Identifying functional subpopulations of IFN-γ, TNF-α, Granzyme B secretors and actively killing effector cells to understand heterogeneity in adoptive cell therapy

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Background

Adoptive Cell Therapy (ACT) relies on the infusion of large quantities of cells with varying functionalities. This heterogeneity is difficult to discern, which hinders a broader understanding of relevant and irrelevant effector functions.

By droplet encapsulation of single cells (NK, TILs or CAR T cells) or single-cell pairs (NK:K562, Tumor cells (TC): TILs, Daudi:CAR T Cells) , we reveal the functional heterogeneity in cells used for ACT.

Identifying a high TNF-α secreting subpopulation of T cells

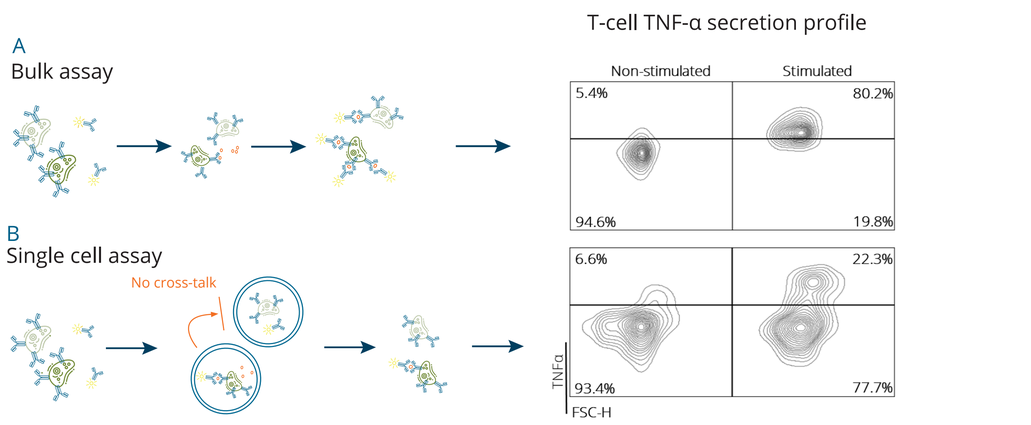

Figure 1: A high TNF-α secretor subpopulation in PBMC-derived T cells was revealed by single-cell analysis (Fig. 1B). This population was masked in bulk assays (Fig. 1A), likely due to crosstalk between cells.

Evaluating the proportion of active killer effector cells

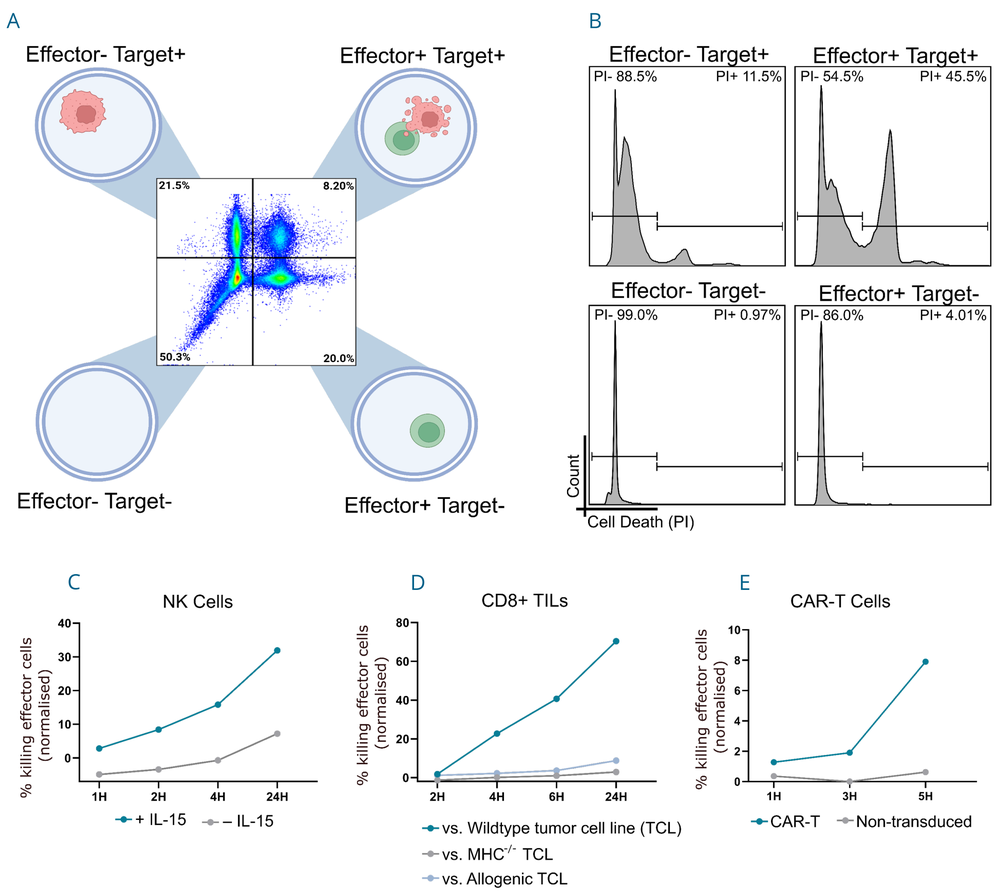

Figure 2: Percentage of killing effector cells was determined by co-encapsulating effector cells (NK, CD8 TILs and CD19 targeting CAR T cells) with target cells (K562, autologous tumor cell line and Daudi cells, respectively (Fig. 2A, C-E). Propidium iodide (PI) was used as a dead cell marker (Fig. 2B), revealing that 30% of NK, 80% of CD8+ TILs, and 8% of CAR T cells were able to kill their respective target cell during the assay.

Conclusion and future perspectives

- A high throughput method is presented to assess the functional heterogeneity of cellular products.
- The results presented here deconvolute masked functions, allowing better characterization and detection of specific subpopulations.
- The possibility to select and isolate cells based on a specific functionality is extremely promising.
- The technology could pave the way for research on rare cell populations and enable the selective expansion of highly potent cells.

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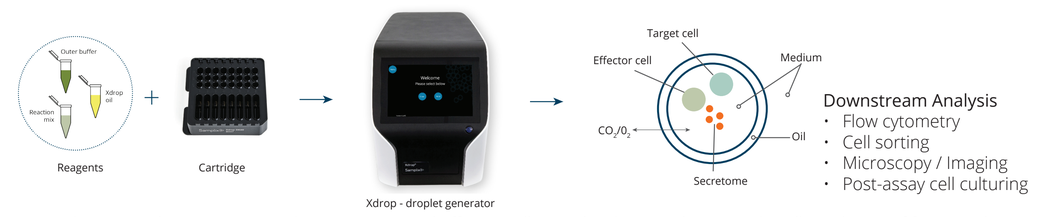
Materials and Methods



Using Xdrop (Samplix) to co-encapsulate 1:1 target and effector cells in a droplet we achieve single cell resolution of functional readouts.

CD19- targeting CAR T cells secrete granzyme B upon target cell recognition

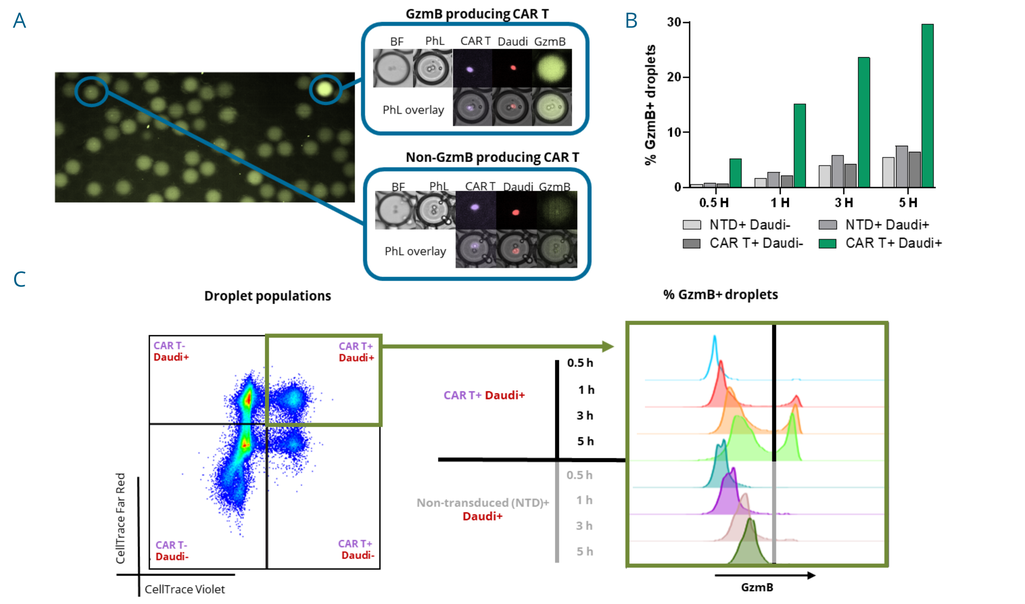


Figure 3: Secretion of Granzyme B by CD19 targeting CAR T cells is detected after just 30 minutes and depend on target cell recognition. The assay reveals the heterogeneity in CAR-T cell response.

Granzyme B secretion succeeds target cell killing

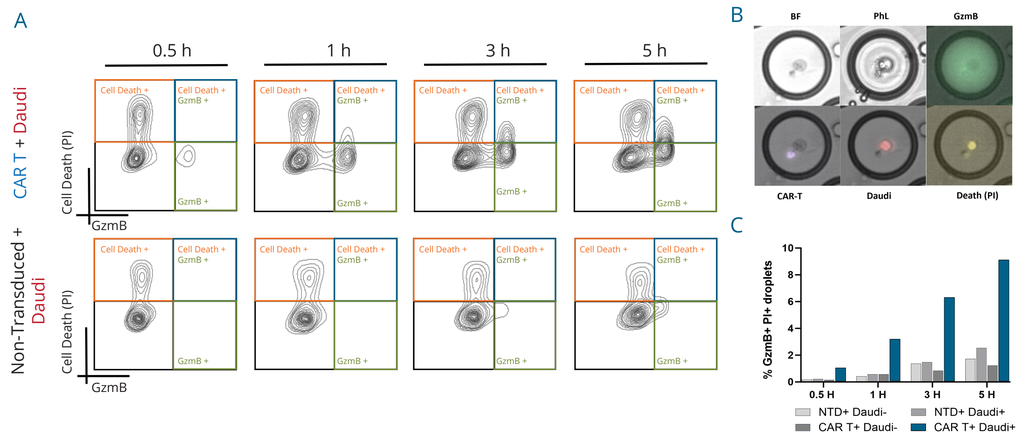


Figure 4: Combining Granzyme B secretion and cell death measurements, revealed that granzyme B secretion from CD19 targeting CAR T cells preceded target cell death (Fig. 4A-C) while cell death without granzyme B secretion were negligible, highlighting the importance of granzyme B mediated killing pathway in these cells.

Selection and recovery of fast-killing NK cells

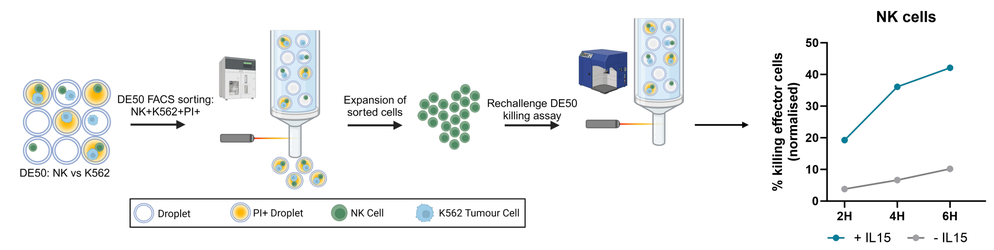


Figure 5: NK and K562 cells were co-encapsulated with the cell death marker PI and droplets positive for the death signal was isolated (FACS), cells recovered and cultured. The fast killing NK cells had retained their phenotype post recovery and expansion.

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