Automated Manufacturing of Tumor-Reactive T cells for Immunotherapy of Glioblastoma

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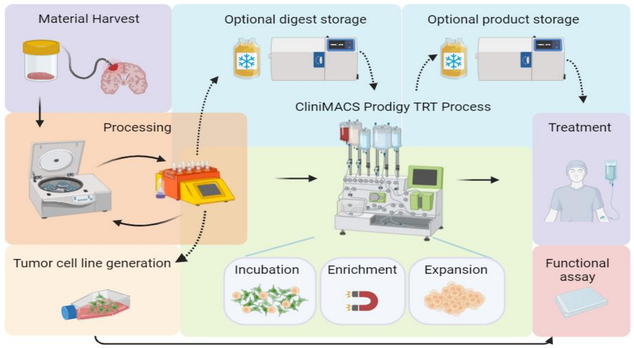
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Introduction

Glioblastoma multiforme (GBM) is the most common and the most aggressive primary brain tumor in adults with a median overall survival of approximately 15 months from diagnosis. Current GBM Standard-of-Care comprises maximal surgical resection, followed by a combination of radiotherapy with an alkylating chemotherapy agent, Temozolomide. Despite intensive multimodal treatment GBM inevitably relapses following therapy, and therefore has a high unmet need for new therapeutic strategies.
GBM has a relatively low mutational burden compared to other malignancies, however, it's tumor antigen profile has a high degree of intra- and interpatient heterogeneity.
Tumor Infiltrating Lymphocyte (TIL) therapy is polyclonal cellular therapy, in which autologous tumor-resident T cells are expanded *ex vivo* and subsequently infused back into the patient. This strategy has been used for decades in other solid tumors, showing durable complete responses and a good safety profile. The first TIL-based drug "Amtagvi" was recently approved by FDA.
In the current study, we sought to develop a clinical strategy focused on using tumor-infiltrating lymphocytes (TILs) to treat GBM

Figure 1

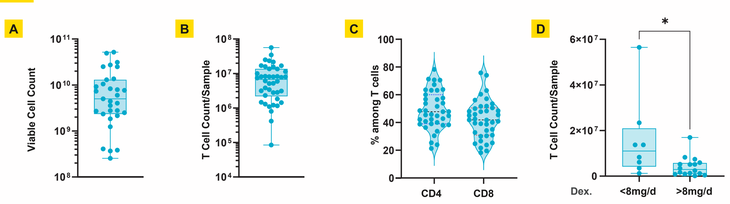


Tumor material from 39 glioblastoma patients was harvested perioperatively, transported in pre-cooled containers, and processed on the same or the following day. Processing involved mechanical dissection using GentleMACS dissociator in the presence of an enzymatic cocktail (Tumor Dissociation Kit, Miltenyi Biotec), followed by several washing and filtration steps. Single-cell suspension was characterized using flow cytometry. After an optional freezing step, TIL were manufactured with the help of CliniMACS Prodigy® product Tumor-Reactive T cell (TRT) Process, which consists of pre-incubation, CD137-based reactive T cell enrichment, and expansion. The final product was analyzed by phenotyping using flow cytometry. The tumor digest was also utilized for the establishment of primary GBM cell lines, which were subsequently used for functional testing of the manufactured TIL products.

Results

1 GBM tumor digest represents a reliable source for high number of TILs

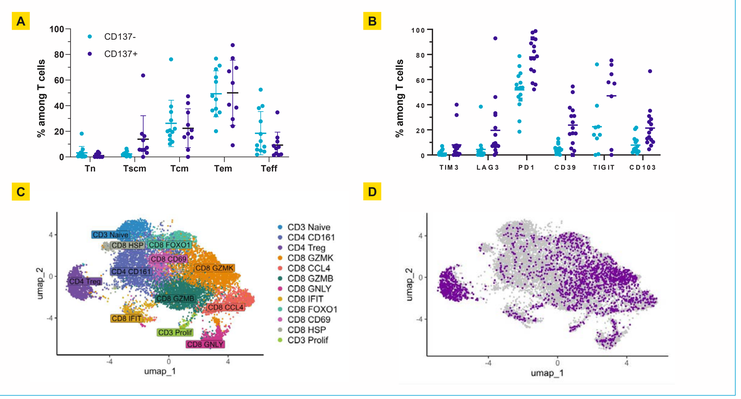
Figure 2



(A) Total count of viable cells derived from GBM samples. (B) Viable T cell count in the single cell suspension after GBM processing. Median = 6,7 million T cells. (C) CD4 and CD8 fractions within GBM-derived T cells. Median values: 47,8% and 41,8% cells for CD4 and CD8 fractions, respectively. (D) Decrease in the total count of infiltrating T cells was associated with steroid treatment. Patients were stratified according to their pre-operative treatment regimens: first group received no or up to 8mg/day dexamethasone (dex.), whereas second group received more than 8 mg/day. T cell counts within resected material were compared. P<0,05.

2 Characterization of GBM-derived TIL

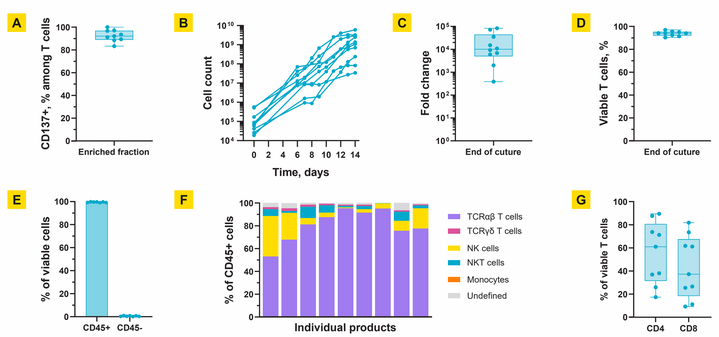
Figure 3



(A, B) Infiltrating T cells within the GBM digests were characterized by flow cytometry with the help of (A) Differentiation and (B) Exhaustion panels. (C, D) TILs from four GBM tumor digest samples were enriched by REAlease CD4/CD8 (TIL) MicroBead kit and their transcriptome and TCR sequences were studied by scRNA-seq (10xGenomics). (C) TILs were clustered into 12 subsets based on their gene expression and visualized in a UMAP, containing: 2 CD4, 8 CD8, and 2 mixed CD4 and CD8 TIL clusters. (D) TNFRSF9 gene (encoding for CD137) was expressed by T cells in different clusters, particularly in CD8 GZMK, CD8 GZMB and CD4 Treg clusters.

3 Reactive TILs can be enriched from GBM digest and expanded with the CliniMACS Prodigy® TRT Process

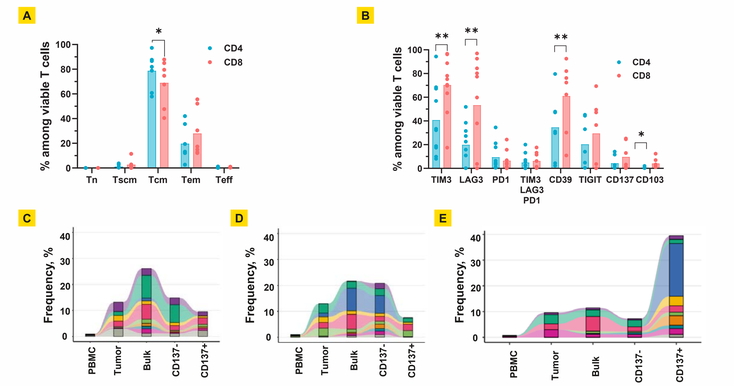
Figure 4



Clinical scale TIL manufacturing was performed with material from ten GBM patients. In flow of the CliniMACS Prodigy® TRT Process, tumor digest was incubated overnight to recover CD137 expression on the surface of potentially reactive TILs, which were subsequently labeled and enriched to prevent bystander T cell expansion. (A) CD137 purity within T cells after enrichment. (B) Enriched T cells were expanded for 15 days yielding up to 5 billion cells. (C) The expansion fold and (D) Viability of the expanded T cell product. (E-G) Upon harvest GBM TIL products were characterized by flow cytometry. (E) Fraction of CD45 negative cells did not exceed 1%. (F) Cell composition of final products. No monocytes were detected in the products. (G) Percentage of T cell subsets.

4 Clinical GBM TRT Product predominantly consists of Tcm skewed TILs

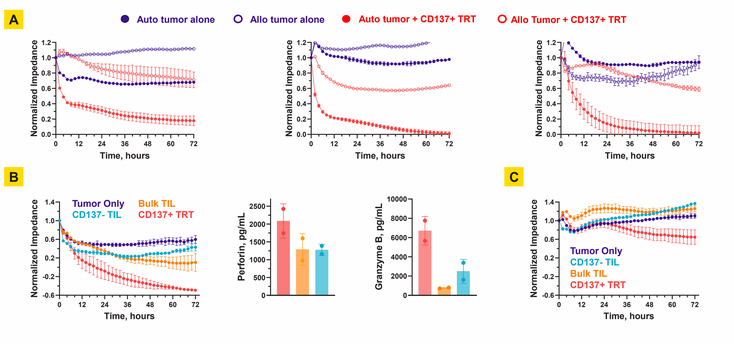
Figure 5



(A, B) Flow cytometry characterization of the GBM TIL products (C-D) Patient T cells were sequenced by bulk TCR sequencing to track TCR clonotypes among blood, tumor, and post-REP samples. The frequency of top 10 dominant TCR clonotypes from (C) unselected TIL product, (D) expanded CD137-, and (E) expanded CD137+ is depicted across samples. Unique, dominant clonotypes were found in the final TRT product (E, blue, yellow, and orange), which were rare or undetectable in the other T cell populations

5 Manufactured GBM TRTs successfully kill autologous tumor cells

Figure 6



(A-C) Harvested TRTs were restimulated with autologous GBM cells for 72 hours. Tumor cell killing was assessed with the help of impedance measurement. (A) Example results from three donors (B,C) Expanded CD137 enriched (CD137+), non-enriched (bulk) or CD137-depleted GBM TIL were co-incubated with either autologous (B) or allogeneic (C) GBM tumor cell line.

Conclusions and outlook

1. 16-day CliniMACS Prodigy® based Tumor-Reactive T cell process can be successfully adapted for manufacturing of therapeutic TIL products for GBM patients
2. The TRT process allows high-rate expansion of the CD137-enriched reactive T cells
3. GBM-derived tumor-reactive T cell product consists of memory T cells with low PD1 expression
4. TILs enriched for CD137 prior to rapid expansion contain unique TCR clonotypes and demonstrate better autologous tumor cell killing compared to unselected and CD137 negative TILs.

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