Functional Dissection of Cytotoxic and Non-Cytotoxic Cellular States of a CD19 CAR T Infusion Product from the DAN-CART 1901 Trial Using Xdrop® Co-Encapsulation

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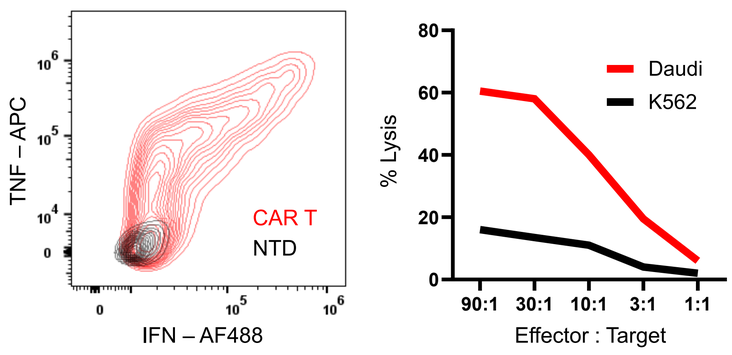
Background

CAR T Functional Heterogeneity

- DAN-CART 1901 (CTIS no: 2024-515174-27-00) is a clinical trial evaluating a second-generation CD19-targeting CAR T cell therapy for patients with acute lymphoblastic leukemia and non-Hodgkin lymphomas.
- Fresh to fresh infusion products are comprised of phenotypically diverse CAR T cells.
- CAR T cell function does not just depend on receptor specificity, but also cellular state, which can significantly impact clinical outcome[1, 2]

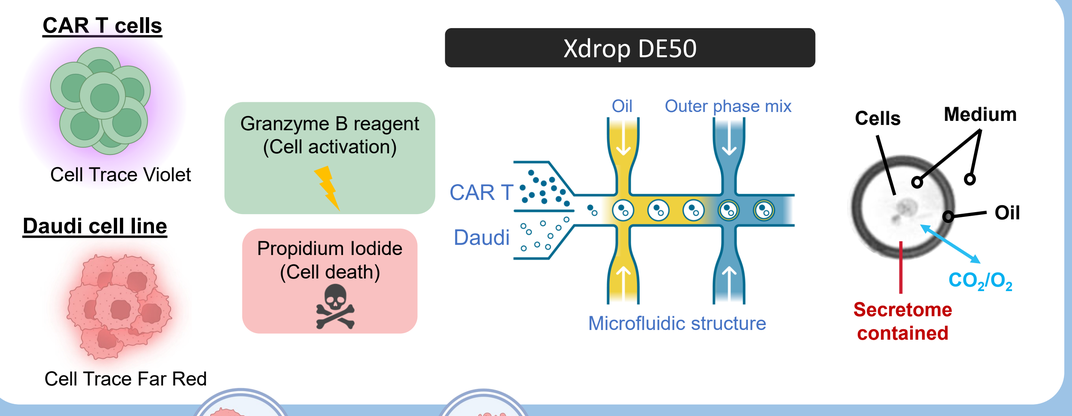


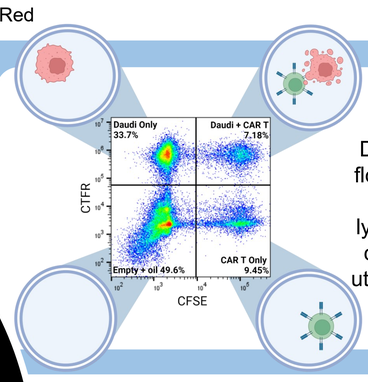
- CAR T functionality confirmed through activation against Daudi cell line (CD19+)
- Non-transduced (NTD) cells showed no reactivity towards Daudi
- K562 cell line (CD19-) does not activate CAR T cells

- Bulk assays cannot resolve which individual CAR T cells mediate tumor killing.
- This limits the ability to link cytotoxic function to transcriptional state, CAR expression, and clonal architecture.

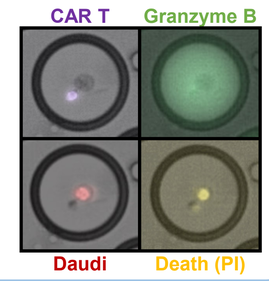
Xdrop DE50 Co-encapsulation

- We previously demonstrated that Xdrop® DE50 enables isolation of individual CAR T-tumor cell interactions in droplets[3].

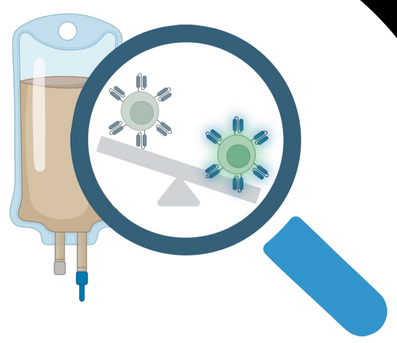




Droplets can undergo flow cytometry assays, microscopy, and be lysed to viably retrieve cells for downstream utilization and analyses



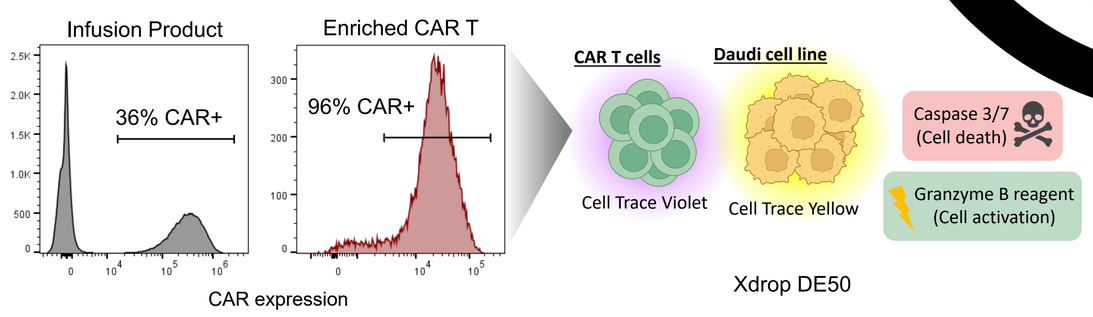
Key Question



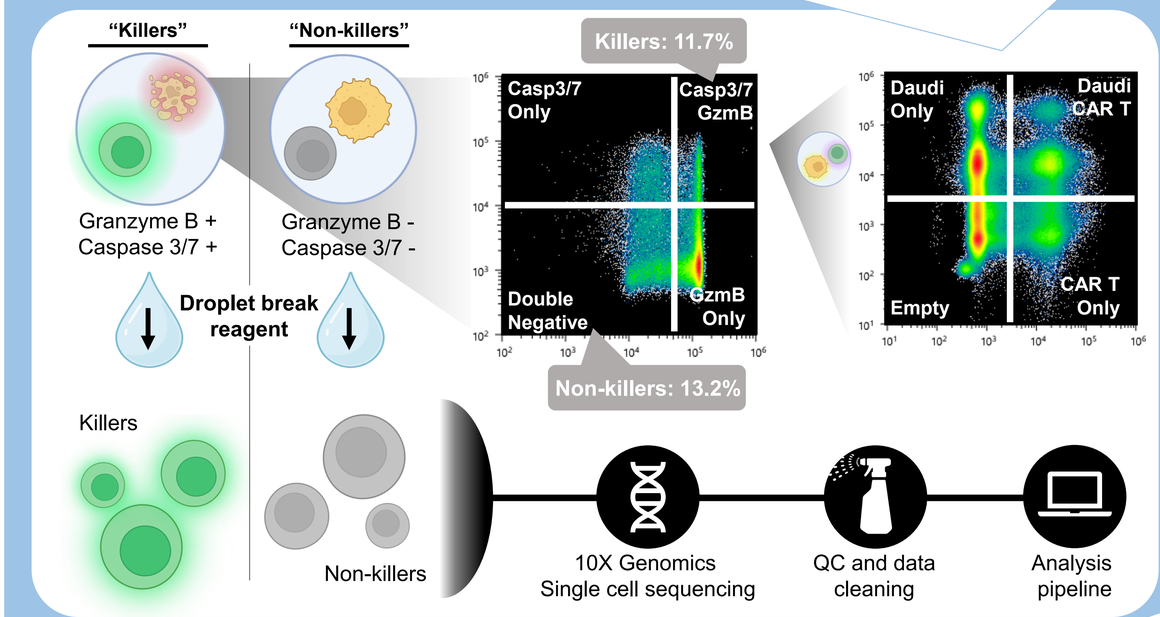
Why are some CAR T cells effective killers and others are not?

Methodology

- One patient with acute lymphoblastic leukemia enrolled in the DAN-CART 1901 trial due to relapse after allogeneic stem cell transplantation
- CAR T cells from the infusion product were enriched based on CAR expression and confirmed via flow cytometry.



Cell Sorting



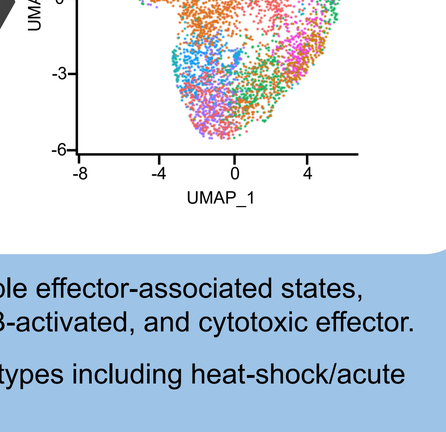
Recovered killer and non-killer fractions were processed for 10x Genomics and single-cell RNA sequencing.

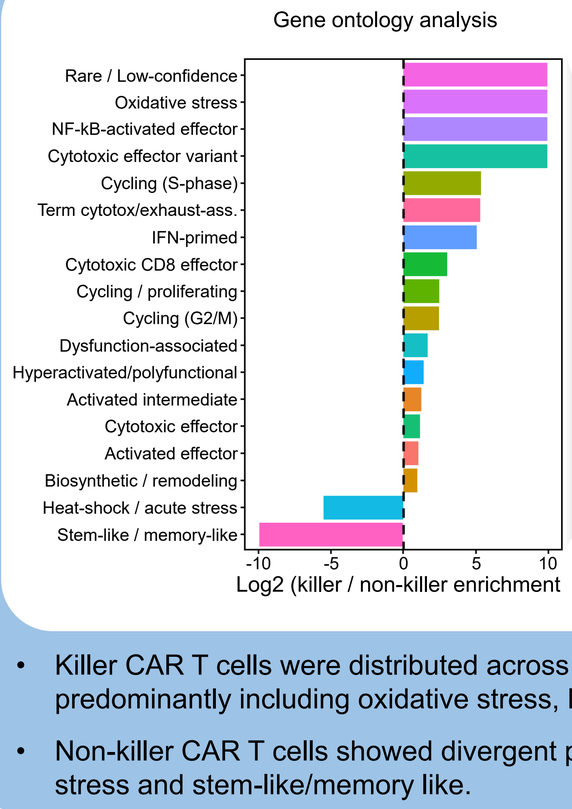
Results

- Enrichment and Xdrop DE50 of CAR T infusion product successfully separated Killer and Non-killer CAR T cells

Final analyzed dataset

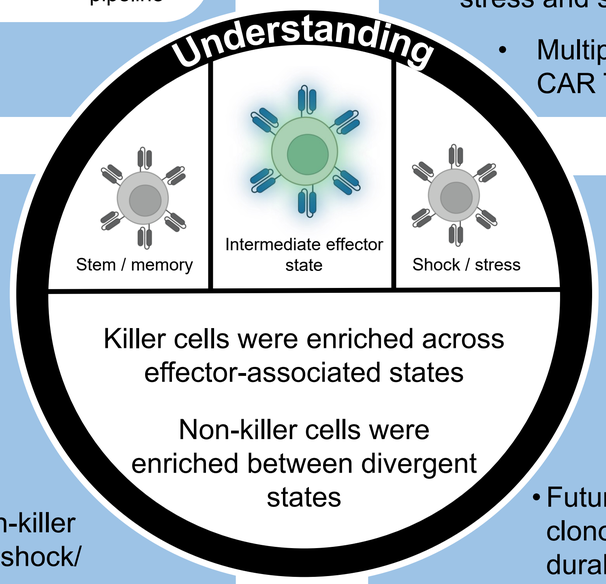
5418 sequenced cells
4172 Killers
1246 Nonkillers





- Killer CAR T cells were distributed across multiple effector-associated states, predominantly including oxidative stress, NF-KB-activated, and cytotoxic effector.
- Non-killer CAR T cells showed divergent phenotypes including heat-shock/acute stress and stem-like/memory like.
- Multiple transcriptional phenotypes are shared by both killer and non-killer CAR T cells, demonstrating the need for further investigation.

Understanding



Summary & Conclusions

- The findings represent one patient and require further validation in additional infusion products.
- Xdrop DE50 enabled direct functional stratification of CAR T cells using granzyme B and caspase-3/7 signals to identify killer and nonkiller CAR T–tumor interactions.
- A subset of CAR T cells displayed no measurable cytotoxic activity, supporting the concept that CAR T cell products contain functionally heterogeneous effector populations.
- Killer-dominant clusters appear to occupy multiple cell states while Non-killer CAR T cells overwhelmingly occupied stem-like/memory-like and heat-shock/ stress-associated programs.
- Together, these data support a state-dependent model of CAR T-cell function, where cytotoxic capacity is potentially shaped by cellular state.

Implications & Future Perspectives

- Identifying killer-associated states may help define CAR T potency biomarkers within infusion products.
- Nonkiller states may reveal targets for CAR T product optimization.
- The analysis will be extended to additional infusion products from the DAN-CART 1901 trial.
- Future work should integrate functional phenotype, transcriptional state, clonotype, CAR expression, and clinical outcome to identify features linked to durable therapeutic efficacy.
- This approach provides a framework for improving ACT products by identifying functional CAR T cells and the programs that distinguish them.

Funding

børne cancer fonden
Danske Regioner

References

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2.Prinzing, B. et al. Deleting DNMT3A in CAR T Cells Prevents Exhaustion and Enhances Antitumor Activity. Sci. Transl. Med vol. 13 https://www.science.org (2021).
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