Society for Immunotherapy of Cancer

T cell-intrinsic and -extrinsic determinants of response to CAR T cell therapy

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Disclosure Information

Jos Melenhorst

I have the following financial relationships to disclose:

Consultant for: Shanghai Unicar Therapy, Simcere Pharmaceutical

Scientific Advisory Board member for: IASO Biotherapeutics

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Patents: Related to T cell engineering, biomarkers

Employee of: University of Pennsylvania, Children's Hospital of Philadelphia

- and -

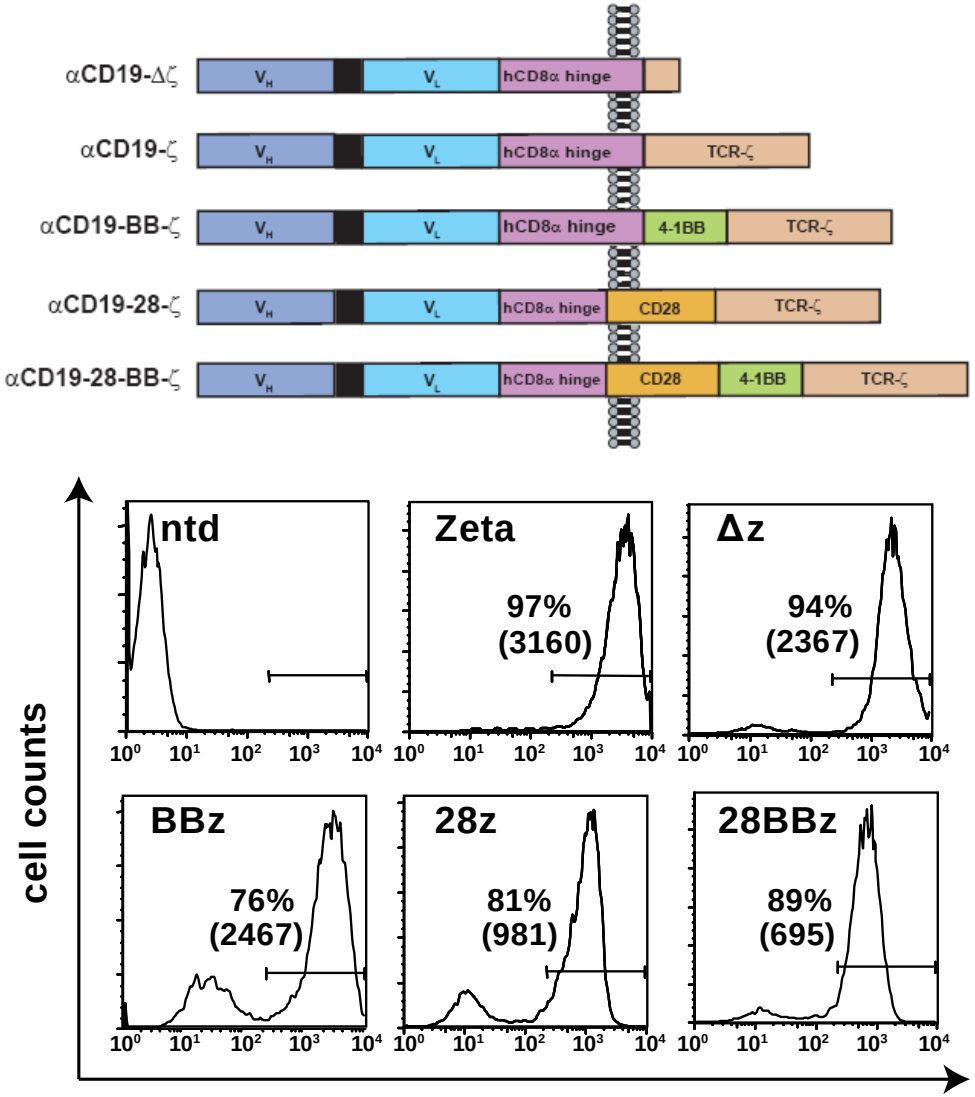
I will discuss investigational use in my presentation: Tisagenlecleucel/Kymriah

CLL Background

- Chronic lymphocytic leukemia (CLL) accounts for 25% of all newly diagnosed leukemias, with 20,940 new cases diagnosed in the US in 2018
- Average at diagnosis: 70 years
- Male:female ratio is 2:1
- Current drug-based therapies e.g.
  - first and second generation Bruton's tyrosine kinase inhibitors (e.g. Ibrutinib),
  - PI3Kδ inhibition (e.g. Idelalisib),
  - Bcl-2 inhibition (e.g. Venetoclax) or
  - antibody-based therapies (e.g. Rituximab; targets CD20)
- ***are not curative and all come with severe clinical and financial toxicities***
- Cell-based therapies, on the other hand, can be curative

ACS 2018 statistics https://www.cancer.org/cancer/chronic-lymphocytic-leukemia/about/key-statistics.html

CAR Costimulatory Domains Do Appear to Influence T Cell Engraftment – mouse data

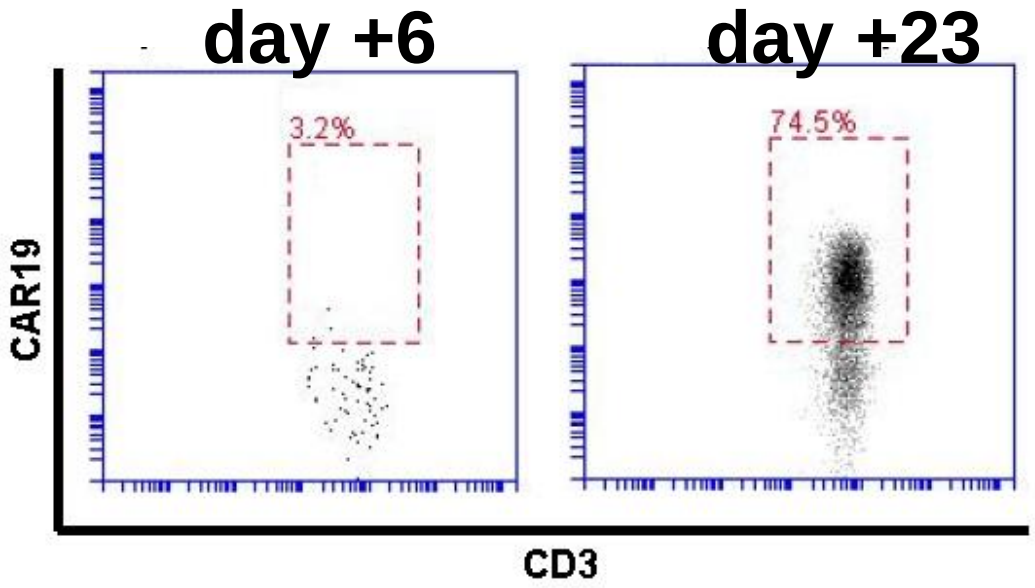


Group	Median Human T-cells/mcL at peak (10/mcL threshold)	Duration of T cell engraftment in peripheral Blood (days)
Mock	26 ± 8	10 ± 4
19-zeta	124 ± 41*	32 ± 5*
19-28-zeta	102 ± 70*	36 ± 5*
19-28-41BB-zeta	327 ± 72*, **	45 ± 3*, **
19-41BB-zeta	6494 ± 1180*, **	35 ± 4*
Meso-41BB-zeta	18 ± 5	7 ± 2
Saline	7 ± 3	0

Carpenito; Milone; Barrett

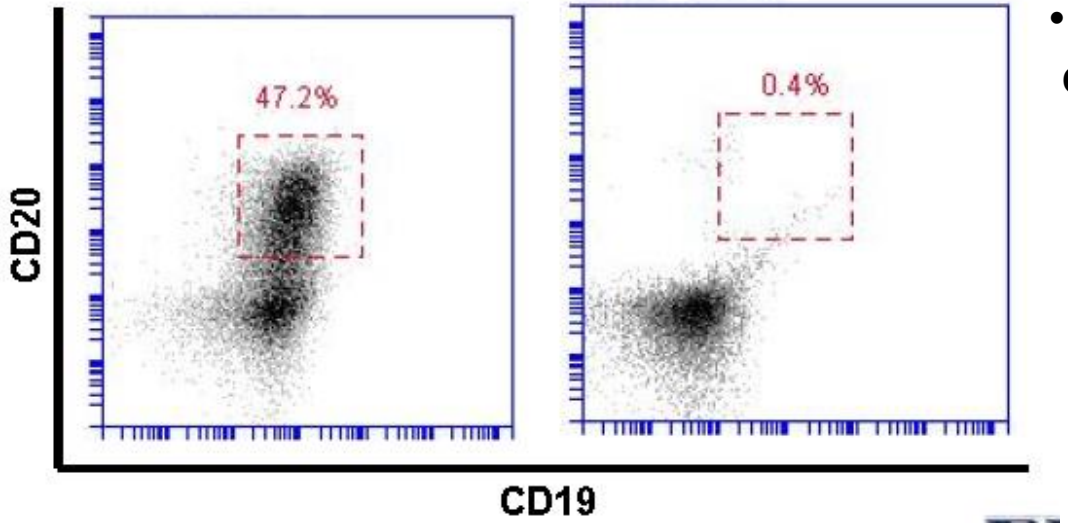
Expansion CAR T Cells & Clinical Efficacy go Hand-in-Hand

T Cells



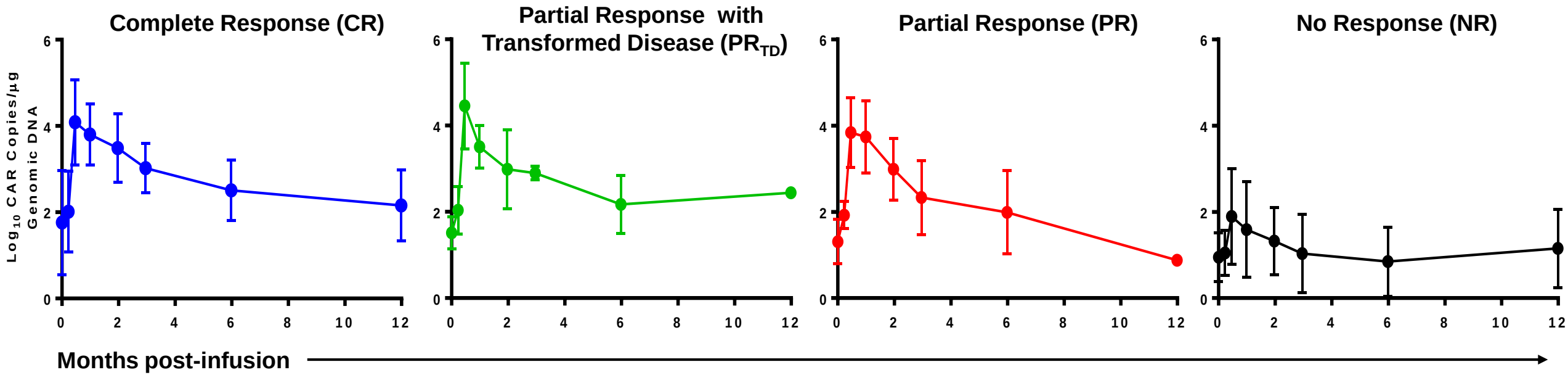
- **Deep remission induced in 23 days**
- **0% blasts seen**
- **flow MRD negative**
- **CR maintained out to 5 mo**

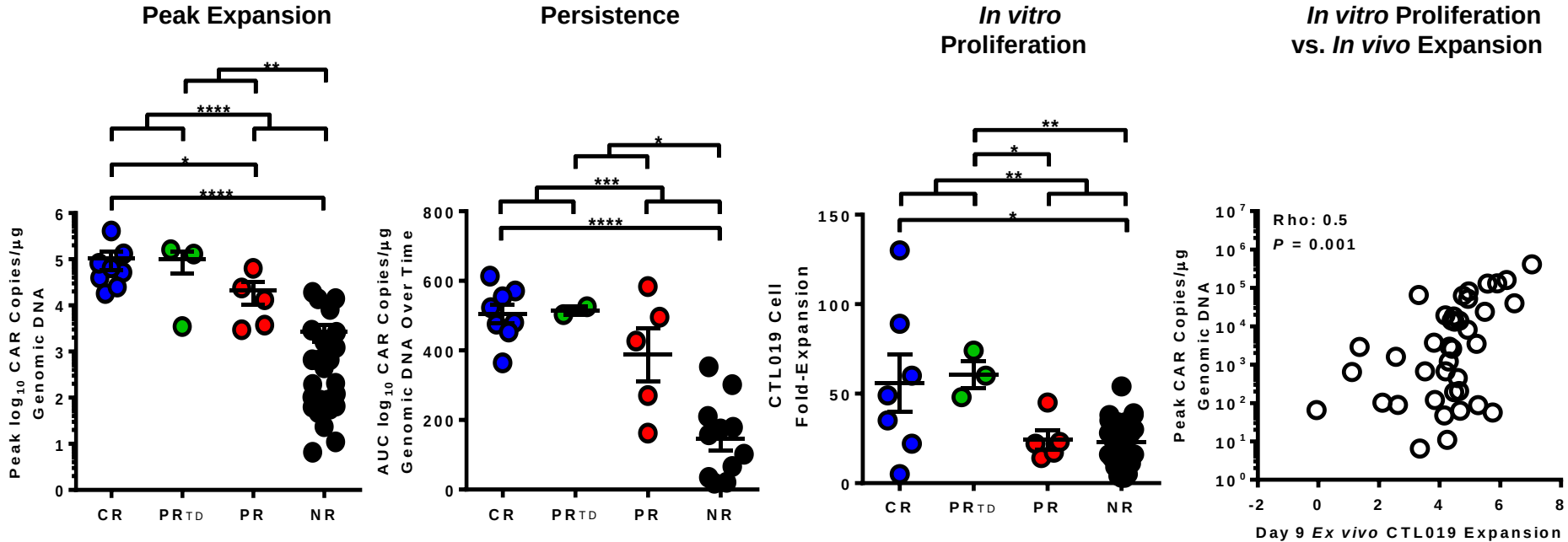
Blasts (ALL)



Grupp et al., 2013, New Engl J Med

Longest Persistence of Functional CAR T-Cells

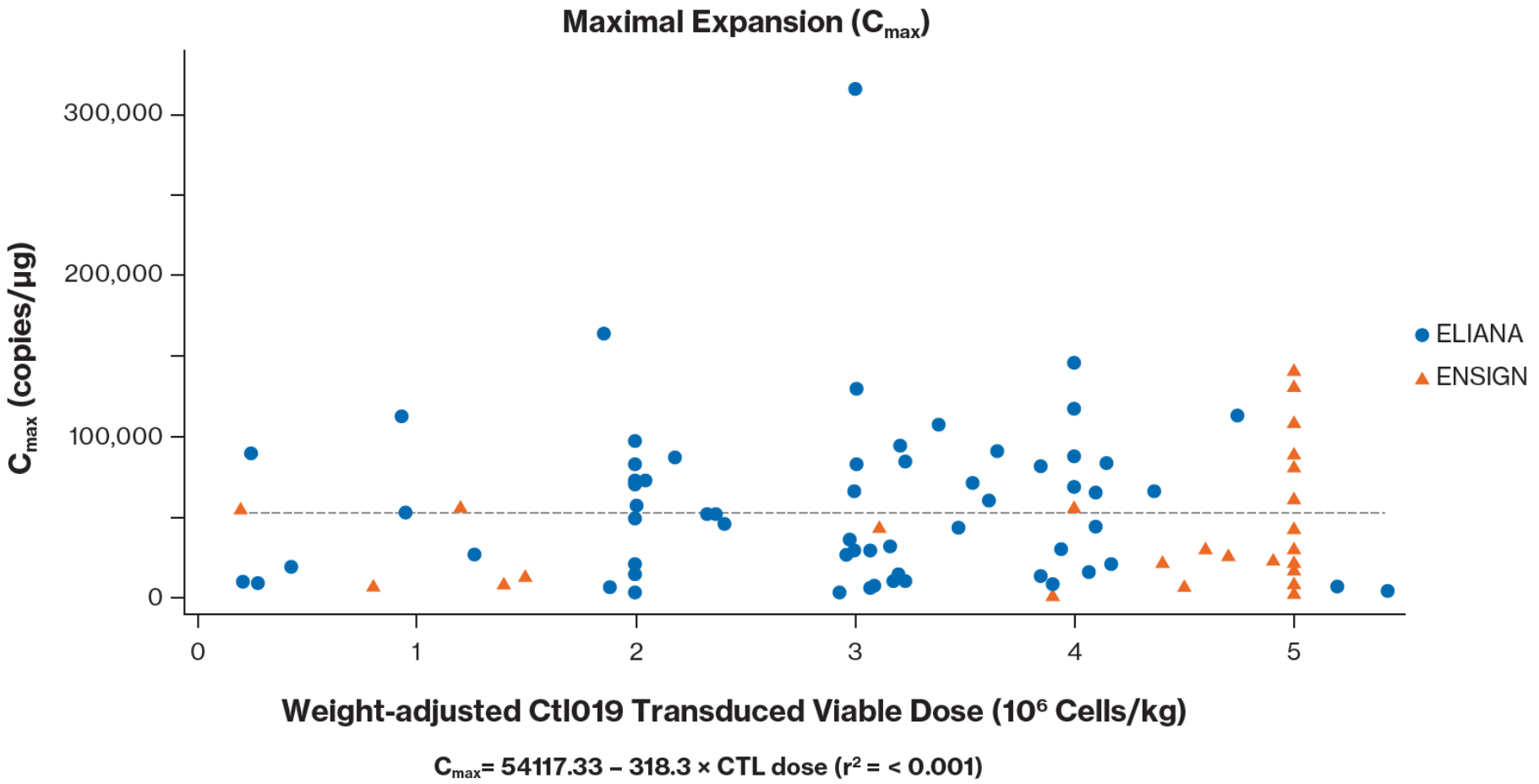




Fraietta et al., *(2018). Nature Med 24:563-571*

Tisagenlecleucel Expansion and Dose

- Across a wide range of doses, in vivo expansion and dose are independent

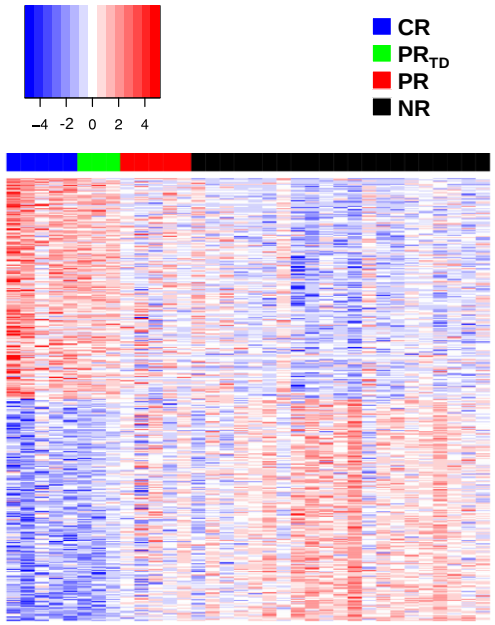


Data pooled from ELIANA AND ENSIGN.

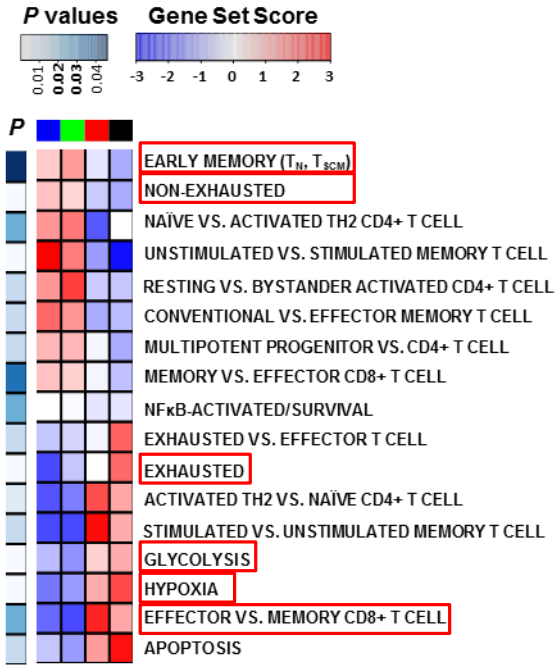
Karen Thudium Mueller, et al. SOHO 2017

Mechanism(s) of Response to CAR T-Cell Therapy

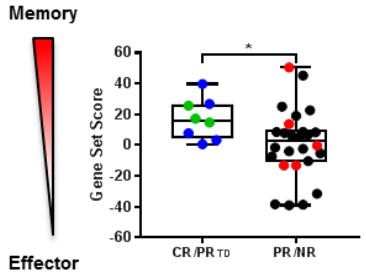
Genes Differentially Expressed

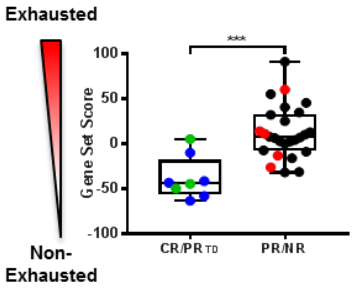


Selected Pathways



GSEA: T cell Memory and Exhaustion

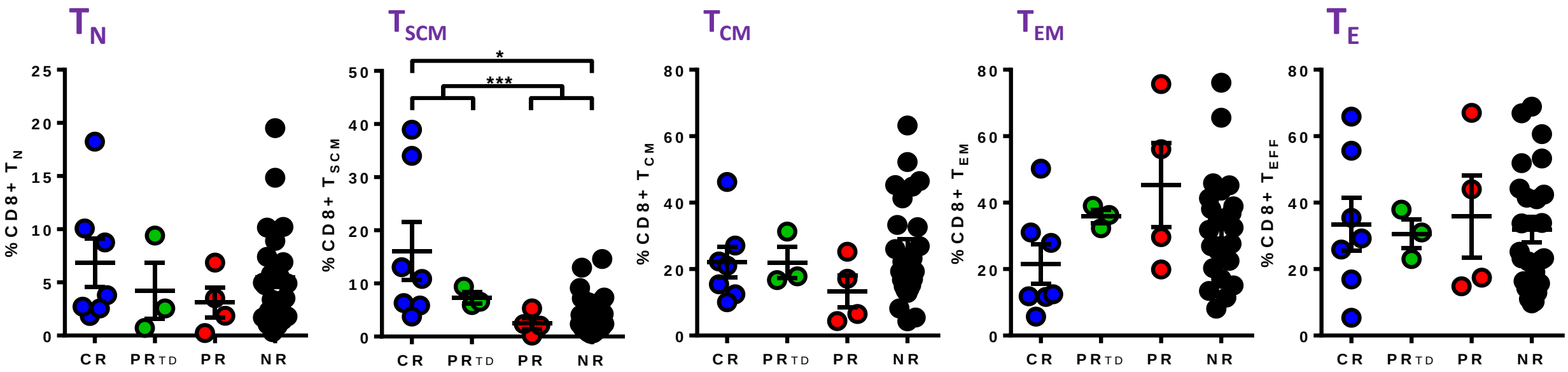




- **Gene expression profiles of CTL019 cells generated from CR and PR$_{TD}$ patients exhibit marked differences compared to those from PR and NR patients**
- **Gene set enrichment analysis (GSEA) revealed that CTL019 cells from CR and PR$_{TD}$ patients were enriched in gene expression profiles involved in early memory differentiation**
- **CTL019 cells from PR and NR patients exhibited increased expression levels of key regulators of late memory cell as well as effector differentiation, pro-apoptotic signaling and exhaustion**

Fraietta et al. *(2018). Nature Med 24:563-571*

Frequencies of Canonical CD8+ T cell Subsets in Pre-manufactured Cells and Response to CTL019

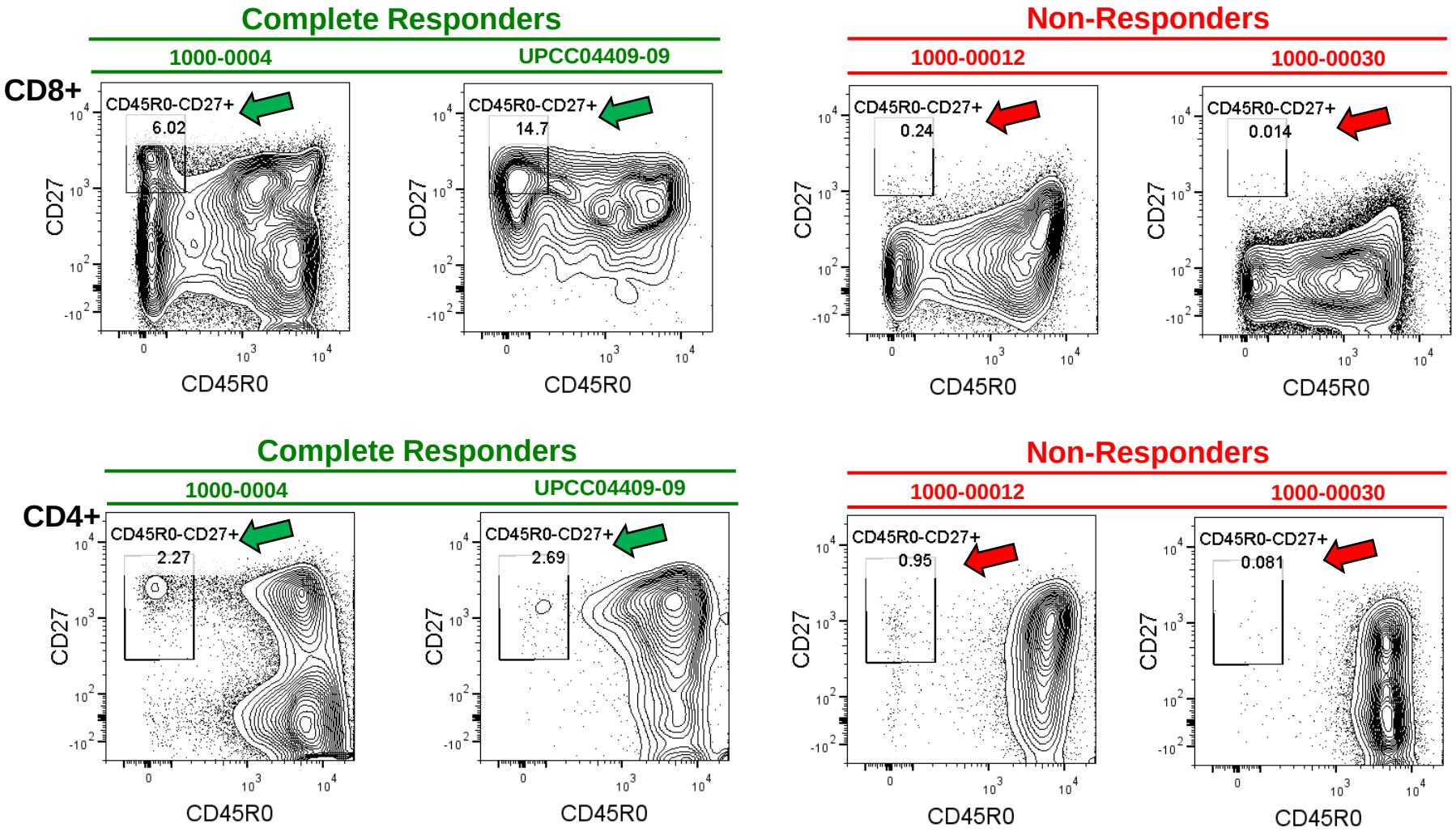


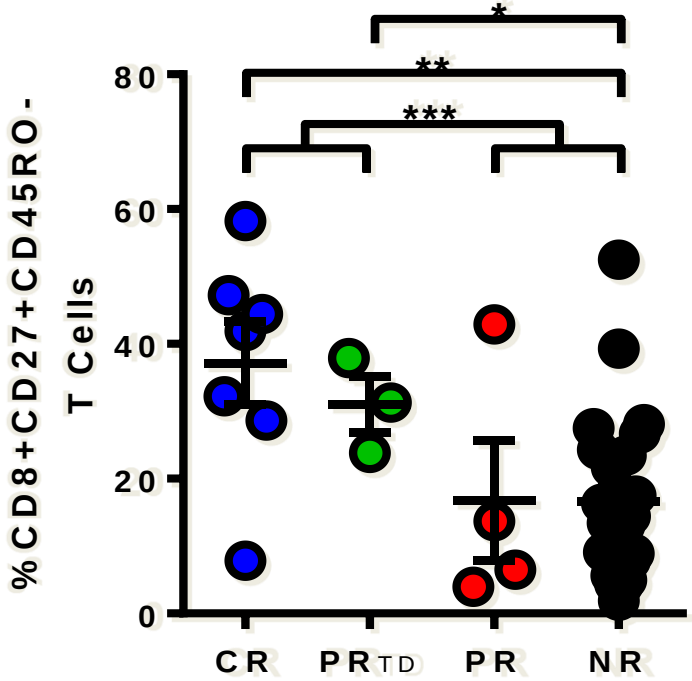
Memory T cell differentiation

Proliferation
Persistence
Memory function

Cytokine production
Target lysis

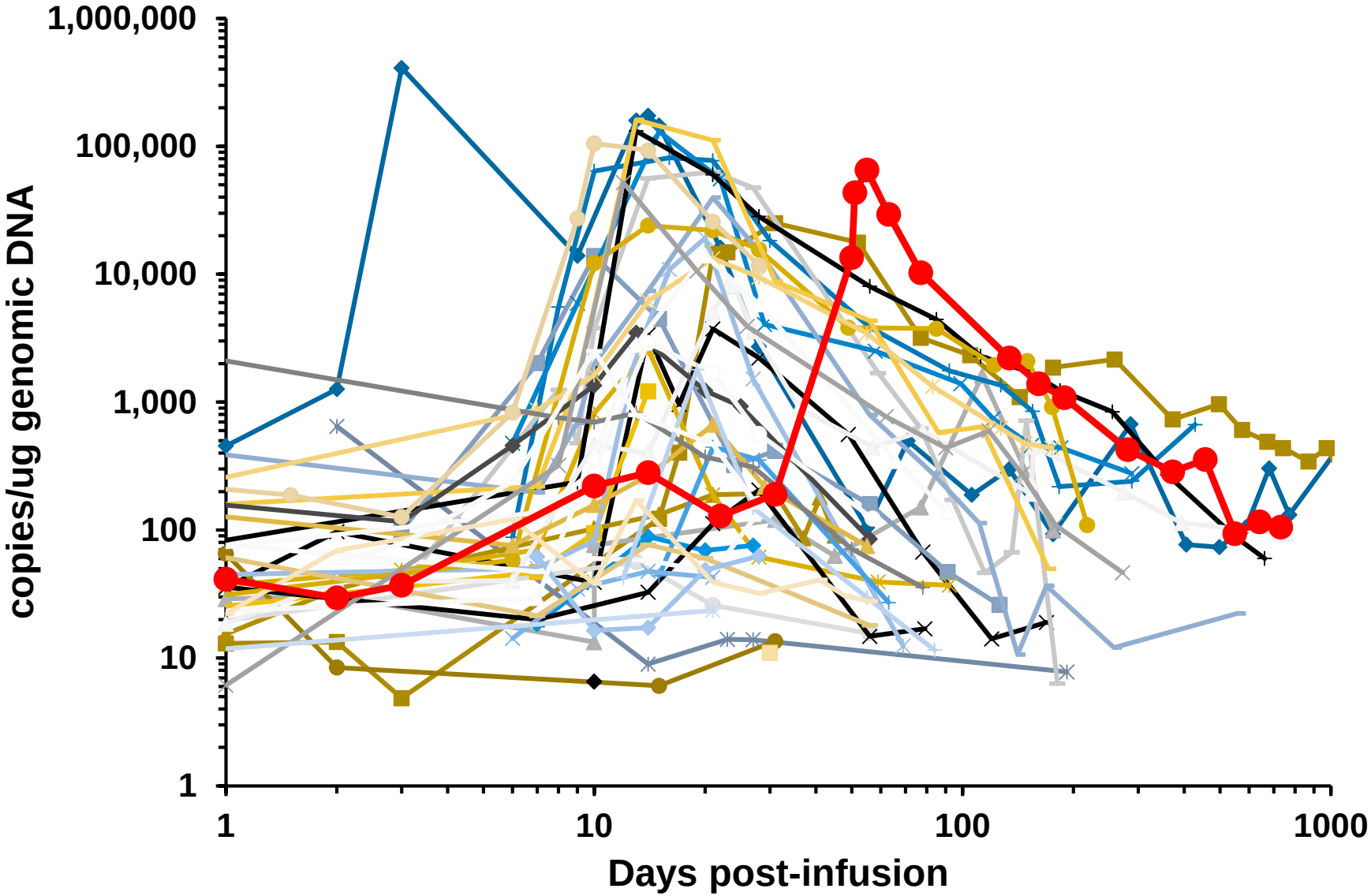
Analysis of Pre-Manufacturing T Cells Identifies an Immunophenotype Predictive of Response to CTL019





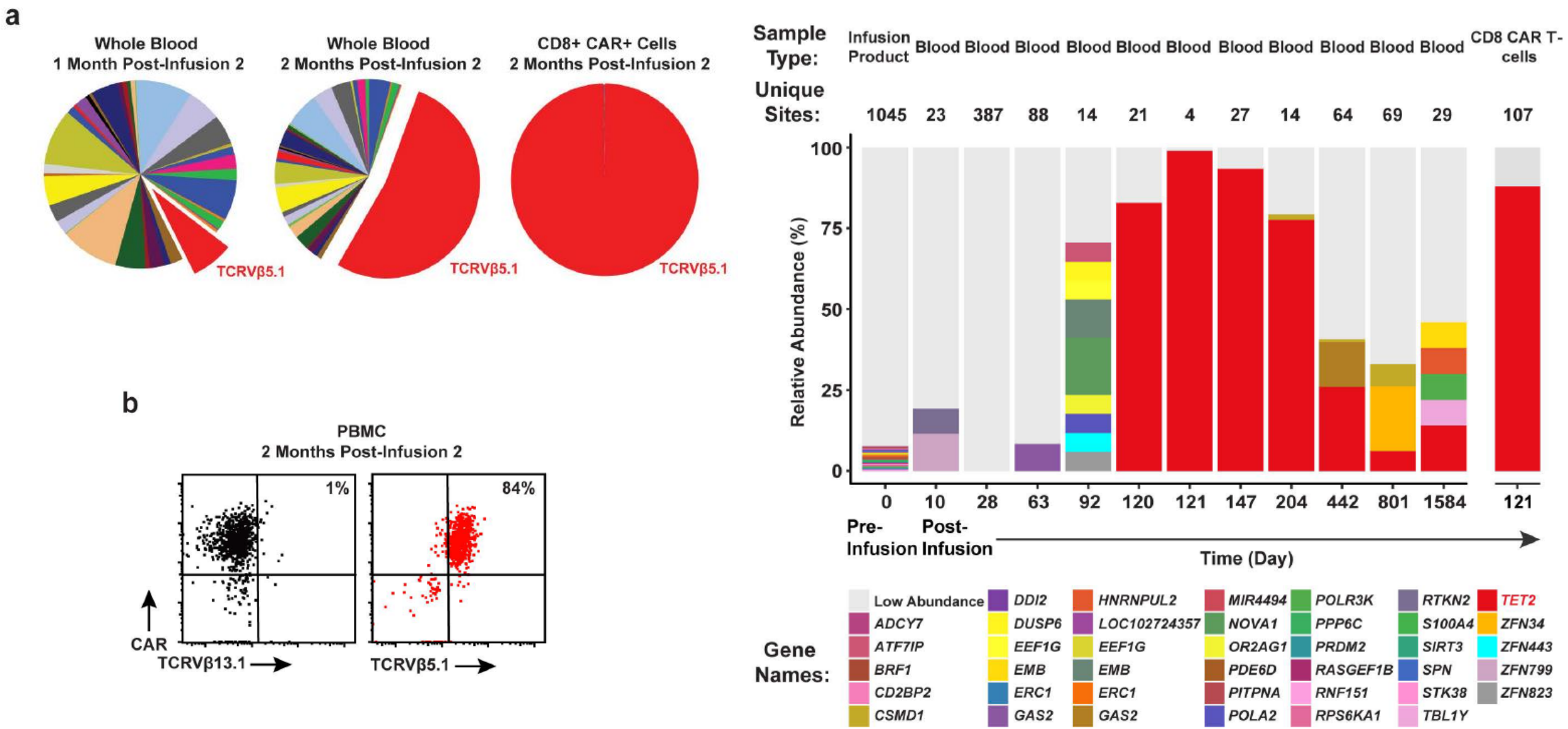
Fraietta et al. *(2018). Nature Med 24:563-571*

Lessons Learned From Exceptional Cases: CLL Patient 10



Fraietta *et al*., *Nature 2018*

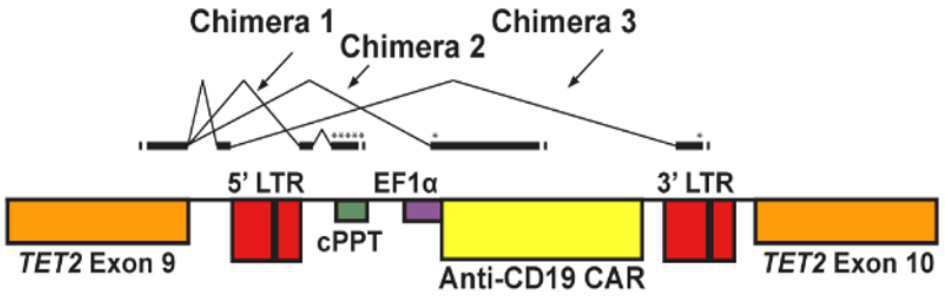
Massive expansion of clonal CART cell population in patient #10



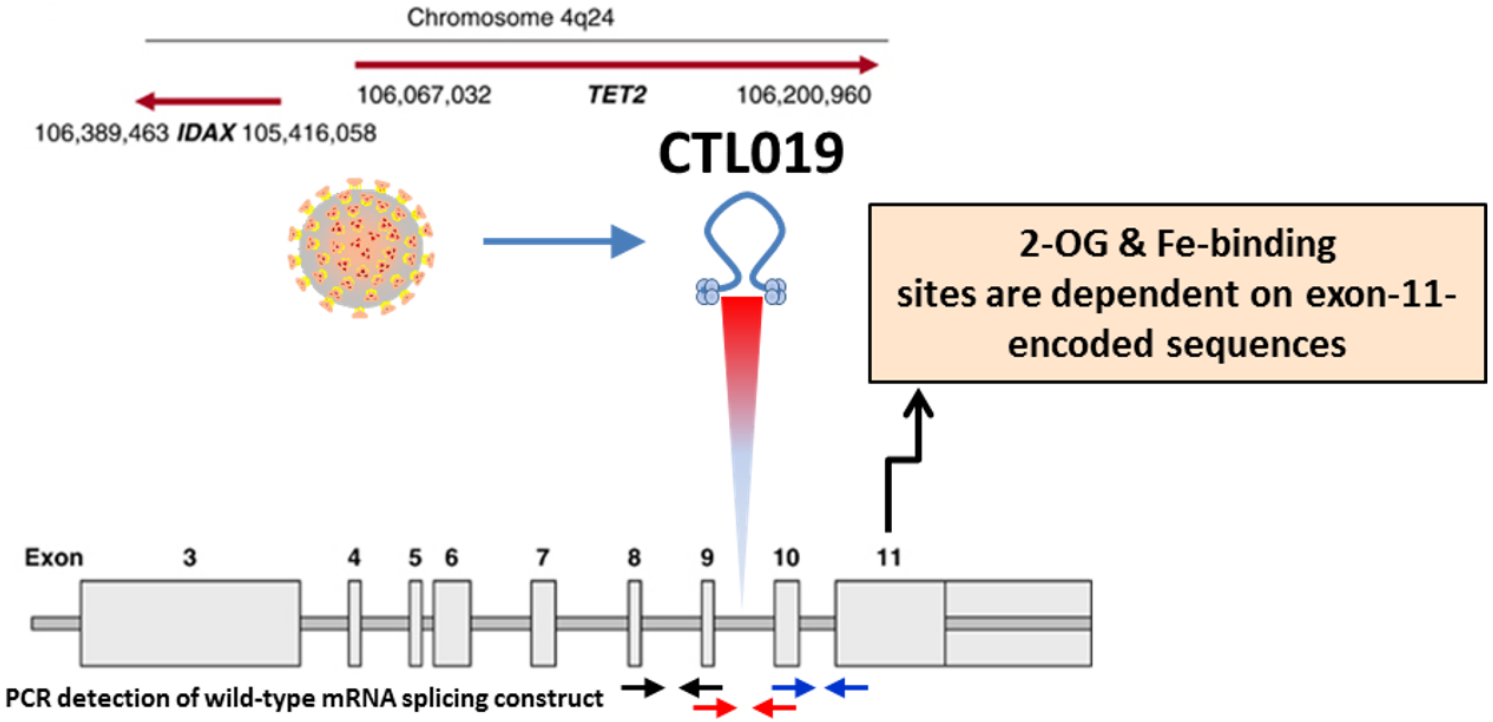
Fraietta *et al*., *Nature 2018*

Mapping CAR Integration Site in Pt #10

Single copy of integrated CAR: 4q24

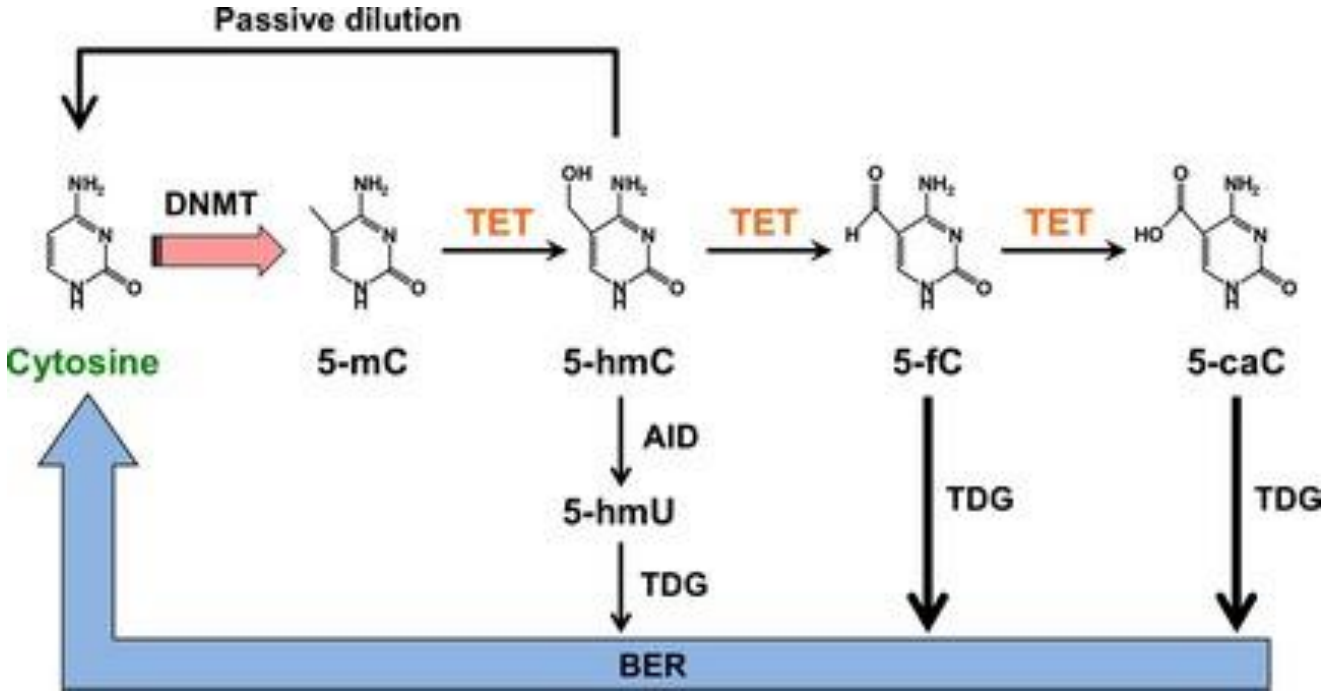


Chimera	Splice Site	Donor Sequence		Acceptor Sequence	
1	1st	ACAUUG	GUAAGU	CUCUAG	CAGUGG
1	2nd	GACUGG	UGAGUA	GUUAGG	CAGGGA
2	1st	ACAUUG	GUAAGU	UUUCAG	GUGUCG
3	1st	ACAUUG	GUAAGU	UAACAG	GUAGGA
3	2nd	CAACUA	AUGUAG	GGGGAC	UGGAAG



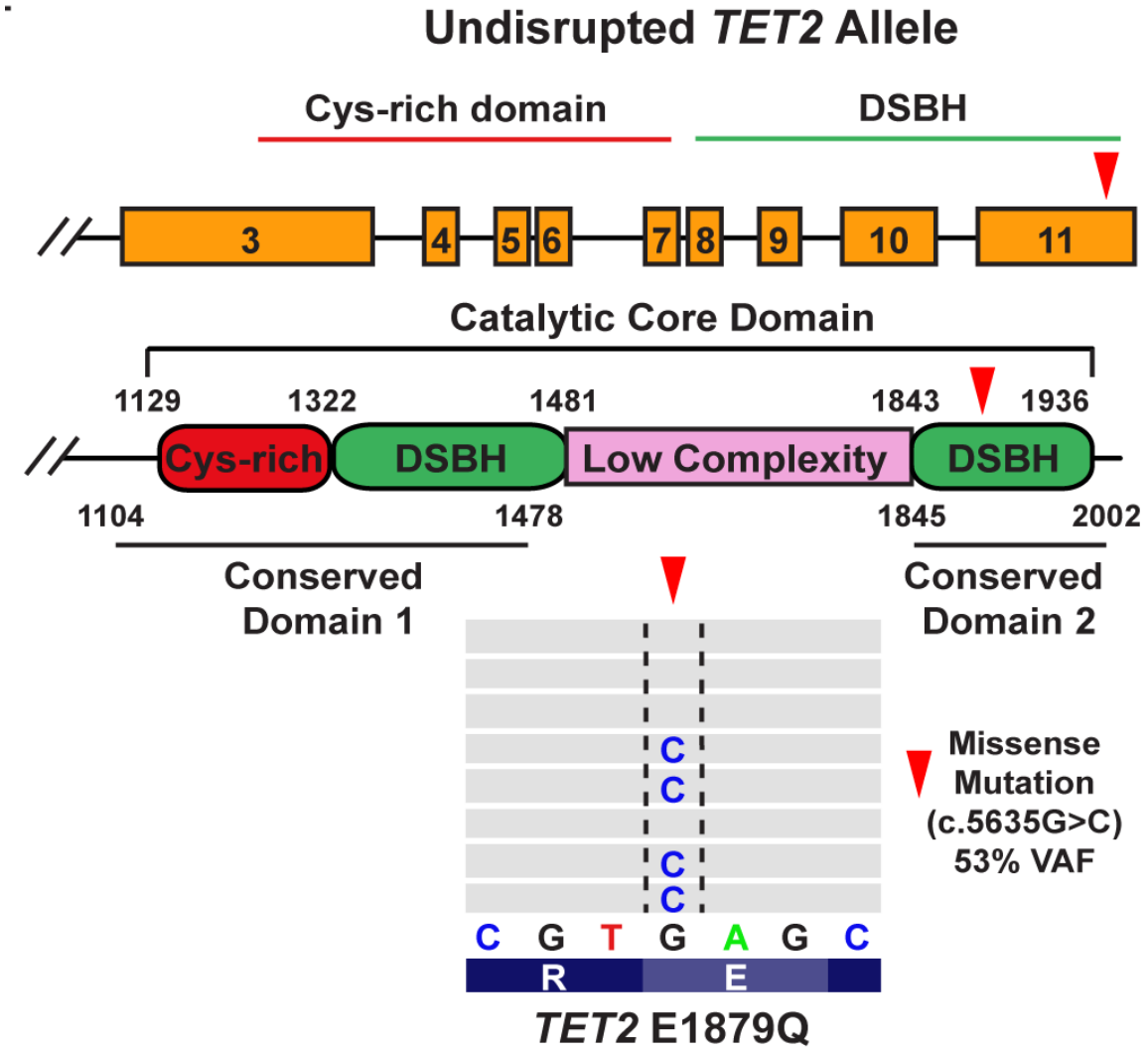
Fraietta *et al*., *Nature 2018*

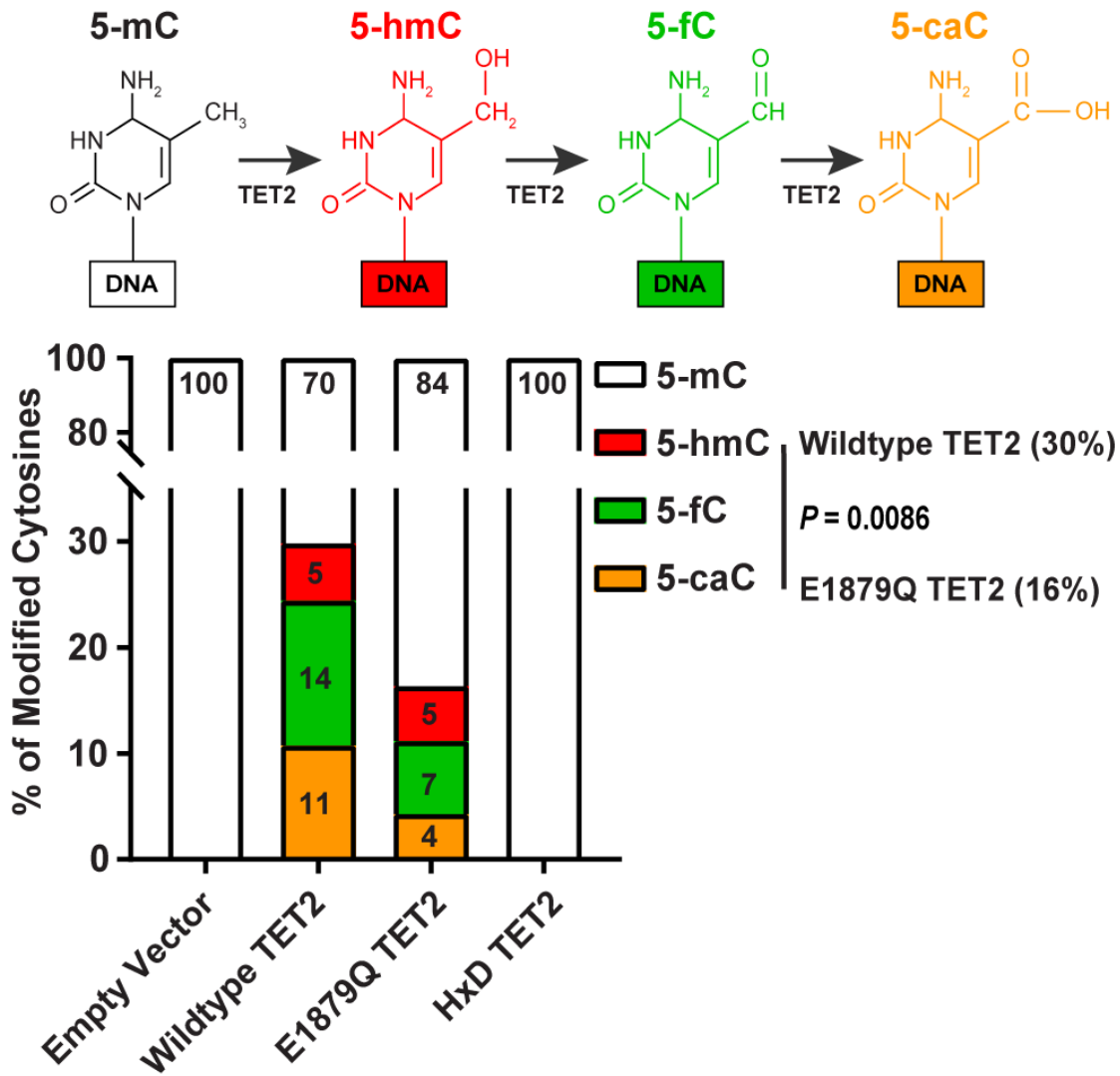
TET (Ten-eleven translocation) Proteins



- All TET enzymes contain a C-terminal catalytic domain (CD) that belongs to the dioxygenase superfamily and oxidizes 5mC in a 2-oxoglutarate- (2-OG) and Fe(II)-dependent manner
- TET2 mutations frequently occur in hematological malignancies, including myeloid malignancies, T cell lymphomas and adult T cell leukemia
- TET2 mutation not sufficient for transformation
- TET2 LOF mutations frequent in clonal hematopoiesis

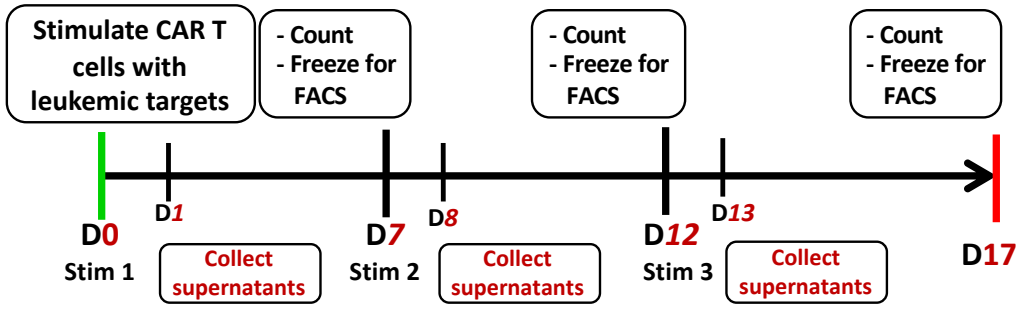
TET2 SNP on Non-Disrupted Allele Creates Hypomorphic Enzyme





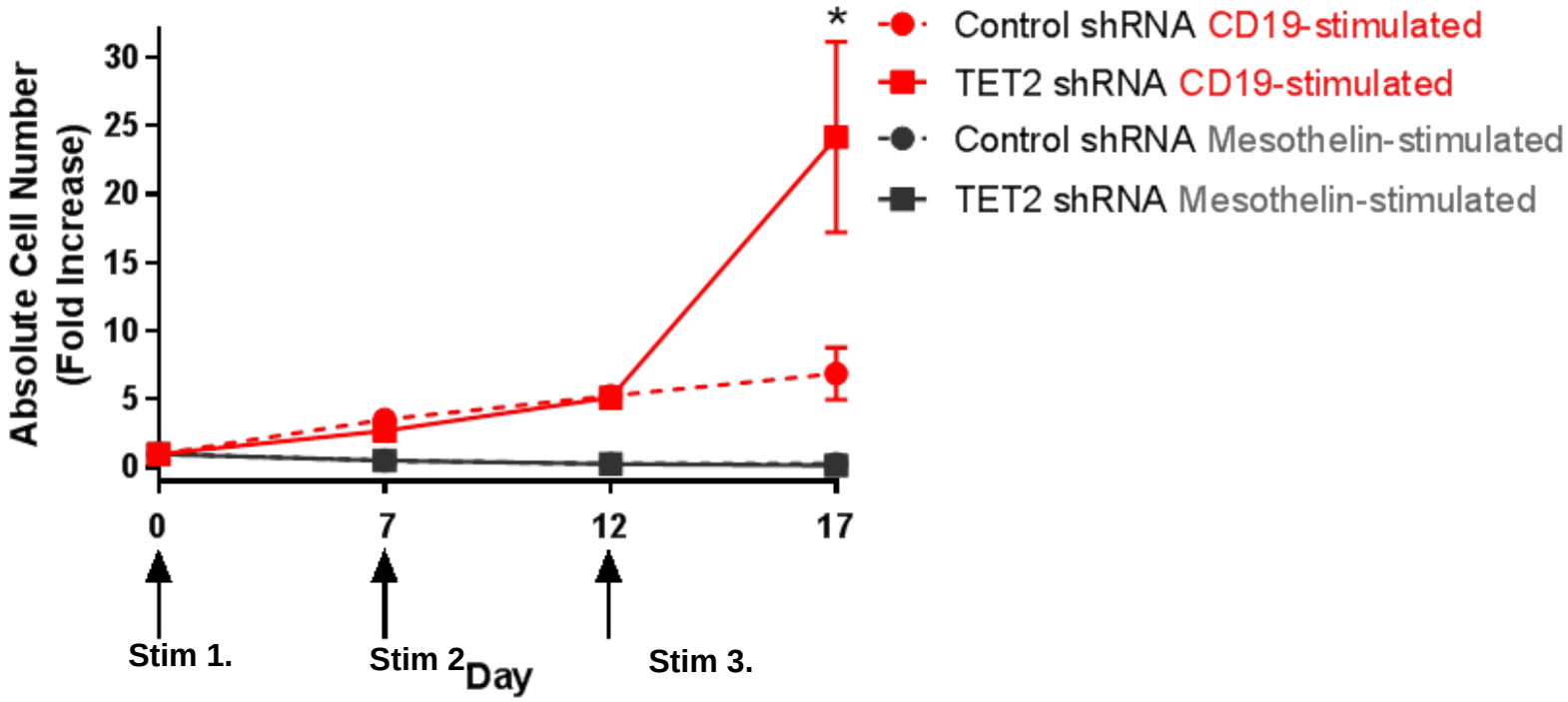
Fraietta *et al*., *Nature 2018*

TET2 Deficiency Increases CAR T-Cell Proliferative Capacity



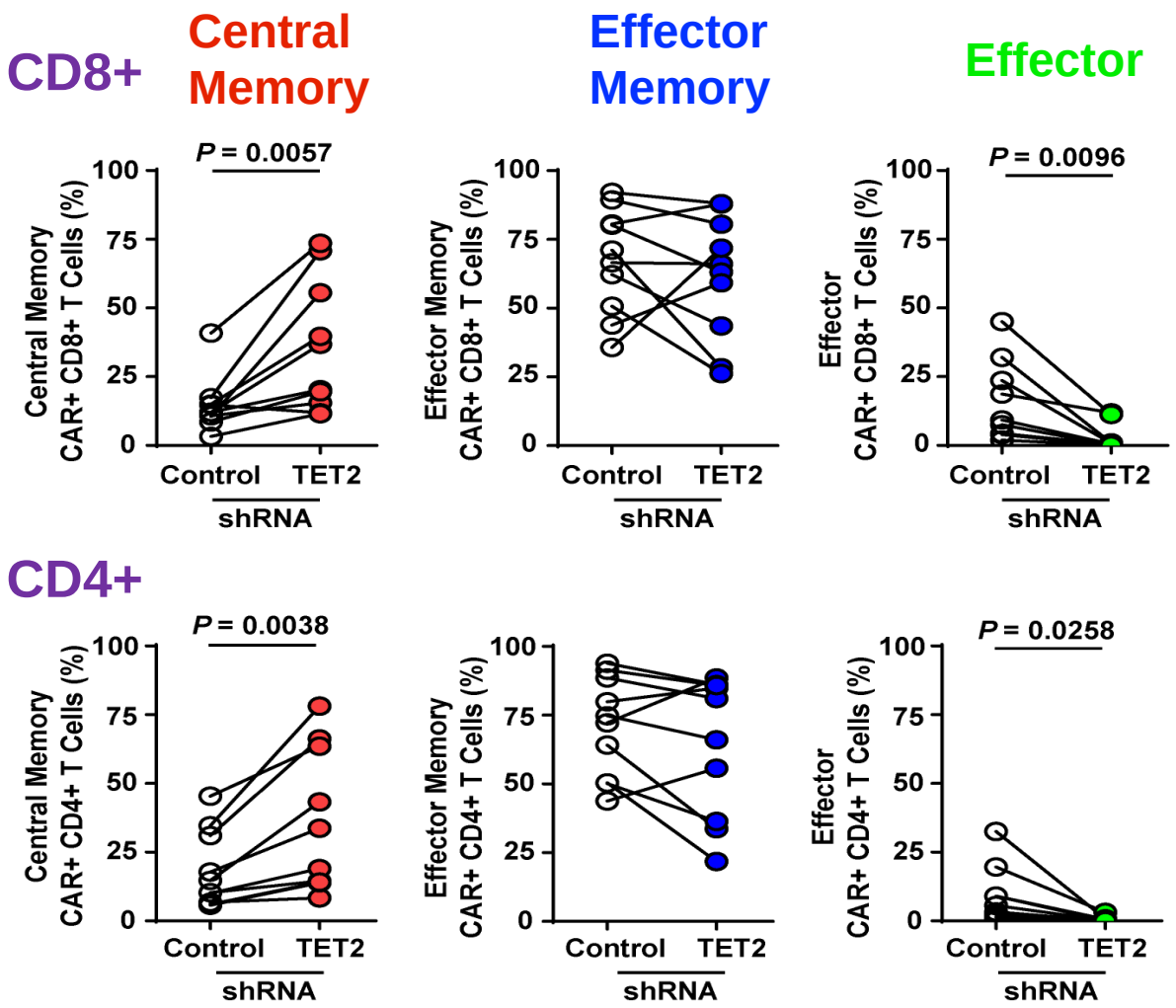
Secretomics
- Cytokine secretion (*sustained broad spectrum cytokine production*)
- Metabolites (*glucose metabolism, lactic acid production*)

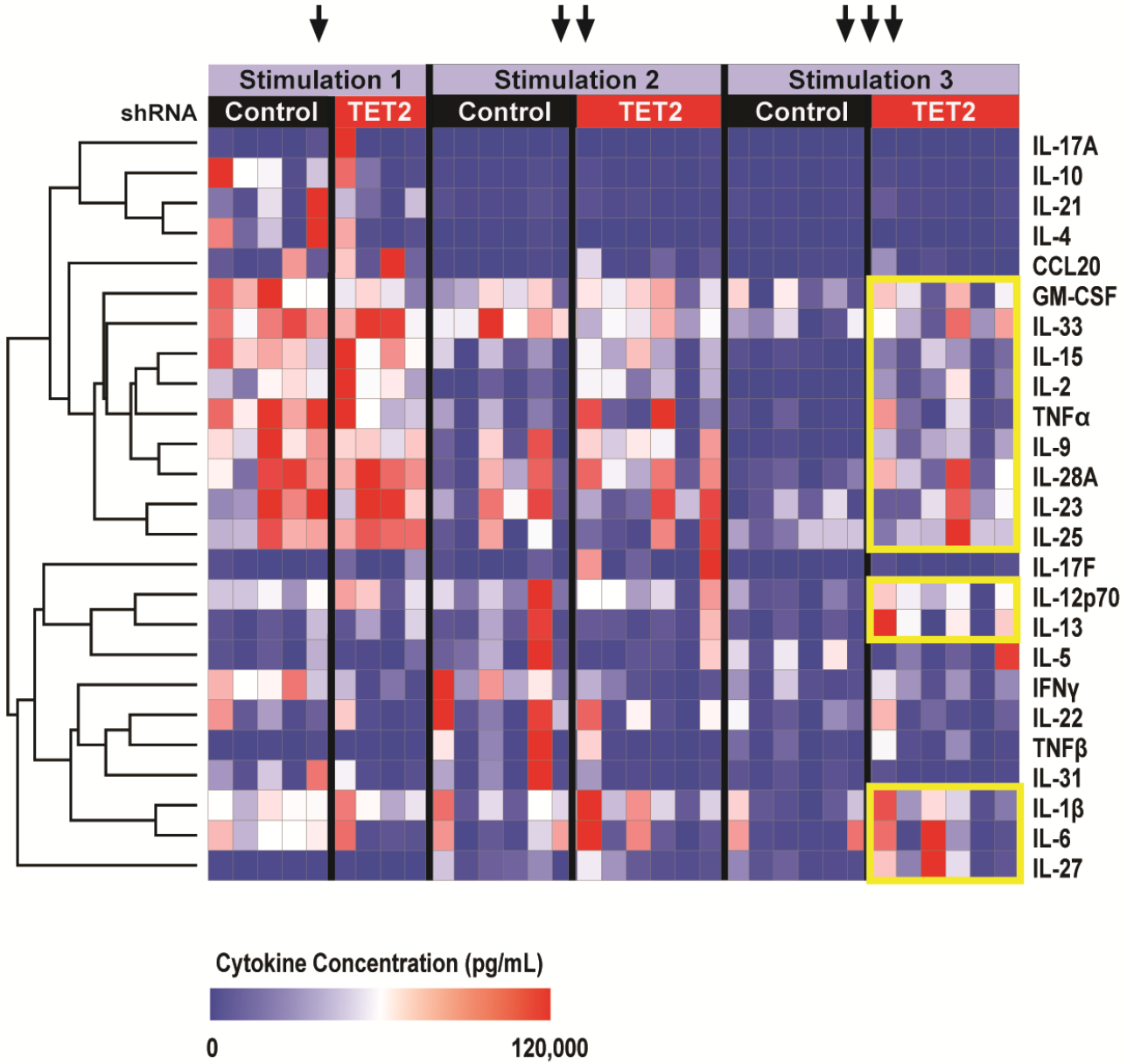
- CAR T cells expressing TET2-targeting or non-targeting shRNA generated in 9d culture
- Cells were sorted prior to restimulation assay



Fraietta, J.A.. *et al*., *Nature 2018*

TET2 Deficiency Alters T-Cell Differentiation





Fraietta, J.A.. *et al*., *Nature 2018*

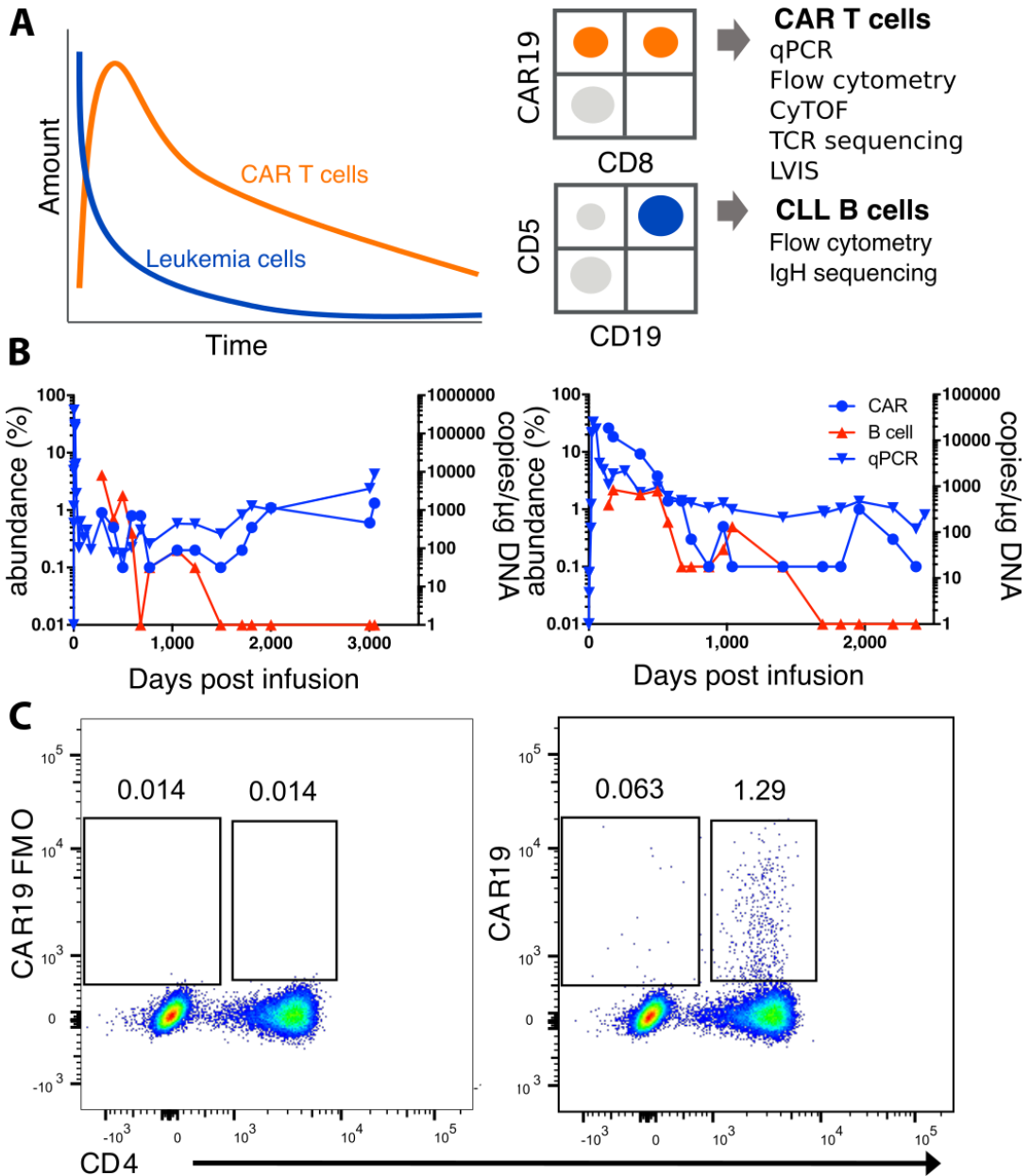
TET2 Disruption in CAR T Cells: Tumor Tamed by Clonal CAR T-Cells[1]

- A CLL patient developed delayed response to CAR T cell re-infusion, 2 months after the first
- CAR T cells peaked by day 50, coincident with significant tumor reduction and cytokine release syndrome
- CAR T cells at the peak of expansion displayed early memory T cell phenotype, unlike typical responders who are predominantly effector-memory T cells
- This CAR T cell population was a) clonal and b) carried a disrupted TET2 allele; second allele was hypomorphic
- Knock-down of TET2 in normal donor T cells recapitulated phenotype and enhanced memory function of T cells
- Q: Does TET2 knockdown prevent T cell differentiation/exhaustion, or possibly reprogram to early memory/non-exhausted state?

[1]Marcela Maus, *New & Views* with Fraietta et al. *Nature 2018*

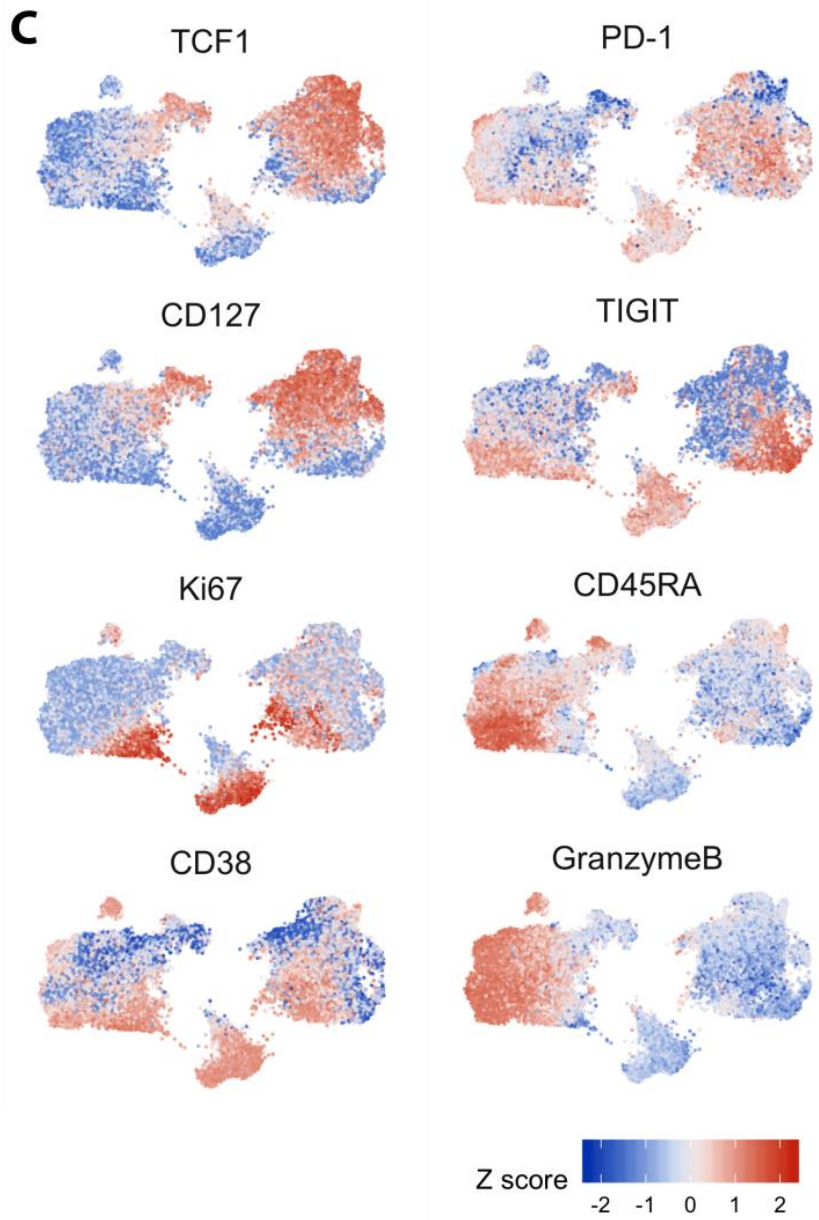
Fate Mapping of CAR T Cells

- Patients with longest follow-up (7-8 years) and persistence studied
  - Both patients had advanced, chemotherapy-resistant CLL, treated with CTL019 in July, 2010
  - Patient 1: 1.1 x 10^9 CAR T cells
  - Patient 2 1.4 x 10^7 CAR T cells; delayed kinetics
  - Both patients in remission
- 40-marker cyTOF panel designed to interrogate T cell differentiation, activation (status), and exhaustion plus anti-CAR19 idiotype mAb
- Use Spearman correlation matrix, UMAP, and Phenograph

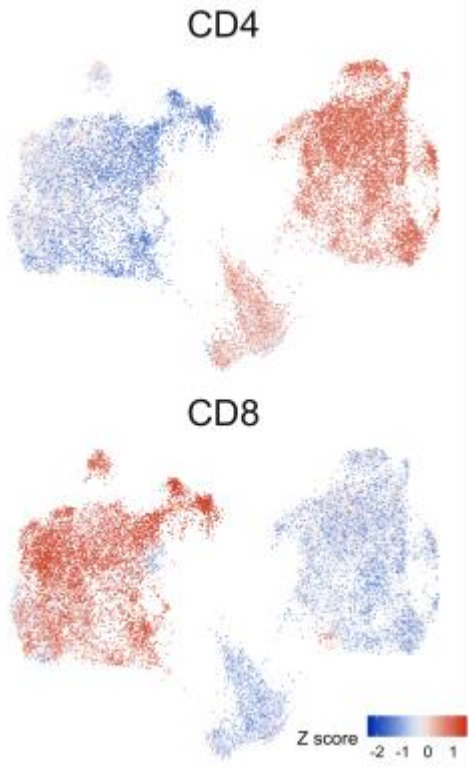


Melenhorst *et al*., unpublished (mns in preparation)

UMAP-Based Dimensionality Reduction Visualization



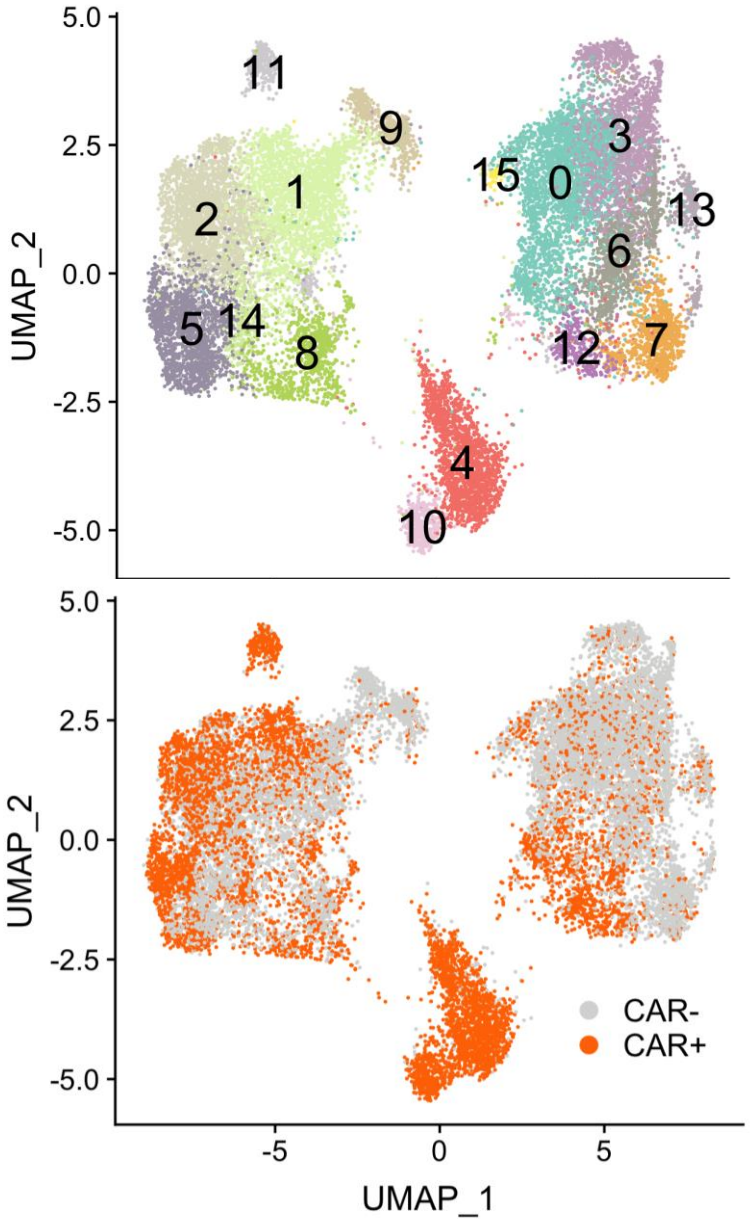
- Clustering of TCF1 with CD127 away from Ki67 and CD38, confirming correlation matrix analysis
- Granzyme B and CD45RA mostly found in the same domain, and some of CD45RA in CD127/TCF1 domain, highlighting bimodal expression pattern for CD45RA during T cell differentiation
- Granzyme B and Ki67 non-overlapping molecules

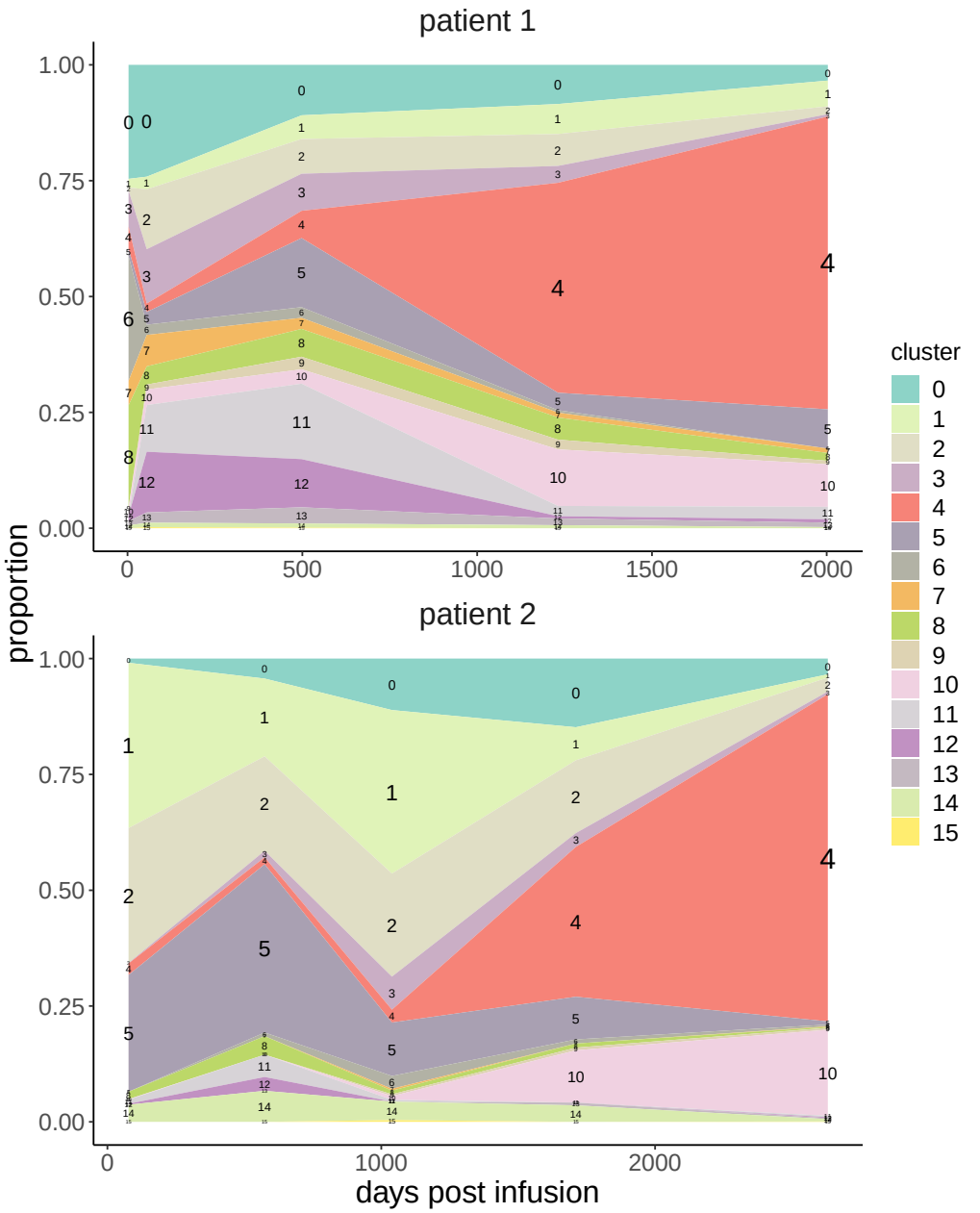


Melenhorst *et al*., unpublished

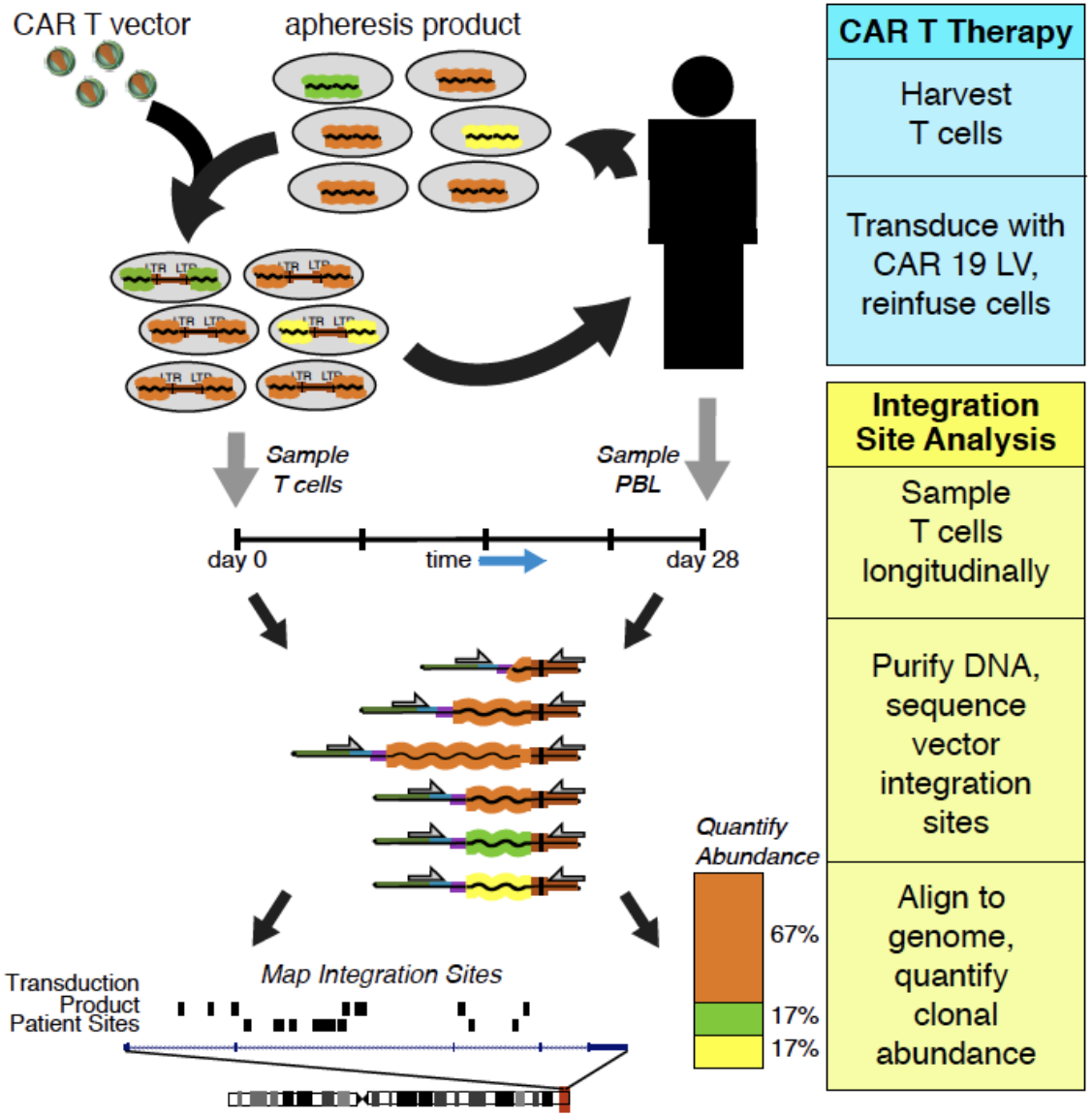
Identification of Phenotypically Stable Clusters with Phenograph

- 16 phenotypically distinct T cell clusters
- Distinct clusters dominated T cell repertoire in both patients
- CD4+ CAR T cells gradually dominated CAR T cell repertoire in both patients, suggesting a prominent role for CD4+ CAR T cells in sustained remissions
- In both patients, clusters 4 and 10 most prominent: Actively cycling, negatively regulated CD4+ CAR T cells
- Low-level persistence of CD8 CAR T cell clusters 2 and 5





Fate Mapping of CAR T-Cells via Vector Integration Site Sequencing



CAR T Therapy

Harvest T cells

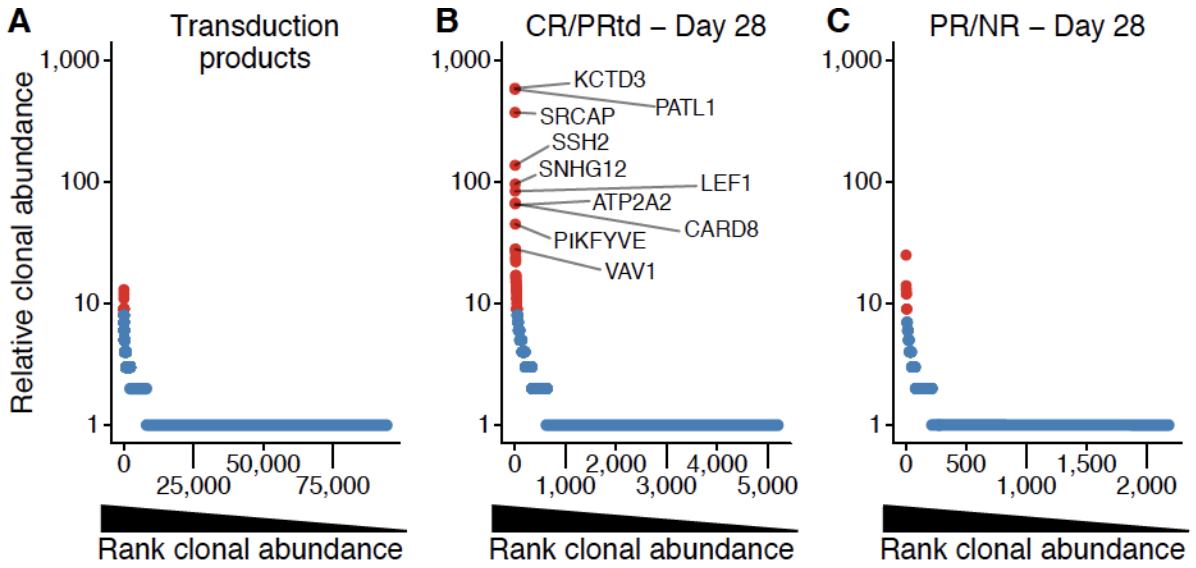
Transduce with CAR 19 LV, reinfuse cells

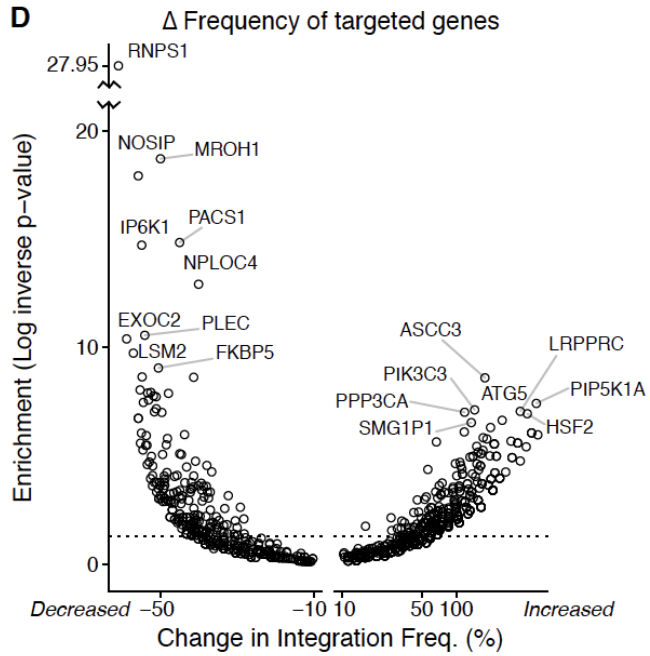
Integration Site Analysis

Sample T cells longitudinally

Purify DNA, sequence vector integration sites

Align to genome, quantify clonal abundance

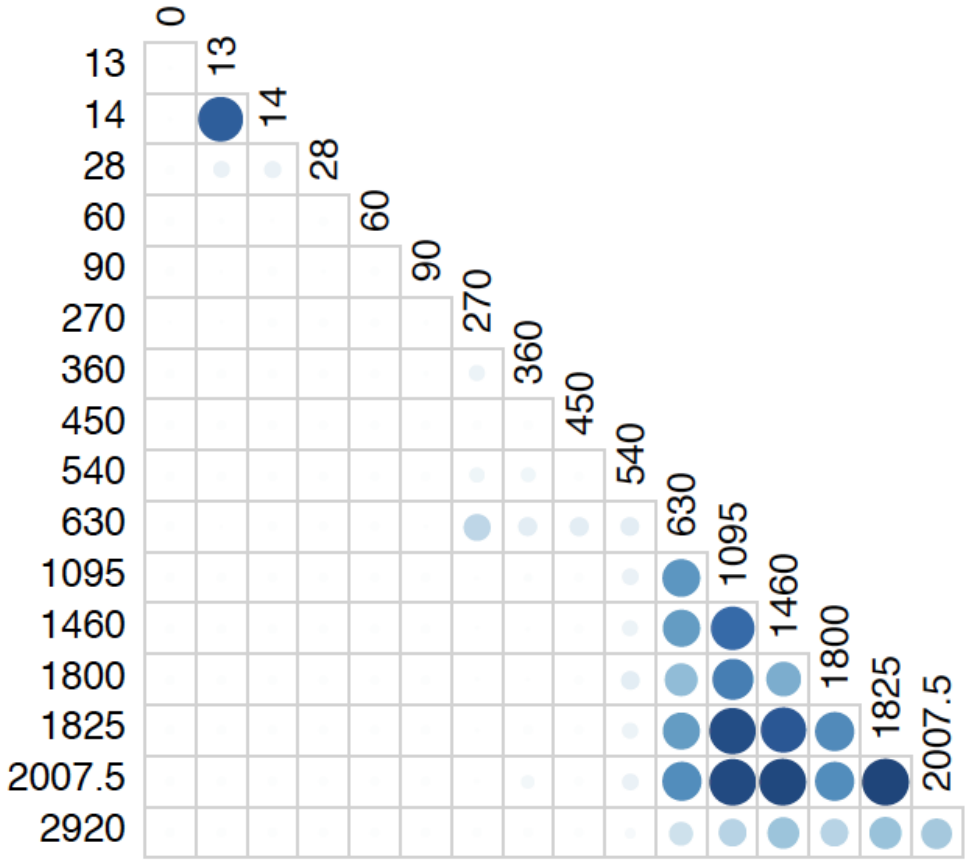




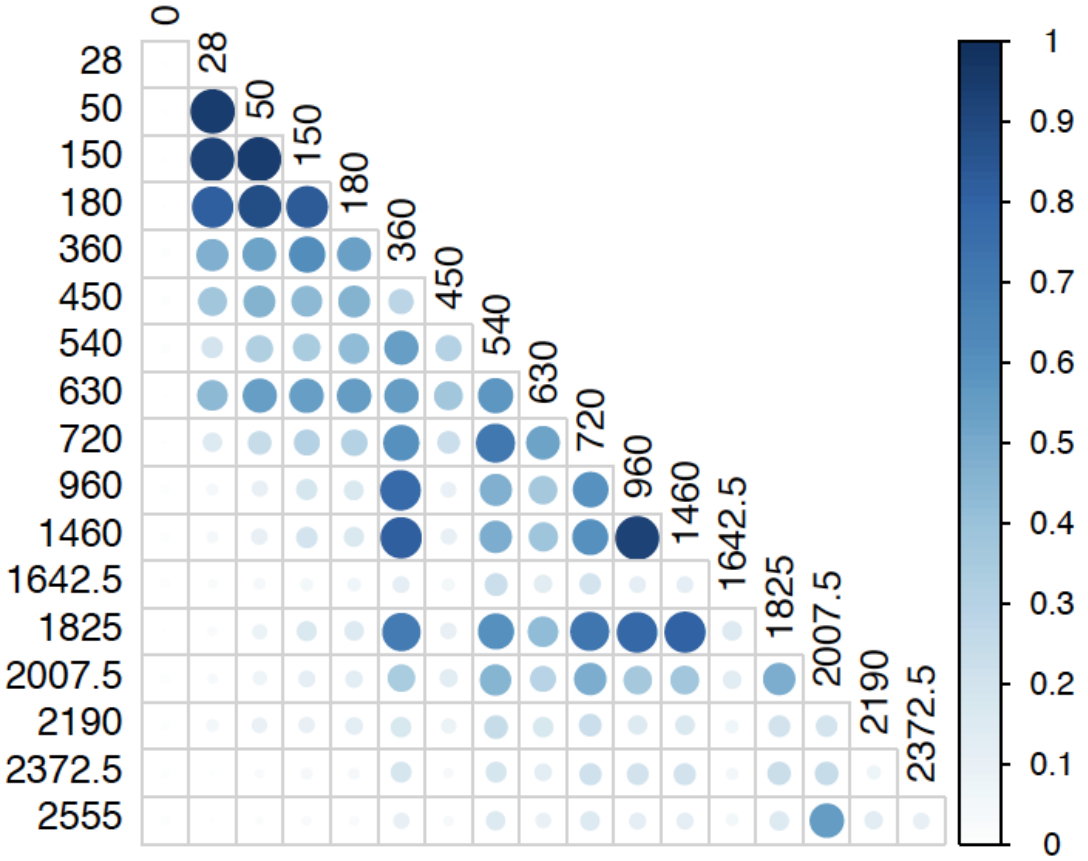
Nobles et al. *(2019). Resubmitted*

Sustained Remission in Pts 1, 2 by Few Persisting CAR T Cell Clones

CLL patient 1



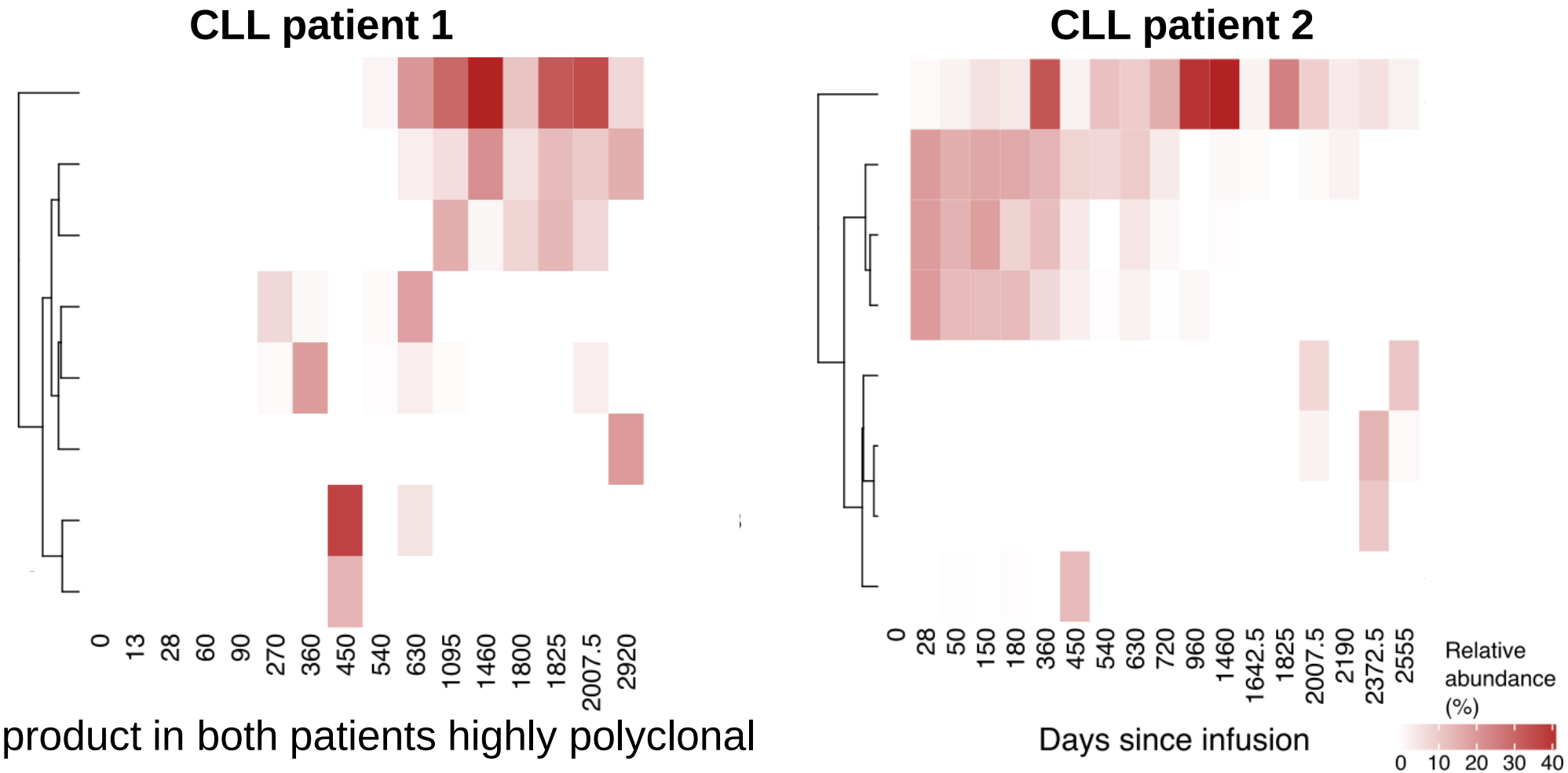
CLL patient 2



- High degree of sharing of the integration sites within each patient but not between
- Same CAR T cells continue to control the tumor

Melenhorst *et al*., unpublished

Oligoclonal Composition of Memory CAR-T Cells



- Infusion product in both patients highly polyclonal
- CAR integration site landscape in both patients demonstrates selective clonal expansion and persistence
- CAR integration site repertoire in both patients appears to come in two separate waves, coincident with switch from CD8 to CD4 dominance

Melenhorst *et al*., unpublished

Sustained Remission of CLL Following CART19 Therapy

- Two patients infused 9 years ago with anti-CD19 CAR T cells with durable molecular remission, B cell aplasia
- Memory function of CAR T cells critical for this clinical efficacy
- Mass cytometry with UMAP and Phenograph-based data analyses revealed initial dominant role of effector CD8+ CAR T cells, followed by CD4+ CAR T cells
- Initial 2-3 years post-infusion showed diverse phenotypes, which converged on actively proliferating, immune checkpoint inhibitor molecule-expressing CD4+ CAR T cells
- CAR T cells sustain high level of activation throughout, but also expression of negative regulatory molecules such as CTLA4, PD1, and TIGIT
- Fate mapping experiments demonstrates rapid clonal focusing after infusion with maintenance of some of the same clones
- This data suggest that remission in CTL019 treated CLL patients is induced and sustained by a pauciclonal repertoire of CAR T cells

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