Bispecific CAR 19-22 and Sequential CAR22 Rescue - the Experience of a Few Prodigies

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Dr. Miklos' Disclosures:

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- CAR-T, AlloHCT, cGVHD, and MRD are evolving fields and this presentation reflects the critical opinion of Dr. Miklos alone
- Scientific and Clinical Advisory Boards:
  - Adaptive Biotechnologies, Novartis, Juno-Celgene-BMS, Kite-Gilead, Pharmacyclics-Abbvie, Janssen, Pharmacyclics, AlloGene, Precision Bioscience, Miltenyi Biotech, Adicet, Takeda
- Industry Contracted Research:
  - Pharmacyclics - Abbvie, Kite-Gilead, AlloGene, Roche, Genentech, Becton Dickinson, Isoplexis, Miltenyi Biotech, Adaptive Biotechnologies
- **Dr. Miklos does not hold equity or stock in any of these companies**

CD19 Antigen Loss is a Common Cause of treatment failure after CAR19 Therapy

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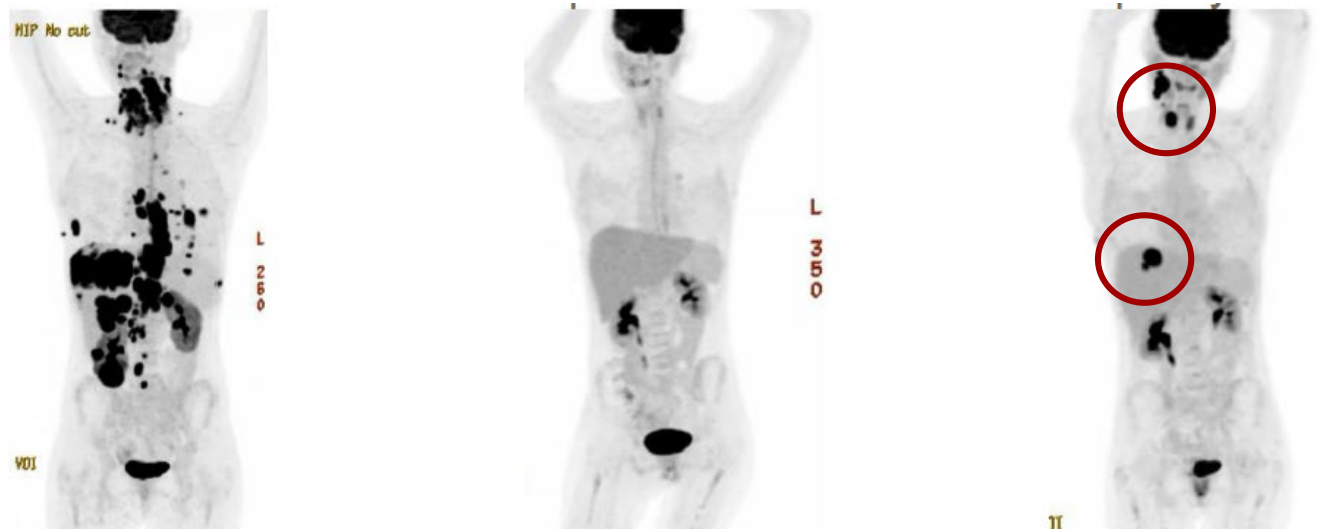
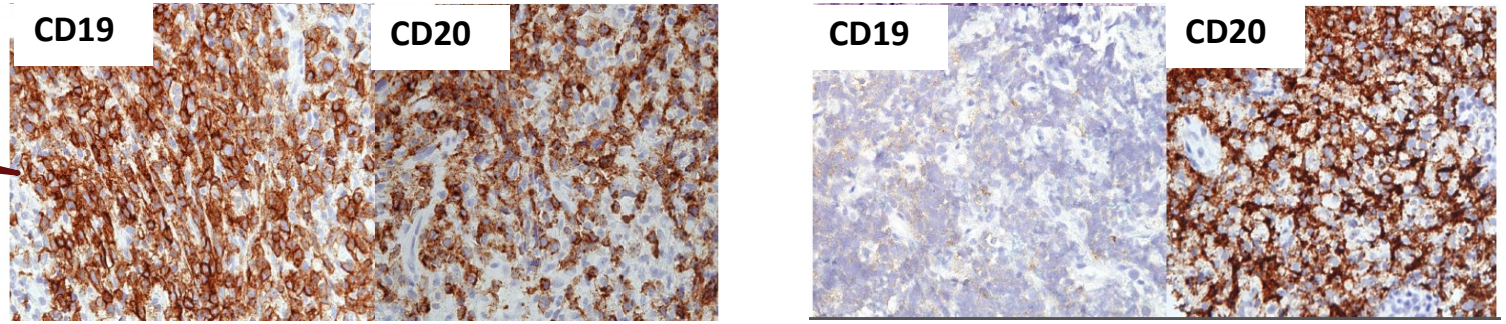
- 7/21 (33%) ZUMA-1 patients w/ disease progression after therapy were CD19 negative#
- 34 patients treated with commercial Axi-Cel at Stanford*
  - 16 developed disease progression
  - 12 were biopsied at time of progression
  - Six showed CD19 loss

PRE-INFUSION ***DAY 28*** ***DAY 60***



Lymph node analysis pre-CAR and at Day 60 highlighted loss of CD19 but preservation of CD20 expression

PRE-THERAPY ***DAY 60 RELAPSE***



#Neelapu et al, ASH2017 Abstract #578

*Jean Oak et. al, ASH2018 Abstract #4656

Pre-treatment CD19 by IHC Does Not Associate with Clinical Outcomes

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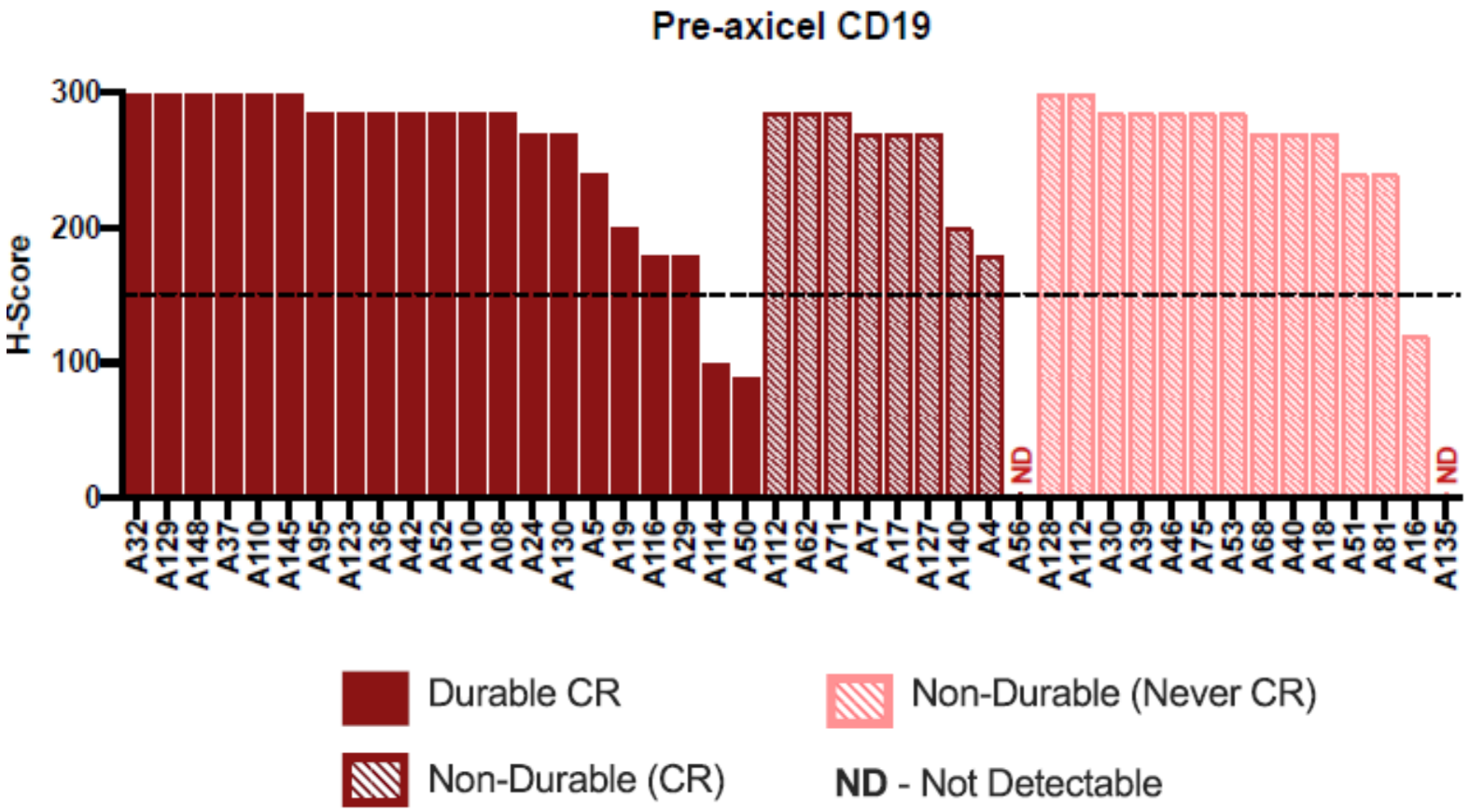
H-score = %tumor cells positive (0-100) x staining intensity (0-3)



CD19 loss or down-regulation occurs after axi-cel

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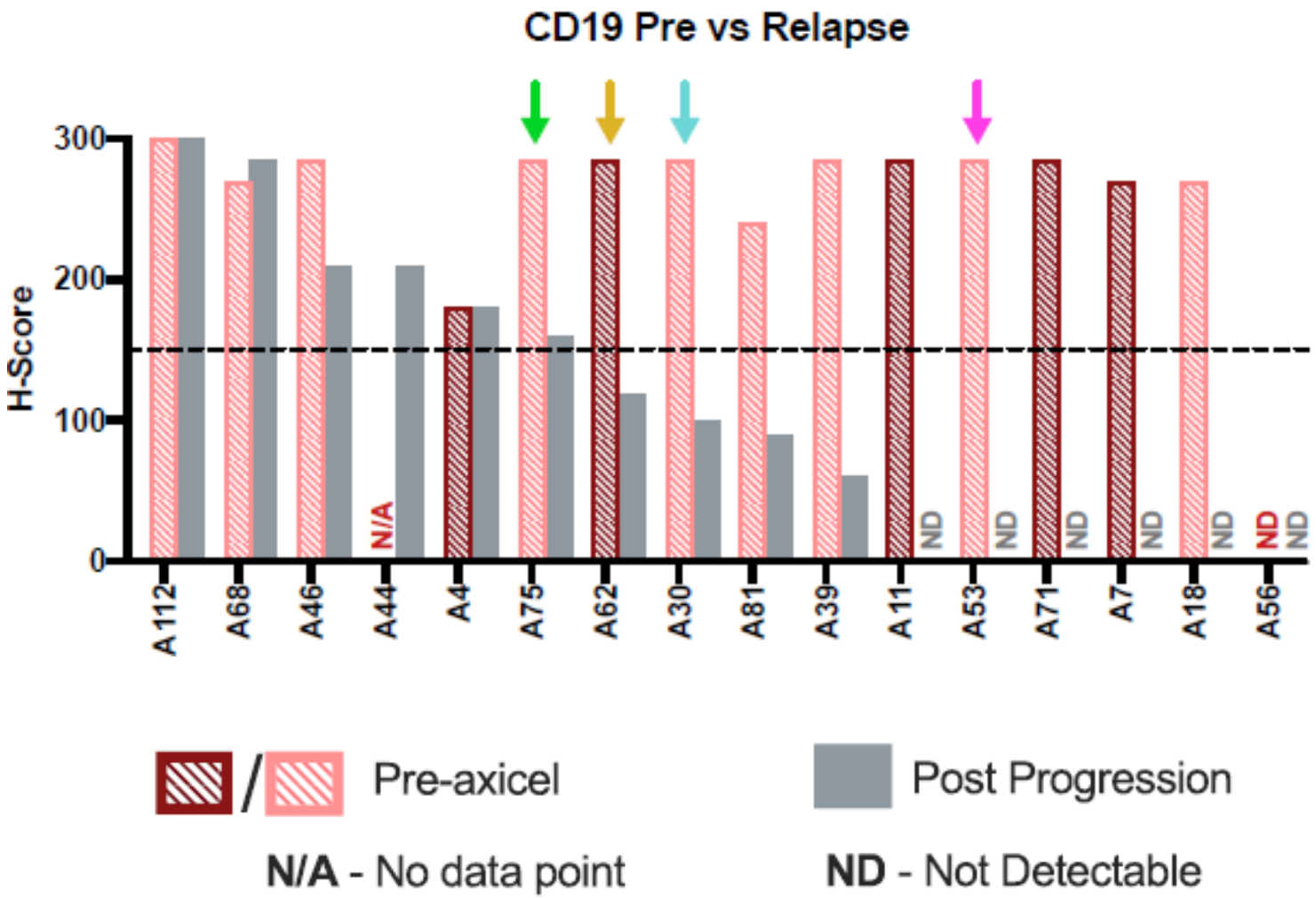

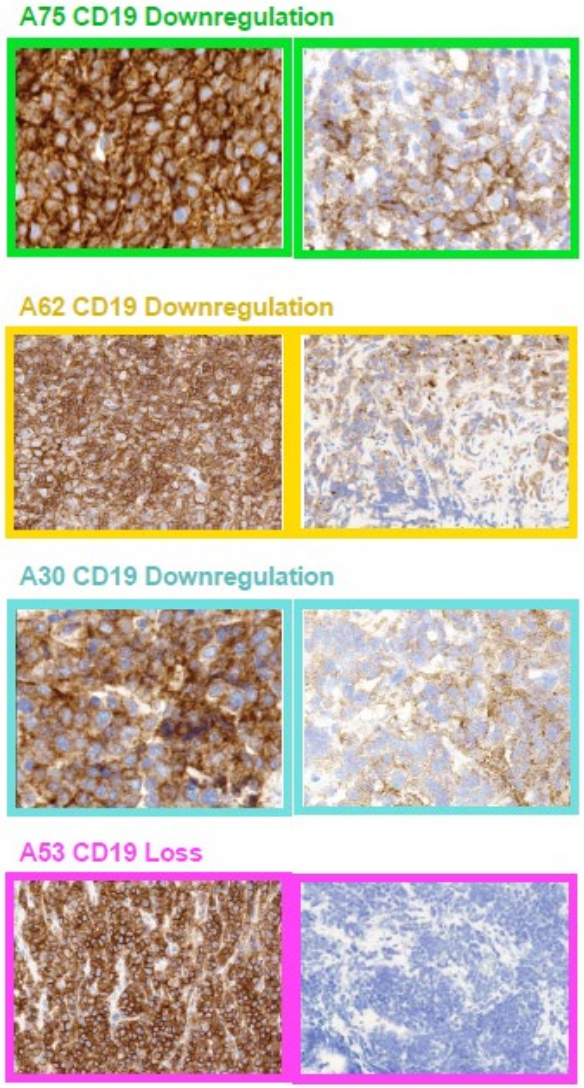

Spiegel, Patel, Muffly…Feldman, Mackall, Miklos. Manuscript accepted for publication

Current Clinical Flow Cytometry Does Not Adequately Assess Antigen Density

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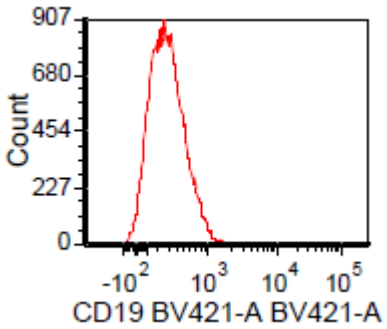
3 patients with large B cell lymphoma considered for immunotherapy

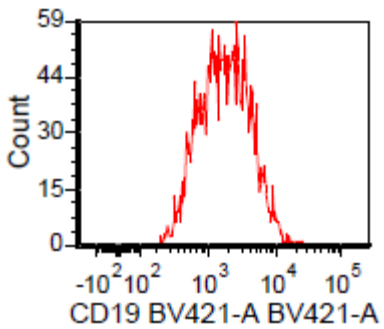
Reported result in pathology report:

30-40% positive



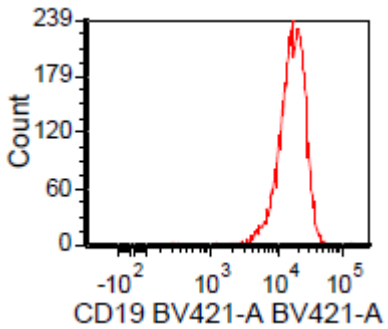
#CD19/cell **88**

>90% positive



646

>90% positive



6539

Both are eligible,
but 10-fold difference in antigen density

Courtesy of Dr. Jean Oak

Pre-treatment quantitative flow may identify patients at risk for treatment failure

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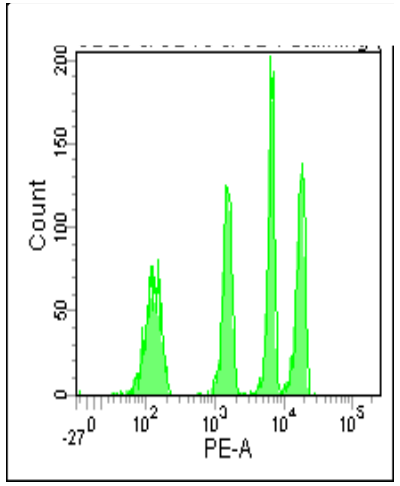
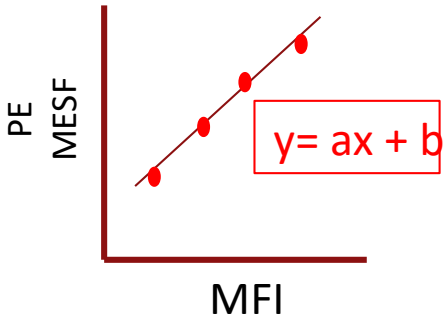
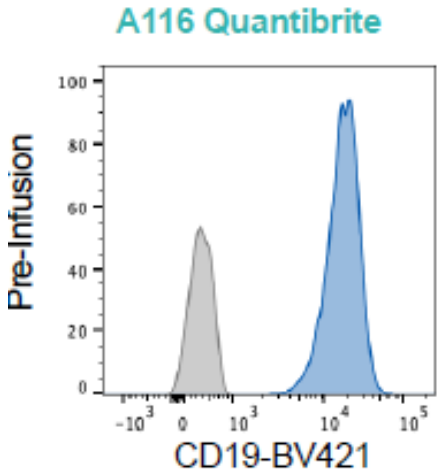
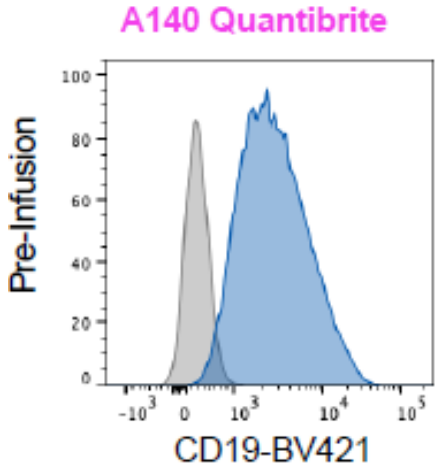
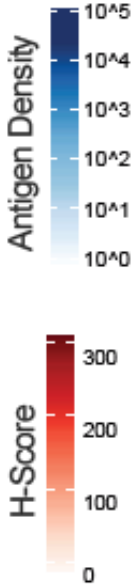




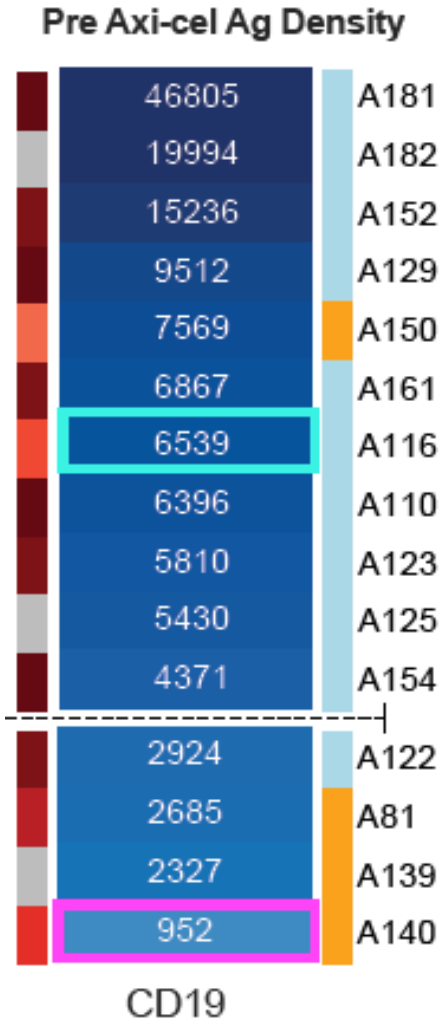
Bead population	PE Molecules
High	81,345
Med-High	29,683
Med-Low	6,573
Low	355







Durable Response
Progressive Disease
*3K molecules/cell cutoff



Post-treatment quantitative flow identifies patients with decreased CD19 density

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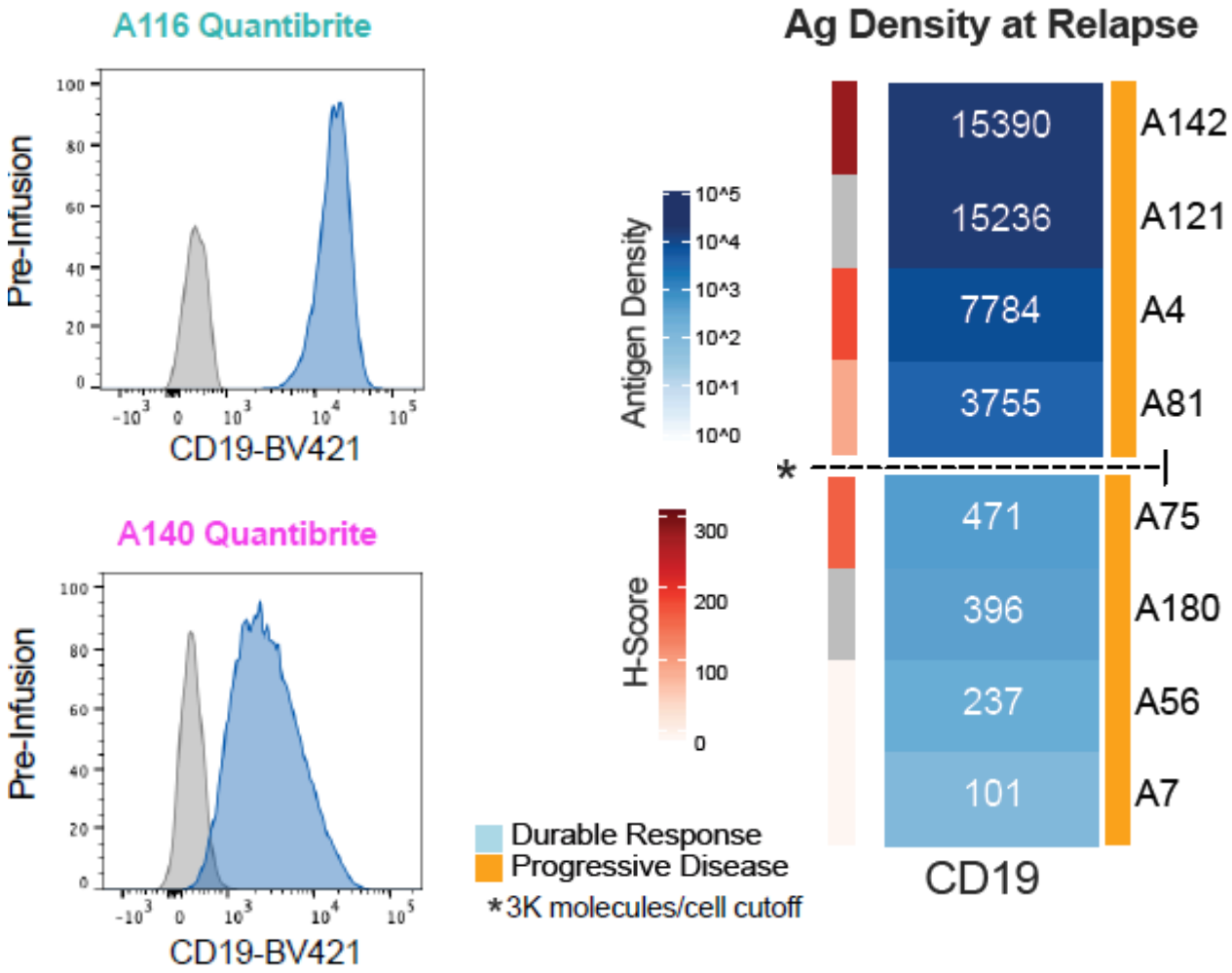

Spiegel, Patel, Muffly…Feldman, Mackall, Miklos. Manuscript accepted for publication

Simultaneous targeting of two tumor antigens may overcome antigen loss and improve efficacy

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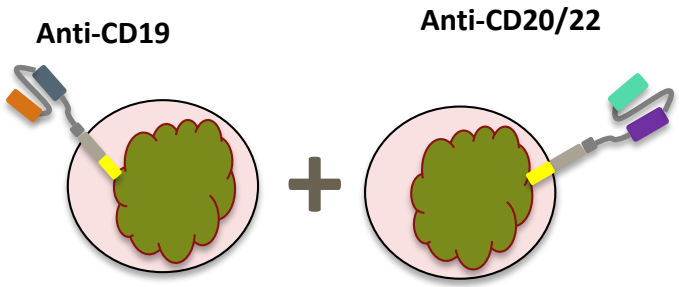
Co-administration

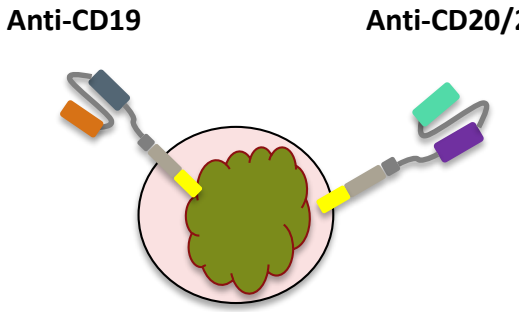


Pros:
- Defined dose for each CAR

Cons:
- Multiple production runs
- Potential competition
- When to infuse 2nd dose

Co-expression

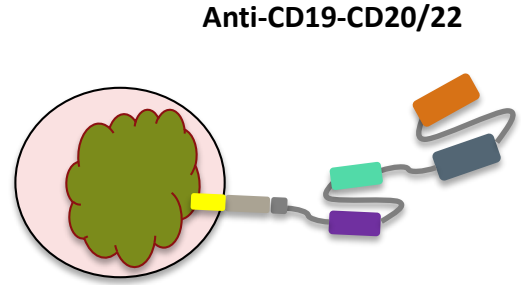
(co-transfection or bicistronic)



Pros:
- Each CAR molecule signals independently
- Reduces steric concerns

Cons:
- Can generate multiple CAR populations

Bivalent-bispecific receptor



Pros:
- Each cell expresses both scFVs

Cons:
- Distal scFV may have signalling deficiencies

Phase I Dose Escalation Study of CAR19-22 in Adults with Relapsed/Refractory DLBCL or B-ALL

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Primary Objectives

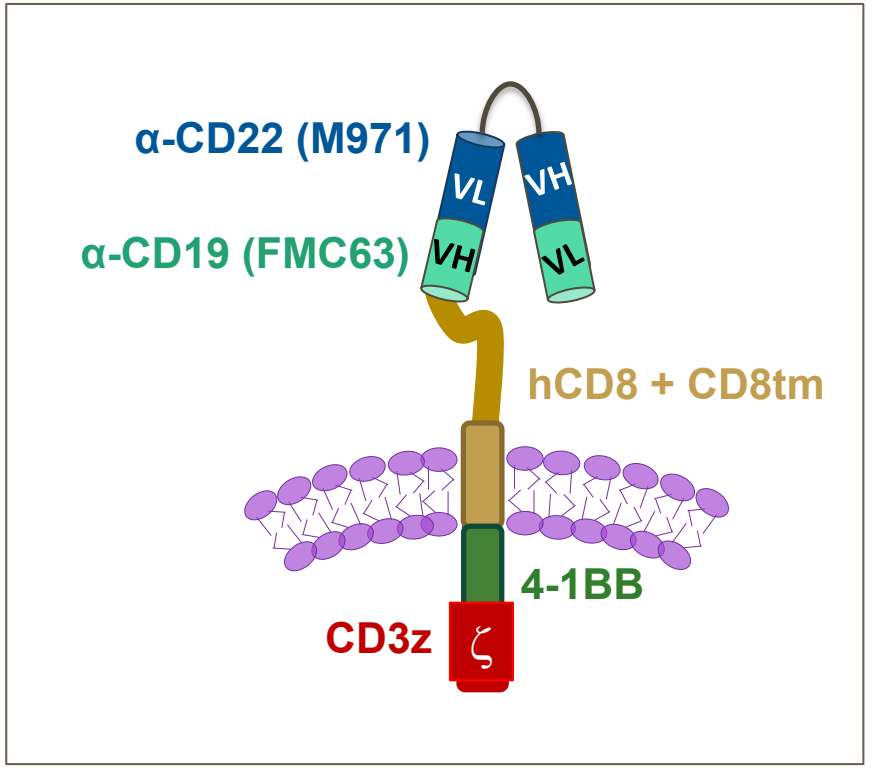
- Determine feasibility of production
- Assess safety

Secondary Objectives

- Response rate and clinical efficacy

Exploratory Objectives

- CAR19-22 persistence
- Antigen remodeling at Relapse



ClinicalTrials.gov NCT03233854

CIRM CALIFORNIA'S STEM CELL AGENCY

Closed System Manufacturing Allowed for Optimization of Product Attributes

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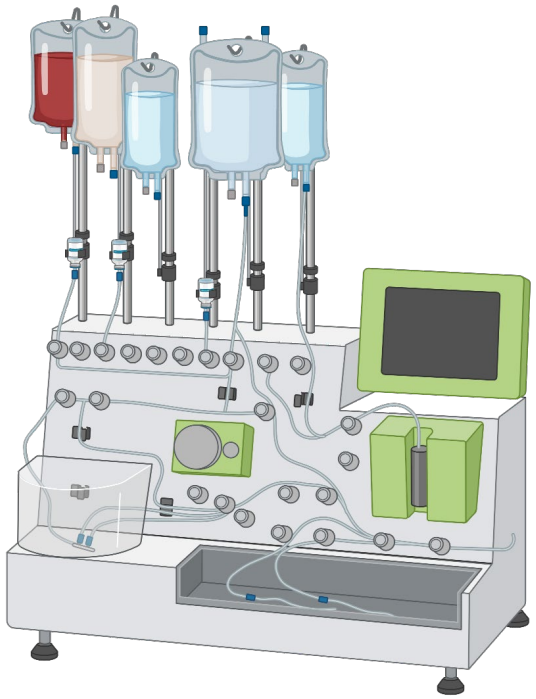
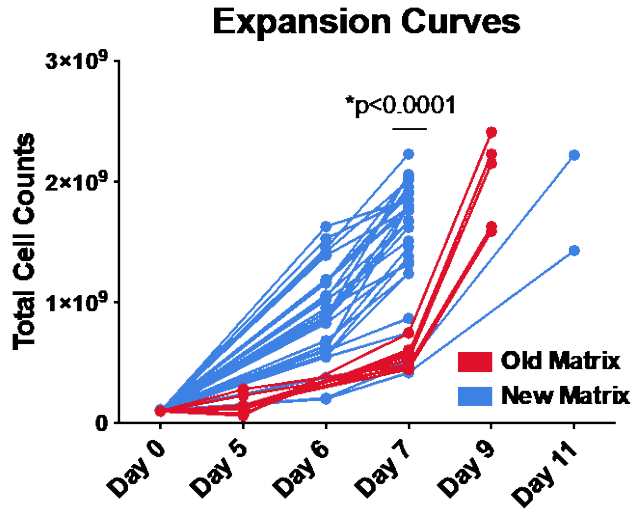
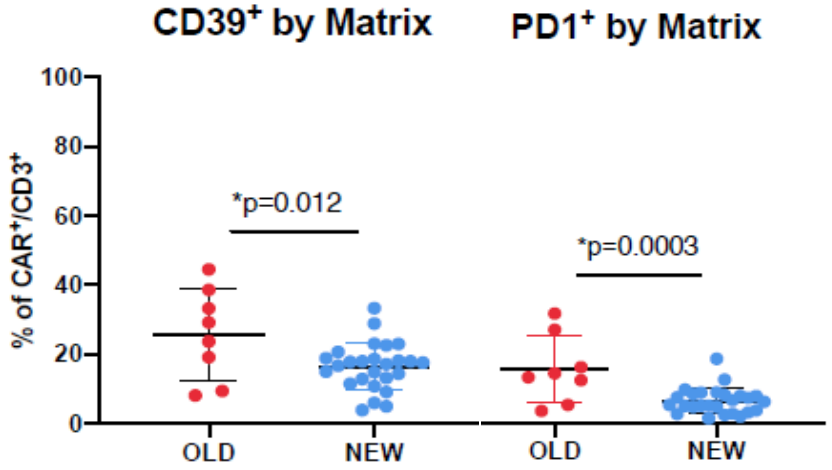
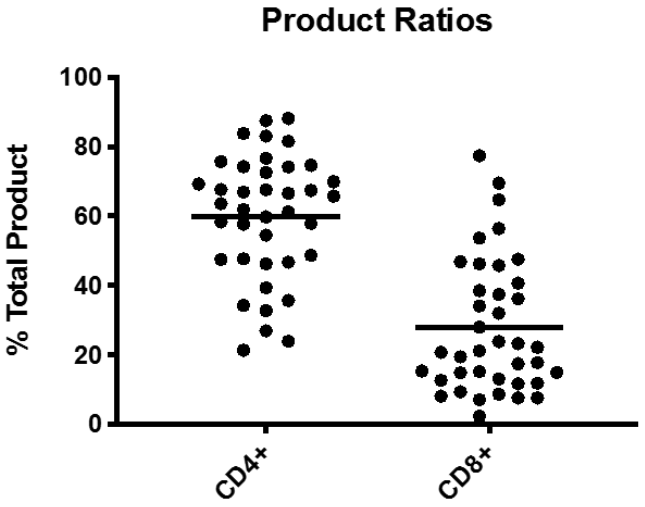
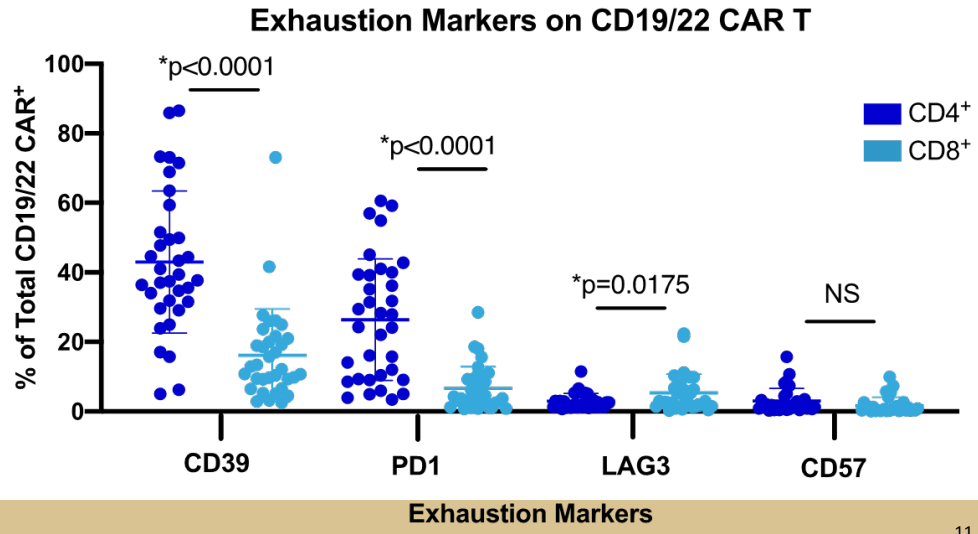


97% of products met protocol specified dose









CAR19-22 Expansion was Predominantly CD8

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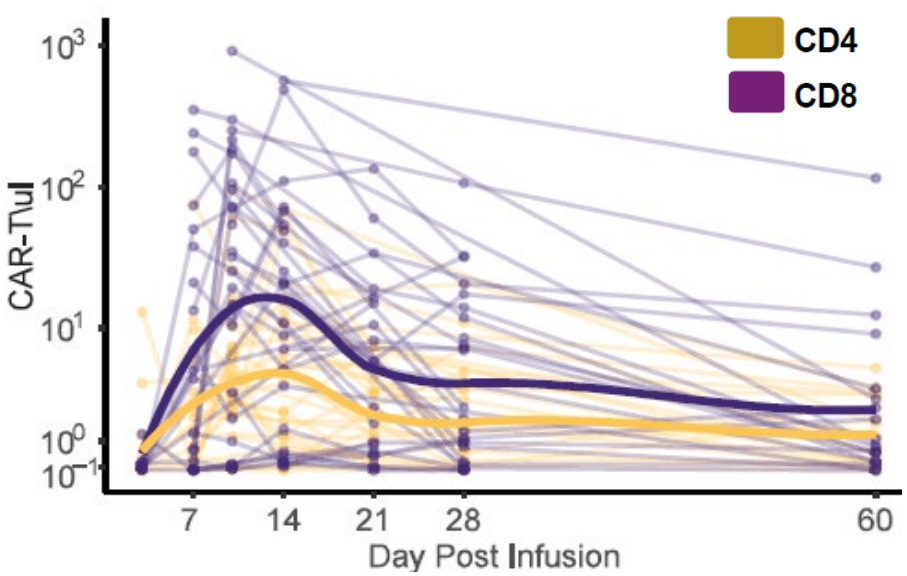

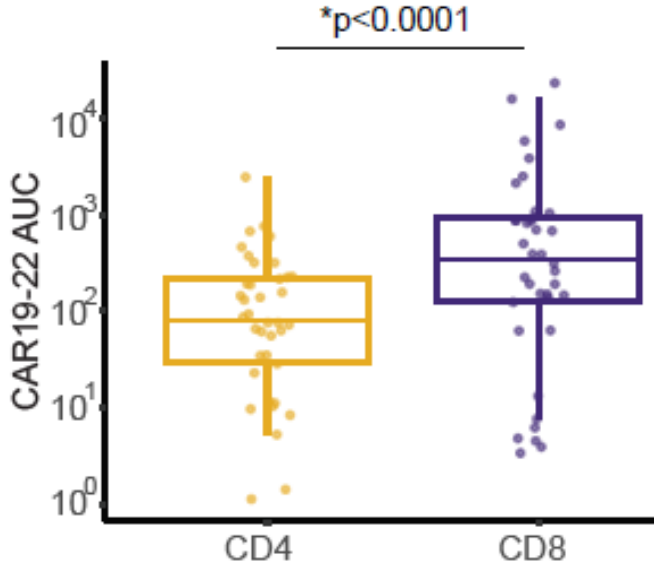

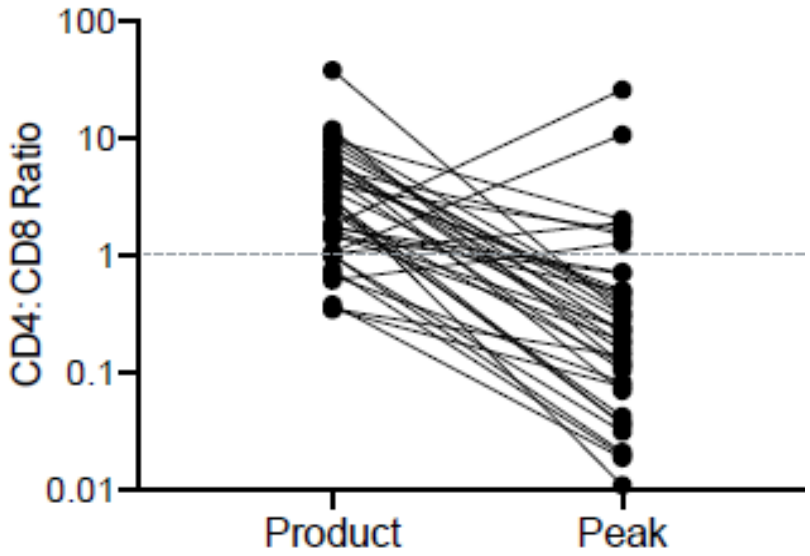

Spiegel, Patel, Muffly…Feldman, Mackall, Miklos. Manuscript accepted for publication

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CAR19-22 Expansion Associated with Neurotoxicity but Not Clinical Response

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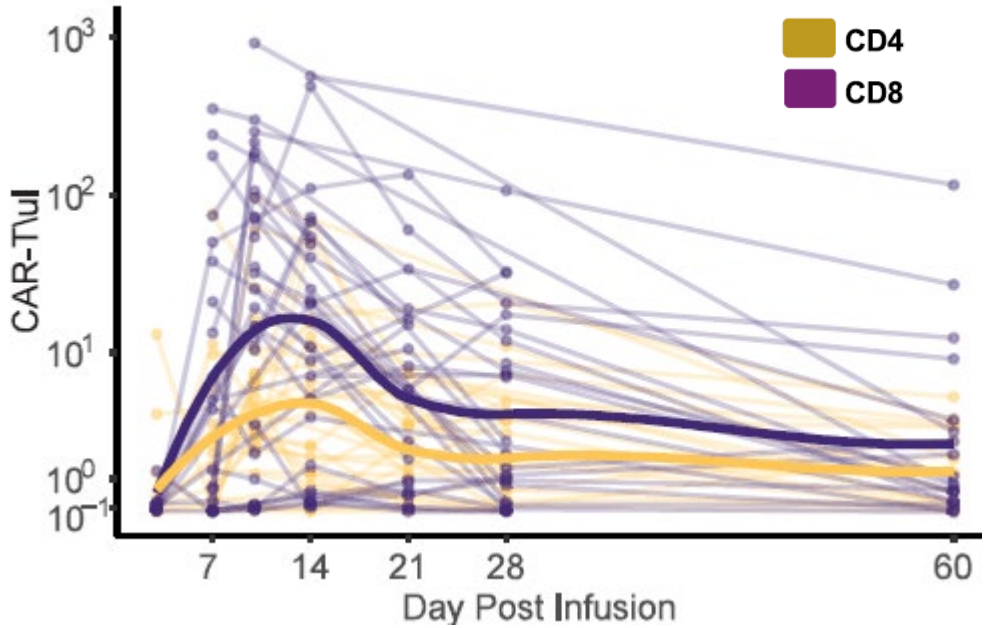

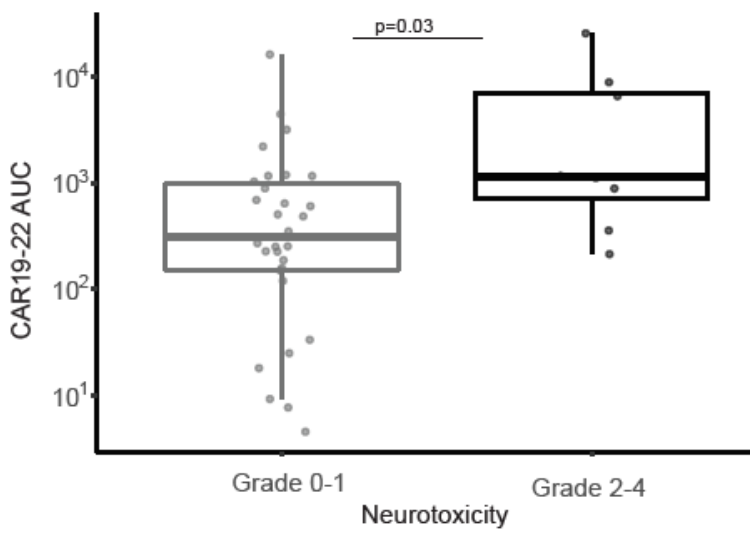

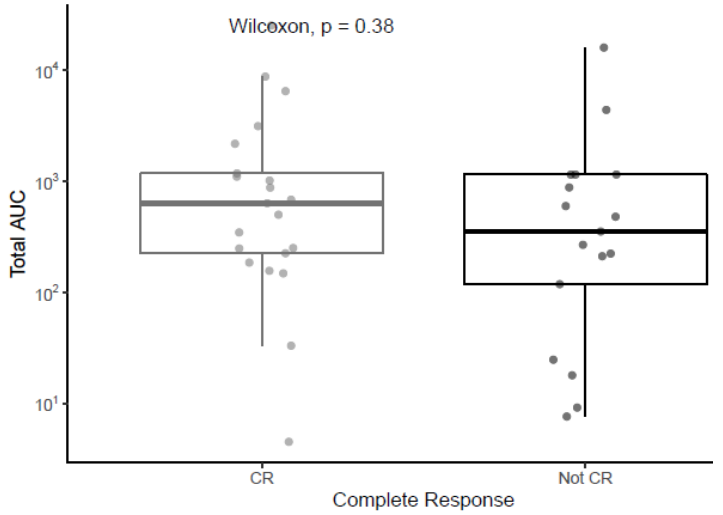

Spiegel, Patel, Muffly…Feldman, Mackall, Miklos. Manuscript accepted for publication

CIRM CALIFORNIA'S STEM CELL AGENCY

Patient Outcomes

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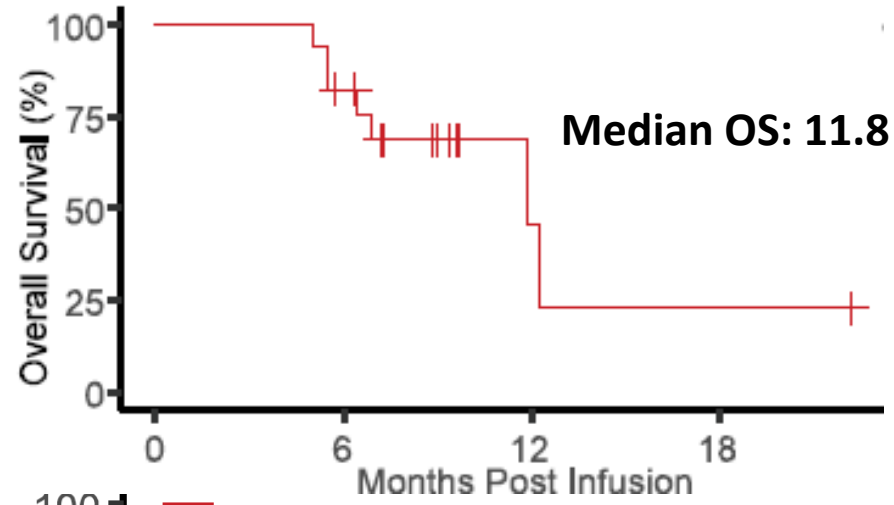
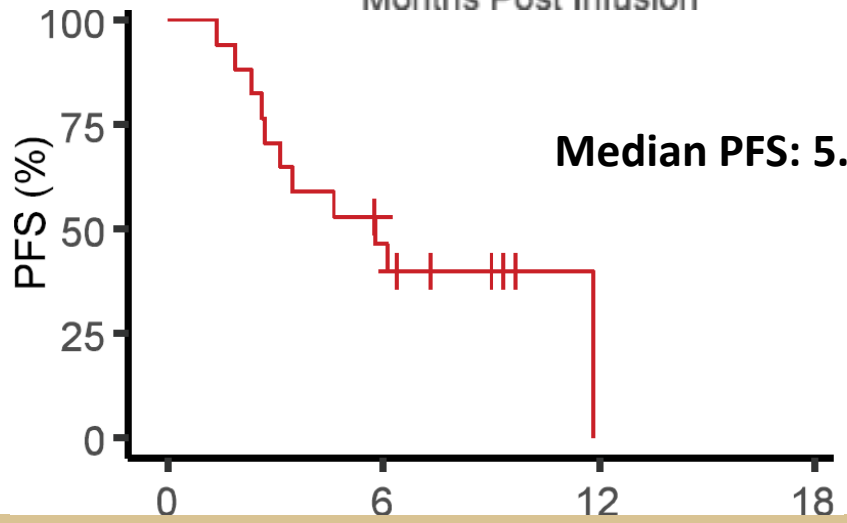
ALL : 100% ORR, 88% CR



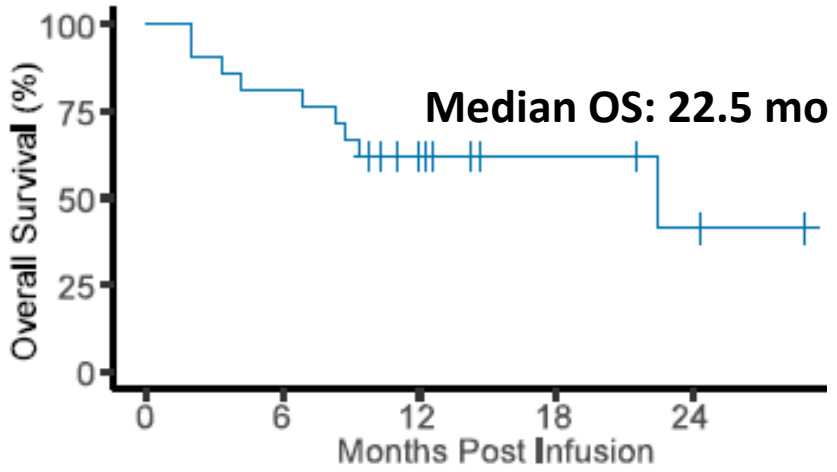
Median OS: 11.8 mo



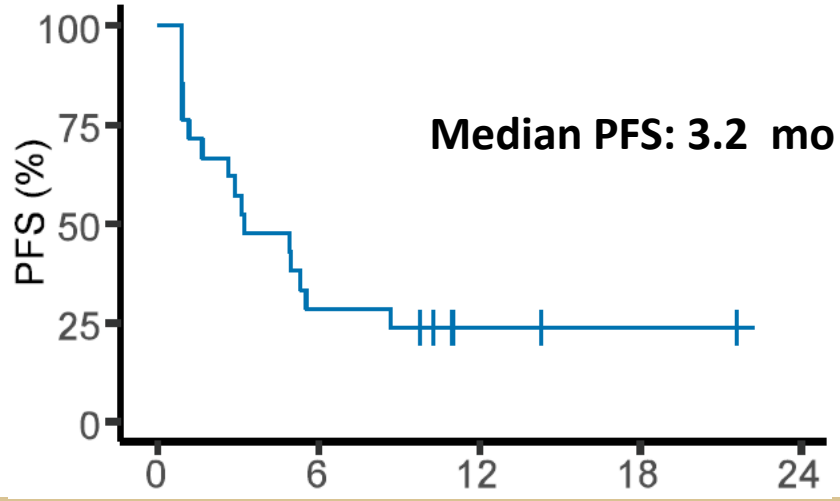
Median PFS: 5.8 mo

ALL n=17

Lymphoma : 62% ORR, 29% CR



Median OS: 22.5 mo



Median PFS: 3.2 mo

LBCL n=21

Spiegel, Patel, Muffly…Feldman, Mackall, Miklos. Manuscript accepted for publication

CD19 Negative Relapse Occurs after Treatment with CAR19-22

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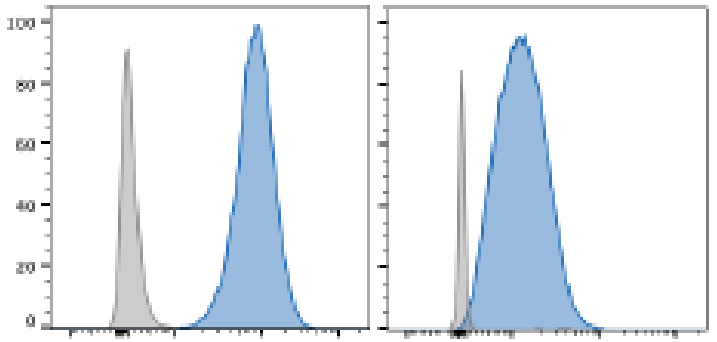
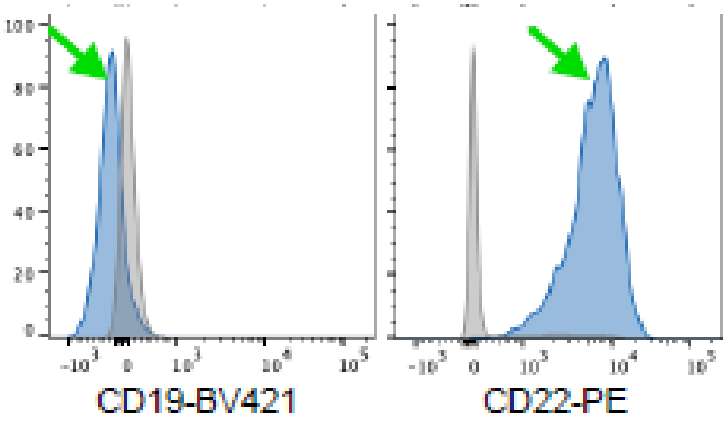
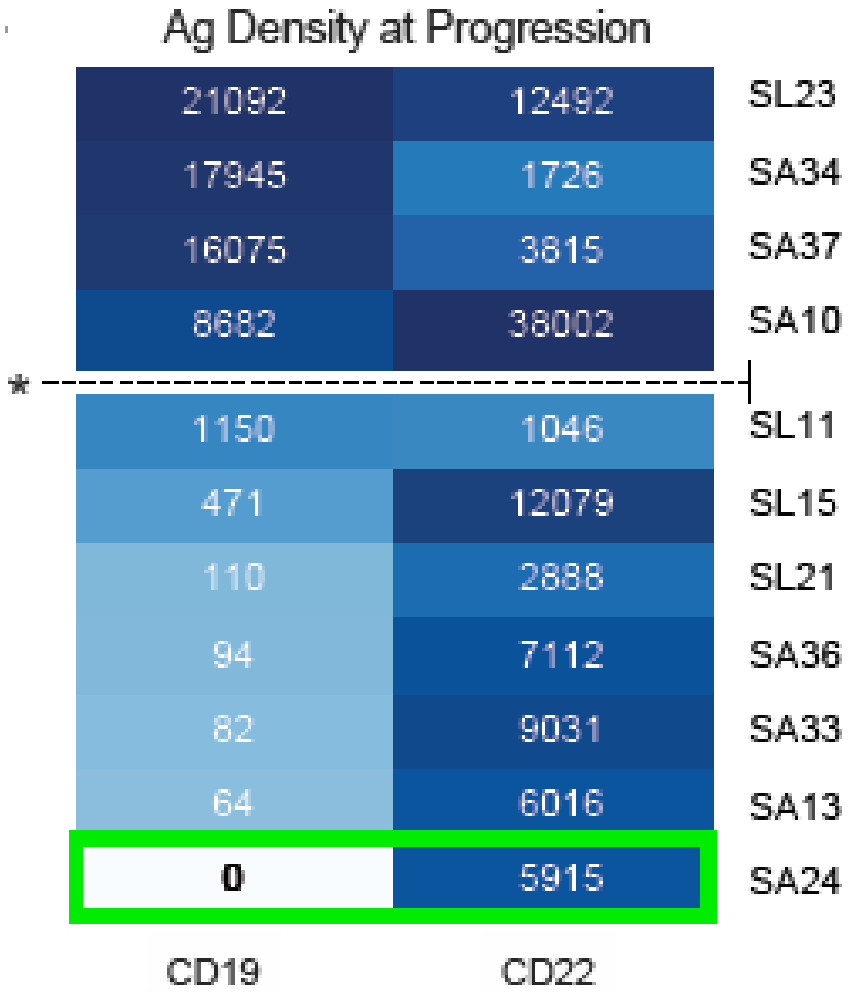
Before Therapy



Relapse after CAR19-22



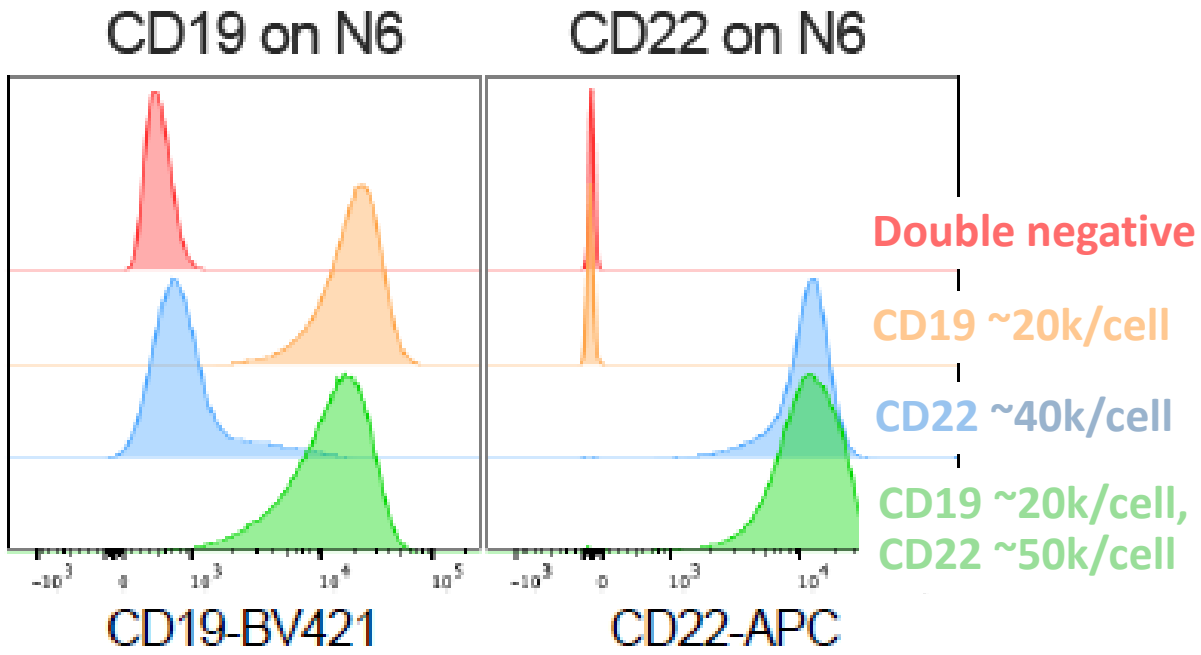
CD19 expression is lost, while CD22 expression is maintained

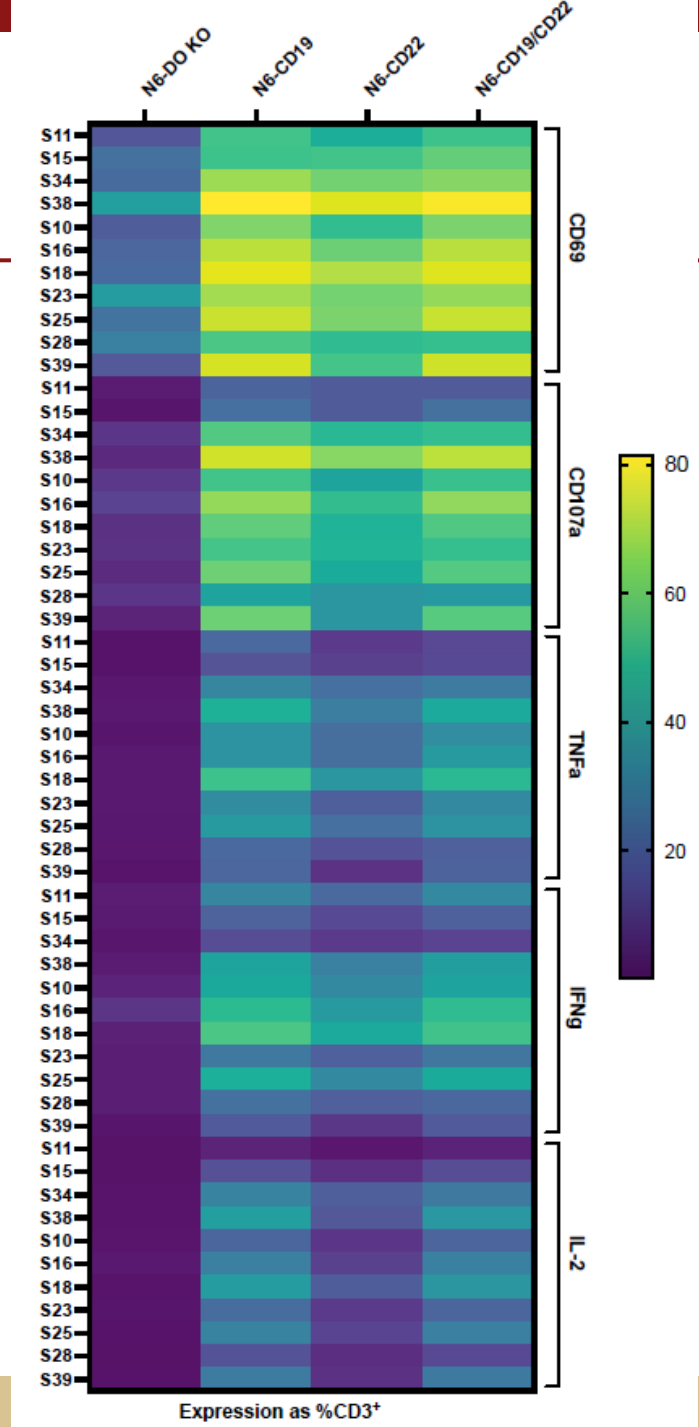


Relapsed Patients (n= 26)	CD19 Positive	CD19 Negative
DLBCL (n= 16)	9/14 (2 not bx)	5/14
ALL (n=10)	5/10	5/10

CIRM CALIFORNIA'S STEM CELL AGENCY

CAR19-22 Product Analysis Shows Different Cell Activation When Comparing CD19 and CD22





Lack of cytokine upregulation with CD22 expressing cell line provides basis for antigen relapse pattern

Spiegel, Patel, Muffly…Feldman, Mackall, Miklos. Manuscript accepted for publication

Mono-CAR22 is more functional than CAR19-22 when stimulated by CD22

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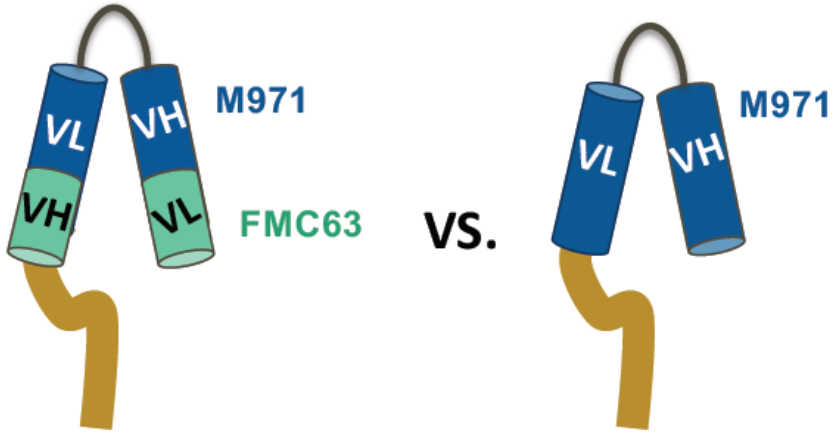
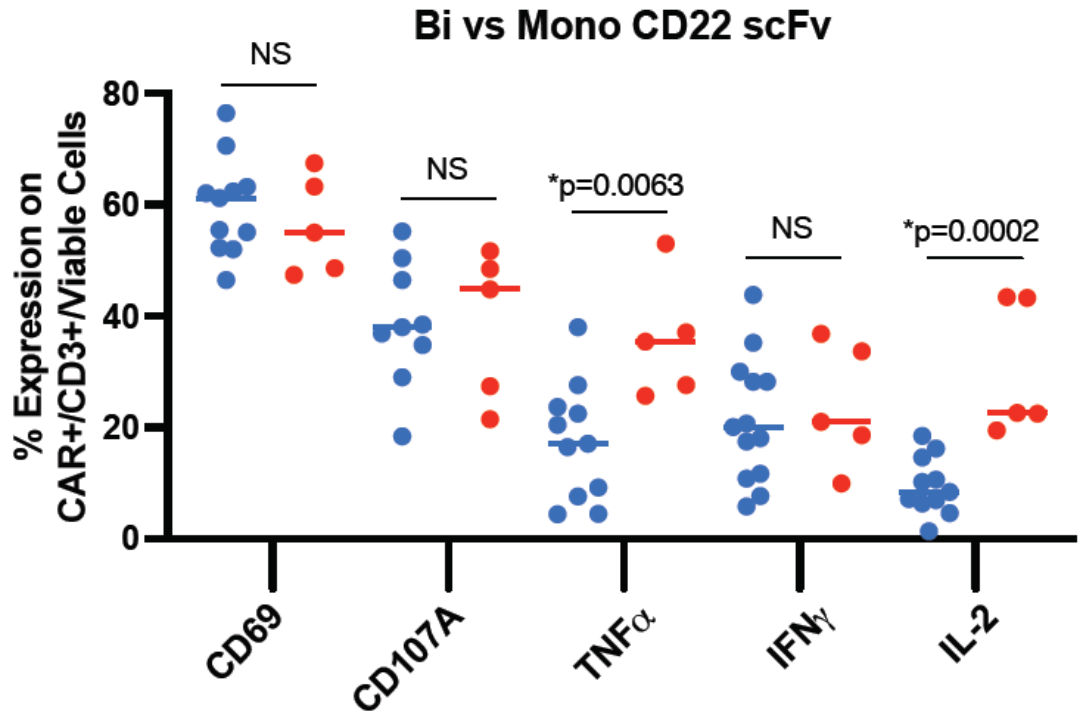
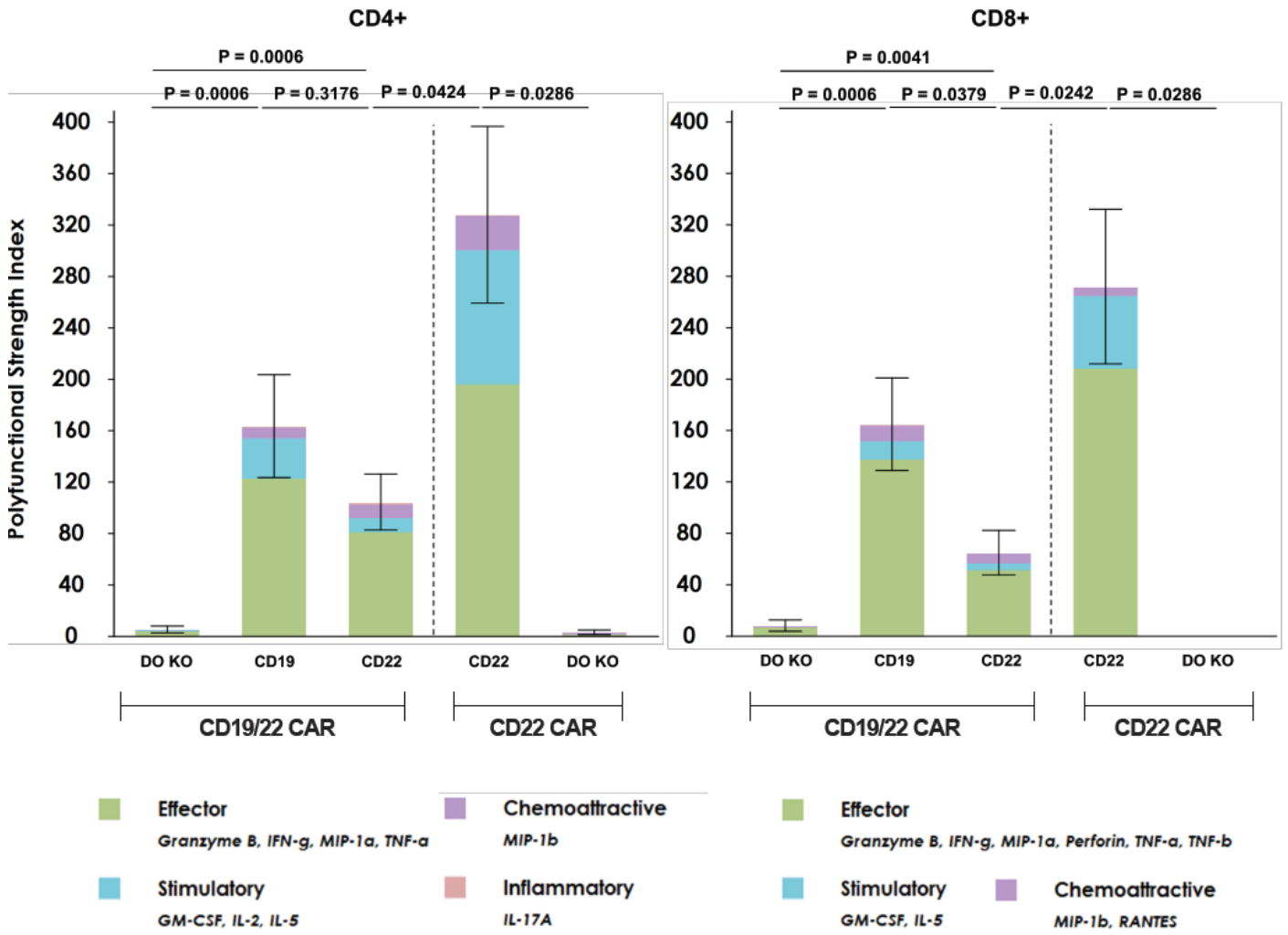


CAR19-22 **Mono-CAR22**



CIRM CALIFORNIA'S STEM CELL AGENCY

Isoplexis: Comparing CD19/22 to CD22 CAR



- Same pattern of higher cytokine secretion against CD19 stim versus CD22 Stim for the CD19/22 CAR
- The CD22 CAR secretes significant more cytokine against CD22HI compared to the CD19/22 CAR

Bispecific CAR-T Summary

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- Decreased CD19 Antigen expression accounts for at least 1/3 of CAR19 Axi-cel disease progression and multi-targeted CAR-T strategies are warrented
- Closed system manufacturing with the prodigy was more than feasible
- CAR19-22 had limited toxicity, one DLT grade 4 CRS and ICANS
  - Beneficial Clinical Outcomes: Overall Response: 69%
    - 20 DLBCL and 2 PMBCL: ORR: 62% → **29%CR**
    - 17 ALL patients: ORR:100% → **88% CR**
  - Unfortunately 36% DLBCL and 50% ALL subjects have relapsed CD19- CD22+, thus we will reapproach multi-antigen targeting with new constructs and strategies
- In the meantime, there is no commercially available therapy for Adults with R/R ALL
- We are optimizing the manufacturing for a more balanced CD4/CD8 cell product and have reopened the trial to treat another 15-20 ALL patients

CIRM
CALIFORNIA'S STEM CELL AGENCY

Sequential CAR-T Therapy:
First CD19 and then CD22

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CAR22.41BB Therapy
Induces durable Complete Remissions in Rel/Ref Large B-Cell Lymphoma who progressed after prior CAR19 therapy

Characteristic	Patient 1	Patient 2	Patient 3
Age at enrollment, years	57	53	51
Sex	Female	Female	Male
ECOG PS	1	1	1
Disease Classification			
2016 WHO classification	TFL	HGBCL	HGBCL
IHC and FISH status*	DEL	DHL	DHL
Cell of origin subgroup**	GCB	GCB	GCB
CD19 expression***	>90%	>90%	0%
CD22 expression***	>90%	>90%	>90%
Prior Therapy			
Prior lines of therapy†, n	5	8	6
Prior CAR T-cell therapy	Yes	Yes	Yes
Product(s) received	Axicabtagene cilocleucel	Lisocabtagene maraleucel	(1) Axicabtagene ciloleucel (2) MB-CART2019.1
Antigen(s) targeted	CD19	CD19	(1) CD19 (2) CD19 & CD20

John Baird, Matt Frank et al. Blood 2021

CAR22.41BB therapy achieved durable CR in patients with rel/ref LBCL after prior CAR19 therapy

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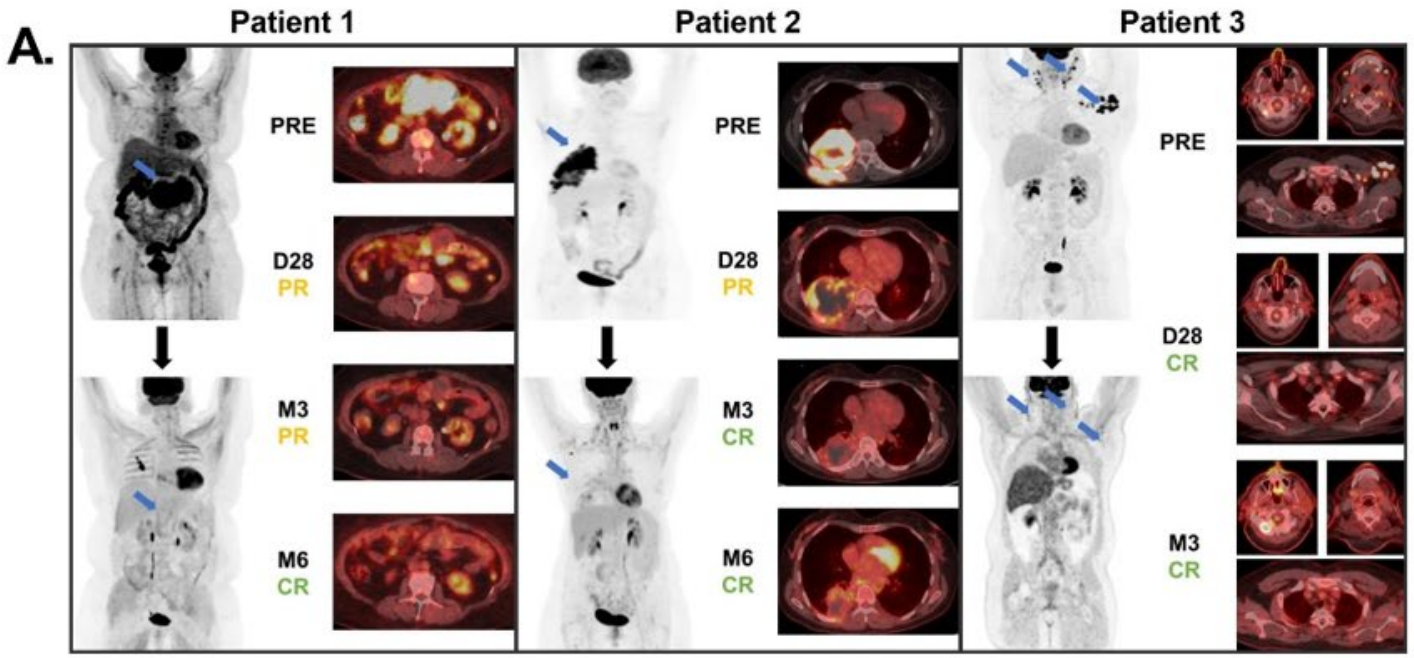

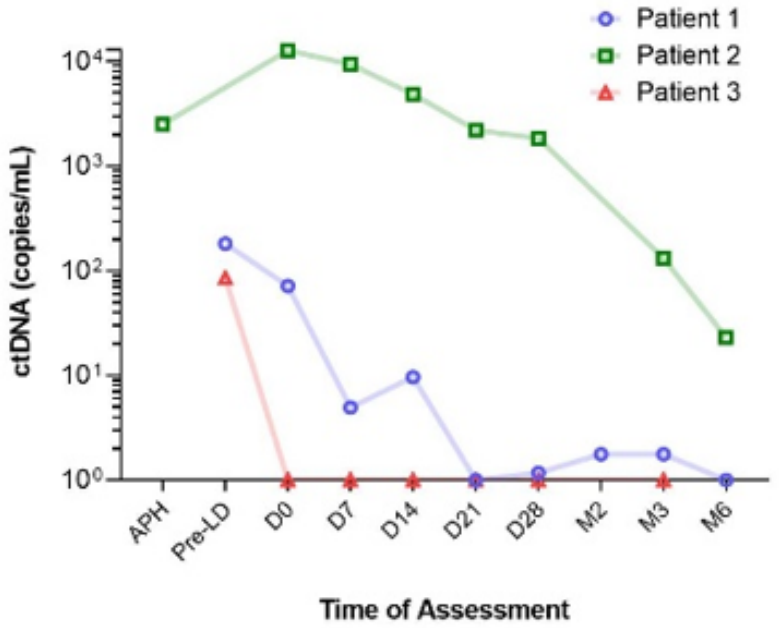

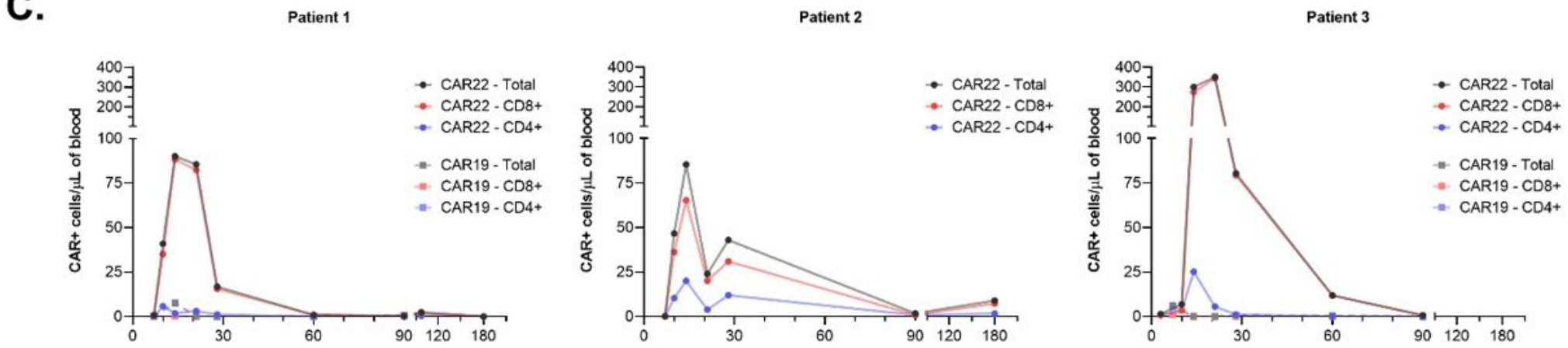

John Baird, Matt Frank et al. Blood 2021

CD20 expression is preserved despite loss of CD19 following Axi-Cel on Zuma-1

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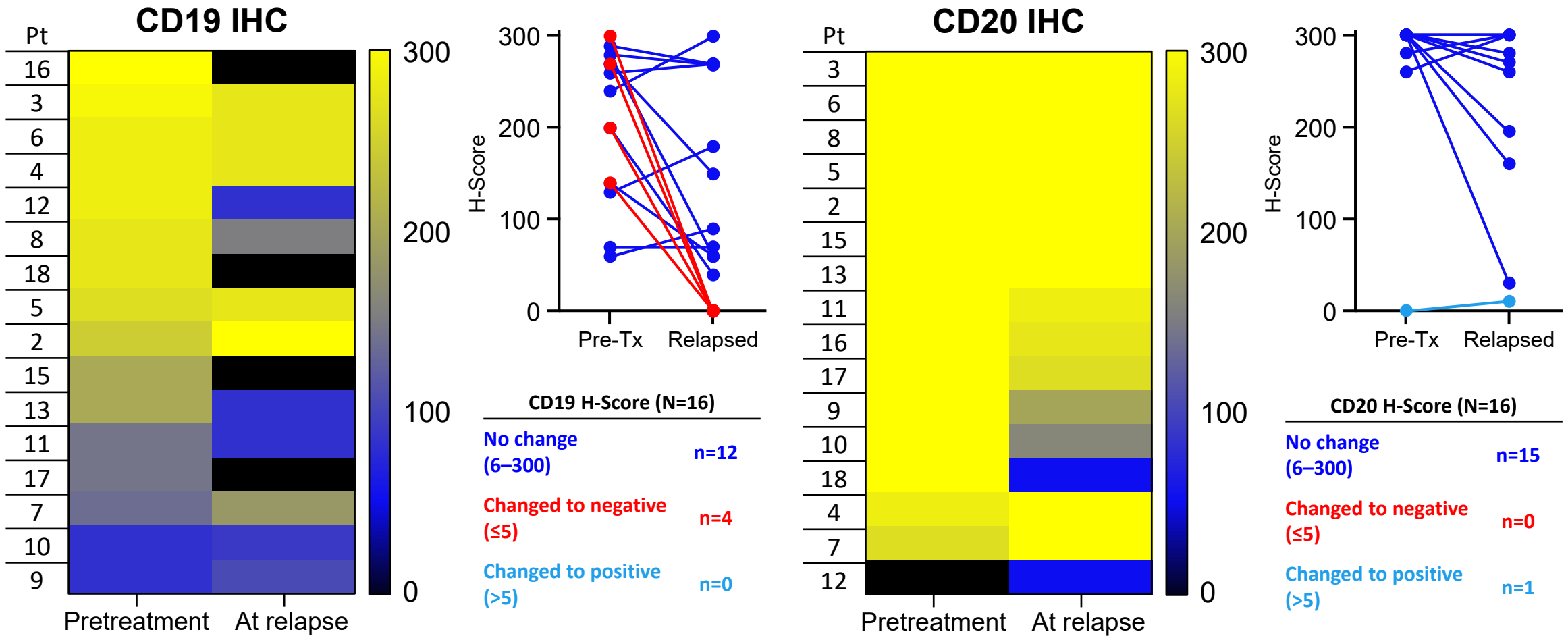

Pt 16 found to have down-regulation of CD19 by RNAseq

IHC: immunohistochemistry; Pt: patient; RNAseq: RNA sequencing; Tx: treatment

Neelapu SS, *et al.* ASH 2019 (Abstract 203; oral).

CAR-T Therapies Targeting both CD19 and CD20 simultaneously

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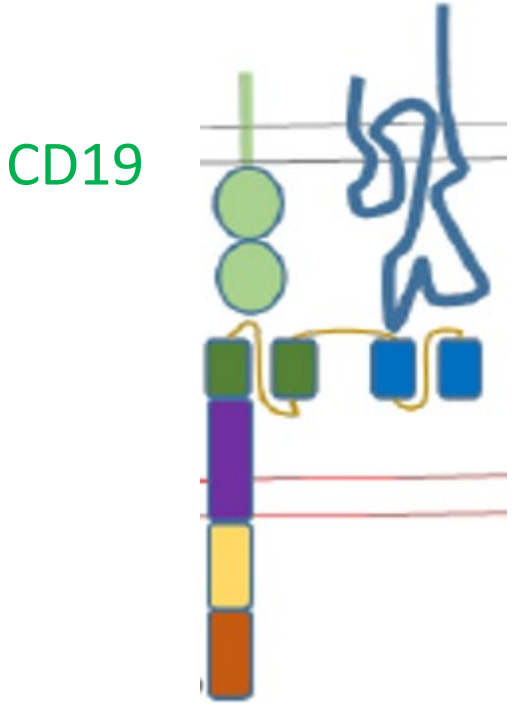
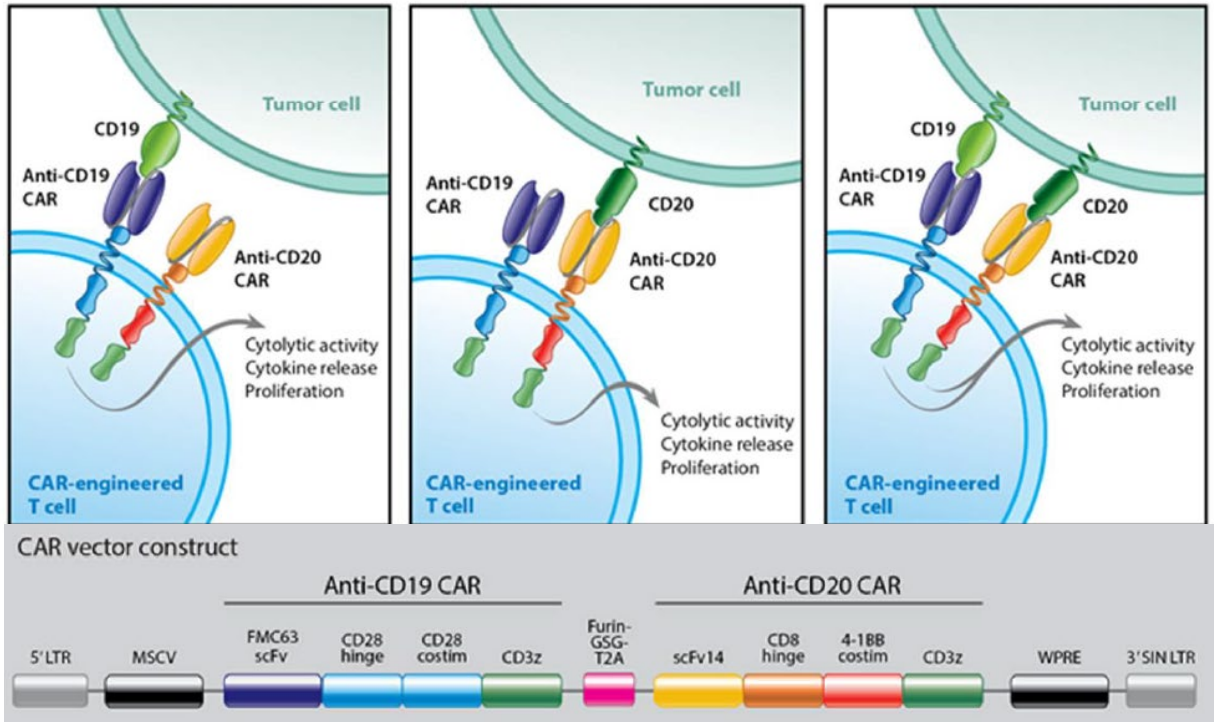
Miltenyi MB-CART2019.1

Single polypeptide bispecific anti-CD19 and CD20 CAR Therapy



Kite 363: Bi-cistronic co-Expression of anti-CD19 and CD20 CAR Therapy



Pro: Anticipate Axi-Cel efficacy without progression due to antigen loss

Con: Potentially more toxicity

Nirav Shah et al. Nature Medicine 2020; NCT04792489

Acknowledgements

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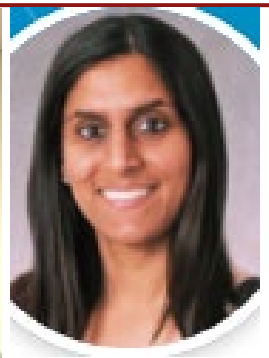
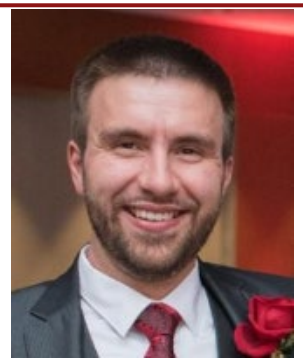
Jay Spiegel



Shabnum Patel

Steve Feldman

Bita Sahaf



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