Tumor-infiltrating lymphocytes-derived CD8 clonotypes infiltrate the tumor tissue and mediate tumor regression in glioblastoma

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Background

Adoptive cell therapy using tumor-infiltrating lymphocytes (TILs) has shown remarkable efficacy in treating melanoma, but it's therapeutic potential in treating cold tumors remains underexplored. Despite the poor outcomes to immunotherapy even in highly mutated gliomas due to defective tumor antigen presentation and suppressive microenvironment, existing clinical evidence advocates for further evaluation of T-cell therapy to treat GBM, including TILs. Expanding TILs with IL-2, IL-15, and IL-21 enhances their proliferation, persistence, infiltration and cytotoxicity, improving the therapeutic potential of TIL products and broadening their application to cold tumors. In this case report, we administered IL-2/IL-15/IL-21-expanded TILs to a patient with recurrent and rapidly progressing glioblastoma and present the clinical and immunological outcomes.

Methods

- TILs were isolated from a resected tumor and expanded with a defined concentration of IL-2, IL-15, and IL-21.
- Patient received lymphodepletion with cyclophosphamide, followed by two TIL infusions two weeks apart and a single IL-2 dose post-infusion.
- TIL characterization was performed by flow cytometry and cytotoxicity assays against autologous and allogeneic tumor cells.
- Multi-omics: Whole-exome sequencing (WES) for mutation profiling, RNA sequencing for transcriptomic changes, and TCR sequencing for clonotype tracking.
- Immune Response: Peripheral blood flow cytometry to track T-cell activation, subset dynamics, and TIL persistence in the tumor microenvironment.

Results

A 75-yo male with highly mutated non-methylated glioblastoma multiforme presented with recurrent disease after 12 months of initial diagnosis. Initial treatment included surgical resection, radiotherapy (34 Gy), 6 cycles of temozolomide and lomustine. Patient presented recurrent disease and received compassionate TIL therapy. Tocilizumab plus etanercept were administered to prevent hyper-inflammatory reactions post-infusion. TIL products presented specific lysis of autologous tumor cell line. Clinical status stabilized over the next 2 weeks post-TIL-1 treatment and cranial MRI showed further significant tumor remission. Sequential cranial MRI showed complete tumor remission after TIL-2 administration (**Fig. 1**). Central memory T-cells expanded over time, alongside with increased PD-1+ and CD95+ pro-apoptotic T cells levels in circulation (**Fig. 2**). Transcriptomic analysis demonstrated an enrichment of genes related to immunological synapse and T-cell effector function parallel to the infiltration of TIL-derived CD8⁺ clonotypes (**Fig. 3-4**), altogether suggesting the T-cell-mediated tumor clearance.

Conclusions

- TILs expanded with IL-2/IL-15/IL-21 can overcome the challenges of glioblastoma's immunosuppressive microenvironment, achieving significant clinical outcomes.
- TIL-derived CD8⁺ T-cells showed tumor infiltration and expansion, driving tumor clearance.

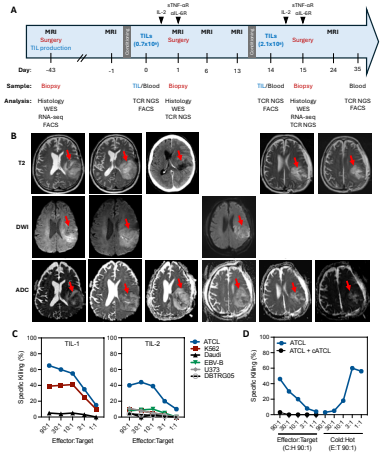

Figure 1. Treatment schedule, sampling scheme, clinical evolution and TIL product killing assay.
(**A**) Six weeks before TIL-1 infusion (day -43), the patient underwent partial resection of the GBM tumor in the occipital lobe to obtain tissue for TIL manufacturing. The patient received 60 mg/kg cyclophosphamide one day before both TIL-1 and TIL-2 infusions. IL-2 (60,000 IU/kg) was administered intravenously 8 hours post-infusion, followed by etanercept (25 mg) subcutaneously and tocilizumab (4 mg/kg) intravenously to prevent hyperinflammatory reactions 24 and 72 hours after TIL-1 and TIL-2, respectively. Serial sampling assessed tumor mutation burden, transcriptomics, and TIL persistence/infiltration. (**B**) MRI and CT imaging of the patient during treatment. (**C**) Cytotoxic potential of TIL products against autologous tumor cell line (ATCL) was assessed using standard Chromium release assays. Controls included allogeneic GBM cell lines (U-373, DBTRG05), Daudi B-lymphoma cell line, and autologous EBV-transformed B-cell line. (**D**) Cold target inhibition assays with a constant E:T ratio of 90:1 and varying cold tumor cell numbers showed highest blocking of TIL activity at higher cold tumor cell numbers. A constant cold:hot tumor cell ratio (90:1) was used to assess TIL activity at varying TIL numbers.

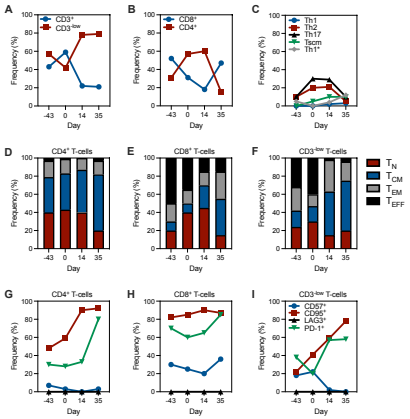

Figure 2. T-cell analysis in the peripheral blood during TIL therapy.
(**A**) Total CD3⁺/CD3^{-low} and (**B**) CD4⁺/CD8⁺ T-cells before and after TIL treatment. CD3^{-low} T-cells have downregulated TCR complex due to activation. (**C**) Changes in CD4⁺ T-helper subtypes post-treatment, categorized by CXCR3/CCR4/CCR6 expression: Th1 (CXCR3⁺), Th2 (CCR4⁺), Th17 (CCR4⁺/CCR6⁺), and Th1* (CXCR3⁺/CCR6⁺). Changes in naïve (TN), central memory (TCM), effector memory (TEM), and fully differentiated effector (TEFF) subtypes before and after TIL therapy in (**D**) CD4⁺, (**E**) CD8⁺, and (**F**) CD3^{-low} T-cells. Expression of CD57, CD95, LAG3, and PD-1 before and after TIL therapy in (**G**) CD4⁺, (**H**) CD8⁺, and (**I**) CD3^{-low} T-cells.

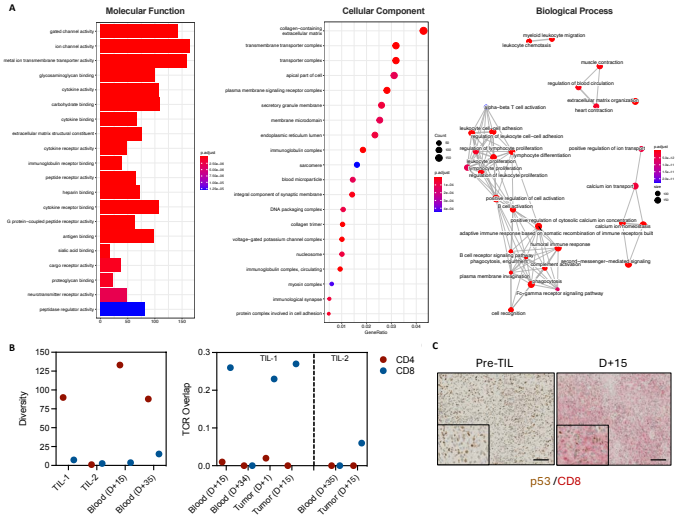

Figure 3. Transcriptomics and TCR NGS.
(**A**) Gene ontogeny analysis comparing differentially expressed genes after TIL treatment (day +15) as compared to baseline. Note the enrichment of processes associated with T-cell activation and effector function. (**B**) Left panel, TCR diversity as calculated by the inverse Simpson's index of sorted CD4+ and CD8+ T-cells from TIL products and blood samples after TIL therapy. Right panel, Morisita-Horn overlap index between CD4/CD8 T-cells isolated from the TIL products and blood samples or tumor-infiltrating T-cells. (**C**) Dual immunohistochemistry of p53 (brown) and CD8 (red) in a tumor sample collected before TIL treatment and at day +15 post infusion (scale bar, 100 µm).

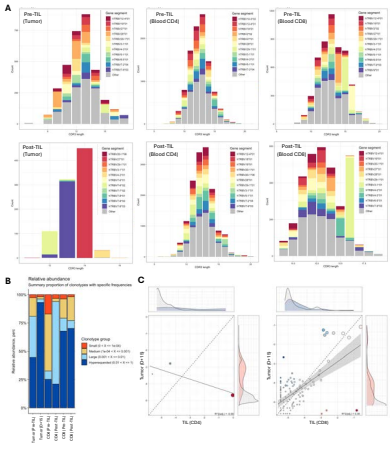

Figure 4. CD8⁺ TIL-derived clonotypes infiltrate tumor tissue after TIL therapy.
(**A**) CDR3 spectratype of tumor and TIL/blood samples (sorted CD4⁺ and CD8⁺ fractions) before and after TIL treatment (day +15), showing counts of unique CDR3 sequences with different amino acid lengths. Most frequent variable gene segments are highlighted. (**B**) Proportion of homeostatic space occupied by clonotypes classified as hyperexpanded (1–100%), large (0.1–1%), medium (0.01–0.1%), and small (0–0.01%) in tumor and TIL/blood samples before and after TIL treatment (day +15). (**C**) TCR repertoire overlap scatterplot showing CDR3 sequence overlap between sorted CD4 or CD8 T-cells from TIL-1 product and infiltrating T-cells within tumor tissue at day +15, with point size scaled to clonotype abundance. R^2 represents the Pearson's correlation coefficient of overlapping clonotypes.

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