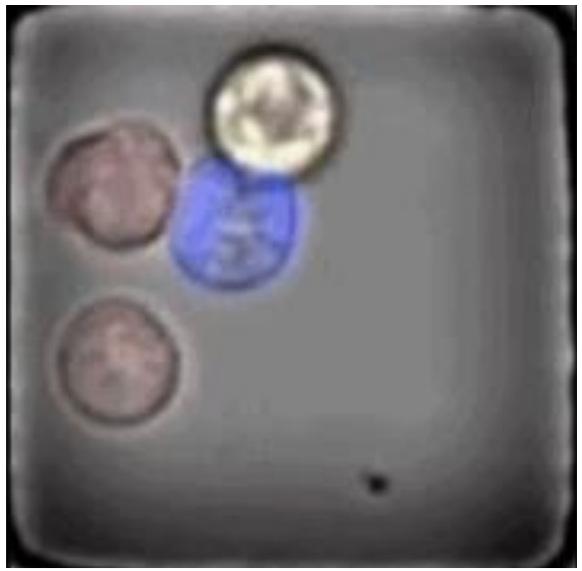
Society for Immunotherapy of Cancer

2019

Designed improvement to T-cell immunotherapy by integrated single cell profiling.

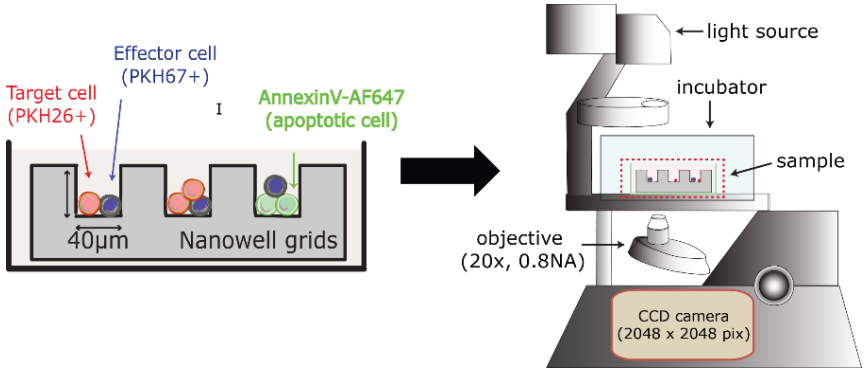


Irfan Naseem Bandey

Goals

Molecular and cellular profiling of T-cell fate and function to enable the design of next generation of immunotherapies.

Aims



Effectors and targets

CD19-specific CAR^{+}

- CD19-IGg4-28TM-CD3z
- CD19-CD8a-CD28-CD3z
- CD19-CD8a-CD28-CD3z-T2A-CD137L

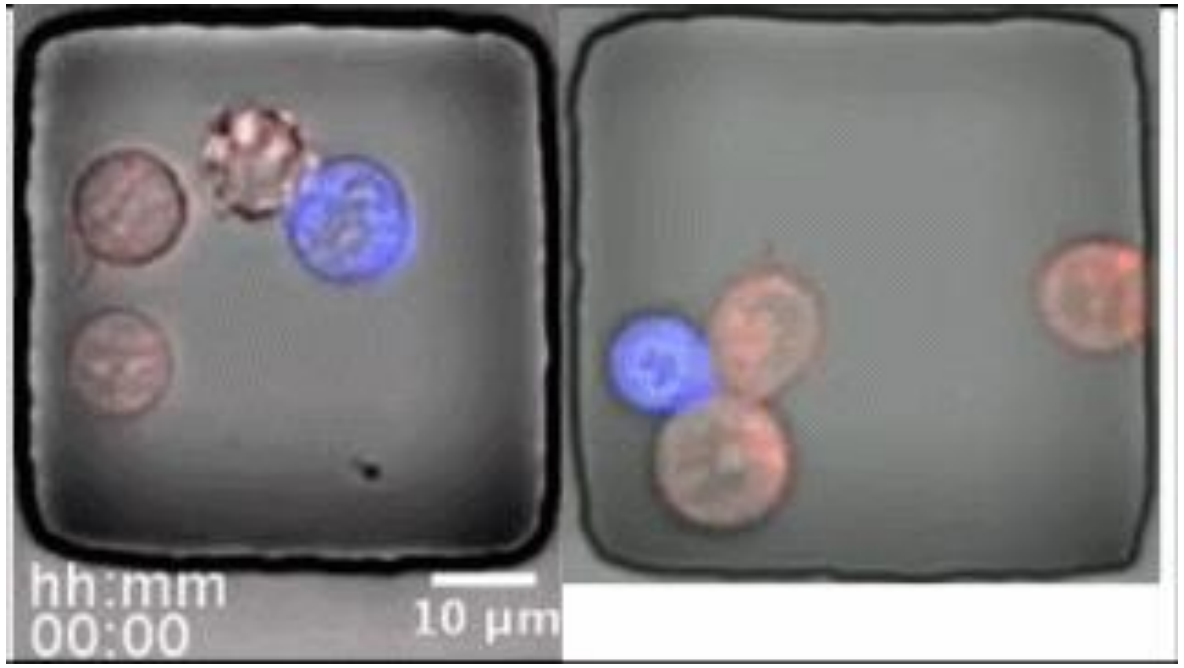
Target Tumors

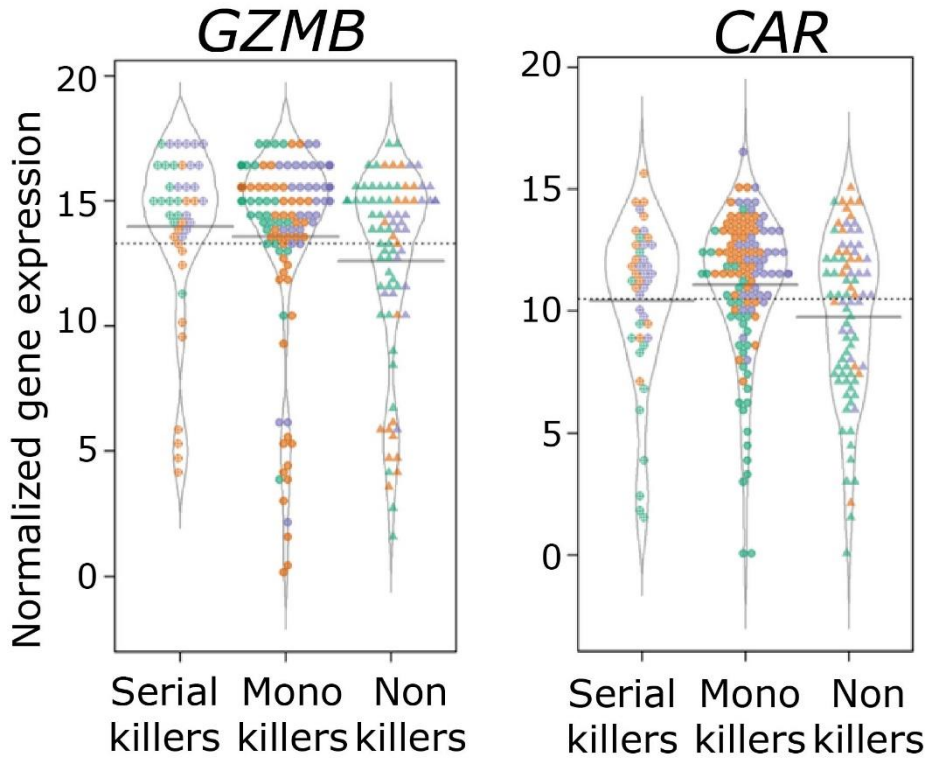
* NALM-6

Mice

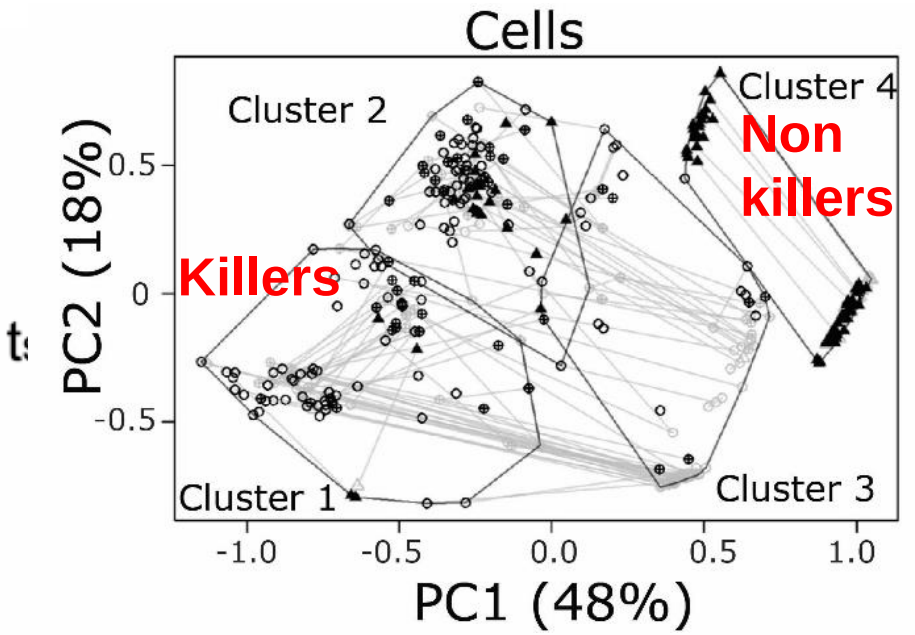
* NOD.Cg-PrkdcscidIl2rgtm1wjl/SzJ (NSG)

CAR and GzmB expression cannot differentiate killers and non-killers.



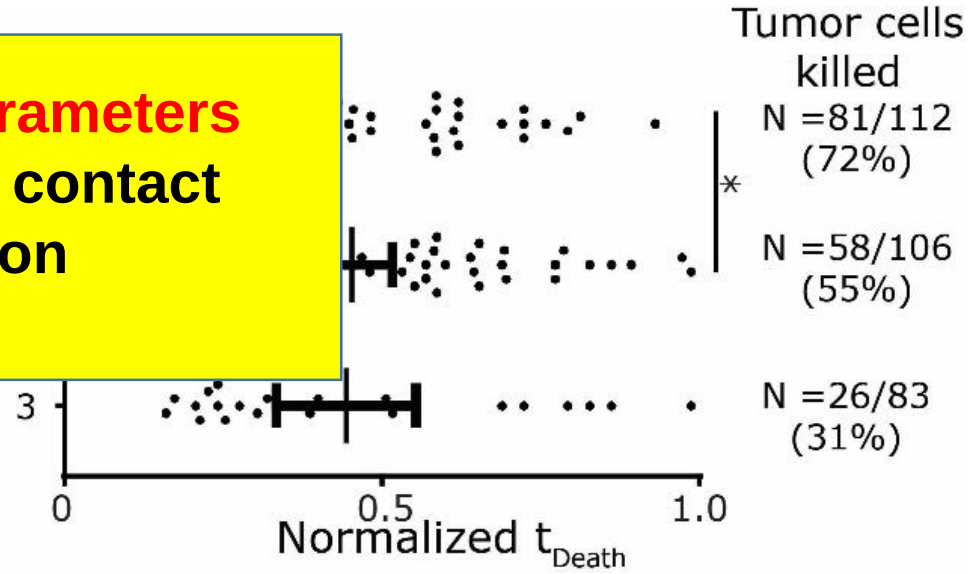


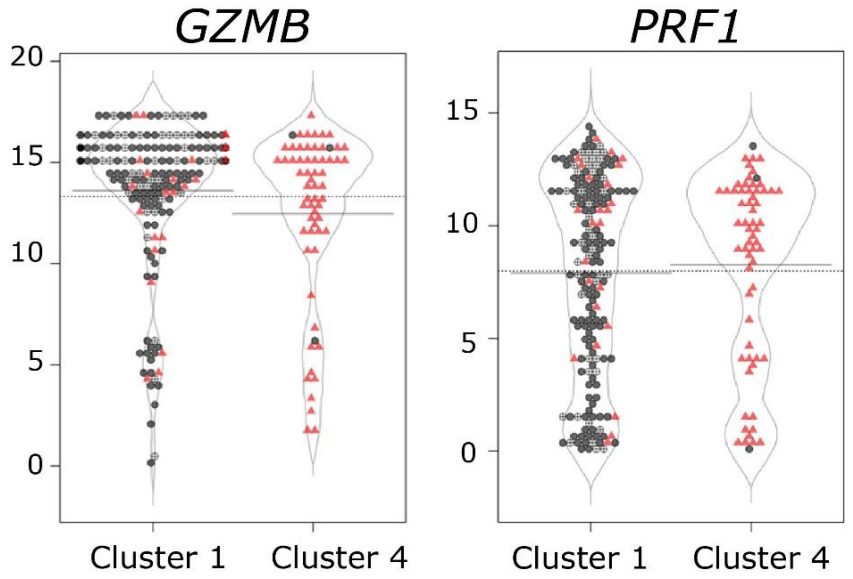
T cells with varying efficiencies of killing could be identified within heterogeneous populations by dynamic profiling.



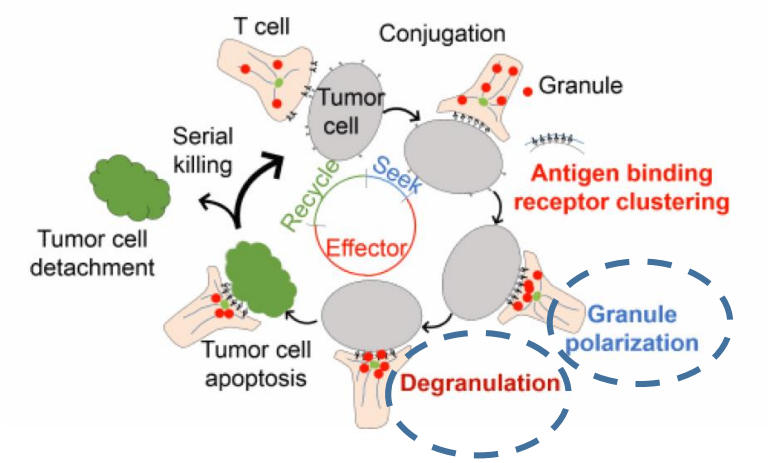
Dynamic parameters

- **Temporal contact**
- **Polarization**
- **Motility**





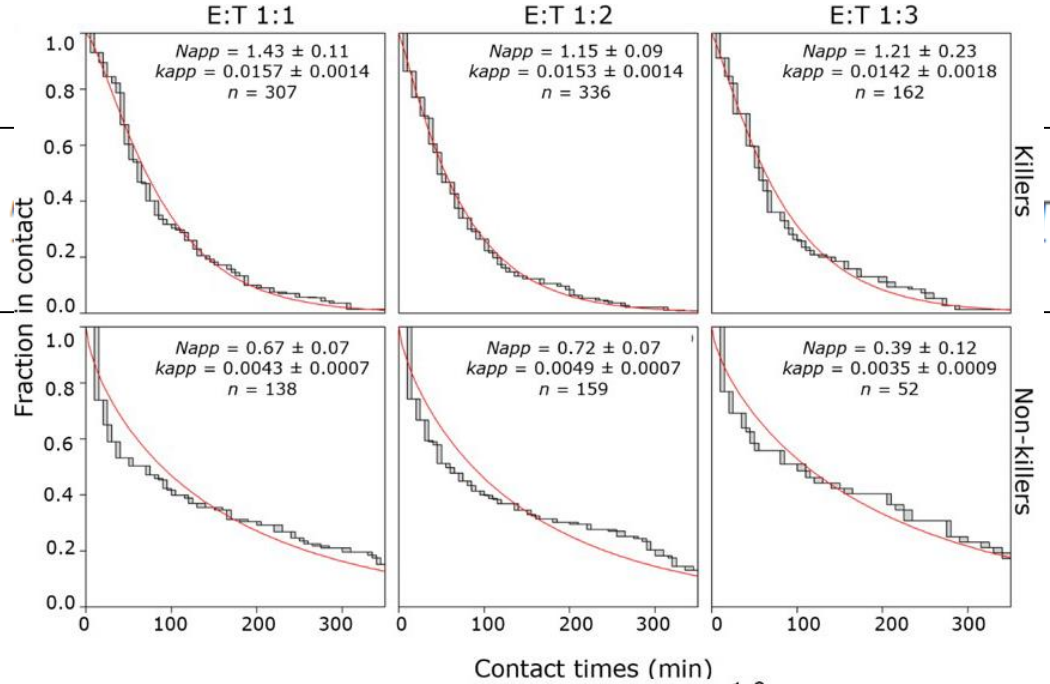
Failure of T cells to kill can arise from failure at multiple steps in lysosome polarization and degranulation prior to killing



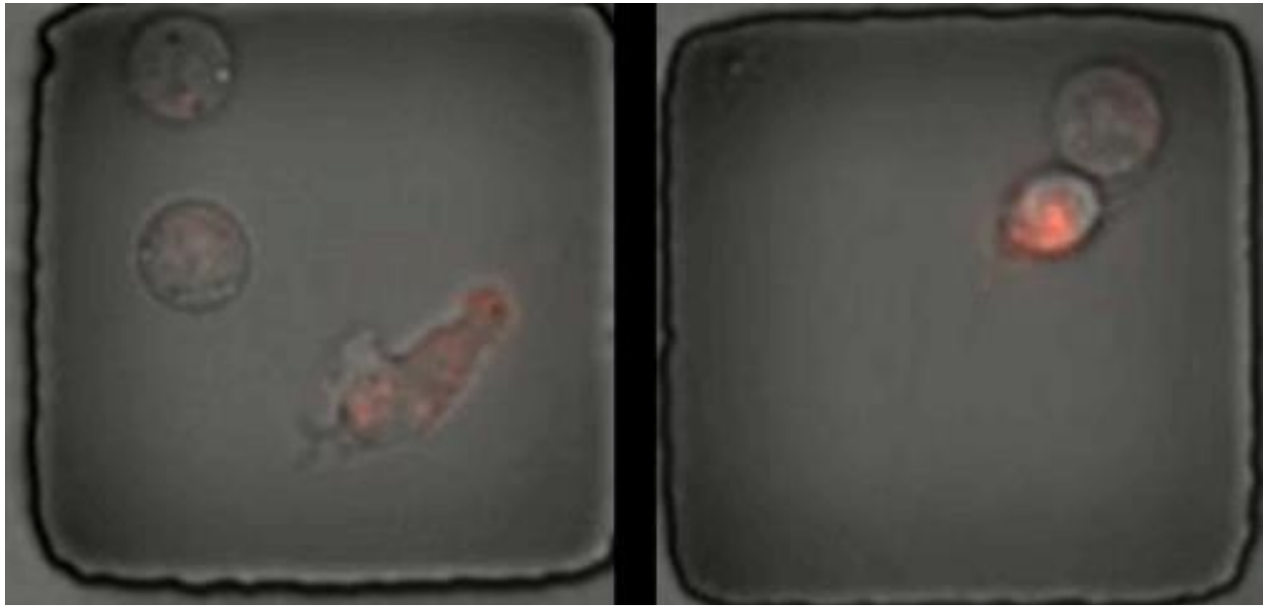
Parameters

- **Receptor ligation and activation**
- **Polarization of the granules towards MTOC**
- **Degranulation, and**
- **Target cell apoptosis**

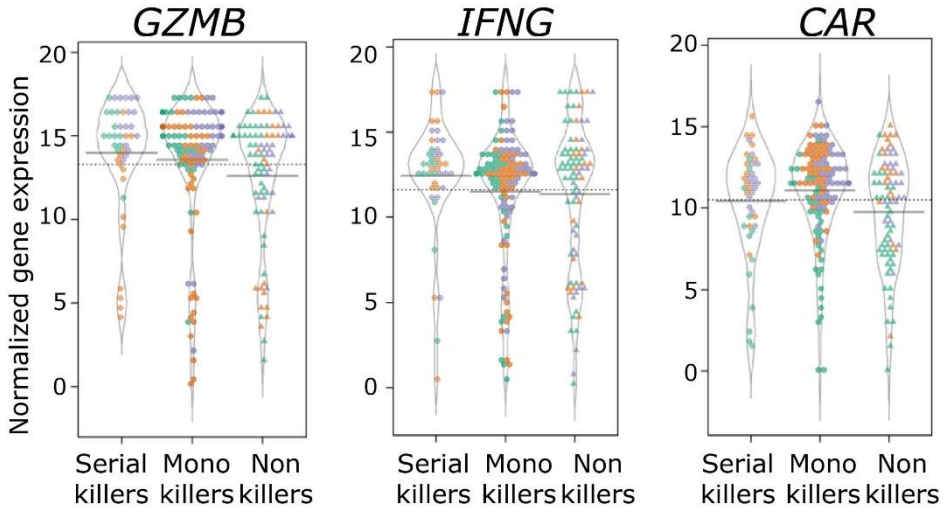
Contact time killers vs non-killers



Lysosomal tracking Killers vs Non-Killers

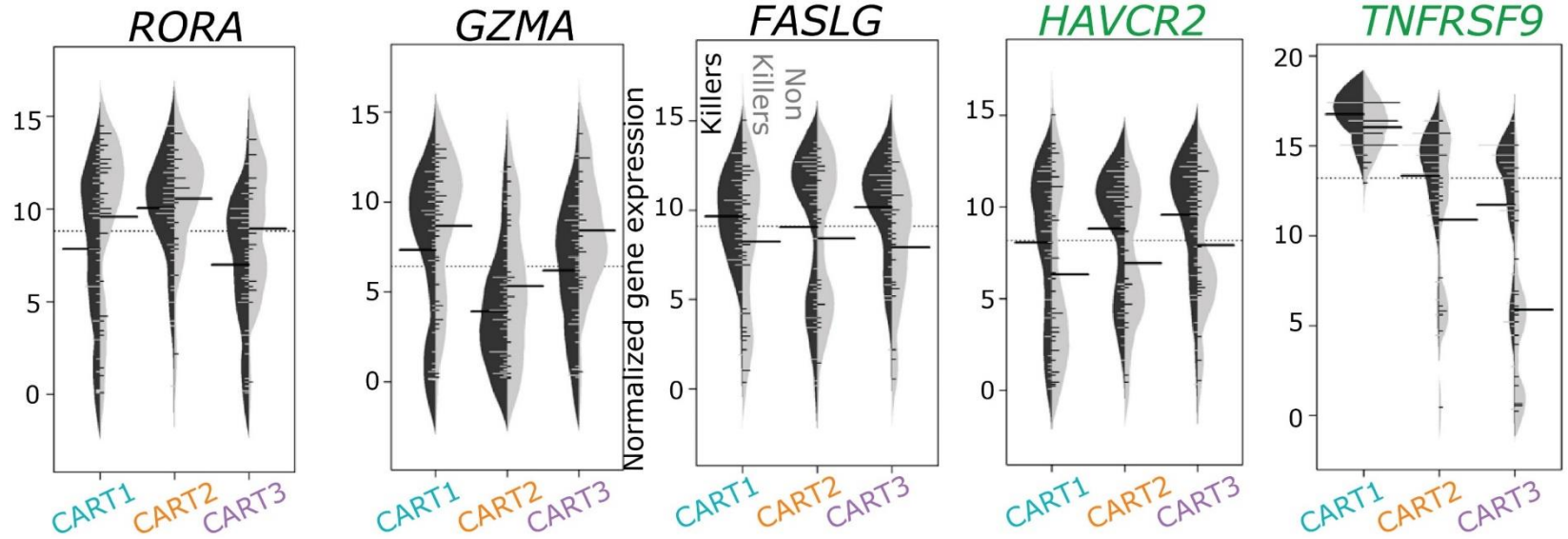


A core set of genes can support functional classifications of killer vs non-killer.

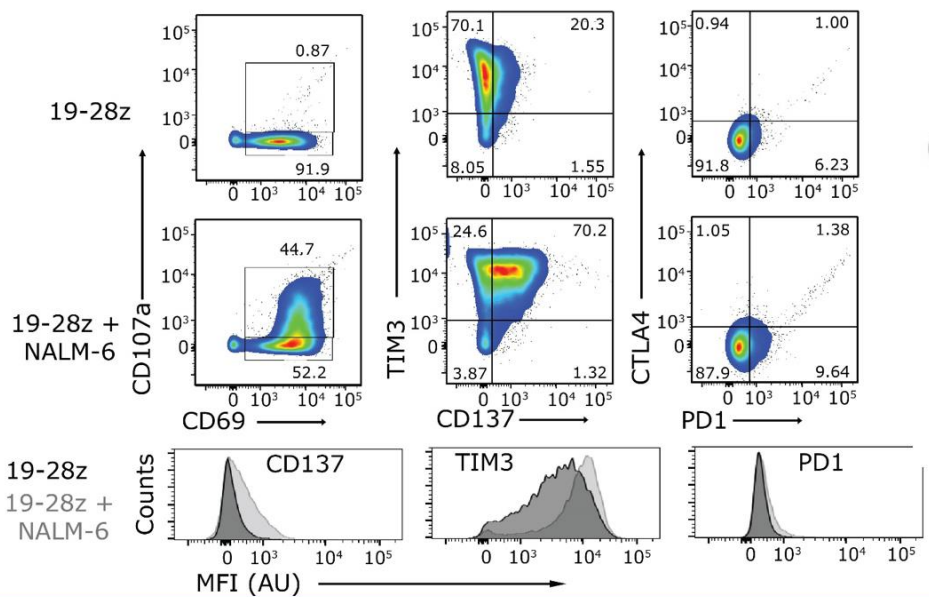


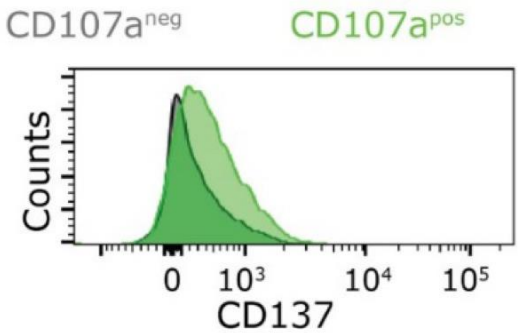
Non-killers T-cells: *RORA* and *GZMA*

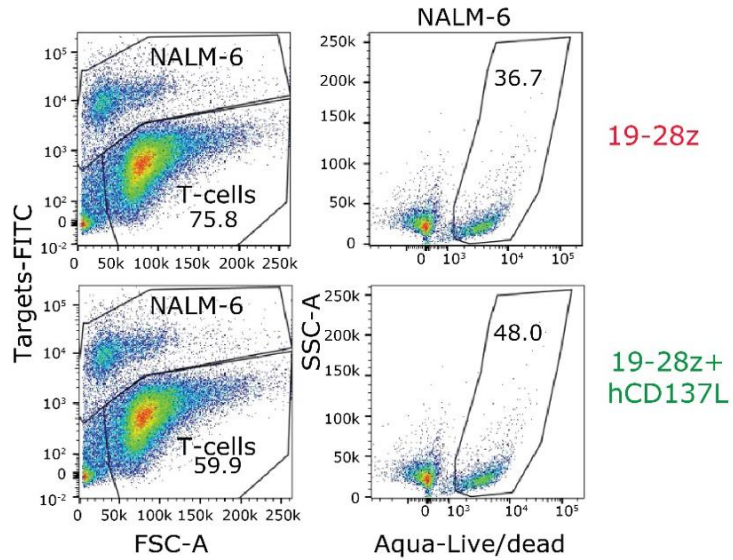
Killer T cells: *FASLG*, *HAVCR2*, AND *TNFRSF9*



Flow-cytometric data validated the induction of CD137 expression within killer T cells.

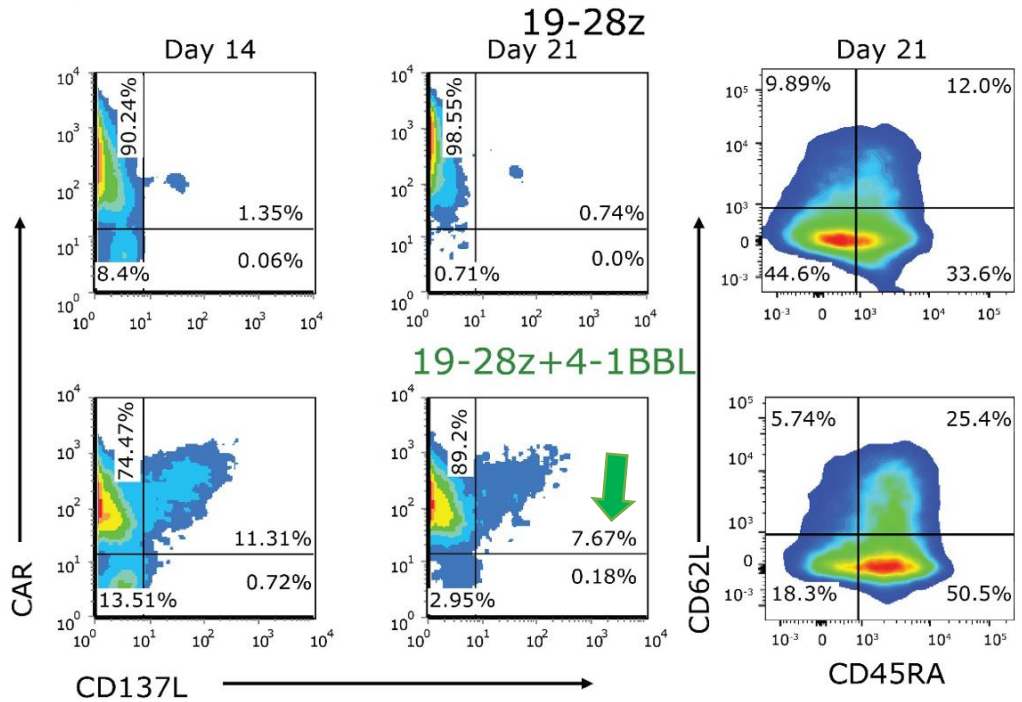


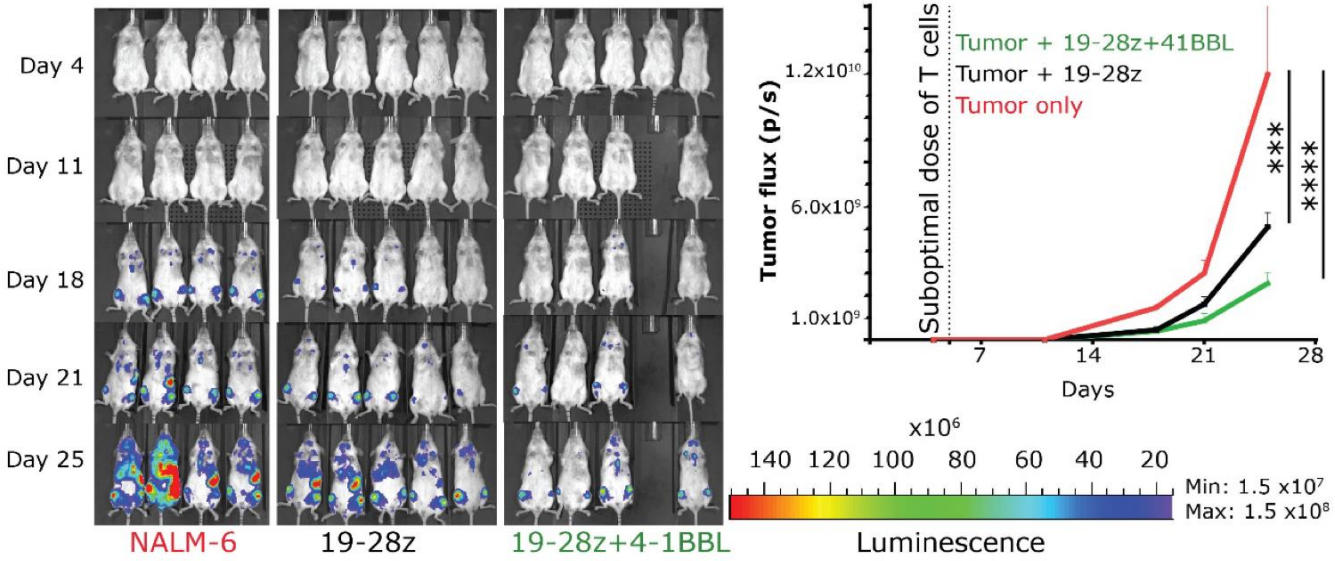


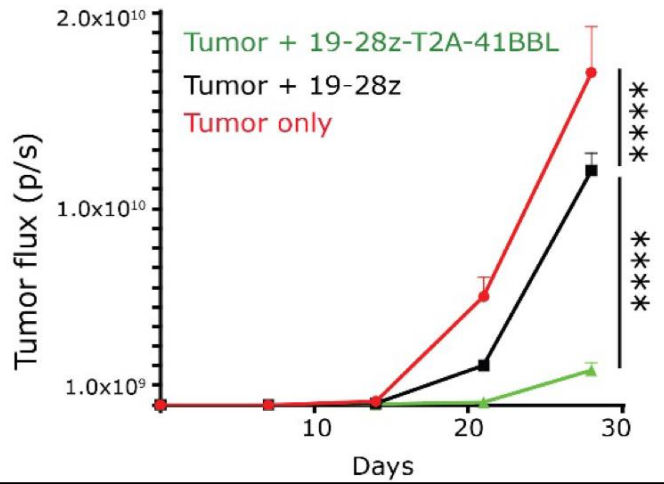


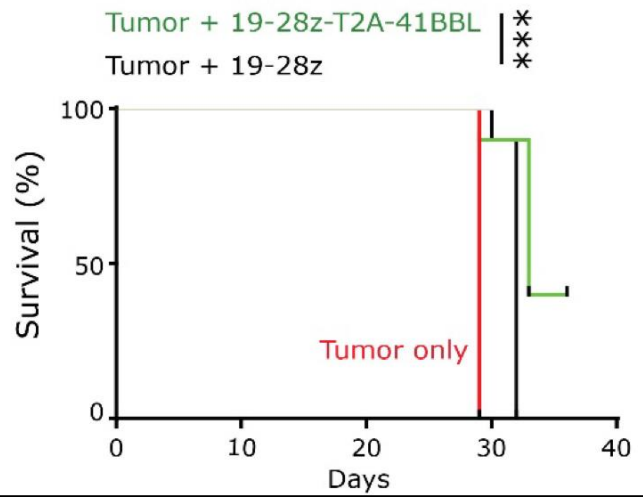
CD137L trimer potentiated serial killing efficiency of CAR T-cells over prolonged time

Genetically engineered CD137L costimulation in 19-28z T cells leads to the expansion of younger cells and enables better control of tumor *in vivo*.









Findings

1. Using TIMING assays, we demonstrate qualitatively different CAR T-cells : serial, mono and non killers.

2. Kinetic modeling and lysosomal polarization experiments illustrate that failed killing events of non-killer T cells resulted in part due to their persistent contact with tumor cells and failure to maintain polarization of lytic granules to immunological synapse.

3. Our mouse xenograft studies show that genetically engineered 19-28z-41BBL T-cells demonstrate better tumor regression in leukemia models, at lower doses compared to 19-28z T-cells.

Significance

- Increasing Granzyme or Perforin expression might not necessarily lead to more killing.

- Inducible expression of CD137 in killer cells could make it a likely candidate for surrogate marker of Killer T cells (in contrast to just an activation marker).

- Overexpression of CD137L in 19-28z CAR T-cells impacted tumor growth in mouse xenograft models. This enhanced CAR design might balance killing and proliferation needed for different tumors. Combined CD28 and CD137L costimulation can balance both cytotoxicity and proliferation.

  Zhao Z *et al.*, Cancer Cell. 2015 Oct 12;28(4):415-428. doi: 10.1016/j.

  Drent E *et al.*, Clin Cancer Res. 2019 Jul 1;25(13):4014-4025. doi: 10.1158/1078-0432.

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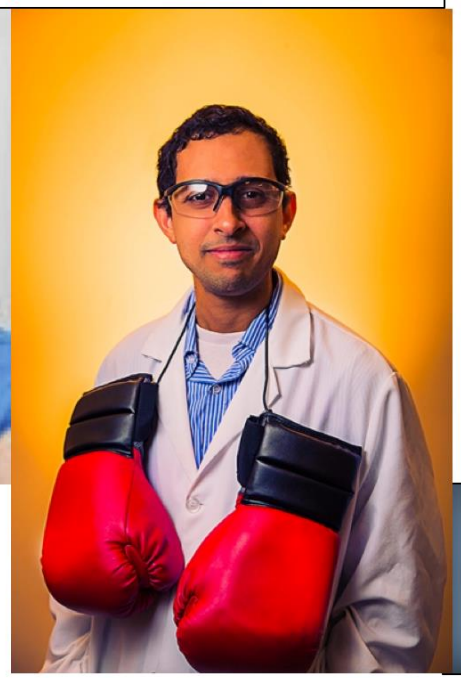
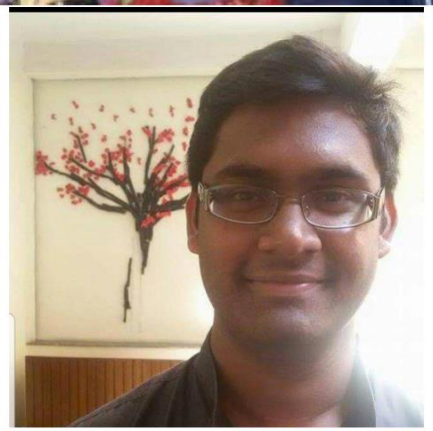


MD Anderson Cancer Center

Harjeet Singh, Tiejuan Mi, Laurence J.N. Cooper, MDACC Flow core



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Thanks

Questions