Supplementary Methods:

Cell Product Characterization:

Confirmation of plasmid vector integration: A single site of plasmid vector chromosomal integration was confirmed by Southern blot analysis of *Xba*I/*Hind*III digested T cell genomic DNA using a 440-basepair Hygro-resistance gene-specific probe generated by PCR using the pMG vector plasmid (InvivoGen) as a template.

Surface phenotype determination: T cell products were evaluated for cell-surface phenotype using standard staining and flow cytometric procedures with fluorochrome-conjugated monoclonal antibodies - all were from BD Biosciences except those specific for NKG2D (R&D Systems) and TIM-3 (eBiosciences) - followed by analysis on either a FACS Caliber (BD Biosciences) or a MACSQuant Analyzer (Miltenyi Biotec Inc., Auburn, CA) and FCS Express version 3 software (De Novo Software).

Assay for anti-lymphoma cytolytic activity: Cytolytic activity of CAR^+ CTL clones against ^{51}Cr-labeled target cells was performed as previously described using a 4-hr chromium release assay (33). Negative control targets were human lymophoma Daudi cells (ATCC, Rockville, MD); IL13Rα2-expressing targets were human glioma U251T or IL13Rα2-transfected Daudi (Daudi-13Rα2) cells [both described in (23)]. Target cells were authenticated for the desired antigen/marker expression by flow cytometry prior to cryopreservation, and thawed cells were cultured for less than 6 months prior to use in these assays.

Sensitivity to ganciclovir ablation: To test for acquired cytocidal sensitivity to ganciclovir (GCV), aliquots of clones were harvested from 5-day REM expansion cultures, then

maintained for 14 days in 37.5 U/mL rhuIL-2 with or without 1μM GCV, at which time cells were harvested and subjected to viability testing.

Adoptive Transfer of T cells

Cryopreserved cell banks of quality control released autologous IL13-zetakine^{+} CTL clones were thawed and expanded using REM stimulation, and reformulated for infusion by washing twice with phosphate buffered saline (PBS) and resuspending in pharmaceutical preservative-free normal saline. Local intracranial delivery of the therapeutic CAR T cells into the glioma resection cavity was achieved using a 9 mm Rickham® reservoir connected to a ventricular catheter containing side openings (Ventriculostomy Reservoir Set, Codman & Shurtleff, Inc., Raynham, MA). The reservoir/catheter system 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 (**Supplementary Fig. S1, data not shown**). Patients were completely tapered off steroids prior to T cell injections. Cells were manually injected into the Rickham reservoir using a 21 gauge butterfly needle to deliver a 2 mL volume over 5-10 minutes, followed by 2 mL flush with preservative free normal saline over 5 minutes. While the absolute delivery efficiency of this system into the brain is unknown, CAR T cell mock infusions through the delivery device into a collection tube yielded a 94±10% (n = 2) cell recovery *in vitro* (data not shown). The intra-patient dose escalation plan is schematically presented in **Figure 1A**.

Detection of T cells in tumor

IHC for T cell markers was performed on 5 µm-sections of formalin-fixed paraffin-embedded (FFPE) specimens that had undergone antigen retrieval with Target Retrieval Solution (Dako) in steam for 20 minutes. Staining was performed with Dako mouse anti-CD3, -CD4, and CD8 antibodies (M7254, M7310 and M7103, at 1:200, 1:40 and 1:90 dilutions, respectively) at room temperature for 30 minutes, followed by peroxidase-conjugated secondary antibody. The slides were washed twice, and the peroxidase was developed with DAB (Dako). The stained slides were scanned on a NanoZoomer 2.0-HT C9600 digitial slide scanner (Hamamatsu), and images of the whole section were enumerated for the CD3, CD4 or CD8 positive cells by two different individuals, and the mean values were reported in **Supplementary Figure S5B**.

To detect CAR-expressing cells by digital droplet PCR (ddPCR), gDNA was isolated from a single 5 µm-section of an FFPE specimen using the ReliaPrep^{TM}FFPE gDNA miniprep system (Promega). 100ng gDNA from each sample was then used as a template for duplicate ddPCR reactions, with a Taqman MGB probe (Lifetechnology) that had been designed to target the fusion sequence of the HyTK transgene within the IL13-zetakine/HyTK expression construct (Lifetechnology, sequences available upon request). 20uL PCR reactions (containing 100ng gDNA, 10uL 2X ddPCR supermix (BioRad), and 1uL 20X probe/primer) involving droplet generation and droplet reads on a Qx200 reader (BioRad) were performed according to the BioRad Droplet Digital PCR protocol, with transgene copies calculated using QuantaSoft software (BioRad).