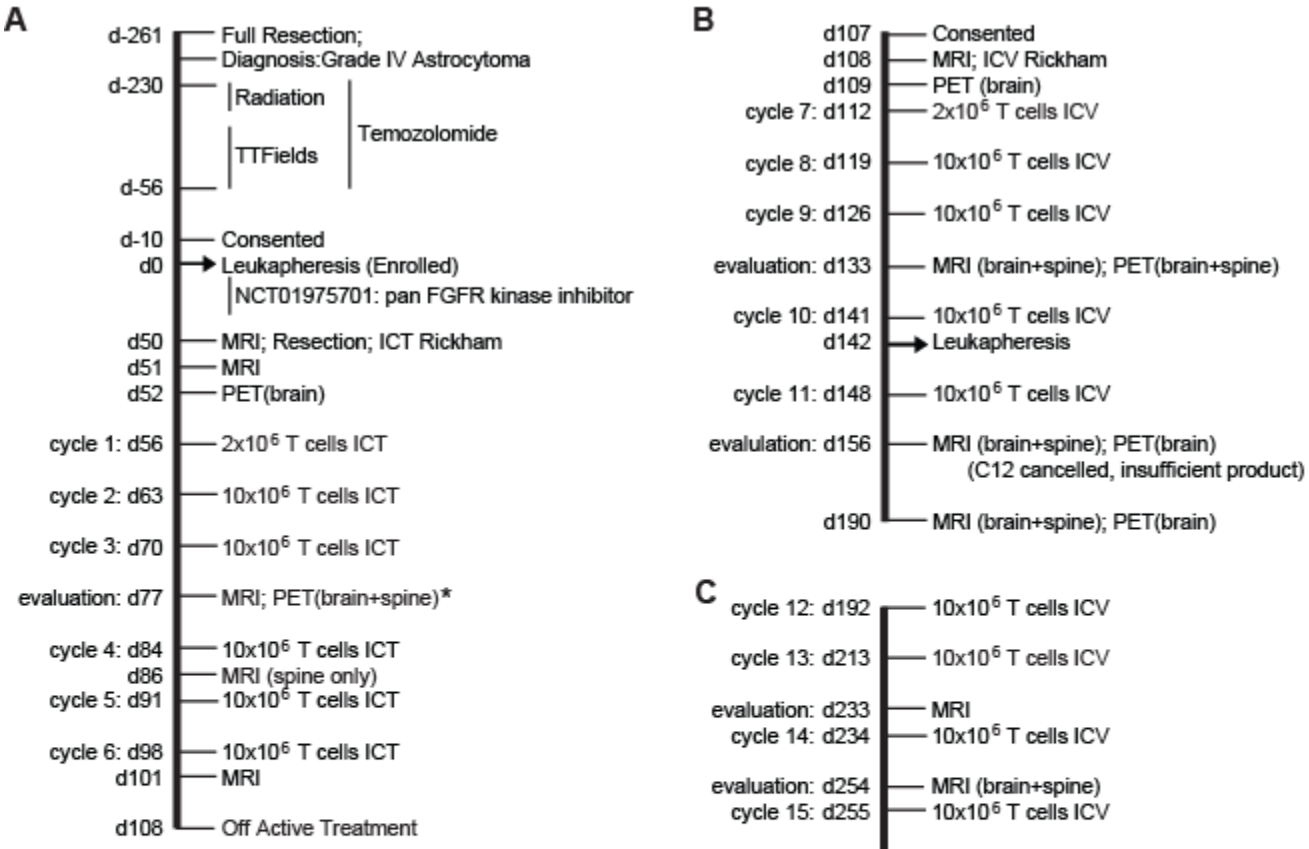


Figure S1. Immunohistochemistry of primary tumor. A, Representative control tissue staining performed on every slide. Testis is positive IL13Rα2 control, prostate is negative IL13Rα2 control. **B,** Tumor resected at initial diagnosis showed typical morphological features of glioblastoma, including mitotically active infiltrating astrocytic cells, micro-endothelial proliferation and pseudopalisading necrosis. Immunohistochemical staining using IL13Rα2-specific DAB with hematoxylin counterstain are depicted, with red boxes outlining the successive magnified images going left to right. IL13Rα2 staining of primary tumor (for enrollment) had an H score of 100 (0:30%, 1+:30%, 2+:20%, 3+:10%). Note that the highest IL13Rα2 expression was often observed in tumor regions of pseudopalisading necrosis (See enlarged panels in **B**), an expression pattern noted by our group for other GBM tumors (data not shown).



D

Dexamethasone Dose	Start Day	Stop Day	Comments
4 mg/day	d53	d67	Concurrent with ICT cycles 1, 2
2 mg/day	d68	d107	Concurrent with ICT cycles 3-6
6 mg/day	d108	d108	Day of ICV Rickham placement
4 mg/day	d109	d123	Concurrent with ICV cycles 7, 8
2 mg/day	d124	d157	Concurrent with ICV cycles 9-11
1 mg/day	d158	d200	Concurrent with ICV cycle 12
0.5 mg/day	d201	d240	Concurrent with ICV cycles 13, 14
0.5 mg every 3 days	d241	d254	
None	d255	N/A	Concurrent with ICV cycles 15, 16; Recurrence documented d284

Figure S2. Treatment regimens with enrollment on NCT02208362 and a compassionate use protocol. Enrollment on NCT02208362 (7/20/15) was set as day 0 (**A**), with initiation of the compassionate use protocol on day 107 (**B**), and use of second cell product for ICV administration starting on day 192 (**C**). Radiation, total dose of 59.4 cobalt Gy equivalent; Temozolomide, 140 mg daily; TTFields, tumor-treating fields, through a portable medical device that delivers low intensity, intermediate frequency, alternating electric fields by means of noninvasive, disposable scalp electrodes; FGFR, fibroblast growth factor receptor; MRI, magnetic resonance imaging, all of which were performed on the brain unless otherwise indicated; ICT, intracavitary; PET, positron emission tomography, performed at the indicated sites; ICV, intraventricular; CT, computed tomography, performed at the indicated sites. **D,** Dexamethasone dose through both protocols, up to the first sign of recurrence on d284. *, First sign of spinal lesion occurred with PET on day 77; **, first signs of recurrence after initiation of ICV administration cycles occurred with MRI/CT/PET scans days 284-298.

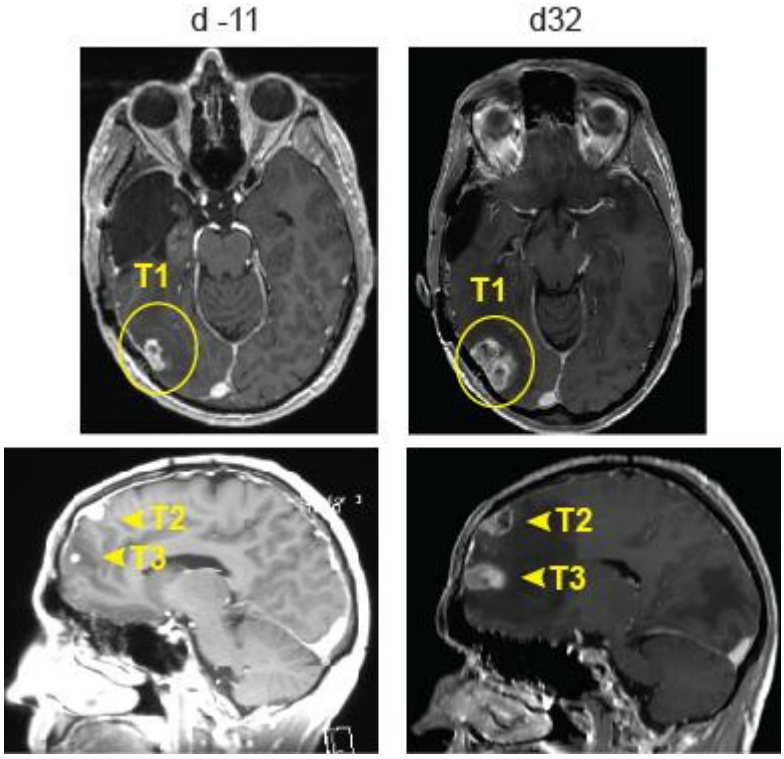


Figure S3. Evidence of rapid GBM progression prior to treatment on NCT02208362. Days of MRI are indicated relative to enrollment on this trial (d0). For reference, ICT Rickham catheter was implanted on d50.

A

Tumor (T)	Anatomical Location	Surgically Resected	Notes
T1	Right posterior temporal-occipital lobe	Yes	
T2	Right frontal lobe, superior	Yes	
T3	Right frontal lobe, inferior	Yes	
T4	Left temporal, superior gyrus	No	
T5	Left temporal, middle gyrus	No	
T6	Right frontal lobe	No	New lesion arising during CAR T cell cycles 1-6, adjacent to resected cavity of T3
T7	Olfactory groove	No	New lesion arising during CAR T cell cycles 1-6
T8	Lumbar Spine	No	1 large tumor (18mm), multiple small tumors (≤ 4mm)

B

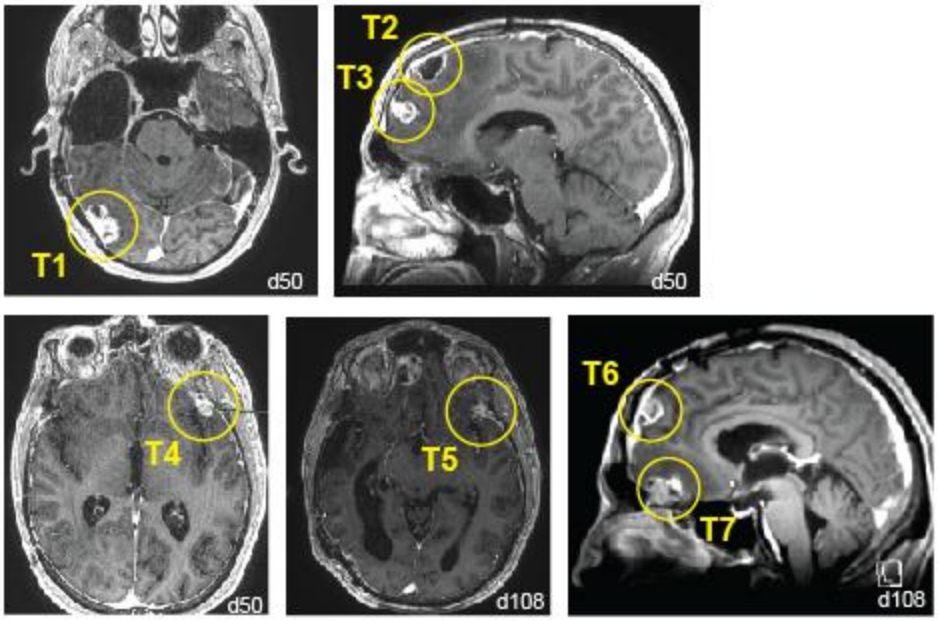


Figure S4. Tumor lesion identification. A, Summary of the patient's multlifocal tumor lesions 1 through 8 (T1-T8), with description of anatomical location, surgical resection and general features. **B,** Brain MRI scans depicting the sites of tumors 1 through 7 (T1-T7) at the days indicated in the lower right corner of each image.

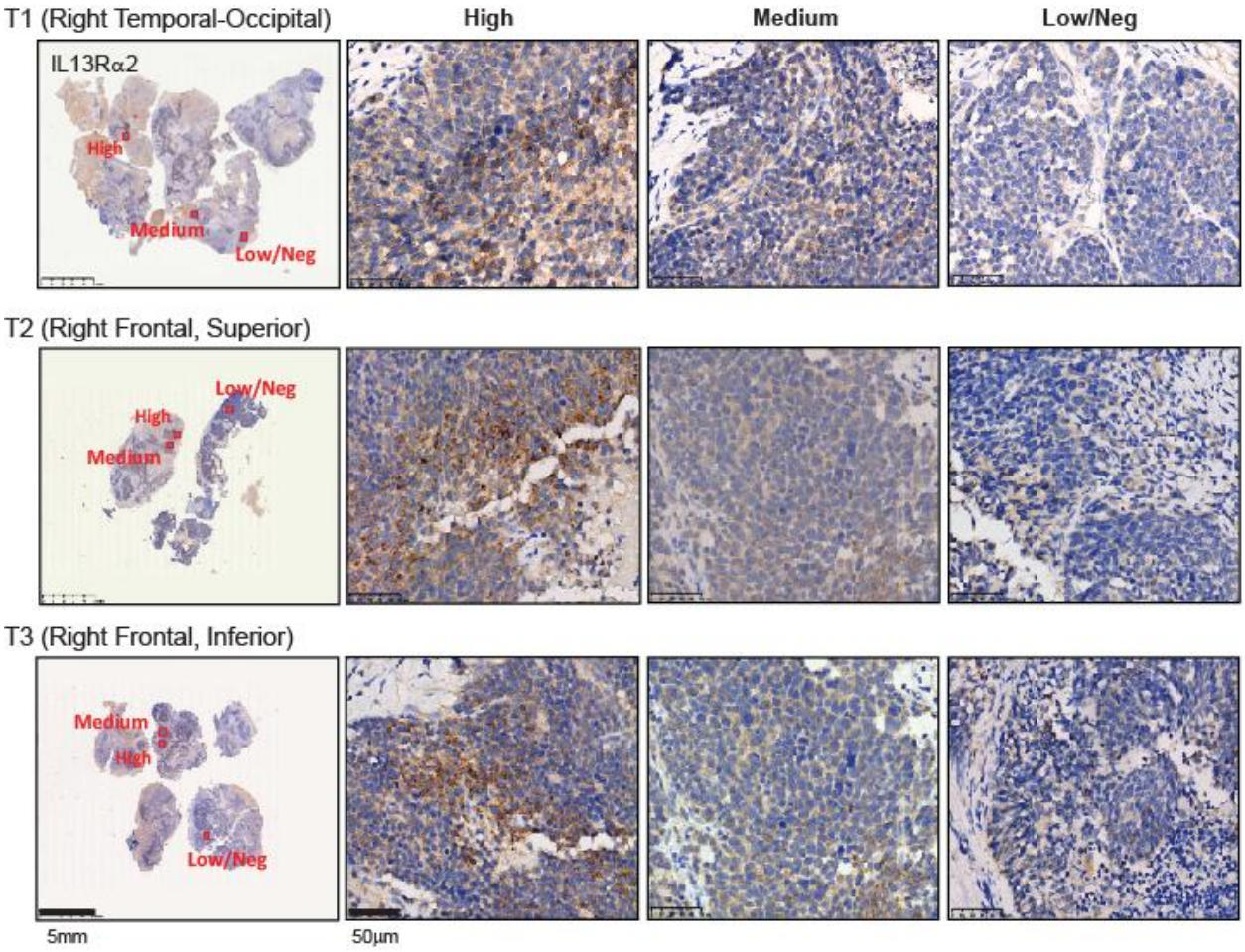


Figure S5. Immunohistochemistry of IL13Rα2 heterogeneity within recurrent tumors. Recurrent tumors 1, 2 and 3 (T1, T2 and T3) resected at time of Rickham placement under NCT02208362 showed an undifferentiated round blue cell phenotype with a multinodular growth pattern and significant areas of necrosis. Immunohistochemical staining using IL13Rα2-specific DAB with hematoxylin counterstain are depicted, with red boxes outlining the High, Medium and Low/Negative areas depicted in the successive magnified images going left to right. Recurrent tumor 1 (T1) H score 100 (0:10%, 1+:80%, 2+:10%, 3+:0%); recurrent tumor 2 (T2) H score 100 (0:10%, 1+:80%, 2+:10%, 3+:0%); recurrent tumor 3 (T3) H score 65 (0:60%, 1+:20%, 2+:15%, 3+:5%).

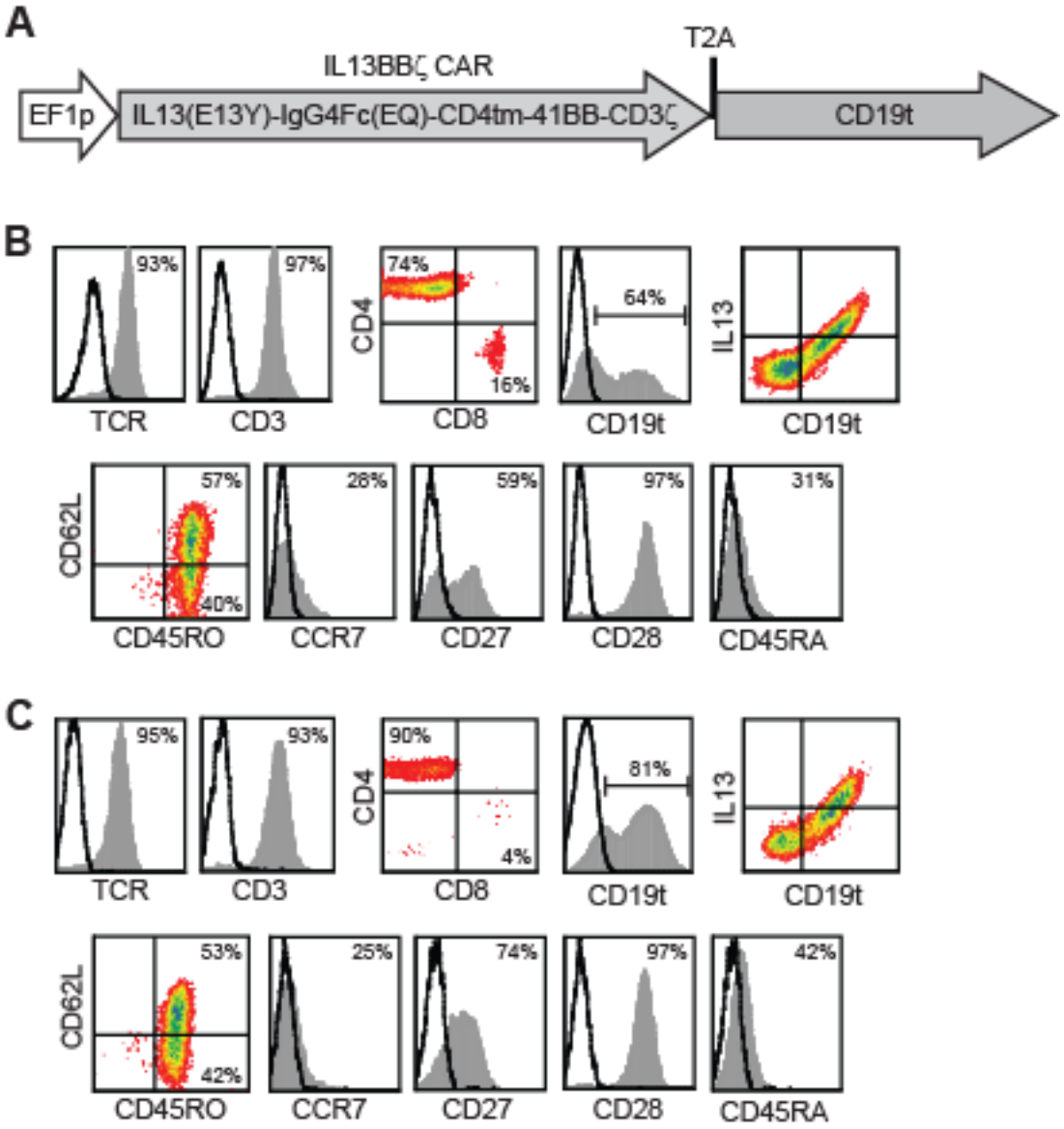


Figure S6. IL13BBζ-CAR T-cell products. A, Diagram of cDNA open reading frame, where transcription of the IL13BBζ CAR (containing the IL13Rα2-specific ligand IL13(E13Y), IgG4Fc(EQ) linker, CD4 transmembrane domain (CD4tm), and the 41BB (CD137) and CD3ζ cytoplasmic signaling domains), as well as the T2A ribosomal skip and truncated CD19 (CD19t) sequences are driven by the human EF1α promoter (EF1p). **B, C** Flow cytometric analysis of the final IL13BBζ-CAR T-cell products showing that these CD3+ and TCR+ T cells consisted of CD4 and CD8 subsets and expressed the IL13BBζ CAR (detected with anti-IL13) and CD19t (detected with anti-CD19) transgenes, with gene modified cells co-staining for both cell surface proteins (top panels). Both the first (**B**) and second (**C**) CAR T-cell products also exhibited a T_{CM} cell phenotype, expressing CD45RO, CD62L, CCR7, CD28 and CD27 (lower panels).

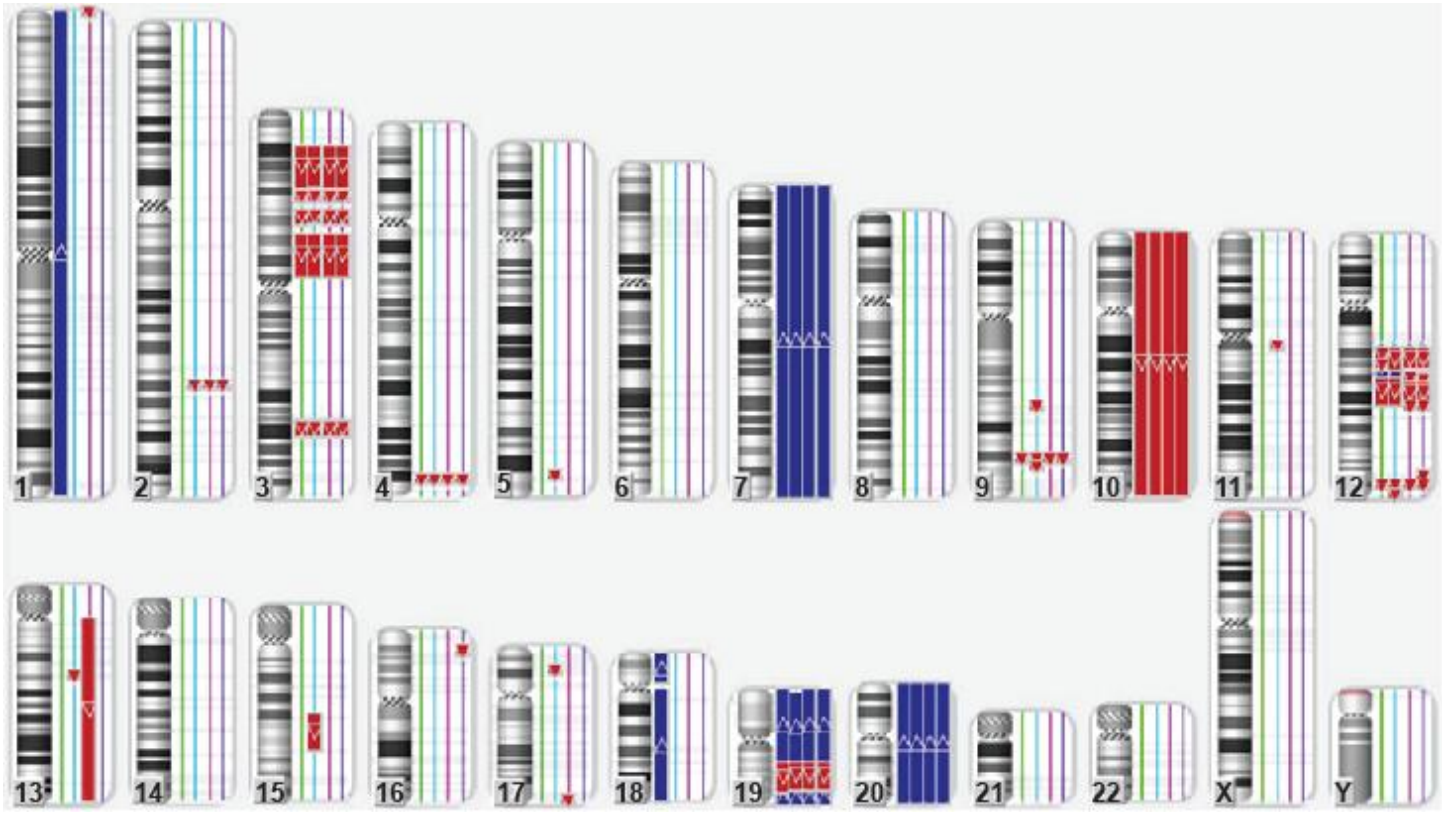


Figure S7. Oncoscan analysis of primary vs recurrent tumors 1, 2 and 3. DNA from microdissected tumor regions was processed and analyzed by Oncoscan® assay array, which uses molecular inversion probe technology to identify copy number changes, loss of heterozygosity and somatic mutations. Chromosome number is indicated at the bottom of each schematic, with order of sample result summaries from left to right: primary GBM (green line), tumor 1 (blue line), tumor 2 (pink line), tumor 3 (purple line). Red indicates areas of loss (e.g., chromosome 10) ; blue indicates areas of gain (e.g., trisomy 7).

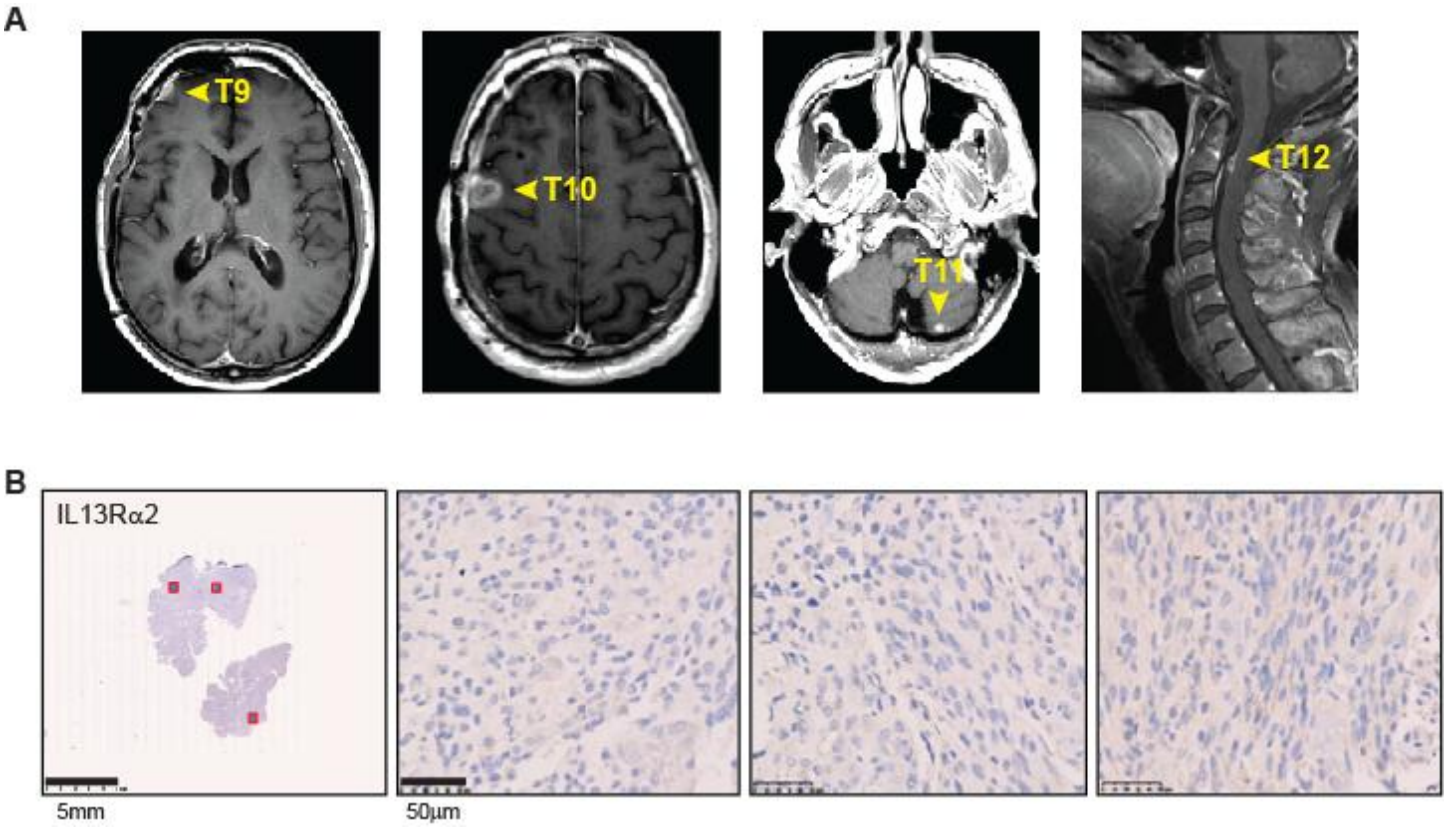


Figure S8. Tumor recurrence after CAR T therapy. A, Patient's disease recurred after cycle 16 at four new locations (non-adjacent to tumors 1 through 8), involving the right frontal lobe (tumors 9 and 10 [T9, T10]; intraparenchymal/leptomeningeal), left cerebellum (tumor 11 [T11]; intraparenchymal) and upper cervical spine (tumor 12 [T12]; leptomeningeal). **B**, Immunohistochemistry of the resected posterior right frontal tumor (T10) demonstrated very low IL13Rα2 staining**.** Tissue was stained using IL13Rα2-specific DAB with hematoxylin counterstain, with red boxes outlining representative Low/Negative areas depicted in the successive magnified images going left to right.

Table S1: Dose Schedule (CAR+)^				
Cycle	**Dose schedule 1de**	**Dose schedule 1**	**Dose schedule 2de**	**Dose schedule 2**
1	A: $2x10^6$	A: $2x10^6$	C: $10x10^6$	C: $10x10^6$
2	B: $5x10^6$	C: $10x10^6$	D: $25x10^6$	E: $50x10^6$
3	B: $5x10^6$	C: $10x10^6$	D: $25x10^6$	E: $50x10^6$
Total Dose	*$12\ x10^6$*	*$22\ x10^6$*	*$60\ x10^6$*	*$110\ x10^6$*
	Rest/Restaging			
Cycle[#] 4, 5, 6	$\leq 5x10^6$	$\leq 10x10^6$	$\leq 25x10^6$	$\leq 50\ x10^6$

^ Infusion doses should not exceed $2.5x10^8$ per 0.5mL ($0.5x10^6/\mu L$)

[#]Up to 3 additional cycles will be administered at ≤ the final cell dose reached in cycle 3, provided that the participant continues to meet eligibility criteria and there are cell doses available from the already manufactured cell product.

de = de-escalation schedule

Table S2. Safety and Tolerability						
Delivery Route	**T-cell Doses**	**Maximum T-cell Dose**	**Cumulative T- cell Dose**	**Adverse Event**	**Worst Grade***	**Longest Duration^**
ICT	6	10^7	5.2×10^7	Chills	1	1 day
				Fatigue	1	< 1 day
				Fever	1	1 day
				Lymphopenia	2	1 day
				Myalgia	1	1 day
				Dizziness	1	2 days
				Headache	1	14 days
				Olfactory Aura	1	1 day
ICV	10	10^7	9.2×10^7	Sinus Tachycardia	1	< 1 day
				Chills	1	< 1 day
				Fatigue	1	4 days
				Fever	1	2 days
				Flushing	1	1 day
				Myalgia	2	1 day
				Headache	2	< 1 day
				Olfactory Aura	1	< 1 day
				Anxiety	1	< 1 day
				Hypertension	1	2 days

*Worst grade of adverse events based on NCI Common Toxicity Criteria for Adverse Events version 4.03 with possible or higher attribution to the T cell administration are reported.

^ Longest duration of the worst grade event is indicated.

Table S3. MRI Evaluation of Non-Resected Lesions (Volume in cm^3, Area in mm^2)													
Tumor	**Anatomical Location**	**Pre Op**	**Post Op i.c.t.**	**Post Cycles 1-3**	**Post Cycles 4-6**		**Post Op i.c.v.**	**Post Cycles 7-9**	**Post Cycles 10-11**		**Post Cycles 12-14**	**Post Cycles 15-16**	
		D50	D51	D77	D86	D101	D108	D133	D156	D190	D254	D289	D295
4	Left temporal, pterion	0.2 cm^3	0.3 cm^3	0.5 cm^3	ND	0.8 cm^3	**1.4 cm^3**	0.3 cm^3	0.1 cm^3	0.1 cm^3	0.1 cm^3	ND	NM
		65 mm^2	98 mm^2	112 mm^2		168 mm^2	**224 mm^2**	80 mm^2	49 mm^2	28 mm^2	24 mm^2		NM
5	Left temporal, apex	0 cm^3	0 cm^3	0.1 cm^3	ND	0.3 cm^3	**0.7 cm^3**	0.1 cm^3	NM	NM	NM	ND	NM
		20 mm^2	20 mm^2	36 mm^2		54 mm^2	**126 mm^2**	33 mm^2	11 mm^2	7 mm^2	NM		NM
6*	Right frontal lobe	0 cm^3	0 cm^3	0.5 cm^3	ND	1 cm^3	1.7 cm^3	**1.8 cm^3**	1.4 cm^3	0.4 cm^3	NM	ND	NM
		0 mm^2	0 mm^2	42 mm^2		176 mm^2	187 mm^2	**300 mm^2**	143 mm^2	64 mm^2	NM		NM
7*	Olfactory groove	0 cm^3	0.1 cm^3	0.4 cm^3	ND	1.4 cm^3	**2.5 cm^3**	1.9 cm^3	1.3 cm^3	0.3 cm^3	NM	ND	NM
		27 mm^2	18 mm^2	60 mm^2		171 mm^2	**360 mm^2**	312 mm^2	98 mm^2	40 mm^2	NM		NM
8	Spinal	ND	ND	ND	**270 mm^2**	ND	ND	35mm^2	18 mm^2	8 mm^2	ND	NM	ND

*, new lesion arising during Cycles 1-6

Bold, largest measured tumor volume/area

NM, Not Measurable: minimal residual dural enhancement may be visible, but value was below that of analysis software parameters and/or manual measurement, and no increase in enhancement was detected for > 100 days; these are graphed as 0 cm^3 in Fig 2E.

ND, imaging was not done

Table S4A. UPN 109 CSF Cytokine Analysis (pg/mL), ICV cycles 7 through 11.												
Cytokine	**C7D0 (Baseline)¥**	**C7D2**	**C8D0**	**C8D1**	**C9D0**	**C9D2**	**C9D8**	**C10D0**	**C10D1**	**C11D0**	**C11D1**	**C11D44**
EGF	OOR < (10.0)	OOR <	OOR <	OOR <	OOR <	OOR <	OOR <	OOR <	OOR <	OOR <	OOR <	10.0
Eotaxin	*2.1	*2.4	*2.4	6.2	3.0	3.8	*2.4	*2.4	6.3	*2.4	6.1	*2.6
FGF	7.1	8.0	8.8	14.1	*4.0	*3.1	*4.7	*6.3	12.2	7.3	13.6	11.5
G-CSF	*25.1	68.6	*43.7	232.6	103.9	137.5	64.9	*23.3	245.6	*13.8	248.9	39.9
GM-CSF	*2.0	*2.4	*2.5	*8.7	*2.5	*4.2	*1.4	*1.3	*3.2	*1.3	*2.7	OOR <
HGF	74.4	113.1	127.7	253.9	162.3	250.8	145.8	110.9	213.6	125.2	241.8	81.0
IFN-α	45.5	56.8	42.0	109.7	59.5	66.1	35.6	17.9	90.0	24.8	74.5	OOR <
IFN-γ	*8.2	*7.0	*3.8	140.8	16.8	32.1	*5.0	*1.8	69.5	*4.0	42.8	*1.0
IL-10	*4.4	*6.0	*2.1	74.6	*20.7	70.4	*16.0	*3.5	147.1	*6.9	167.5	OOR <
IL-12	16.7	23.5	24.7	92.4	41.5	82.7	62.4	35.5	57.0	42.6	85.7	12.7
IL-13	*15.8	*15.3	*13.1	29.9	*15.9	18.1	*4.8	OOR <	22.7	OOR <	18.8	OOR <
IL-15	OOR < (*7.1)	OOR <	OOR <	OOR <	OOR <	OOR <	OOR <	OOR <	OOR <	OOR <	OOR <	*7.1
IL-17	*2.4	*2.8	*0.9	*9.2	*5.4	*5.1	*2.3	*1.2	*8.5	*1.0	*8.1	OOR <
IL-1Rα	*50.1	*35.7	*56.9	405.9	238.6	699.3	358.0	605.2	1113.0	*53.4	1141.9	259.9
IL-1β	*5.0	10.1	*6.2	22.1	*3.0	12.9	*6.7	*6.7	15.6	*8.0	17.0	*4.69
IL-2	OOR < (*0.6)	*4.2	*0.8	55.4	*1.0	*2.7	OOR <	*0.6	10.8	*0.6	*5.5	OOR <
IL-2R	43.8	81.0	51.2	223.7	89.6	243.1	67.7	*13.1	219.3	*18.3	241.5	54.2
IL-4	*2.5	*3.8	*2.9	*17.0	*5.5	*8.3	*3.9	*2.3	*13.8	*1.3	*10.5	OOR <
IL-5	OOR < (*0.5)	*1.3	*0.5	14.7	*2.6	9.1	*1.0	OOR <	7.9	OOR <	7.7	OOR <
IL-6	56.5	78.4	40.9	1062.5	106.5	318.4	47.0	33.2	688.5	31.4	857.3	23.2
IL-7	OOR < (6.3)	6.3	OOR <	42.7	20.0	19.9	OOR <	6.3	23.4	OOR <	22.0	28.0
IL-8	226.2	231.0	253.4	4904.6	827.4	1591.0	677.8	283.2	1023.9	84.4	794.9	66.0
IP-10/ CXCL10	161.4	766.7	307.3	6213.7	916.7	59779.1	510.1	156.9	393430.8	345.3	305579.5	79.2
MCP-1/ CCR2	1660.6	1752.3	1280.8	18439.9	4437.4	1939.1	791.9	1598.9	10868.4	420.0	3157.4	888.7
MIG/CXCL9	82.9	302.1	179.1	4500.5	1360.6	3621.2	1342.1	380.7	3423.0	288.2	3823.6	29.3
MIP-1α	22.0	28.0	20.7	68.1	31.9	50.8	19.7	*14.8	68.6	*14.6	64.4	*8.8
MIP-1β	26.3	33.8	26.1	213.8	49.7	106.1	24.2	16.8	126.8	22.3	52.6	13.6
RANTES	*15.5	OOR <	OOR <	41.7	25.7	OOR <	OOR <	OOR <	68.5	*1.0	*12.5	OOR <
TNF-α	OOR < (*1.6)	OOR <	OOR <	19.9	*1.6	*6.3	OOR <	OOR <	11.0	OOR <	*5.1	OOR <
VEGF	17.0	21.8	16.7	90.2	25.5	38.6	10.9	7.8	65.5	OOR <	70.0	14.1

OOR <, Out of Range (below)

*, Value extrapolated beyond standard range

¥, to allow for fold change calculations in Table S4B, baseline 'OOR<' values were replaced with the lowest measurable value for that cytokine as indicated in parentheses

Table S4B. UPN 109 CSF Cytokine Fold Change Analysis, ICV cycles 7 through 11.												
Cytokine	**C7D0¥**	**C7D2**	**C8D0**	**C8D1**	**C9D0**	**C9D2**	**C9D8**	**C10D0**	**C10D1**	**C11D0**	**C11D1**	**C11D44**
EGF	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Eotaxin	1.0	1.1	1.1	3.0	1.4	1.8	1.1	1.1	3.0	1.1	2.9	1.2
FGF	1.0	1.1	1.2	2.0	0.6	0.4	0.7	0.9	1.7	1.0	1.9	1.6
G-CSF^	1.0	2.7	1.7	9.3	4.1	5.5	2.6	0.9	9.8	0.5	9.9	1.6
GM-CSF	1.0	1.2	1.3	4.4	1.3	2.1	0.7	0.7	1.6	0.7	1.4	0.7
HGF	1.0	1.5	1.7	3.4	2.2	3.4	2.0	1.5	2.9	1.7	3.3	1.1
IFN-α	1.0	1.2	0.9	2.4	1.3	1.5	0.8	0.4	2.0	0.5	1.6	0.5
IFN-γ*	1.0	0.9	0.5	17.2	2.0	3.9	0.6	0.2	8.5	0.5	5.2	0.1
IL-10*	1.0	1.4	0.5	17.0	4.7	16.0	3.6	0.8	33.4	1.6	38.1	0.5
IL-12^	1.0	1.4	1.5	5.5	2.5	5.0	3.7	2.1	3.4	2.6	5.1	0.8
IL-13	1.0	1.0	0.8	1.9	1.0	1.1	0.3	0.3	1.4	0.3	1.2	0.3
IL-15	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
IL-17	1.0	1.2	0.4	3.8	2.3	2.1	1.0	0.5	3.5	0.4	3.4	0.4
IL-1Rα*	1.0	0.7	1.1	8.1	4.8	14.0	7.1	12.1	22.2	1.1	22.8	5.2
IL-1β	1.0	2.0	1.2	4.4	0.6	2.6	1.3	1.3	3.1	1.6	3.4	0.9
IL-2*	1.0	7.0	1.3	92.3	1.7	4.5	1.0	1.0	18.0	1.0	9.2	1.0
IL-2R^	1.0	1.8	1.2	5.1	2.0	5.6	1.5	0.3	5.0	0.4	5.5	1.2
IL-4^	1.0	1.5	1.2	6.8	2.2	3.3	1.6	0.9	5.5	0.5	4.2	0.5
IL-5*	1.0	2.6	1.0	29.4	5.2	18.2	2.0	1.0	15.8	1.0	15.4	1.0
IL-6*	1.0	1.4	0.7	18.8	1.9	5.6	0.8	0.6	12.2	0.6	15.2	0.4
IL-7^	1.0	1.0	1.0	6.8	3.2	3.2	1.0	1.0	3.7	1.0	3.5	4.4
IL-8*	1.0	1.0	1.1	21.7	3.7	7.0	3.0	1.3	4.5	0.4	3.5	0.3
IP-10/CXCL10*	1.0	4.8	1.9	38.5	5.7	370.4	3.2	1.0	2437.6	2.1	1893.3	0.5
MCP-1/CCR2*	1.0	1.1	0.8	11.1	2.7	1.2	0.5	1.0	6.5	0.3	1.9	0.5
MIG/CXCL9*	1.0	3.6	2.2	54.3	16.4	43.7	16.2	4.6	41.3	3.5	46.1	0.4
MIP-1α	1.0	1.3	0.9	3.1	1.5	2.3	0.9	0.7	3.1	0.7	2.9	0.4
MIP-1β^	1.0	1.3	1.0	8.1	1.9	4.0	0.9	0.6	4.8	0.8	2.0	0.5
RANTES	1.0	0.1	0.1	2.7	1.7	0.1	0.1	0.1	4.4	0.1	0.8	0.1
TNF-α*	1.0	1.0	1.0	12.4	1.0	3.9	1.0	1.0	6.9	1.0	3.2	1.0
VEGF	1.0	1.3	1.0	5.3	1.5	2.3	0.6	0.5	3.9	0.5	4.1	0.8

¥, baseline for fold change calculations was the C7D0 value in Table S4A.

Red values, 'OOR<' value from Table S3A was replaced with the lowest measurable value for that cytokine to allow for fold change calculation

*, yellow shading, Cytokines in which a > 10 fold increase was observed at least once

^, grey shading, Cytokines in which a > 5 fold increase was observed at least once

Table S5. UPN 109 Serum Cytokine Analysis (pg/mL), ICT cycles 1 through 6.																		
Cytokine	**C1D0**	**C1D2**	**C1D4**	**C2D0**	**C2D2**	**C2D4**	**C3D0**	**C3D2**	**C3D3**	**C4D0**	**C4D2**	**C4D4**	**C5D0**	**C5D2**	**C5D4**	**C6D0**	**C6D2**	**C6D3**
EGF	94	76	77	71	108	64	119	99	91	93	111	134	77	178	140	86	141	142
Eotaxin	102	135	135	104	169	172	157	149	123	143	118	128	94	110	136	115	133	143
FGF	OOR <	OOR <	*4.72	OOR <	OOR <	OOR <	*1.36	*3.03	*1.36	OOR <	OOR	OOR <	OOR	OOR	OOR	OOR	OOR	*3.89
G-CSF	221	238	228	189	204	199	212	252	199	258	235	214	154	222	226	242	258	286
GM-CSF	*1.61	*1.87	*2.02	*1.73	*1.84	*1.55	*1.72	*1.86	*1.55	*1.69	*1.66	*1.67	*1.49	*1.70	*1.58	*1.55	*1.82	*2.08
HGF	466	408	392	358	493	428	381	431	332	352	469	443	319	389	434	365	406	505
IFN-α	44	46	46	43	42	44	46	50	45	46	46	44	32	44	47	45	48	53
IFN-γ	41	49	51	46	50	46	54	54	52	53	53	50	32	47	58	51	57	56
IL-10	*1.19	*3.22	*3.43	*1.19	*2.48	*0.98	*2.37	*5.23	*0.81	*0.64	*1.19	*3.08	OOR	*1.07	*0.98	*1.39	*1.43	*4.01
IL-12	177	197	209	209	202	193	224	228	216	229	229	231	185	197	220	249	226	239
IL-13	23	34	28	*14.64	20	22	26	31	*13.12	28	19	*15.90	OOR	20	19	*17.44	25	32
IL-15	OOR <	OOR <	OOR <	OOR <	OOR <	OOR <	OOR <	OOR	OOR	OOR <	OOR	OOR <	OOR	OOR	OOR	OOR	OOR	OOR
IL-17	*2.33	*3.08	*3.59	*1.14	*2.99	*1.39	*3.13	*3.40	*5.81	*4.09	*5.98	*2.99	*4.31	*6.28	*3.95	*1.64	*5.98	*6.75
IL-1Rα	226	*90.07	*68.92	*91.42	*61.56	128	*79.59	140	*88.21	*71.27	197	110	120	*89.90	*85.48	*92.60	162	107
IL-1β	OOR <	OOR <	*2.88	OOR <	OOR <	OOR <	OOR <	*4.40	OOR	OOR <	OOR	OOR <	OOR	OOR	*0.24	*0.81	OOR	*4.58
IL-2	OOR <	*0.09	*0.72	OOR <	*0.18	OOR <	*0.23	*0.48	*0.09	*0.32	OOR	OOR <	OOR	OOR	*1.64	OOR	*0.64	*1.56
IL-2R	280	292	313	259	294	246	292	332	293	314	338	287	256	323	314	357	390	383
IL-4	*9.51	*10.41	*10.77	*8.42	*10.50	*7.73	*10.14	*11.66	*9.33	*12.37	*12.72	*10.37	*8.51	*10.99	*11.26	*10.41	*12.23	*15.73
IL-5	*0.90	*1.81	*1.96	OOR <	*0.76	*0.29	*0.71	*2.48	*0.80	*1.65	*0.90	*1.07	*0.29	*1.96	*0.90	*0.90	*2.33	*4.43
IL-6	6	*0.76	*0.87	*0.03	*0.65	*0.61	*2.72	*2.25	*2.28	*1.26	*0.40	OOR <	*0.44	*1.24	*1.90	*1.71	*0.47	*0.81
IL-7	OOR <	OOR <	OOR <	OOR <	OOR <	OOR <	OOR <	OOR	OOR	OOR <	OOR	OOR <	OOR	OOR	OOR	OOR	OOR	OOR
IL-8	34	*6.94	*7.37	12	13	10	35	26	25	24	10	*7.53	88	33	33	17	106	19
IP-10/ CXCL10	49	14	18	29	24	25	56	44	41	36	27	27	20	17	32	47	38	40
MCP-1/ CCR2	718	587	474	566	587	656	928	576	847	851	604	525	617	755	902	582	700	740
MIG/ CXCL9	29	29	31	31	34	33	98	83	74	50	57	39	41	37	97	245	105	81
MIP-1α	51	54	53	45	52	54	57	55	52	54	54	50	47	59	56	53	58	63
MIP-1β	74	77	68	63	79	74	105	77	84	77	75	67	78	92	98	92	100	94
RANTES	5118	10437	10893	9282	9725	11965	13148	11387	8938	12677	10660	9160	2631	6272	8789	9347	11698	13910
TNF-α	*1.86	*3.57	*2.95	*1.03	*3.02	*1.65	*2.37	*4.27	*3.47	*3.71	*2.81	*2.02	OOR	*2.95	*2.14	*2.21	*2.52	*5.05
VEGF	OOR <	OOR <	OOR <	OOR <	OOR <	OOR <	OOR <	OOR	OOR	OOR <	OOR	OOR <	OOR	OOR	OOR	OOR	OOR	OOR

OOR<, Out of Range (below)

*, Value extrapolated beyond standard range

Table S6. UPN 109 Serum Cytokine Analysis (pg/mL), ICV cycles 7 through 11.														
Cytokine	**C7D0**	**C7D2**	**C7D4**	**C8D0**	**C8D1**	**C8D4**	**C9D0**	**C9D2**	**C10D0**	**C10D1**	**C10D3**	**C11D0**	**C11D1**	**C11D2**
EGF	148.1	166.7	171.9	168.8	132.2	118.3	105.9	73.8	154.8	158.9	121.9	114.8	152.2	151.8
Eotaxin	110.1	116.8	112.6	101.2	83.7	133.8	152.1	156.4	172.1	167.9	143.2	147.3	197.7	168.3
FGF	*5.3	8.3	6.8	OOR<	OOR<	OOR<	OOR<	14.7	17.4	22.7	20.2	14.8	15.3	15.9
G-CSF	211.6	236.7	284.5	229.3	208.2	208.2	210.8	230.1	216.7	334.3	221.8	282.9	207.4	241.6
GM-CSF	*2.0	*2.1	*2.4	*1.9	*1.6	*2.0	*1.9	*1.7	*2.0	*3.1	*2.3	*1.9	*1.8	*1.7
HGF	471.5	596.1	611.6	420.1	403.0	508.1	362.4	400.0	385.9	502.5	456.6	395.3	476.1	451.6
IFN-α	43.9	47.4	49.9	43.8	42.2	47.1	43.9	40.0	41.1	64.5	43.1	50.4	43.3	43.4
IFN-γ	53.0	52.5	56.4	52.2	52.1	55.5	52.2	44.8	45.6	58.5	47.0	54.8	49.7	50.7
IL-10	*2.9	*3.9	*3.4	*0.8	OOR<	*2.4	*1.0	*3.3	*2.6	*9.4	*3.5	*1.8	*1.9	*0.6
IL-12	211.7	192.3	195.0	187.2	182.3	190.9	192.8	223.3	227.9	241.6	254.4	220.2	240.5	219.1
IL-13	21.1	27.4	30.7	23.1	36.8	31.0	28.0	24.2	22.3	38.3	31.4	32.2	25.8	34.8
IL-15	OOR<	OOR<	OOR<	OOR<	OOR<	OOR<	OOR<	OOR<	OOR<	OOR<	OOR<	OOR<	OOR<	OOR<
IL-17	*3.5	*4.1	*5.2	OOR<	*1.8	*0.7	*0.8	*3.3	*4.6	*11.5	*5.2	*7.3	*4.4	*4.4
IL-1Rα	112.7	145.3	*68.2	*94.3	*73.8	*64.5	*58.2	*64.9	101.1	133.8	96.3	*68.7	107.9	105.8
IL-1β	*2.1	*4.6	*4.9	*1.0	OOR<	OOR<	OOR<	11.3	15.9	30.6	18.2	12.7	14.3	14.7
IL-2	*0.1	*0.9	*1.3	*0.3	OOR<	*0.2	*0.3	*0.4	*0.9	*5.2	*1.2	*1.8	*0.9	*1.0
IL-2R	372.0	391.2	438.5	352.6	273.9	272.7	241.9	304.9	312.2	363.8	314.8	338.0	296.4	314.5
IL-4	*8.9	*11.5	*13.5	*9.0	*10.4	*10.5	*10.1	*10.2	*8.7	*20.9	*11.1	*13.8	*9.7	*10.8
IL-5	*1.6	*2.0	*3.2	*0.2	*1.5	*1.1	*0.8	OOR<	OOR<	5.1	*0.2	*2.2	OOR<	OOR<
IL-6	OOR<	*1.6	*0.4	OOR<	*0.7	OOR<	OOR<	*2.5	*2.6	7.1	*4.2	*2.9	*3.9	*3.0
IL-7	OOR<	OOR<	OOR<	OOR<	OOR<	OOR<	OOR<	OOR<	OOR<	OOR<	OOR<	OOR<	OOR<	OOR<
IL-8	49.4	130.2	88.4	96.4	56.3	17.1	43.6	*9.4	32.8	93.1	112.1	18.3	30.1	59.0
IP-10/CXCL10	33.9	23.5	17.2	11.7	9.0	11.5	15.0	12.7	17.4	33.6	16.3	15.3	23.2	18.9
MCP-1/CCR2	459.6	610.9	475.9	426.2	414.8	561.0	944.8	538.5	848.2	1074.3	703.0	954.9	950.0	826.3
MIG/CXCL9	141.3	108.1	50.6	8.0	OOR<	10.0	28.5	34.0	41.2	79.1	42.6	47.3	42.2	44.2
MIP-1α	58.4	58.4	62.2	51.1	49.2	53.4	53.1	47.1	55.8	81.2	57.7	64.6	52.7	54.1
MIP-1β	103.3	93.4	92.0	78.1	64.4	76.8	83.1	57.3	84.5	157.1	86.3	90.0	87.6	90.0
RANTES	11127.1	11965.0	14328.5	10584.5	12610.1	12415.5	12937.9	9221.7	8567.4	10428.1	8886.3	11117.8	9782.5	9771.1
TNF-α	*1.2	*2.1	*4.5	*2.3	*2.6	*2.1	*2.3	OOR<	OOR<	7.2	OOR<	*2.1	OOR<	OOR<
VEGF	OOR<	OOR<	OOR<	OOR<	OOR<	OOR<	OOR<	OOR<	OOR<	OOR<	OOR<	OOR<	OOR<	OOR<

OOR<, Out of Range (below)

*, Value extrapolated beyond standard range

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