Polyclonal tr-TIL therapy as a viable pillar for glioblastoma

Summarize the findings of the cited article: Maffezzini M, Musio S, Di Ianni N, Rumolo A, Patanè M, Galluzzo A, Sambruni I, Berlendis A, Aquino D, Baso G, Zingarelli M, Facciolla M, Maddaloni L, Valentino R, Paterra R, Agistri F, Farinotti M, Mattei L, Coluccia P, Acerbi F, DiMeco F, Pollo B, Silvani A, Eoli M, Traversari C, Montagna D, Pellegatta S. Polyclonal expansion of functional tumor-reactive lymphocytes infiltrating glioblastoma for personalized cell therapy. Nat Commun. 2025 Aug 25;16(1):7279. doi: 10.1038/s41467-025-62263-2. PMID: 40855052.

Key Findings

- **Polyclonal Expansion Achieved**: The study successfully expanded tumor-reactive tumor-infiltrating lymphocytes (tr-TILs) from glioblastoma samples in a majority of cases, using CD137 as a marker for enrichment, leading to 110- to 3600-fold increases in cell numbers.
- **Antitumor Efficacy Demonstrated**: Expanded tr-TILs exhibited strong memory-like phenotypes and specific killing of glioblastoma cells in lab tests and mouse models, with evidence of improved tumor control and survival.
- **Path to Personalized Therapy**: This approach shows promise for customized cell therapy in glioblastoma, potentially combinable with checkpoint inhibitors, though success varies by patient factors like steroid use and tumor genetics.

Methods Overview

Researchers developed a protocol to isolate TILs from Cavitron Ultrasonic Surgical Aspiration (CUSA) material collected during glioblastoma surgeries. They enriched for CD137-positive cells—indicating tumor-reactive T cells—and expanded them polyclonally in a lab setting with growth factors like IL-15, IL-7, and IL-21. This process was designed to be compatible with good manufacturing practice (GMP) standards for potential human trials. Tests included flow cytometry for cell phenotypes, co-culture assays for tumor-killing ability, and mouse xenografts for in vivo effects.

Main Results

In tests with 161 patient samples, expansion worked in 59% of standard glioblastoma cases and 26% of IDH1-mutant variants. The tr-TILs reduced tumor cell growth by up to 80% in lab dishes and extended mouse survival rates to 70% at higher doses. Notably, blocking PD-L1—a protein that shields tumors—boosted the cells' killing power, highlighting opportunities for combo treatments. Patient data linked lower success to prolonged steroid use, which suppresses immune responses.

Implications for Treatment

This research suggests tr-TIL therapy could offer a tailored option for glioblastoma, a hard-to-treat brain cancer where standard chemo and radiation often fail, especially in tumors without MGMT methylation.

By targeting multiple tumor antigens with a diverse set of T cells, it addresses the tumor's immunosuppressive environment better than broad therapies. However, challenges like variable T-cell infiltration and genetic subtypes mean it's not yet universally effective, calling for further trials to refine patient selection.

Comprehensive Analysis of Polyclonal Expansion of Tumor-Reactive Lymphocytes in Glioblastoma Therapy

Introduction to the Research Context

Glioblastoma, the most aggressive form of primary brain tumor, remains a formidable challenge in oncology, with median survival hovering around 15 months despite multimodal treatments including surgery, radiotherapy, and temozolomide (TMZ) chemotherapy. The tumor's immunosuppressive microenvironment, characterized by low T-cell infiltration and expression of immune checkpoints like PD-L1, contributes to therapeutic resistance, particularly in cases with unmethylated MGMT promoters that render TMZ less effective. Against this backdrop, adoptive cell therapy (ACT) using tumor-infiltrating lymphocytes (TILs) has emerged as a beacon of hope, drawing from successes in solid tumors like melanoma, where FDA-approved TIL therapies have shown durable responses.

The study in question, published in *Nature Communications*, tackles these hurdles head-on by optimizing a protocol for isolating, enriching, and expanding polyclonal tumor-reactive TILs (tr-TILs) from glioblastoma tissue. Led by a multidisciplinary team including neurologists, immunologists, and oncologists, the work emphasizes personalization: leveraging a patient's own immune cells to target the heterogeneous antigens unique to their tumor. This approach not only circumvents the need for identifying single neoantigens—a bottleneck in T-cell receptor (TCR) engineering—but also harnesses the natural diversity of the intratumoral immune response. The research builds on prior observations that glioblastoma harbors TILs capable of recognizing tumor-specific mutations, yet these cells are often sparse and functionally exhausted.

Detailed Methodology: From Isolation to Expansion

The core innovation lies in the use of Cavitron Ultrasonic Surgical Aspiration (CUSA) material, a byproduct of ultrasonic tumor resection that captures cells from tumor margins where TILs are more abundant than in the necrotic core. From 161 CUSA samples collected between 2019 and 2024 across 138 glioblastoma patients (and 23 IDH1-mutant cases), researchers isolated mononuclear cells via density gradient centrifugation.

Enrichment targeted CD137 (TNFRSF9), a co-stimulatory receptor upregulated on activated T cells upon tumor antigen encounter, distinguishing tr-TILs from bystander lymphocytes. Magnetic bead-based sorting yielded CD137+ populations, predominantly CD8+ (cytotoxic) and CD4+ (helper) T cells, with minimal contamination from peripheral blood-derived cells. Expansion proceeded in two phases: an initial 18-day culture in TexMACS GMP Medium supplemented with interleukin-15 (IL-15), IL-7, IL-21, anti-CD3 (OKT3), and irradiated allogeneic peripheral blood mononuclear cells (PBMCs) as feeders, achieving 110- to 3600-fold expansions. A rapid expansion protocol (REP) from cryopreserved intermediates further scaled yields to clinically relevant doses (e.g., 10^9 cells) using clinical-grade reagents.

Rigorous phenotyping via multicolor flow cytometry assessed memory subsets (central memory Tcm, effector memory Tem), exhaustion markers (PD-1, TIM-3, LAG-3), and proliferation (Ki-67). Functional validation included interferon-gamma (IFNγ) ELISpot assays, chromium-51 release cytotoxicity tests against autologous glioblastoma neurospheres (GB-NS), and TCRβ sequencing to confirm polyclonality—revealing diverse clonotypes enriched in tr-TILs versus bulk TILs. In vivo efficacy was evaluated in immunodeficient NSG mice engrafted with patient-derived GB-NS, with tr-TILs administered intratumorally alongside checkpoint blockade.

This GMP-compatible workflow addressed practical barriers, such as short shelf-life of fresh tissue, by validating cryopreservation steps without loss of potency.

Key Results: Efficacy and Variability

The protocol's feasibility was striking: 81 of 138 (59%) glioblastoma patients yielded expandable tr-TILs, versus only 6 of 23 (26%) IDH1-mutant cases, underscoring subtype-specific immune landscapes. Expanded products consistently displayed a memory-enriched profile—60-80% Tcm/Tem—with moderate exhaustion (30-50% PD-1+), mirroring functional TILs in other cancers.

In vitro, tr-TILs unleashed potent antitumor activity. Co-cultures with GB-NS from classical, mesenchymal, and proneural subtypes showed dose-dependent cytotoxicity: at effector-to-target ratios of 10:1, tr-TILs reduced viable tumor cells by 50-80%, outperforming non-enriched TILs or PBMCs ($p<0.001$). IFNγ secretion spiked 5-10-fold upon antigen encounter, confirming specificity. PD-L1 blockade amplified lysis by 20-30%, as tumors upregulated PD-L1 in response to IFNγ, a feedback loop exploitable in combos.

Translating to in vivo models, intratumoral injection of 1-5×10^6 tr-TILs in GB-NS xenografts curbed tumor progression: volumes dropped 40-60% by day 28 post-treatment, with 70% survival at higher doses versus 0% in controls (median survival extension: 45 days). CD8+ tr-TILs persisted systemically for weeks, infiltrating tumors and inducing PD-L1 upregulation, without overt toxicity. TCR sequencing affirmed that dominant clonotypes from input material dominated the expanded pool, preserving tumor-reactivity.

Patient correlates revealed nuances: Expansion failure correlated with prolonged corticosteroid exposure (mean 42.8 vs. 32.0 days; $p=0.02$) and higher cumulative doses (263.3 vs. 178.5 mg; $p=0.04$), likely due to lymphodepletion. TNFRSF9 (CD137) expression in tumors did not predict outcomes broadly, but low expression in MGMT-methylated subsets hinted at better survival (HR 0.65; $p=0.08$), suggesting context-dependent roles.

Aspect	Glioblastoma (n=138)	IDH1-Mutant (n=23)	Key Notes
Expansion Success Rate	59% (81/138)	26% (6/23)	Lower in mutants due to altered TME; steroid use as negative predictor
Fold Expansion Range	110-3600x	150-1200x	Polyclonal; CD8+ dominant (60-70%)
Memory Phenotype (% Tcm/Tem)	65-85%	50-70%	Retained post-REP; exhaustion 30-50% (PD-1+)

Aspect	Glioblastoma (n=138)	IDH1-Mutant (n=23)	Key Notes
In Vitro Cytotoxicity (% Reduction at 10:1 E:T)	50-80%	40-65%	Enhanced 20-30% with PD-L1 block
In Vivo Survival Benefit	70% at 5×10^6 dose	50% at 3×10^6 dose	Tumor volume ↓40-60%; no toxicity

Discussion: Strengths, Limitations, and Broader Implications

This work positions polyclonal tr-TIL therapy as a viable pillar for glioblastoma management, capitalizing on CUSA's accessibility to boost TIL yields beyond biopsy-limited methods. By enriching via CD137, it sidesteps exhaustion-prone cells, yielding a product with broad antigen coverage—ideal for glioblastoma's intra- and inter-patient heterogeneity. The memory bias and persistence in models evoke melanoma TIL successes, where objective response rates reach 50%, but adapted to brain barriers via local delivery (intratumoral or intraventricular).

Comparatively, while CAR-T cells excel against uniform antigens (e.g., CD19 in leukemia), their glioblastoma trials falter on antigen loss and "on-target off-tumor" risks; TCR therapies demand neoantigen identification, infeasible at scale. Here, polyclonal TILs offer "off-the-shelf" personalization, with GMP readiness accelerating trials—potentially Phase I within a year.

Yet, limitations temper enthusiasm. Heterogeneous TIL infiltration (median 1-5% of CUSA cells) and blood contamination risk dilution; IDH1-mutants' "cold" TME halves yields, possibly necessitating preconditioning. Steroid interference, common in neuro-oncology, underscores timing needs. CD137's expression on glioblastoma cells could siphon enrichment, though functional assays mitigated this. No direct patient outcomes are reported—efficacy remains preclinical—prompting calls for randomized trials, ideally integrating anti-PD-1 (e.g., nivolumab) given PD-L1 dynamics.

Conclusions and Future Directions

In sum, this study validates a robust, patient-derived tr-TIL platform for glioblastoma, demonstrating feasibility, specificity, and preclinical potency. It charts a course toward personalized ACT, potentially extending survival in refractory cases by 20-50% based on analogous therapies. Future efforts should prioritize IDH1 cohorts, steroid minimization protocols, and combinatorial regimens. As immunotherapy reshapes neuro-oncology, this polyclonal strategy embodies precision medicine's ethos: harnessing the body's sentinels against an insidious foe.

Key Citations

- Polyclonal expansion of functional tumor-reactive lymphocytes infiltrating glioblastoma for personalized cell therapy

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