Flow cytometry in CAR-T therapy

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NIH NATIONAL CANCER INSTITUTE

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Disclosure

I have no conflict of interest in relation to this presentation.

2

About the NIH Clinical Center

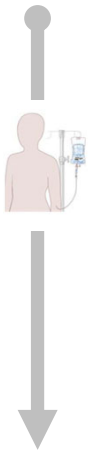
Opened in July 1953

NIH Clinical Center, Bethesda, Maryland

"The nation's largest hospital devoted entirely to clinical research."

3

What Information Can Flow Cytometry Provide?



- Is the patient a good candidate?
  - Disease burden
  - Target antigen expression
- Did infusion work?
- Did the disease respond?
- CAR-related toxicity?
  - MRD detection
  - CAR T monitoring
  - Depletion of normal cells
- Did the disease recur?
- Why recurrence?
- What can be done next?
  - MRD detection
  - CAR T monitoring
  - Target antigen
  - Alternative targets

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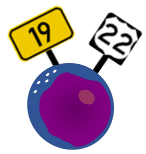
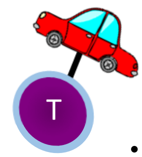
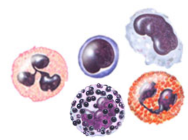
What Information Can Flow Cytometry Provide?



- MRD detection
- Antigen determination



- CAR T monitoring
- CAR T characterization



- Normal cell counterpart
- Microenvironment

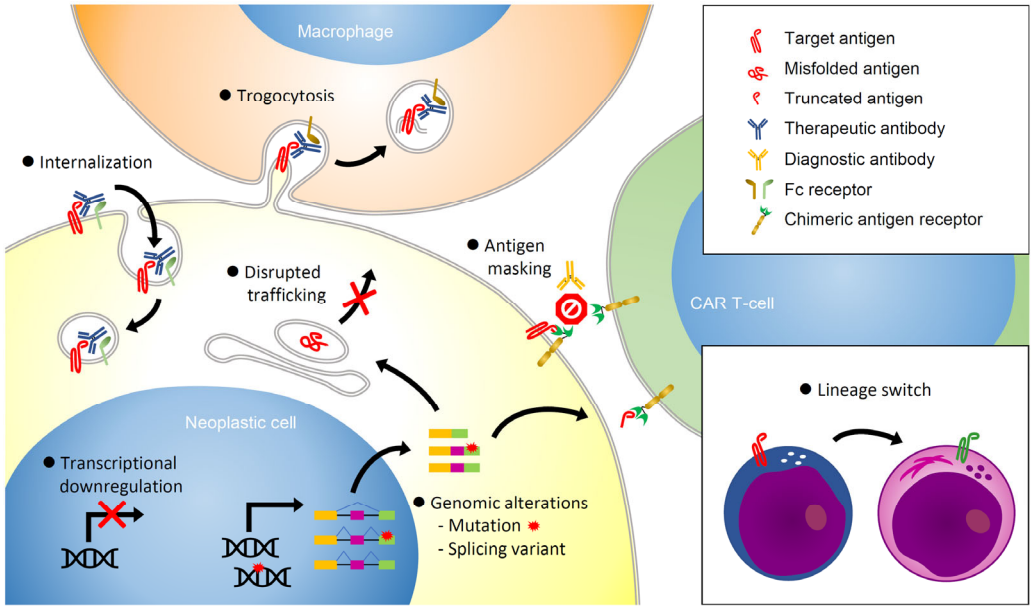
5

Challenges of MRD Detection in Post-immunotherapy Setting

- Antigen changes post-therapy (e.g. CD19-neg)
- Expansion of normal progenitor cells (e.g. CD19-neg)
- Lineage switch

6

Antigen Changes Post-immunotherapy



Zhou T & Wang HW. Clin Lab Med. 2021

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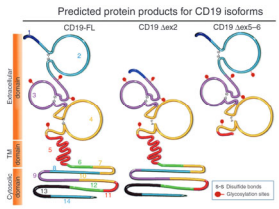
CD19 Modulation post CD19 CAR Therapy

Table 1. A summary of antigen escape in CD19 CAR trials for ALL

Trial	Population	CD19 CAR construct	Relapse rate	CD19-negative relapse rate	References
Children's Hospital of Philadelphia phase I	Pediatric	FMC63-4-1BB-ζ	36% (20/55)	24% (13/55)	8, 19
Novartis phase II (ELIANA)	Pediatric	FMC63-4-1BB-ζ	33% (20/61)	25% (15/61)	17
Seattle Children's Research Institute phase I	Pediatric	FMC63-4-1BB-ζ	45% (18/40)	18% (7/40)	33
NCI phase I	Pediatric	FMC63-CD28-ζ	29% (8/28)	18% (5/28)	9, 20
Memorial Sloan Kettering phase I	Adult	SJ25C1-CD28-ζ	57% (25/44)	9% (4/44)	21
Fred Hutchinson Cancer Center phase I	Adult	FMC63-4-1BB-ζ	31% (9/29)	7% (2/29)	11

Majzner R. & Mackall C. Cancer Discovery 2018



Alternative splicing is a common mechanism of CD19 modulation post CD19 CAR therapy

Sotillo E et al. Cancer Discovery 2015

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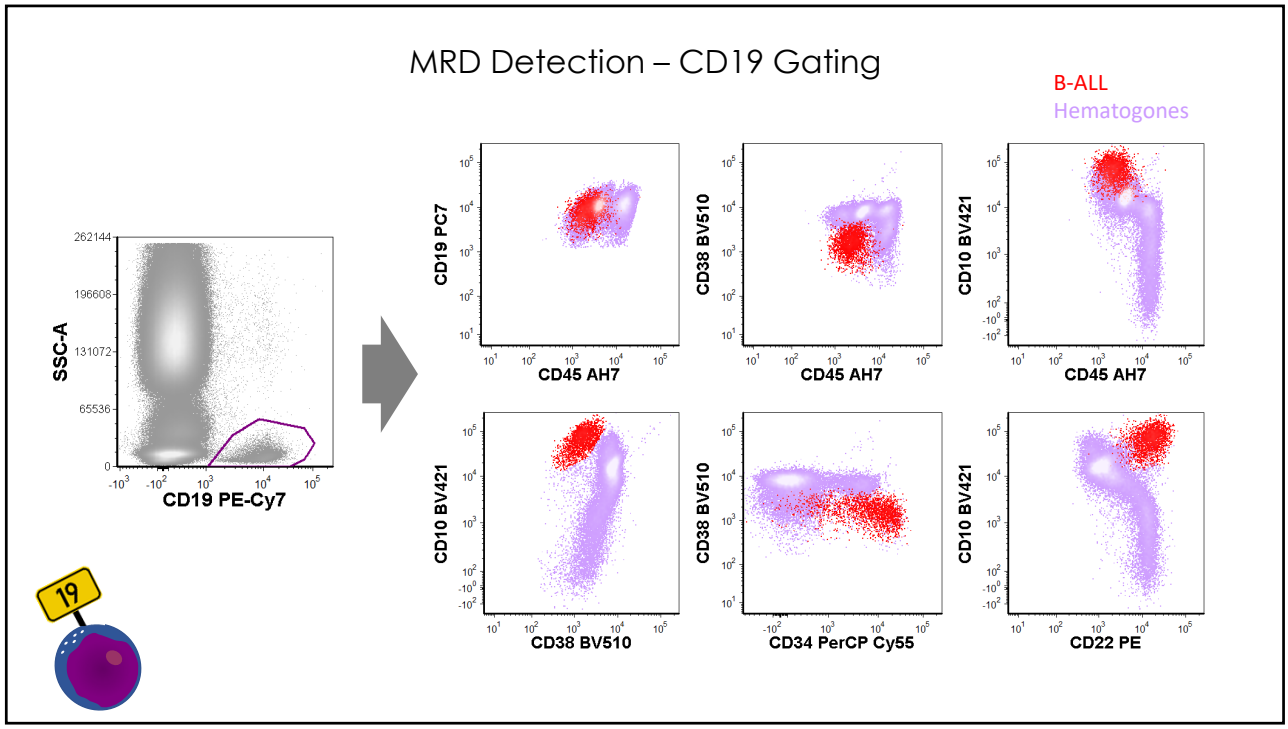
MRD Detection – CD19 Gating



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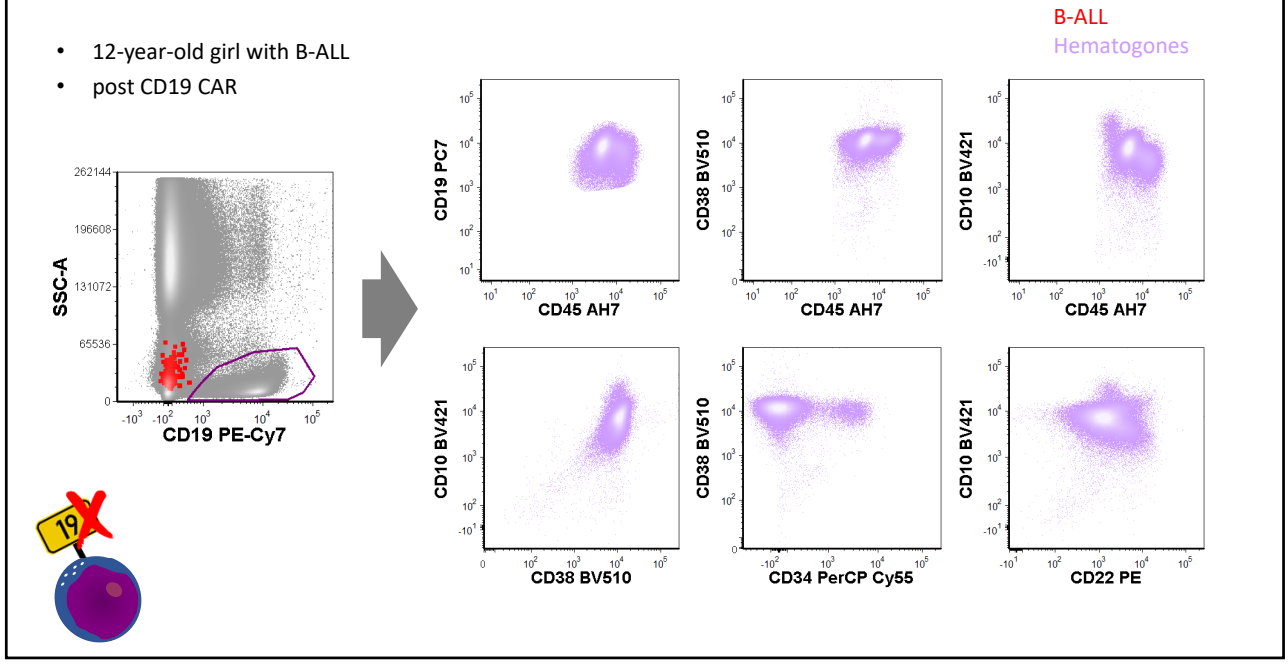
MRD Detection – Post anti-CD19 Therapy

- 12-year-old girl with B-ALL
- post CD19 CAR



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Alternative B-cell Gating

Original Article

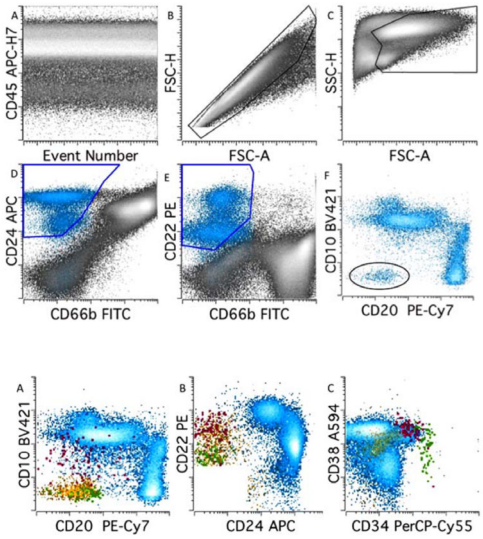
A Novel Flow Cytometric Assay for Detection of Residual Disease in Patients with B-Lymphoblastic Leukemia/Lymphoma Post Anti-CD19 Therapy

Sindhu Cherian,* Valerie Miller, Vivian McCullouch, Katy Dougherty, Jonathan R. Fromm, and Brent L. Wood
Department of Laboratory Medicine, University of Washington, Seattle, WA, USA

B-LL MRD Tube 2

CD10	HI10a	BV421	Biolegend
CD66b	80H3	FITC	BC
CD22	eBio4KB128	PE	eBioscience
CD34	8G12	PerCP-Cy5.5	BD
CD20	B9E9 (HCR20)	PE-Cy7	BC
CD38	HB-7	A594	BD
CD24	ALB9	APC	BC
CD45	2D1	APC-H7	BD

CD22+CD24+CD66b- gate contains:
- B-cell lineage (●)
- CD19-negative progenitors (●●)
- Basophils, dendritic cells, etc.



Cherian S. et al. Cytometry Part B: Clinical Cytometry 2018

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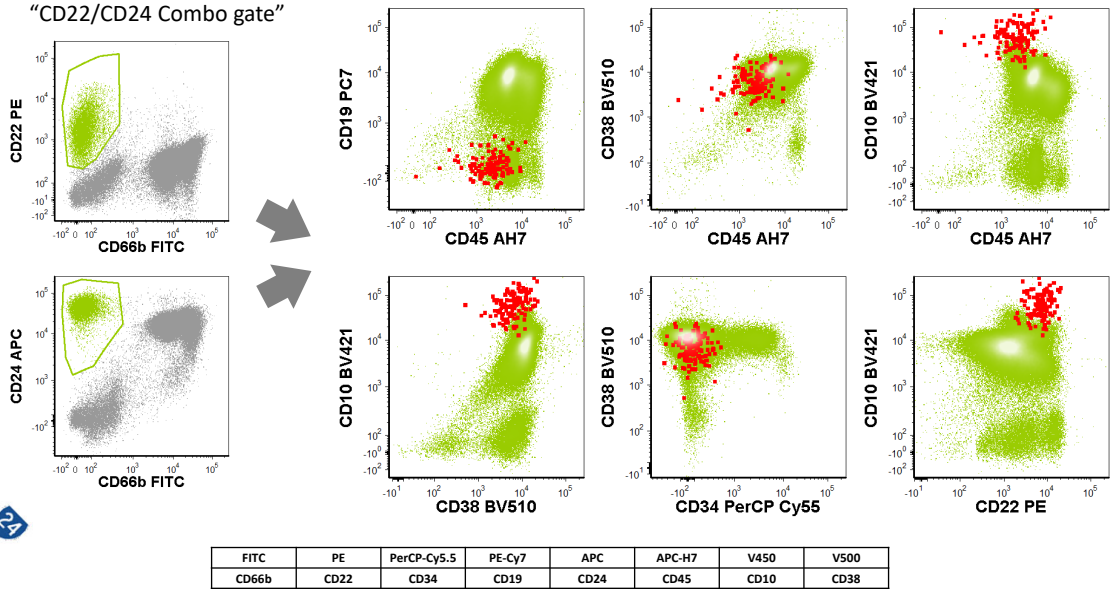
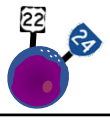
- 12-year-old girl with B-ALL
- post CD19 CAR

MRD Detection – CD22/CD24 Gating

"CD22/CD24 Combo gate"





FITC	PE	PerCP-Cy5.5	PE-Cy7	APC	APC-H7	V450	V500
CD66b	CD22	CD34	CD19	CD24	CD45	CD10	CD38

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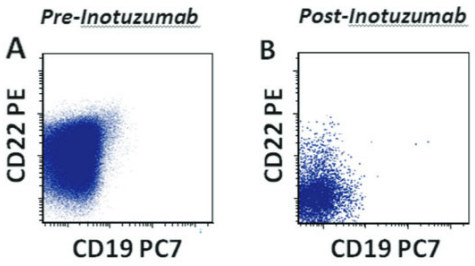
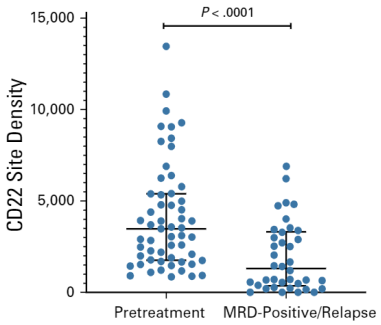
Down-regulation of CD22 post CD22-targeting

Inotuzumab (anti-CD22 antibody)



Bhojwani et al. Leukemia 2019

CD22 CAR T-cell therapy



Shah NN et al. Journal of Clinical Oncology 2020

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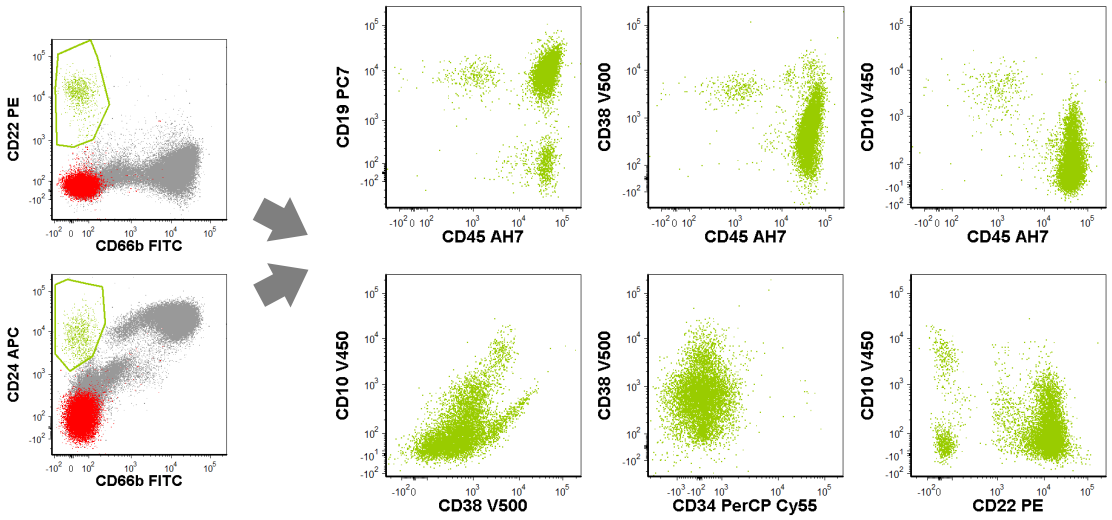
MRD Detection – Post anti-CD22 Therapy

- 15-year-old girl
- B-ALL post Inotuzumab and Blinatumomab
- 7 months post CD22 CAR T-cell therapy



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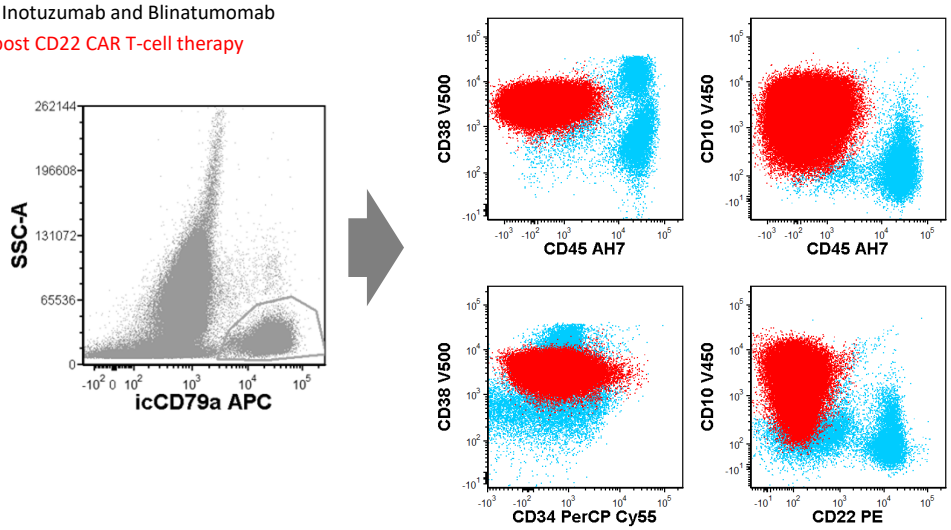
MRD Detection – icCD79a Gating

- 15-year-old girl
- B-ALL post Inotuzumab and Blinatumomab
- 7 months post CD22 CAR T-cell therapy



FITC	PE	PerCP Cy5.5	PE-Cy7	APC	APC-H7	V450	V500
CD20	CD22	CD34	CD19	icCD79a	CD45	CD10	CD38

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Challenges of MRD Detection in Post-immunotherapy Setting

- Antigen changes post-therapy (e.g. CD19-neg B-ALL)

Solution: Multiple-strategy approach

- CD19 gating
- CD22/CD24 gating
- iCD79a gating
- Ungated analysis
- Learn new strategies by attending ESCCA and ICCS meetings

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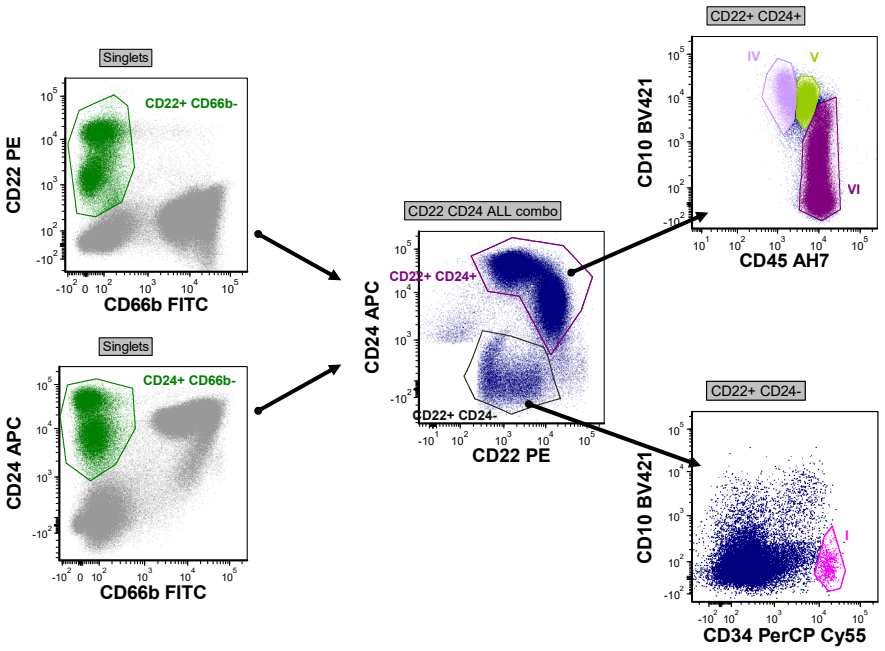
Normal Progenitor Population that May Mimic B-ALL



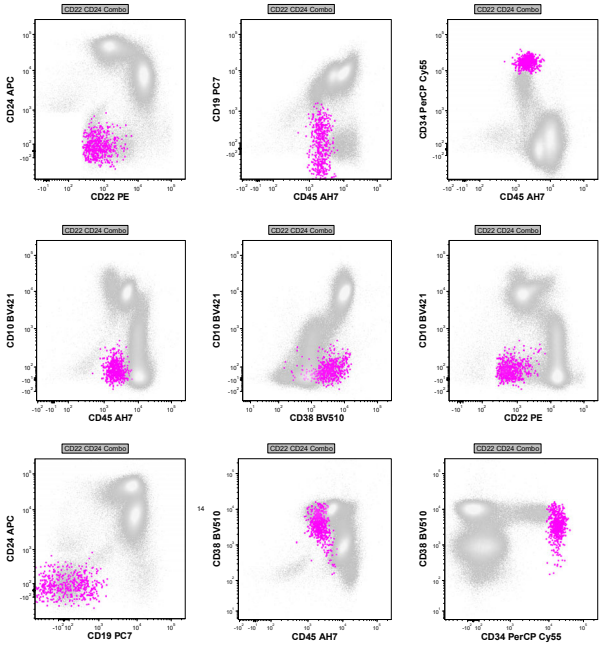
I CD22+ CD24- CD19- CD34b CD10-

IV CD22+ CD24+ CD19+ CD34+ CD10+

V CD22+ CD24+ CD19+ CD34- CD10+

VI CD22+ CD24+ CD19+ CD34- CD10dim/-

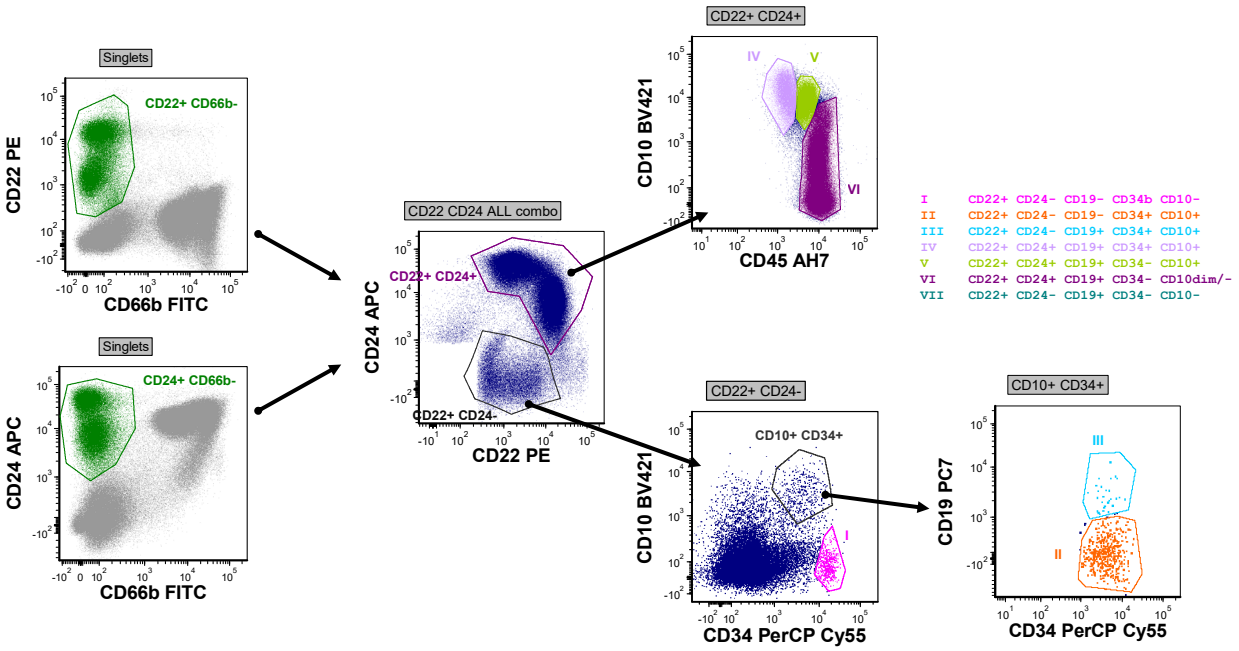
17



I CD22+ CD24- CD19- CD34b CD10-

18

Normal Progenitor Population that May Mimic B-ALL



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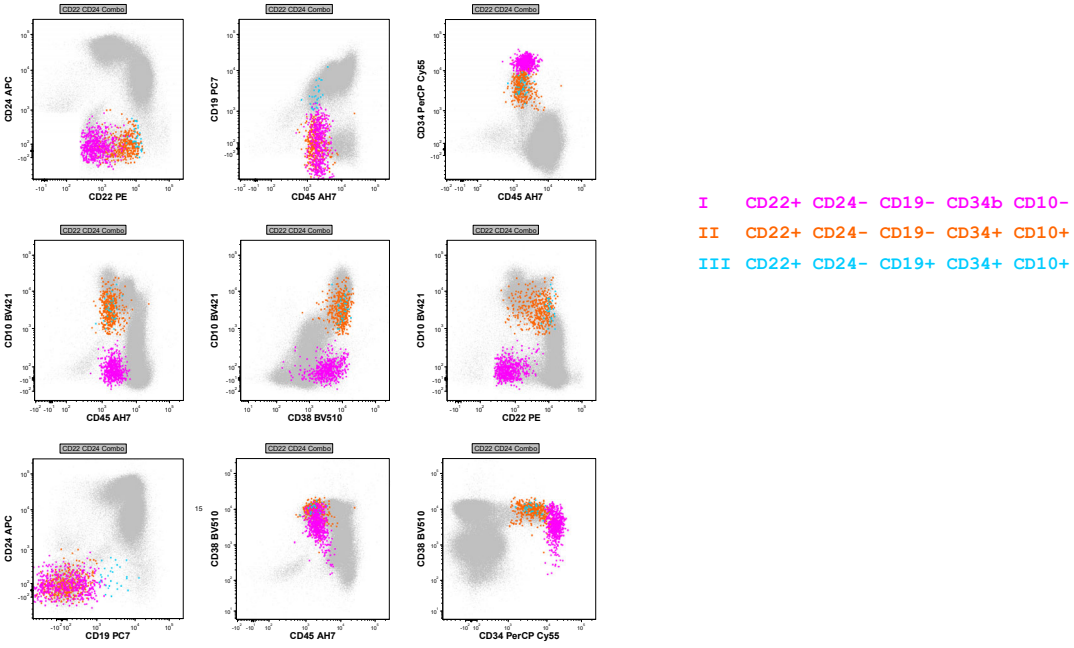

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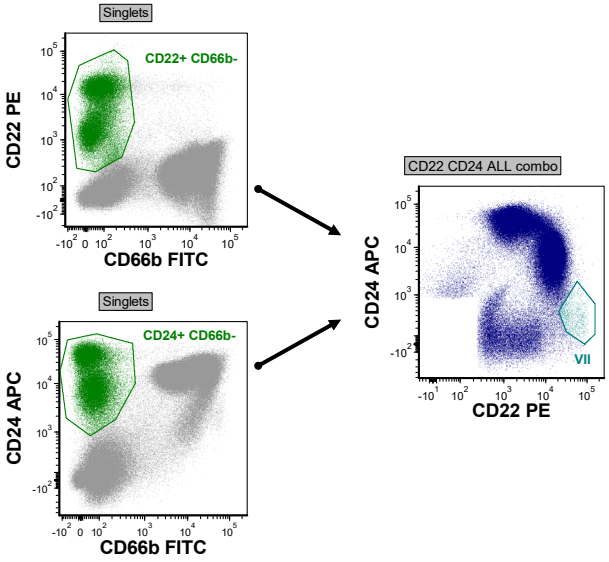
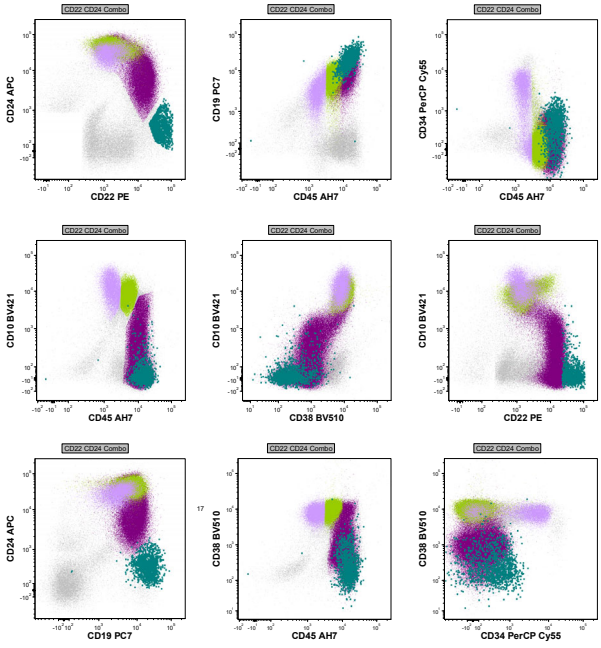
Normal Population that May Mimic B-ALL



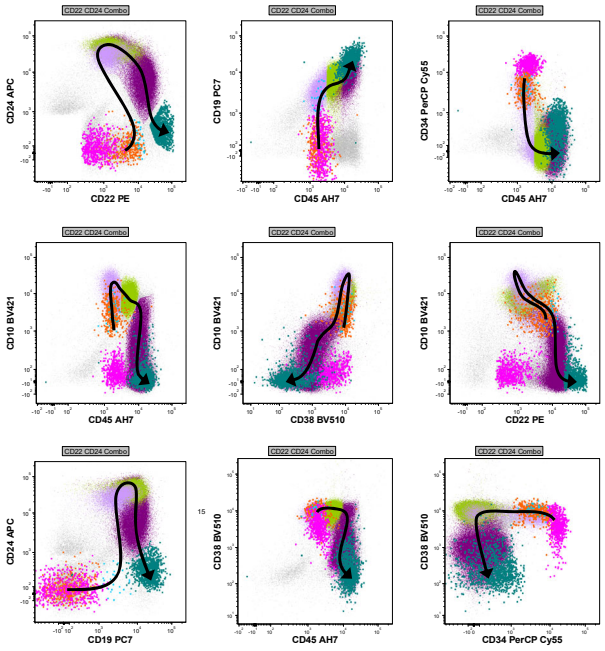
I CD22+ CD24- CD19- CD34b CD10-
II CD22+ CD24- CD19- CD34+ CD10+
III CD22+ CD24- CD19+ CD34+ CD10+
IV CD22+ CD24+ CD19+ CD34+ CD10+
V CD22+ CD24+ CD19+ CD34- CD10+
VI CD22+ CD24+ CD19+ CD34- CD10dim/-
VII CD22+ CD24- CD19+ CD34- CD10-

21



IV CD22+ CD24+ CD19+ CD34+ CD10+
V CD22+ CD24+ CD19+ CD34- CD10+
VI CD22+ CD24+ CD19+ CD34- CD10dim/-
VII CD22+ CD24- CD19+ CD34- CD10-

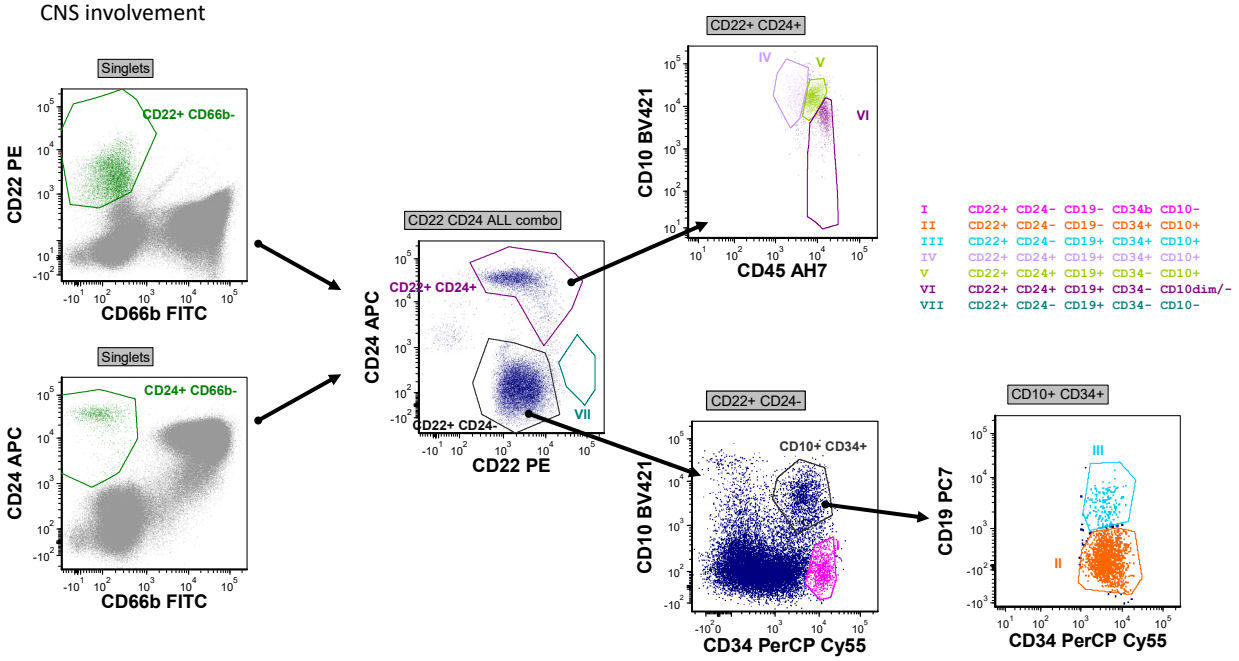
22



I	CD22+	CD24-	CD19-	CD34b	CD10-
II	CD22+	CD24-	CD19-	CD34+	CD10+
III	CD22+	CD24-	CD19+	CD34+	CD10+
IV	CD22+	CD24+	CD19+	CD34+	CD10+
V	CD22+	CD24+	CD19+	CD34-	CD10+
VI	CD22+	CD24+	CD19+	CD34-	CD10dim/-
VII	CD22+	CD24-	CD19+	CD34-	CD10-

23

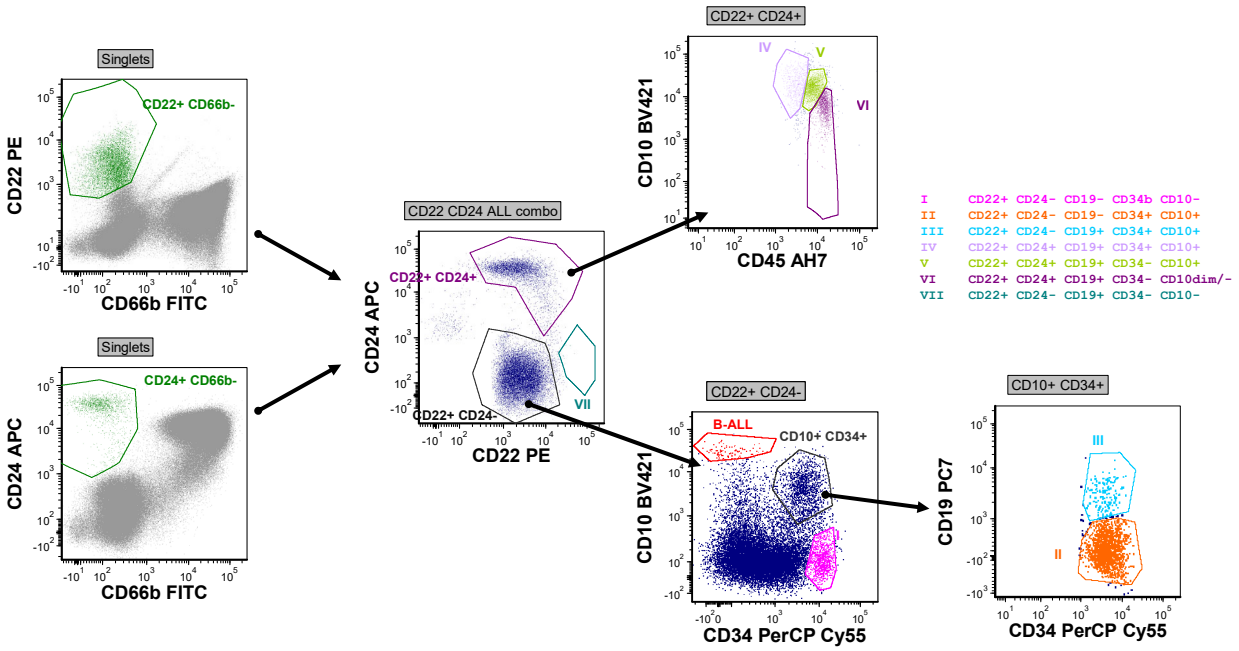
- BM from a 5-year-old boy with relapsed B-ALL and CNS involvement



I	CD22+	CD24-	CD19-	CD34b	CD10-
II	CD22+	CD24-	CD19-	CD34+	CD10+
III	CD22+	CD24-	CD19+	CD34+	CD10+
IV	CD22+	CD24+	CD19+	CD34+	CD10+
V	CD22+	CD24+	CD19+	CD34-	CD10+
VI	CD22+	CD24+	CD19+	CD34-	CD10dim/-
VII	CD22+	CD24-	CD19+	CD34-	CD10-

24

- 5-year-old boy, relapsed B-ALL with CNS involvement



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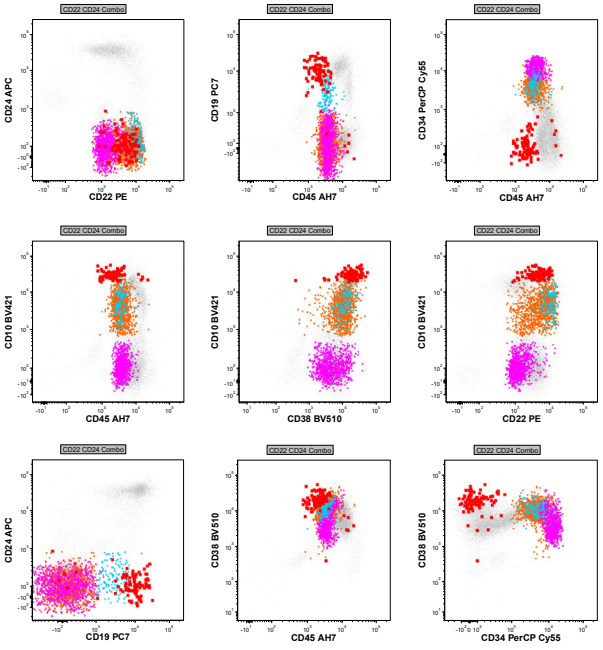

I	CD22+	CD24-	CD19-	CD34b CD10-
II	CD22+	CD24-	CD19-	CD34+ CD10+
III	CD22+	CD24-	CD19+	CD34+ CD10+
B-ALL	CD22+	CD24-	CD19+	CD34- CD10b

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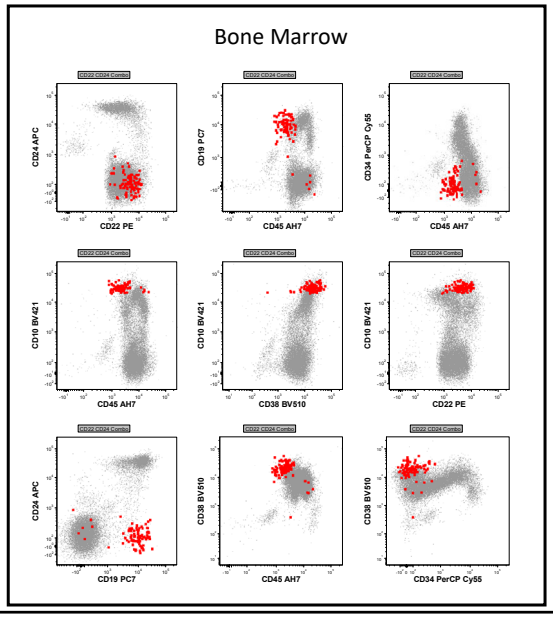
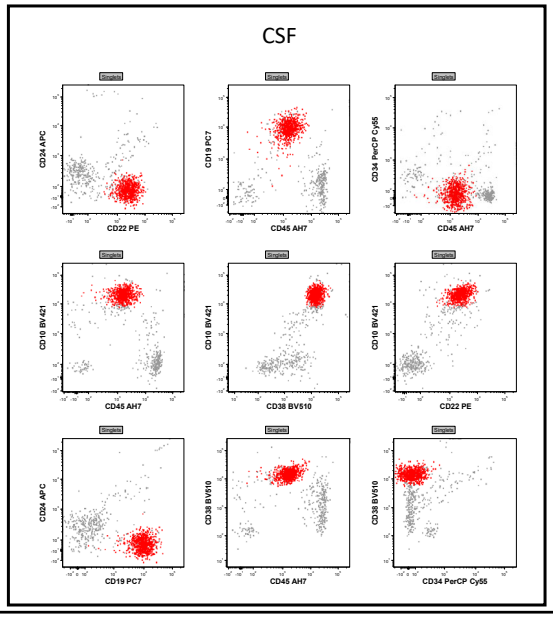
- 5-year-old boy, relapsed B-ALL with CNS involvement
- Screening for CD22 CAR





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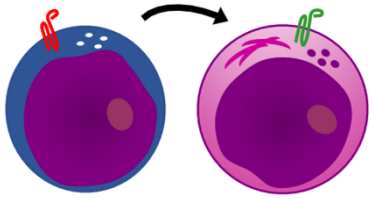
B-ALL Lineage Switch post Immunotherapy

- Rare but real
- Most commonly from B-cell to myeloid / monocytic lineages
- Associated genomic abnormalities reported:
  - KMT2A (MLL1) rearrangement
  - DUX4 rearrangement
  - ZNF384 rearrangement
  - PAX5-P80R mutation



Jacoby et al. Nat Commun. 7, ncomms12320 (2016)
Gardner et al. Blood. 127, 2406–2410 (2016)
Rayes et al. Pediatr Blood Cancer. 63, 1113–5 (2016)
Novakova et al. Haematologica. 106, 2066–2075 (2021)

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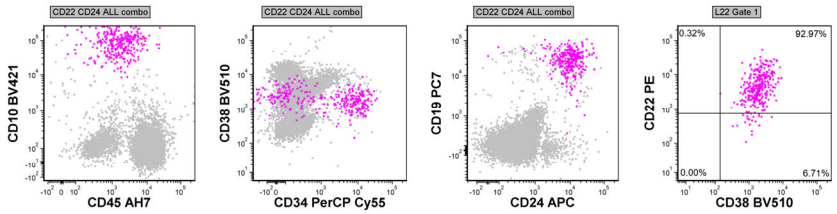
- 19-year-old male with relapsed/refractory B-ALL with extramedullary involvement
- s/p AALL-1821 & AALL-1331 (**blinatumomab**)

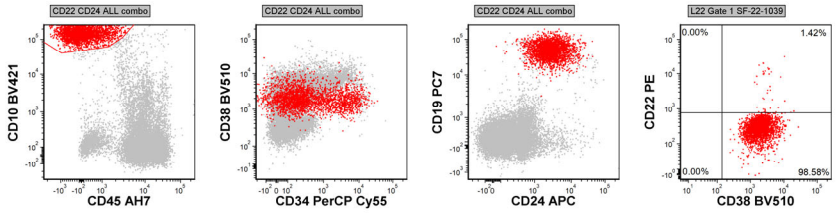
March 2022



CD22 site density:
5,462 molecules/cell

- AALL-1632 (**Inotuzumab**) x 2 cycles

May 2022



CD22 negative

➢ CD19/CD22 bi-specific CAR T-cell therapy (NCT03448393)

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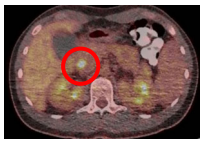
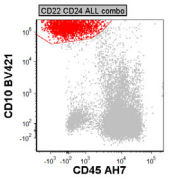
SUV=15.5



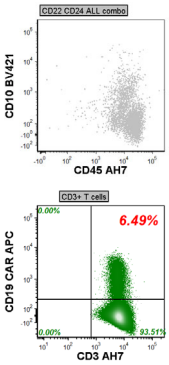
Day +30 PET/CT

CAR infusion

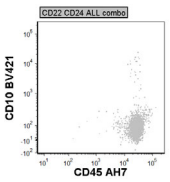
Pre-CAR BM



Day +21 BM:
MRD neg

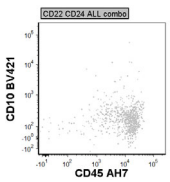


Day +33 PB:
MRD neg



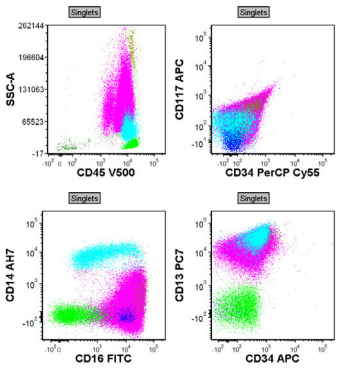
ClonoSEQ MRD+
(1.5×10^{-3})

Day +43 PB:
MRD neg



ClonoSEQ MRD+
(3.0×10^{-3})

Day +47 PB:
MRD neg



30

CAR infusion

Day +49 BM

Rare clusters of blasts

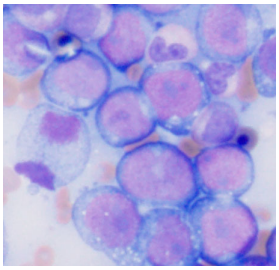
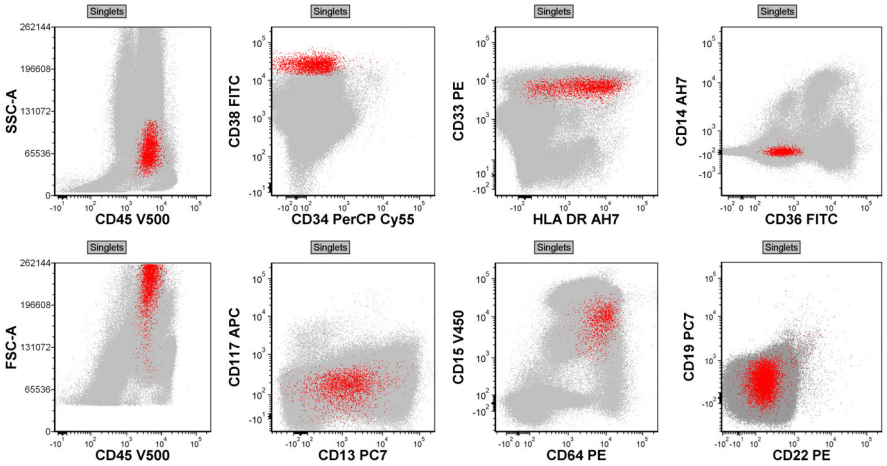


Image courtesy of Dr. Nisha Patel



ClonoSEQ:
MRD+ (1 x 10^{-2}), **same IGK clonotype as in B-ALL**

Lineage-switched acute monoblastic leukemia

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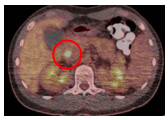
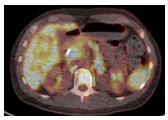


Day +30 PET/CT

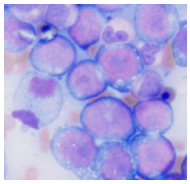


Day +62 PET/CT

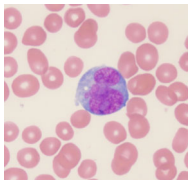
Patient passed away at Day +90 despite 2 lines of AML therapy

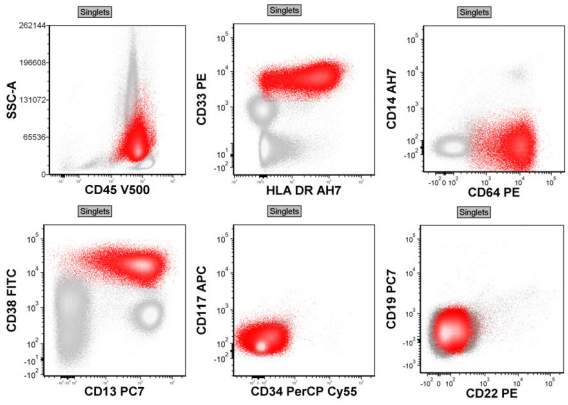
CAR infusion

Day +49 BM



Day +64 PB





BM and PB smear images courtesy of Dr. Nisha Patel

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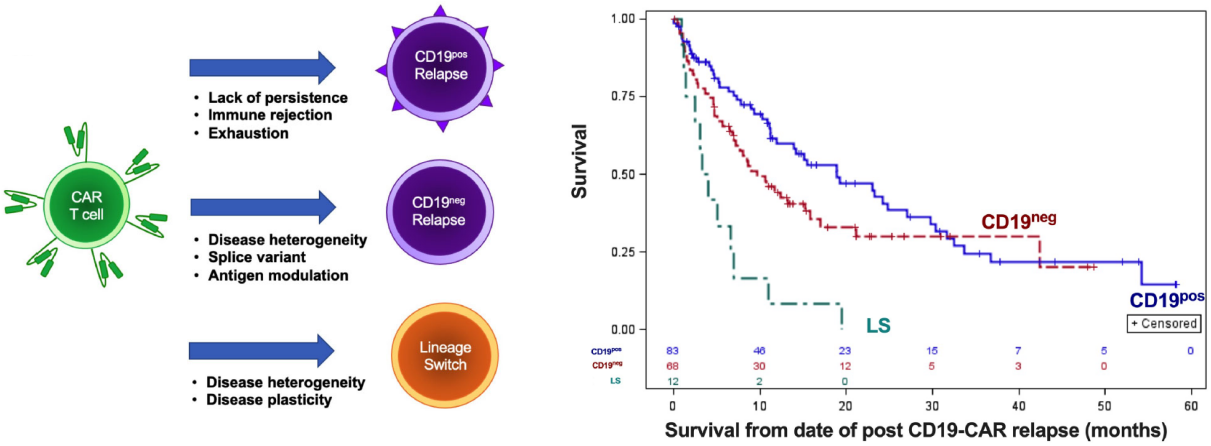
Relapse Phenotype vs Treatment Outcome



Lamble, AJ et al. Blood Adv 2022

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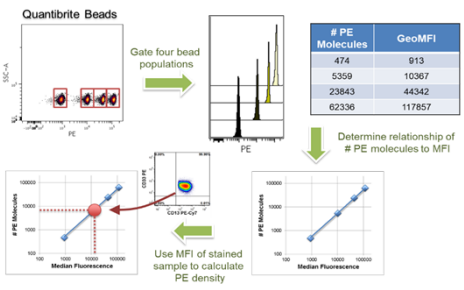
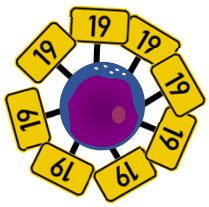


Key points:

- Bright fluorophore
- Available Beads
- Standard curve QC
- Need info about antibody F/P
- Saturated antibody concentration



Antigen Site Density or Antibody Binding Capacity "ABC"
Antigen / cell

Saturated condition

Antibody / cell

"F/P ratio" antibody information
Fluorochrome / protein (antibody)

Fluorochrome / cell

"MFI"
Fluorescent intensity

Jasper, G. et al. Cytometry Part B: Clinical Cytometry 2011: 80B(2), 83-90.
Salem, D. et al. Leukemia Research 2018: 71, 106-111.

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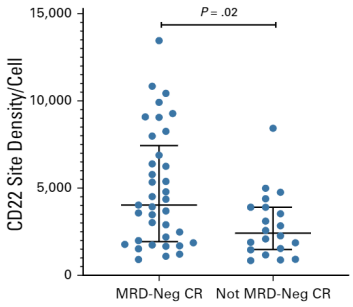
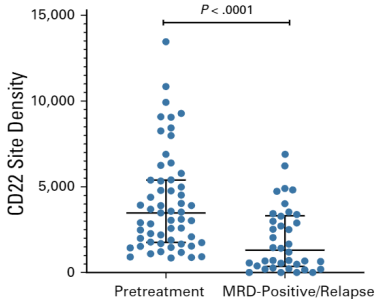
Target Antigen Density vs with Response/relapse



CD22 level correlates with MRD-negative remission



CD22 loss/downregulation is common in relapse

Shah NN et al. *J Clin Oncol* 2020

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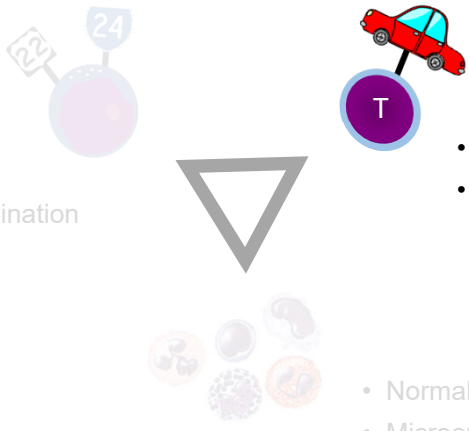

- MRD detection
- Antigen determination

- CAR T monitoring
- CAR T characterization

- Normal cell counterpart
- Microenvironment

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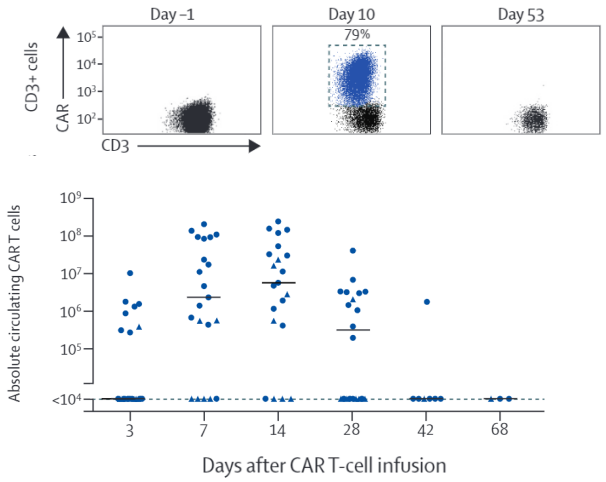
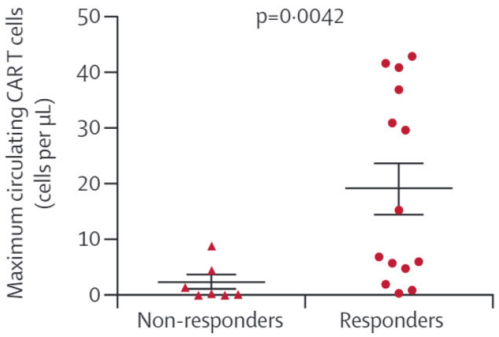
CAR T-cell Expansion as a Determinant of Response to CAR Therapy

- CD19 CAR for B-ALL, NCI data





Lee D et al. *Lancet* 2015

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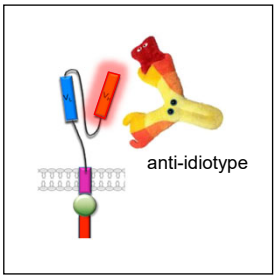
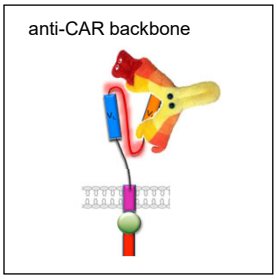
Flow Cytometric Detection of CAR T Cells

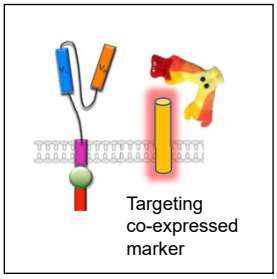
Anti-CD19 idiotype (anti-FMC63)
- Tisagenlecleucel (Kymriah™)
- Axicabtagene ciloleucel (Yescarta™)

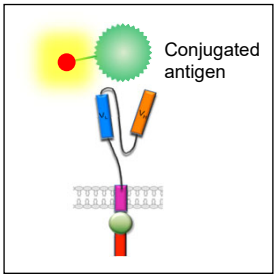




Anti-Whitlow (Kip1, KITE Pharma)
- Axicabtagene ciloleucel (Yescarta™)
- Anti-CD30 CAR (Kochenderfer)
- Anti-CD19/CD20 CAR (Kochenderfer)

Anti-EGFRt
- TSLPR CAR





- CD22 (Siglec-2)-Fc
- BCMA (TNFRSF17)-Fc
- CD319 (SLAMF-7)-Fc
- CD33 (Siglec-3)-Fc

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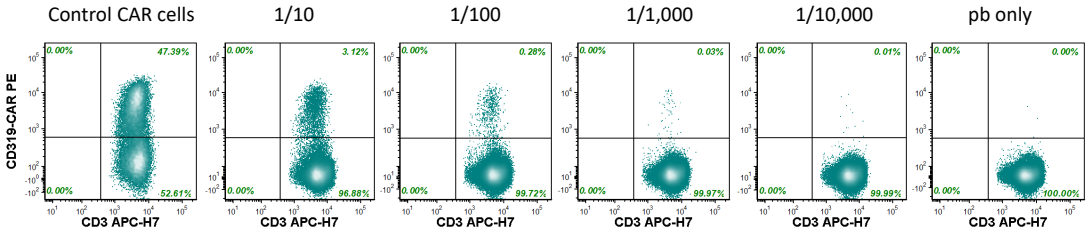
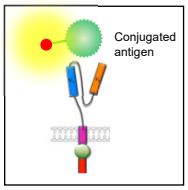
CAR T Cell Detection by Flow Cytometry - LOD Determination

Control CAR cell : peripheral blood serial dilution





CD319 (SLAMF-7)-Fc-PE (Kochenderfer)

Wang HW. ICCS e-Newsletter Volume XI, No. 2, 2020

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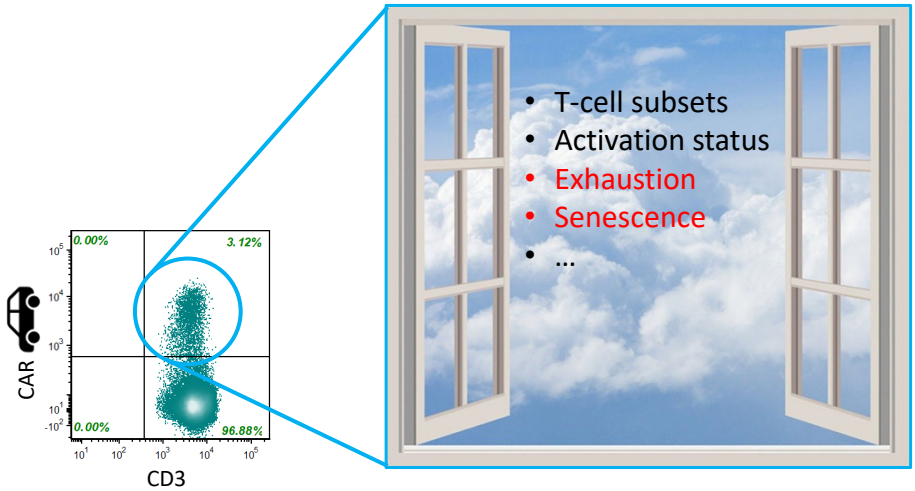
Phenotypic Characterization of CAR T Cells



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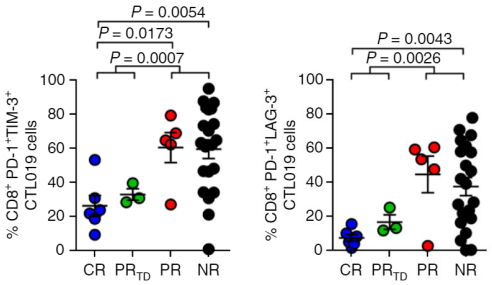
Exhaustion Phenotype in CAR T cells is Associated with Resistance

University of Pennsylvania

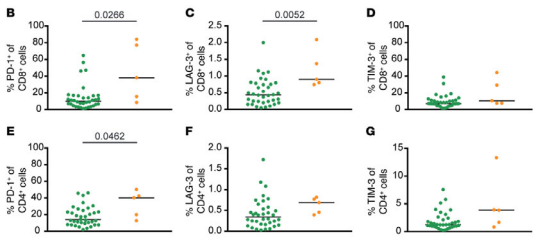
- CD19 CAR for CLL
- 4-1BB costimulatory domain



Fraietta JA et al. Nature Medicine 2018

Seattle Children's

- CD19 CAR for B-ALL
- CD28/4-1BB costimulatory domain



Finney OC et al. Journal of Clinical Investigation 2019

- Exhaustion Phenotype in CAR product is associated with unfavorable response
- Need longitudinal data
- Identify candidates for combined checkpoint inhibitor?

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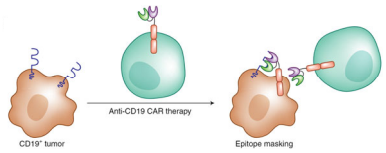
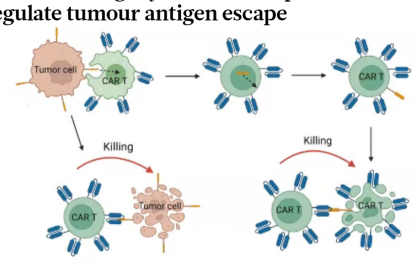
Capricious CARs Causing Resistance

Induction of resistance to chimeric antigen receptor T cell therapy by transduction of a single leukemic B cell

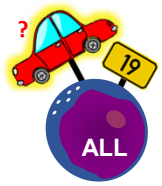


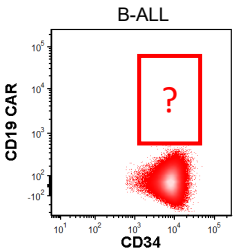
Ruella M et al. Nature Medicine 2018
Rafiq S & Brentjens R. Nature Medicine 2018

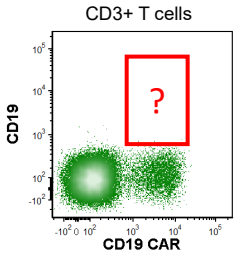
CAR T cell trogocytosis and cooperative killing regulate tumour antigen escape



Hamieh, M et al. Nature 2019







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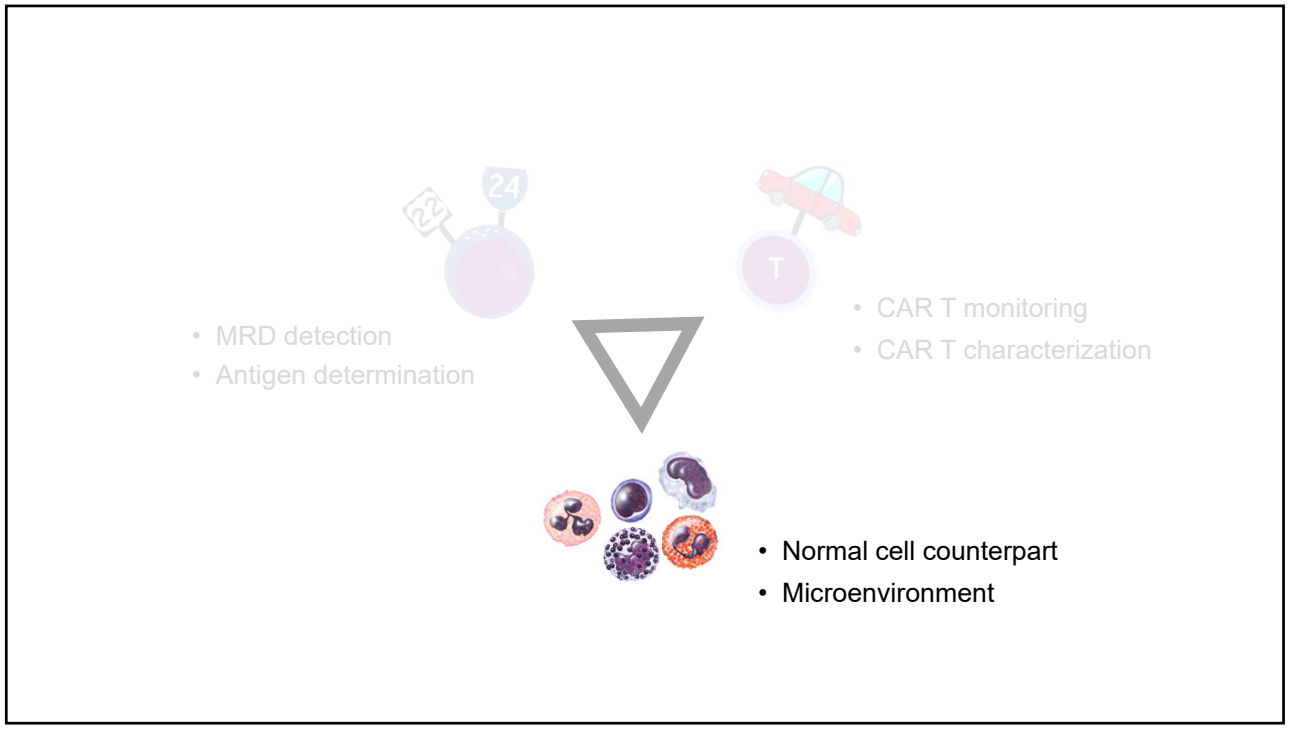

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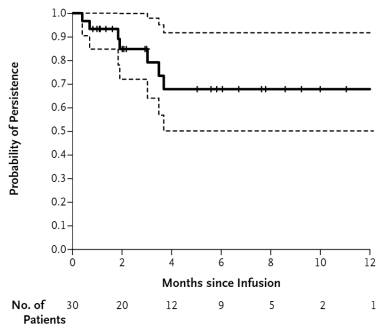
B-cell Aplasia (BCA) after CD19 CAR Therapy

Children's Hospital of Philadelphia

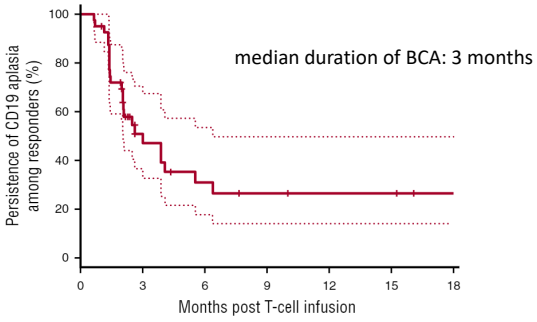
- 4-1BB costimulatory domain
- Definition of "Loss of BCA":
  - >3% CD19+ cells in pb or >1% CD19+ hematogones in bm



Maude, SL et al. The New England Journal of Medicine 2014

Seattle Children's

- CD28/4-1BB costimulatory domain
- Definition of "Loss of BCA":
  - >1% CD19 + cells in bm or pb at two consecutive time points



Gardner, RA et al. Blood 2017

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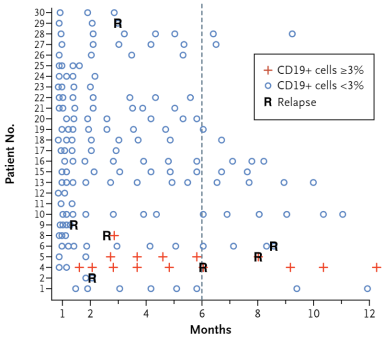
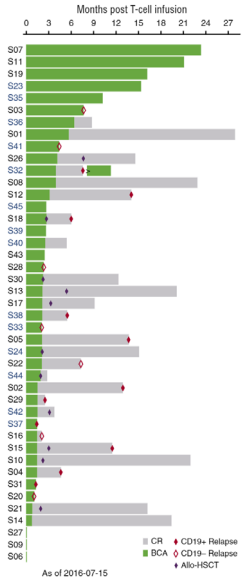
Early Loss of B-cell Aplasia (BCA) Increases Risk of CD19+ Relapse

Children's Hospital of Philadelphia



Maude, SL et al. The New England Journal of Medicine 2014

Seattle Children's



Gardner, RA et al. Blood 2017

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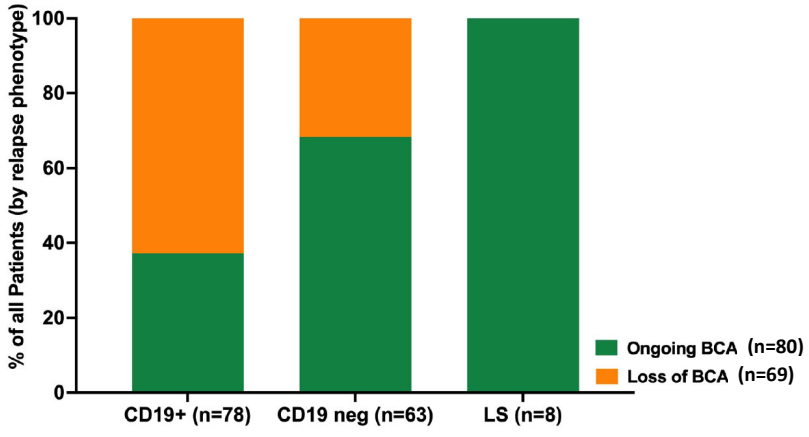
Early Loss of B-cell Aplasia (BCA) Increases Risk of CD19+ Relapse



Lamble, AJ et al. Blood Adv 2022

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Summary – Take Home Messages

- Multiple-strategy approach is needed for antigen-loss disease
  - CD19
  - CD22/CD24/CD66b
  - iCD79a
  - ungated
- (CD19-negative) normal progenitors can mimic B-ALL MRD
- Lineage switch leukemia needs to be always kept in mind
- Antigen quantification is better than MFI, and correlates with response/relapse
- CAR T monitoring is not difficult, but you need the right reagent
- CAR T functional characterization will provide key information to improvement of CAR T in the future
- Early loss of B-cell aplasia is associated with CD19-post relapse

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Acknowledgements

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Jake Wellek
Dan Moyer
Aaron Nelson
Tyler Lowe
Naomi Hniang

NCI Investigators

Nirali Shah
Haneen Shalabi
James Kochenderfer
Jennifer Brudno

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hao-wei.wang@nih.gov

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