Two-step selection strategy improves TIL manufacturing from cold tumors

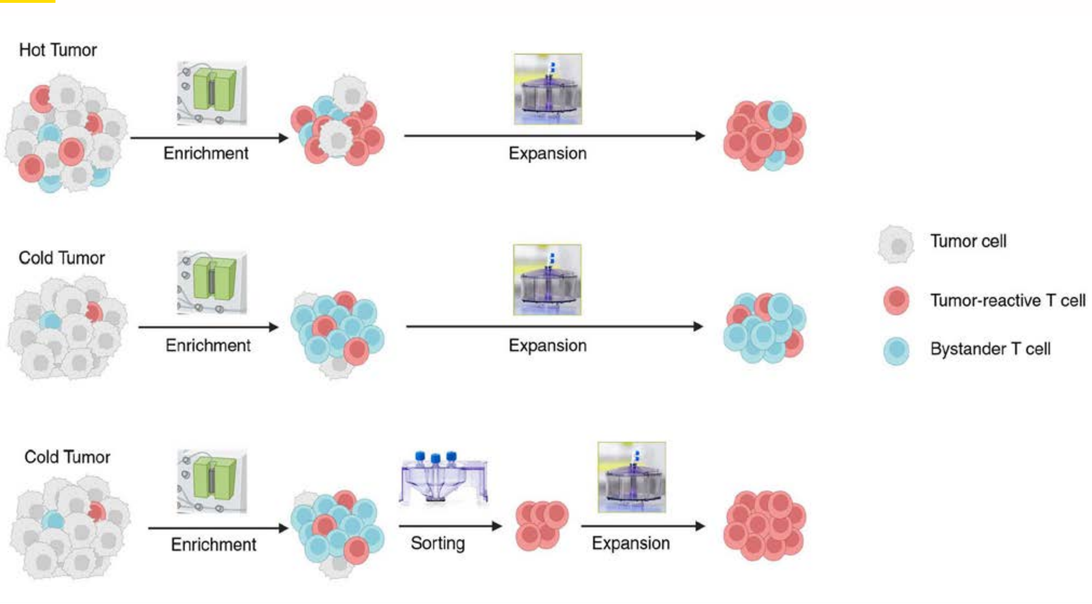
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Introduction

Tumor-Infiltrating Lymphocyte (TIL) Therapy demonstrated promising results in a number of clinical studies which paved the way to FDA approval of the first TIL-based drug, Amtagvi as well as the decision of Dutch and Danish governments to reimburse TIL treatment provided by dedicated hospitals. Despite these encouraging advances, the feasibility of extending TIL therapies into "cold" tumors, such as pancreatic ductal adenocarcinoma or gastrointestinal cancers, remains to be determined. The success rate for TIL manufacturing from colder tumors is quite low, while successful expansion might often yield tumor-unspecific or highly exhausted product leading to the lack of a therapeutic response. In the current study we hypothesized that increasing purity of reactive TIL population prior to expansion would preserve tumor-reactive TCR clones and also potentially improve the TIL phenotype, by limiting the presence of inhibitory signals coming from the tumor cells present during TIL outgrowth (Figure 1). To achieve this, we performed a two-step selection, first magnetically enriching CD4+ and CD8+ T cells followed by a GMP-like MACSQuant® Tyto® based flow sorting for CD45, CD4 & CD8, as well as CD137.

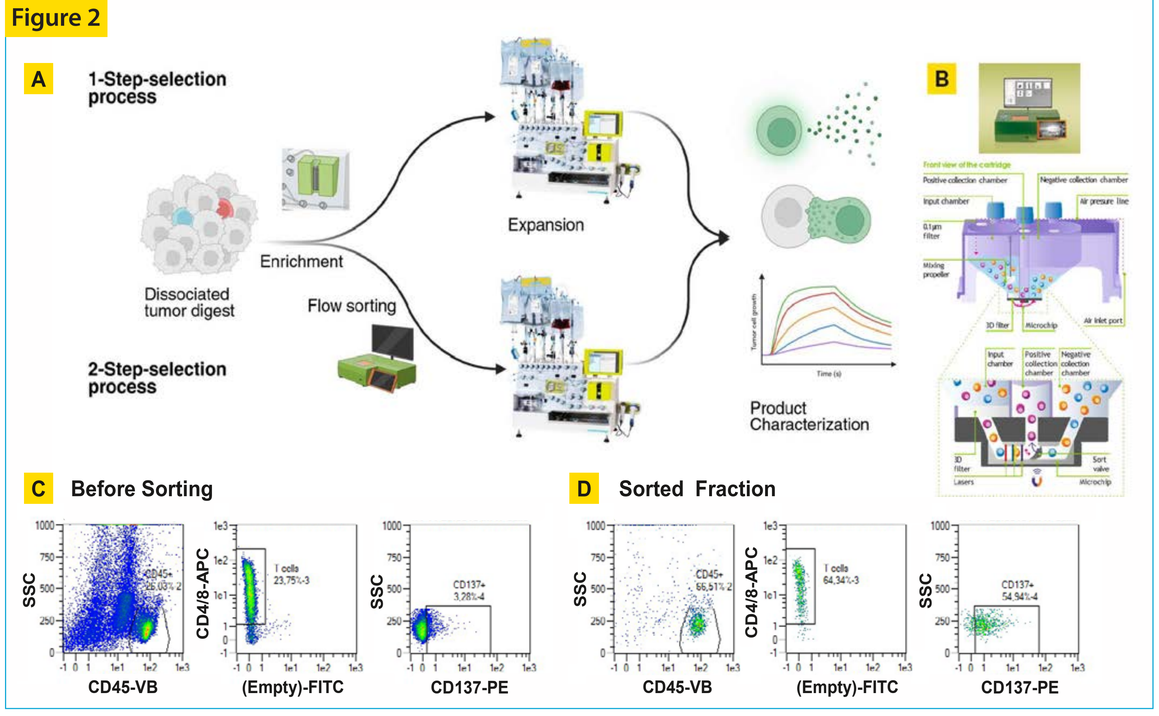
Figure 1



Methods & Results

1 Experimental Setup

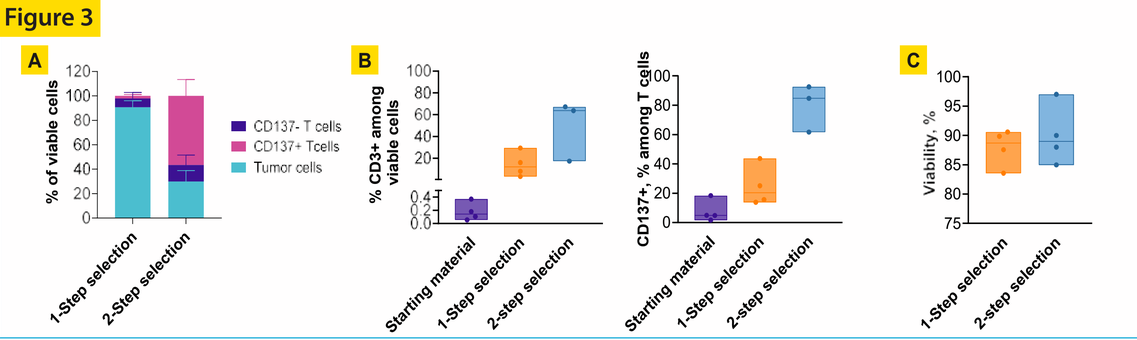
Figure 2



(A) Experimental workflow. Tumor material from glioblastoma patients was processed mechanically dissected with GentleMACS™ Octo Dissociator with Heaters in the presence of an enzymatic cocktail (Tumor Dissociation Kit, Miltenyi Biotec) to generate single-cell suspensions. Processed tumor digests were labeled with CliniMACS® CD4 and CD8 reagents, followed by magnetical enrichment of the T cells on a CliniMACS Prodigy column. Enriched cells were either used for REP (rapid expansion protocol) initiation, or further sorted with the MACSQuant® Tyto® Cell Sorter in a MACSQuant® Tyto® Cartridge HS (B) and expanded thereafter. The final TIL products were phenotypically characterized and functionally tested in co-culture with autologous tumor cells. (C-D) Gating strategy with representative plots before (C) and after (D) sorting.

2 MQ Tyto Sorting yields highly pure and viable TIL

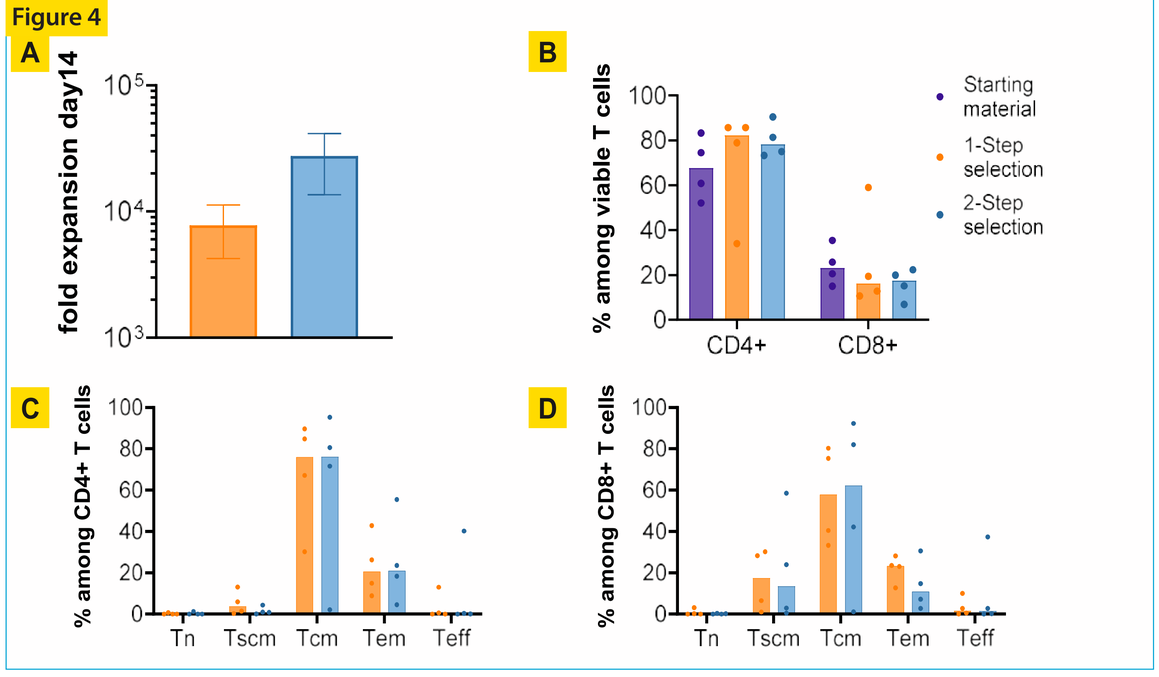
Figure 3



For MACSQuant® Tyto® Cell Sorting pre-enriched cells were labeled with CD45-, CD4-, CD8- and CD137- antibody fluorochrome conjugates. (A) Cell composition of the products following 1-Step or 2-Step selection. (B) Left: Median CD3 purity after pre-enrichment (15%) and enrichment-sorting combination (65%). Right: Median CD137+ percentage among T cells after pre-enrichment (20%) and enrichment-sorting combination (85%). (C) T cell viability following two different selection strategies.

3 2-Step selected TIL show high expansion rates and comparable phenotype to TIL enriched in 1 step

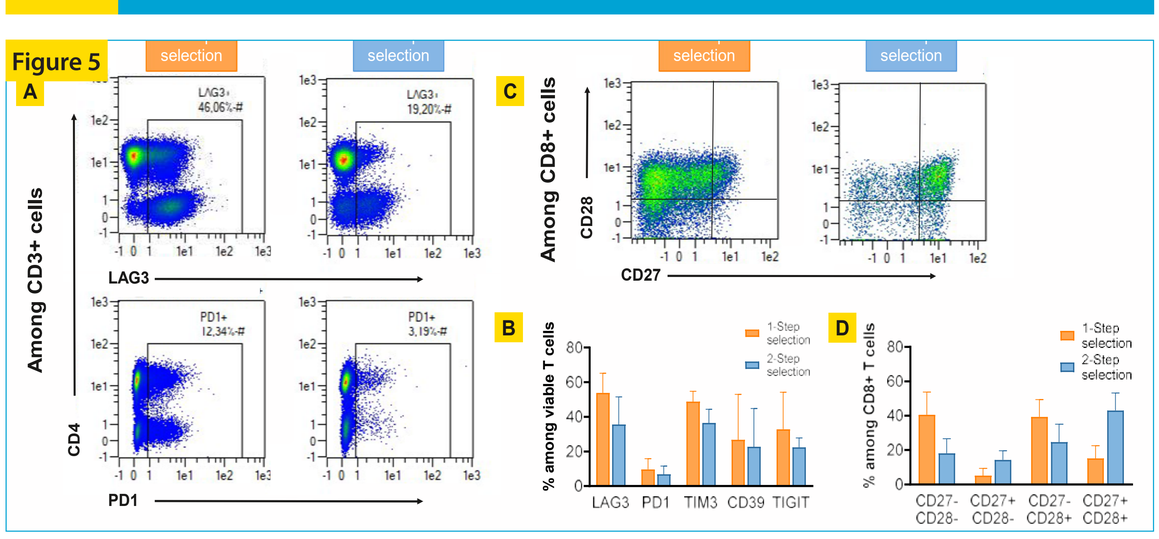
Figure 4



(A) Expansion rates following 14 days in automated culture. 1-Step selected TIL expanded up to 15000 fold, 2-Step selected TIL expanded up to 58000 fold. (B) CD4+ and CD8+ T cell fractions in the starting material and final products following the two protocols. (C, D) T cell memory subsets in the TIL products based on CD62L, CD45RA and CD95 marker expression.

4 2-Step selection strategy leads to expansion of less exhausted cells

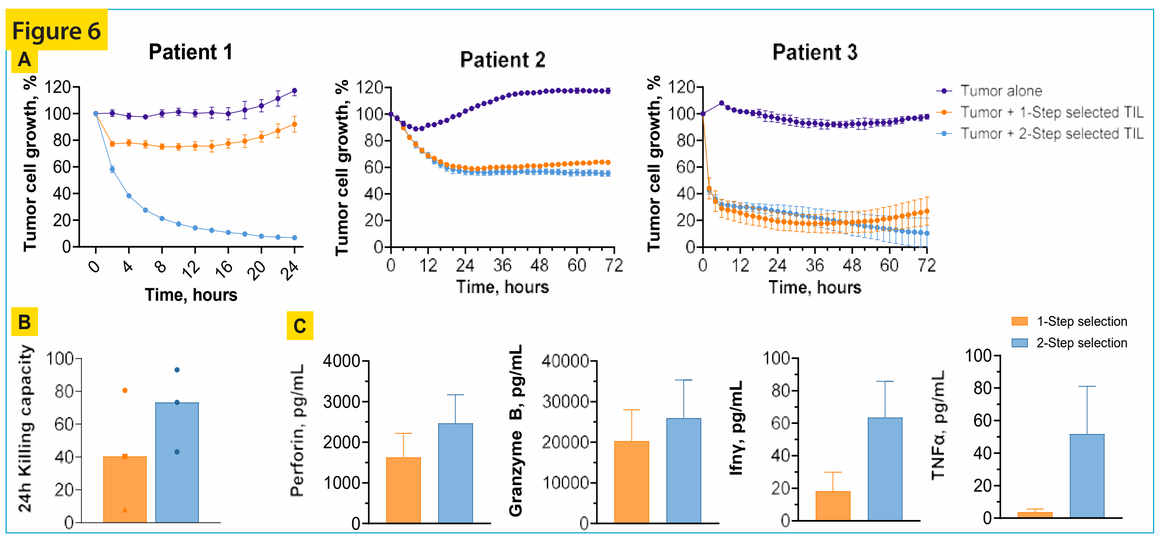
Figure 5



(A-B) Representative LAG3 and PD1 stainings from the products of manufactured 1-Step selected and 2-Step selected TIL (A) and quantification of exhaustion marker stainings (B). (C-D) Representative stainings for CD27 and CD28 memory markers (C) and quantification of the stainings (D).

5 Optimized selection can improve TIL functionality

Figure 6



Final cell products were restimulated with autologous GBM cell lines and tumor cell killing was assessed in an impedance-based RTCA assay. (A) Real time tumor cell killing dynamics. (B) Quantification of cytotoxicity for the three donors. (C) Cytokine secretion analysis with a MACSPlex Assay.

Summary

1. We developed a workflow enabling a precise selection of tumor-reactive cells, with increased T cell purity in the beginning of expansion
2. Introduction of GMP-like sorting with MACSQuant® Tyto® is feasible for the automated workflow and enables refined sorting of a desired T cell population using multiple markers at a time
3. The optimized protocol involving 2-Step reactive TIL selection yields products with improved phenotype and functionality

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