# A workflow for generating tumor-reactive T cells from GBM-derived cavitron ultrasonic aspirator (CUSA) fluids

Poster Session II: 109

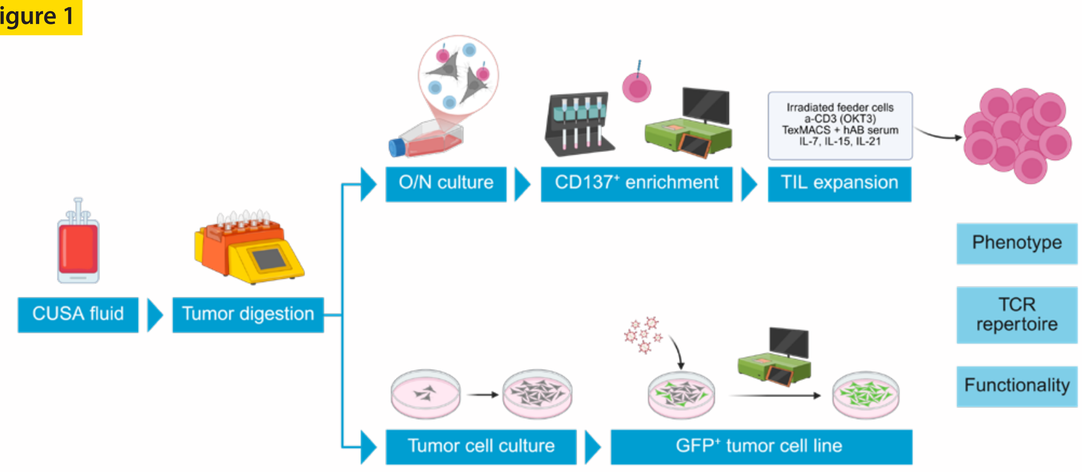
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## Introduction

Glioblastoma (GBM) is an aggressive brain cancer with limited treatment options and a poor prognosis, highlighting the need for more effective therapies. Tumor-infiltrating lymphocyte (TIL) therapy has shown promise and represent a viable strategy for GBM treatment. To support the development of TIL-based therapies, we established a workflow to generate tumor-reactive T cells and autologous tumor cell lines using the fluid collected during surgical resection with a cavitron ultrasonic aspirator (CUSA).

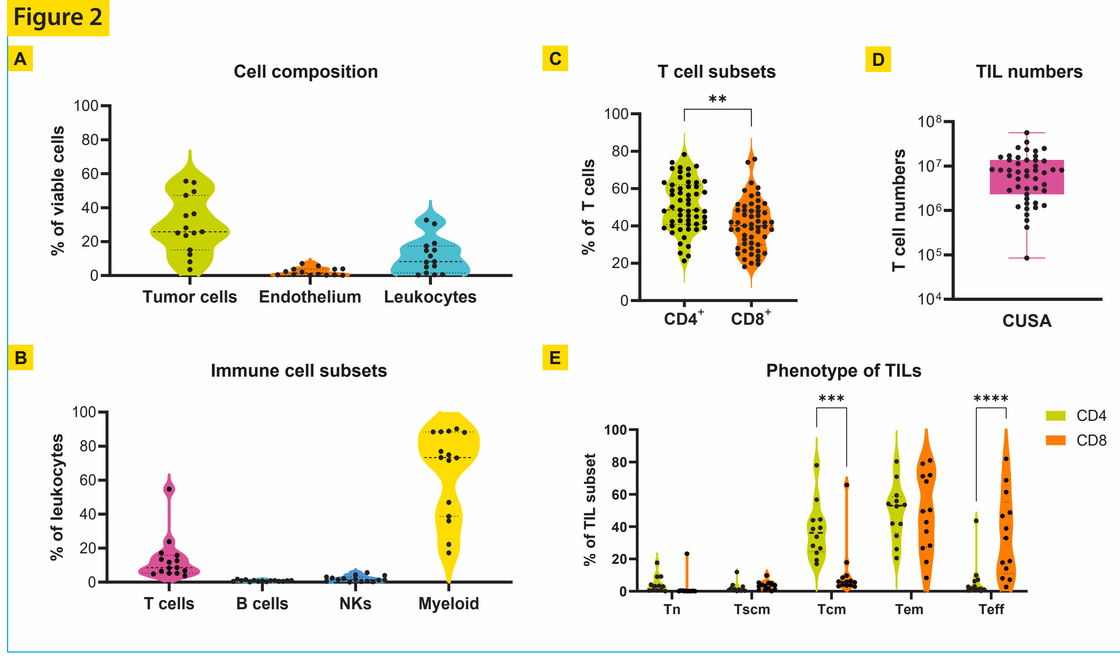
**Figure 1**




## Results

### 1 Composition of GBM-CUSA digests

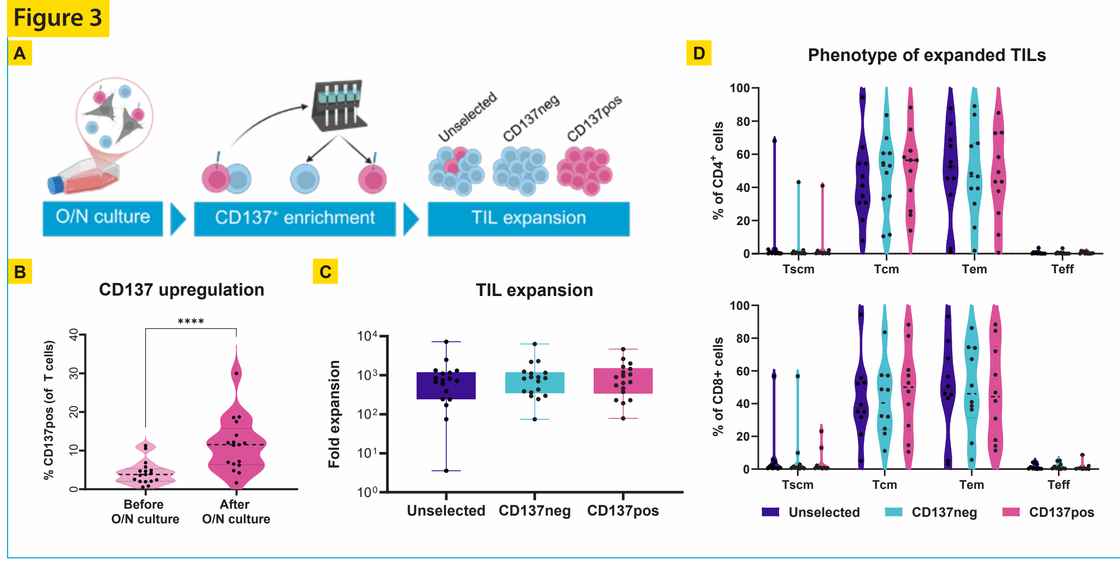
**Figure 2**




We first characterized the cellular composition of CUSA fluid after tumor dissociation, identifying GLAST$^+$ tumor cells, CD31$^+$ endothelial cells, and diverse CD45$^+$ immune cell subsets, including CD4$^+$ and CD8$^+$ T cells, CD20$^+$ B cells, CD56$^+$ NKs, and CD11b$^+$ myeloid cells (A-C). On avarage, 1 x $10^7$ T cells were obtained from GBM-derived CUSA digests (D). T cells were phenotypically classified into naive (Tn), stem-cell memory (Tscm), central and effector memory (Tcm and Tem), and effector (Teff) subsets based on CD45RA, CD62L, and CD95 expression (E).

### 2 CD137 enrichment and TIL expansion

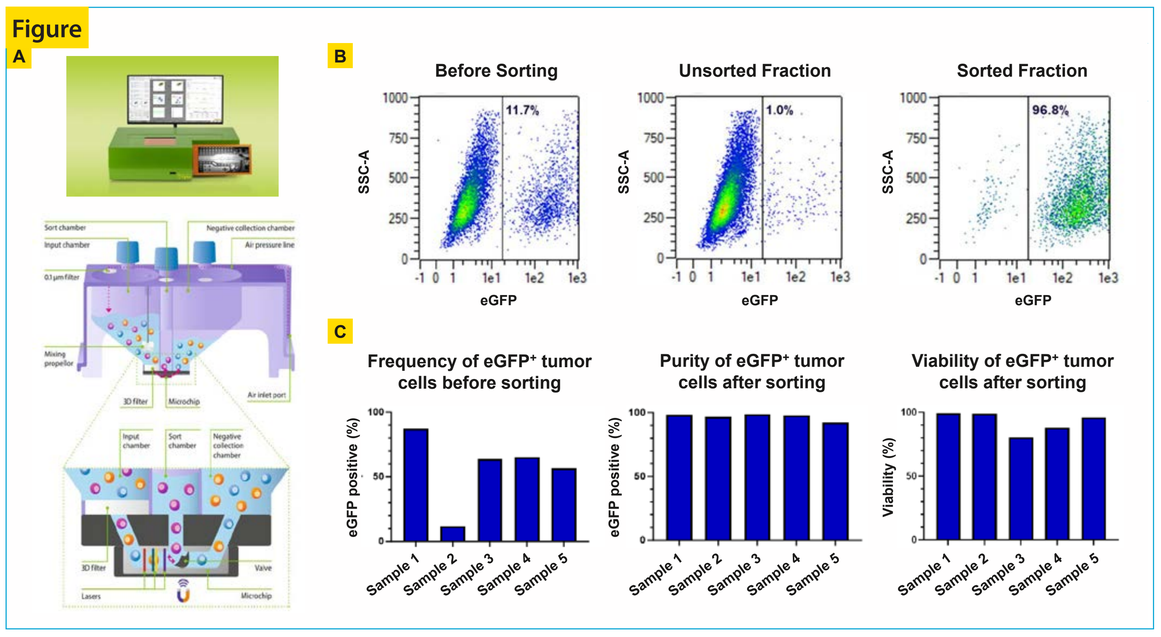
**Figure 3**




To enrich for tumor-reactive T cells, we magnetically enriched CD137$^+$ T cells following an overnight incubation of the GBM CUSA dissociate (A, B). These CD137$^+$ TILs expanded rapidly over 14 days, with expansion folds comparable to those of unselected and CD137$^-$ TILs (C). Phenotypic analysis of the expanded TILs revealed the presence of both Tcm and Tem subsets (D).

### 3 Generation of eGFP$^+$ tumor cell lines

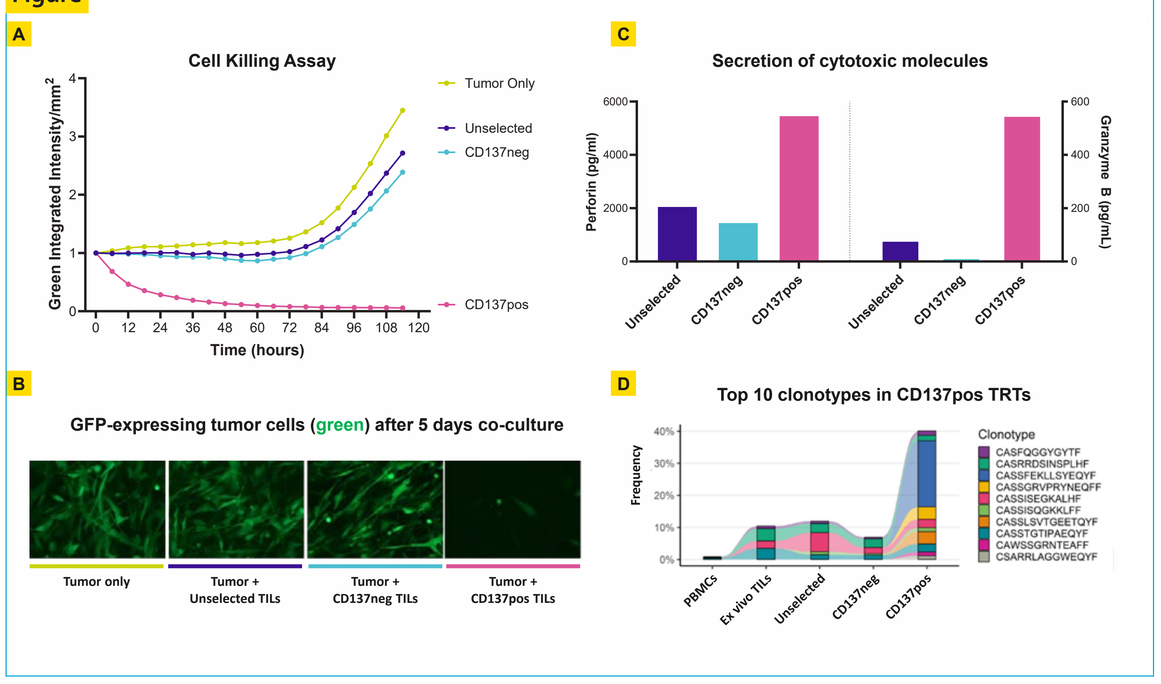
**Figure**




We successfully established primary autologous GBM tumor cell lines *in vitro* from fresh CUSA tumor dissociates. To enable their use in fluorescence-based killing assays, tumor cells were transduced to express eGFP and subsequently sorted using the MACSQuant® Tyto® cell sorter. This instrument uses a closed cartridge system with a mechanical valve for steril, gentle cell sorting (A). Sorting large GBM cells required a newly developed cartridge, designed to support the enrichment of large, fragile cell types while maintaining cell viability and purity (B, C).

### 4 Funcionality of expanded TILs

**Figure**




Functional validation confirmed the tumor reactivity of CD137$^+$ T cells. Co-culture of expanded CD137$^+$ T cells with autologous eGFP$^+$ tumor cells for five days resulted in significant tumor control or elimination, associated with the secretion of cytotoxic molecules such as perforin and granzyme B (A-C). In contrast, unselected or CD137$^-$ TILs did not mediate tumor clearance. Additionally, expanded CD137$^+$ TILs contained dominant TCR clonotypes that were not detected in unselected or CD137$^-$ subsets, as well as in the initial tumor repertoire and the patient´s peripheral blood, indicating selective enrichment and expansion of potential tumor-reactive TCRs within the CD137$^+$ population (D).

## Summary

- We developed a workflow for the generation of tumor-reactive T cells from GBM-derived CUSA fluids, using the activation marker CD137.
- This workflow also enabled the successful generation and transduction of primary autologous tumor cell lines, which were used for the functional validation of TILs.
- Integration of the MACSQuant® Tyto® cell sorter, along with newly developed cartridges, facilitated the gentle enrichment of diverse cell types, including GBM tumor cells. It can also be utilized to sort tumor-reactive T cells.
- CD137 can be substituted with alternative markers to support the analyis of other TIL subsets of interest.
- This workflow provides a valuable tool for both translational research and the clinical development of TIL-based therapies.

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Figures 1 and 3A were generated with BioRender.com

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