



ALMA MATER STUDIORUM  
UNIVERSITÀ DI BOLOGNA  
DIPARTIMENTO DI MEDICINA SPESIALE  
DIAGNOSTICA E SPERIMENTALE

POLICLINICO DI  
**SANT'ORSOLA**

SERVIZIO SANITARIO REGIONALE  
EMILIA-ROMAGNA  
Azienda Ospedaliero - Universitaria di Bologna

# New Drugs in Hematology



**CAR  
T-cell**

President: Pier Luigi Zinzani

Co-President: Michele Cavo

Bologna,  
Royal Hotel Carlton

**May 18-20, 2022**

**BOLOGNA** BOLOGNA, ROYAL HOTEL CARLTON



**Penn Medicine**  
Center for Cellular Immunotherapies

*CAR-T cells for adult  
ALL*

**Marco Ruella, MD**

Assistant Professor of Medicine

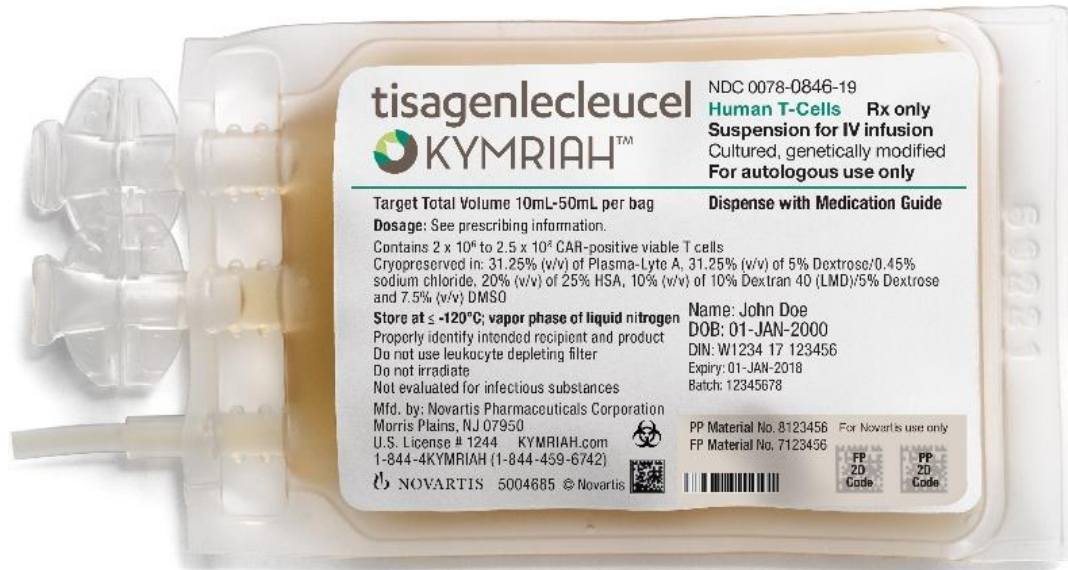
Scientific Director, Lymphoma Program

## *Presenter disclosure information*

- **Inventor:** *CART technologies, Univ. of Pennsylvania, partly licensed to Novartis, Tmunity, and viTToria bio*
- **Research Funding:** *AbClon, Beckman-Coulter, Lumicks, ONI, Novartis*
- **Consultancy/Honoraria:** *nanoString, GLG*
- **Advisory Board:** *AbClon, Bayer, Sana, BMS, GSK, viTToria bio*
- **Scientific Founder:** *viTToria biotherapeutics*

# US FDA-approved CAR T cell products for B-ALL

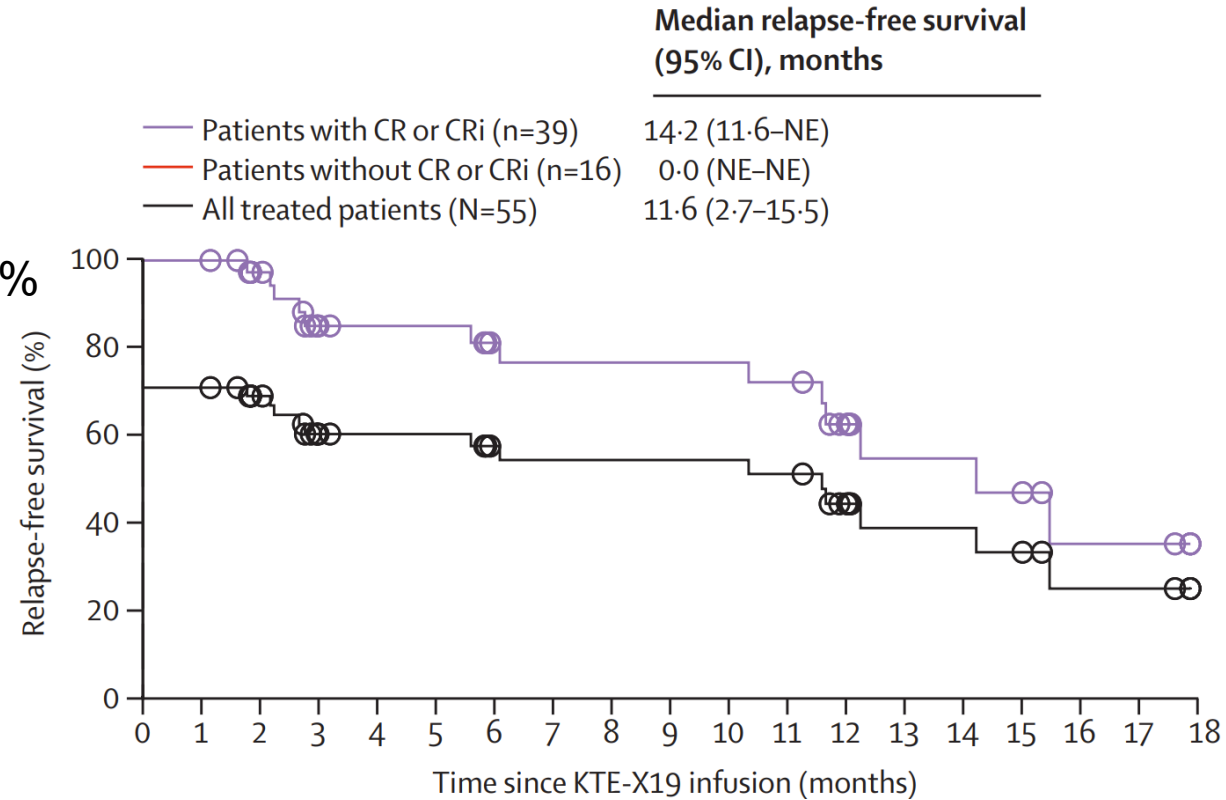
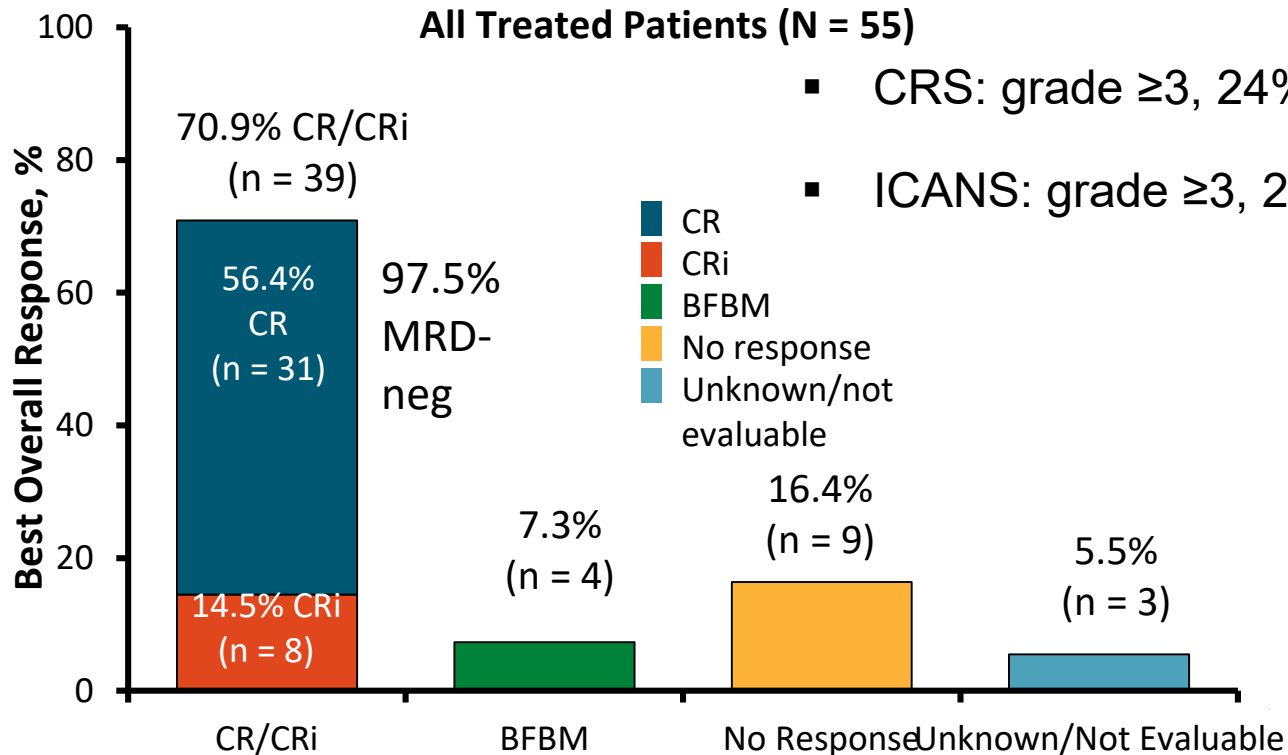
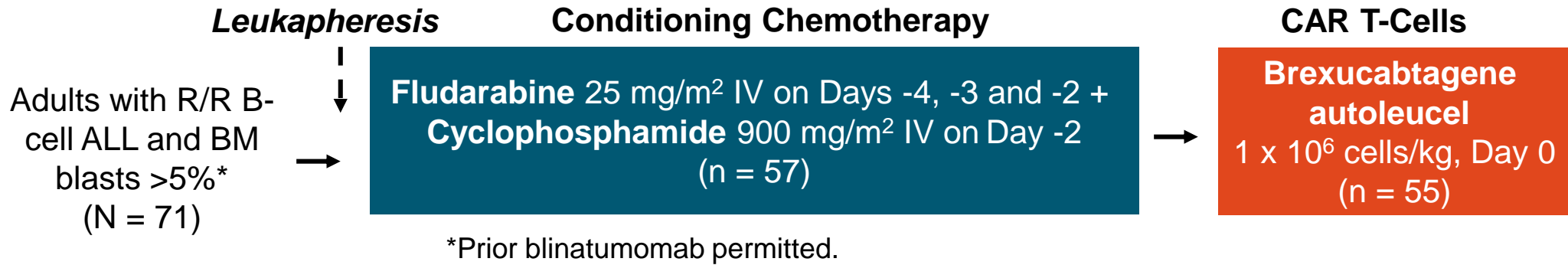
August 2017: ped. and AYA B-ALL



Oct 2021: adult B-ALL



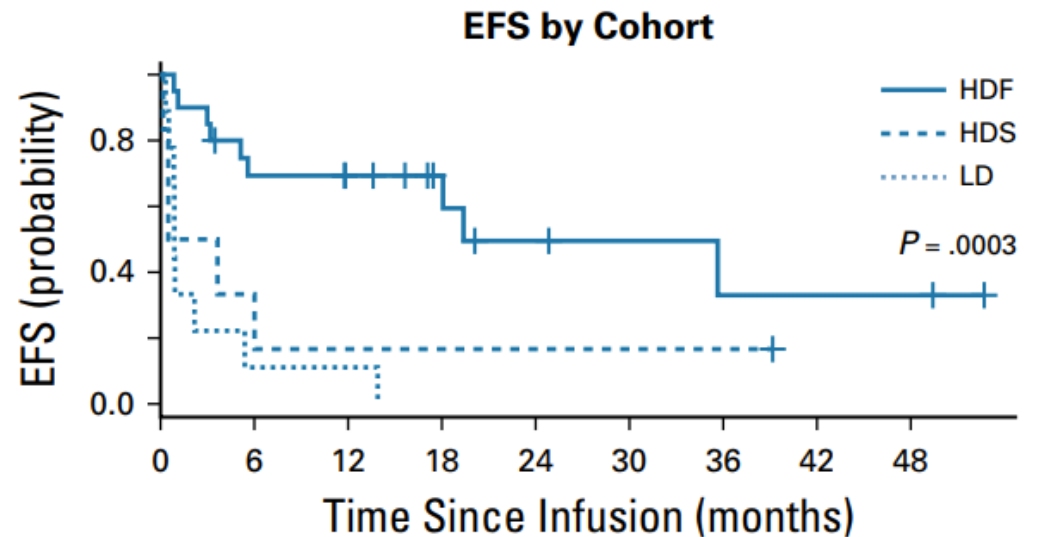
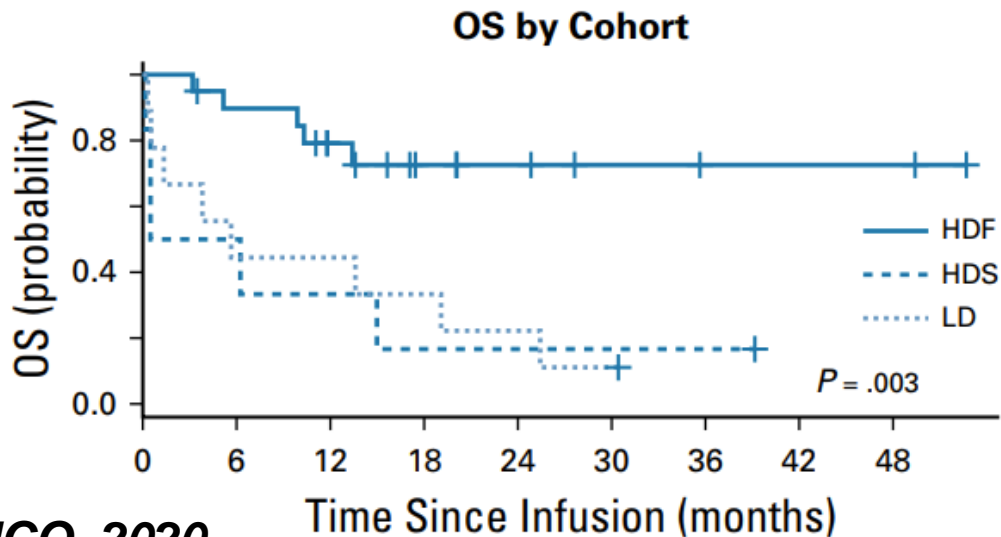
# Phase II ZUMA-3: Brexucabtagene Autoleucel (KTE-X19) for Adults With Relapsed/Refractory ALL



# CTL019 for r/r adult B-ALL (single center trial UPenn)

Outcome	All (N = 35)	Cohort			P <sup>a</sup>
		HDS (n = 6)	LD (n = 9)	HDF (n = 20)	
Day 28 response					
CR	24 (69)	3 (50)	3 (33)	18 (90)	.0038
NR	11 (31)	3 (50)	6 (67)	2 (10)	
Median survival, months (95% CI)					
OS	19.1 (6.2 to NE)	3.4 (0.2 to NE)	5.7 (0.3 to 25.4)	Not reached	.0030
EFS	5.6 (2.2 to 19.4)	2.1 (0.2 to NE)	0.9 (0.3 to 5.4)	19.4 (5.1 to NE)	.0003
Two-year survival rate, % (95% CI)					
OS	47 (28 to 63)	17 (0.8 to 52)	22 (3 to 51)	73 (46 to 88)	
EFS	31 (15 to 49)	17 (0.8 to 52)	0 (0 to 33) <sup>b</sup>	49.5 (21 to 73)	
CRS grade 4/5	6 (17)	3 (50)	2 (22)	1 (5)	.0170

All  
MRD-  
neg

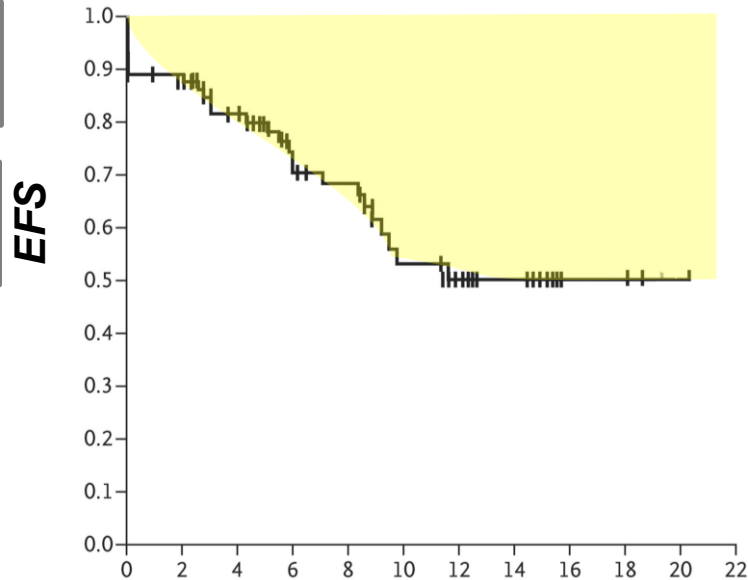


# Building Upon the Success of CART19

## Ped. and AYA B-cell Acute Lymph. Leukemia (ELIANA)

NR=  
~10%

Relapse=  
~40%

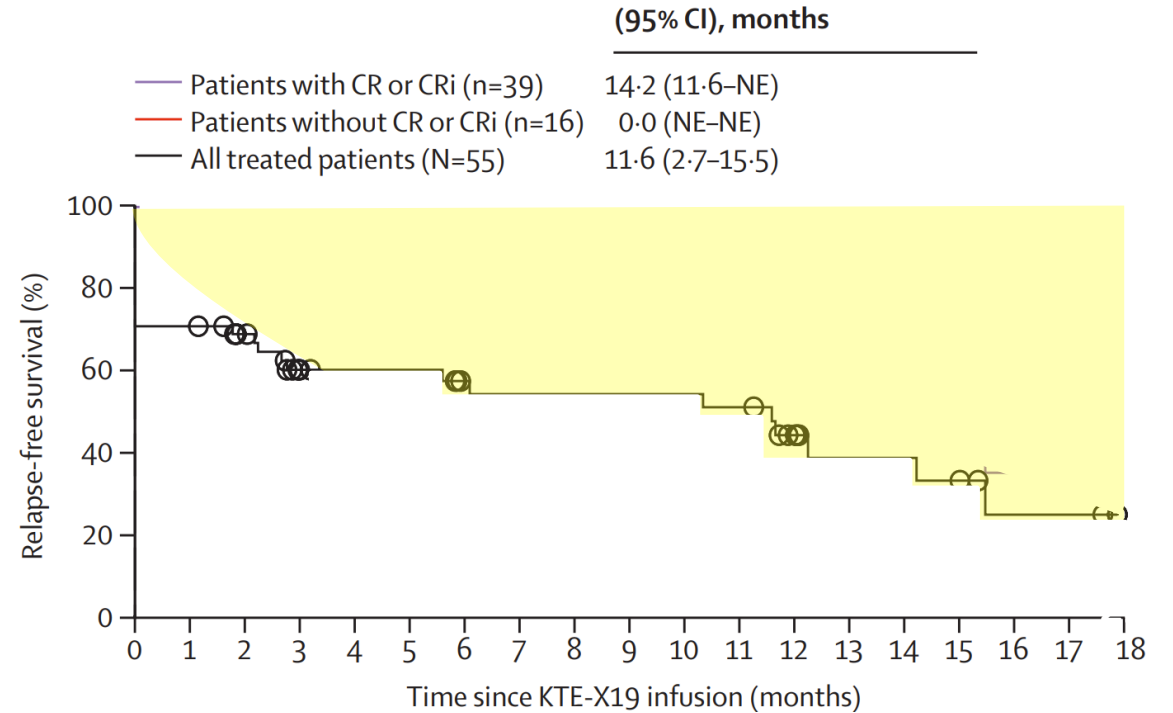


NR=  
~30%

Relapse=  
~40%

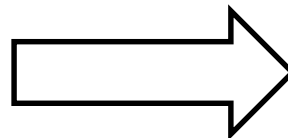
*Maude SL, NEJM, 2018*

## Adult B-cell Acute Lymph. Leukemia (ZUMA-3)



*Shah BD, Lancet, 2021*

**Resistance Mechanisms**



**Novel Effective Approaches**

# Causes of Failure of CART19 Immunotherapy

## Pre-infusion barriers

Low lymphocyte counts

Manufacturing failure

Progression during manufacturing

High Costs

### Legend

-  Antigen
-  CAR
-  CTLA-4
-  LAG-3
-  New Antigen
-  PD-1
-  PD-L1
-  TIM-3

## CART dysfunction

Exhausted T cell phenotype

Central memory T cell phenotype

Low cytotoxicity

High cytotoxicity

Tumor cell

CART

PD-L1 over-expression

Antigen loss

Lineage switch

## Tumor-intrinsic mechanisms

Physical barrier

TGF- $\beta$

IL-10

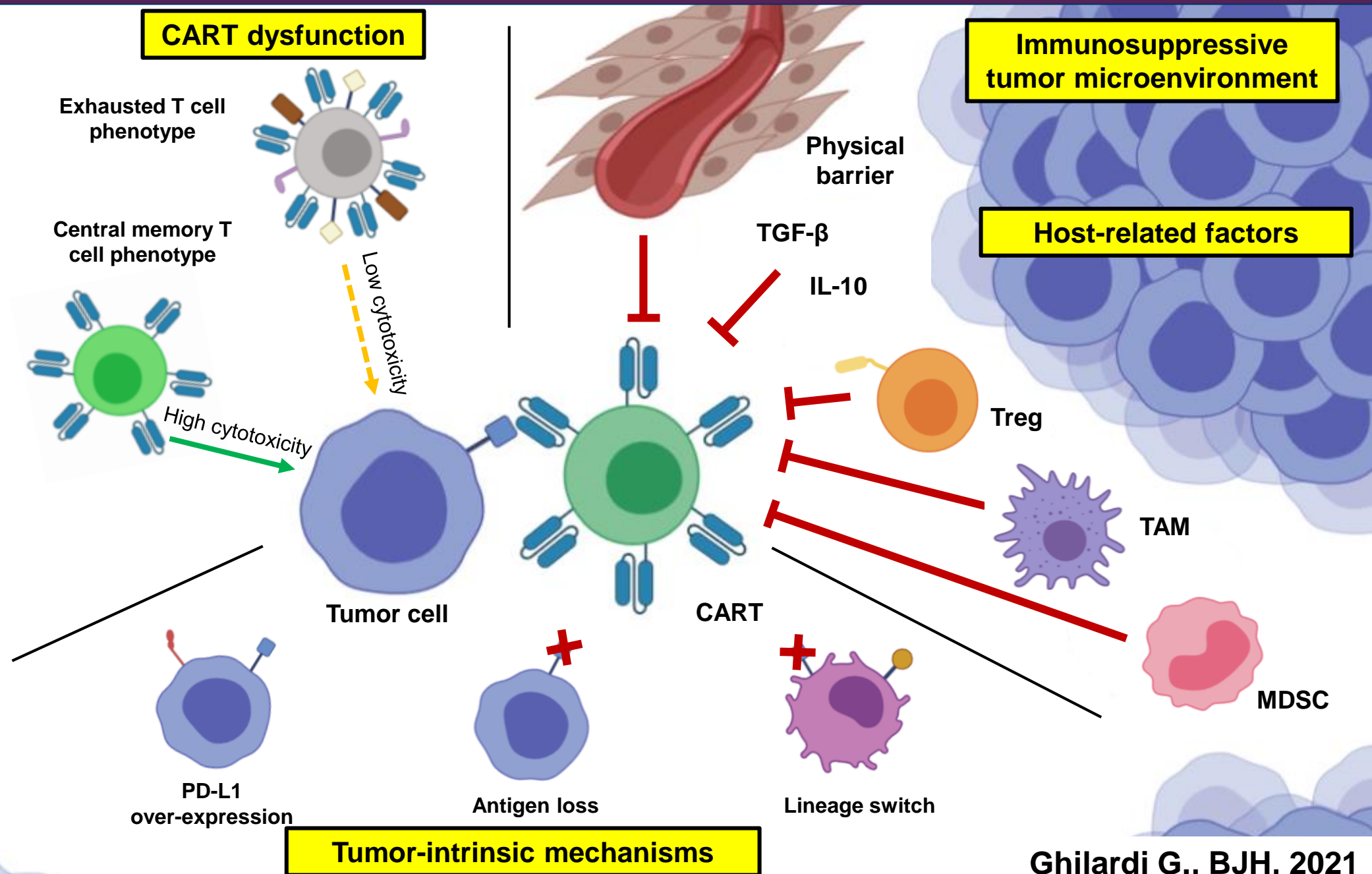
## Immunosuppressive tumor microenvironment

## Host-related factors

Treg

TAM

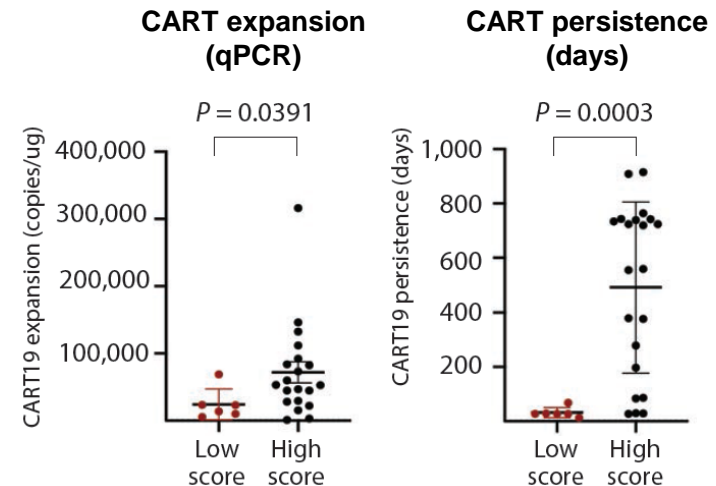
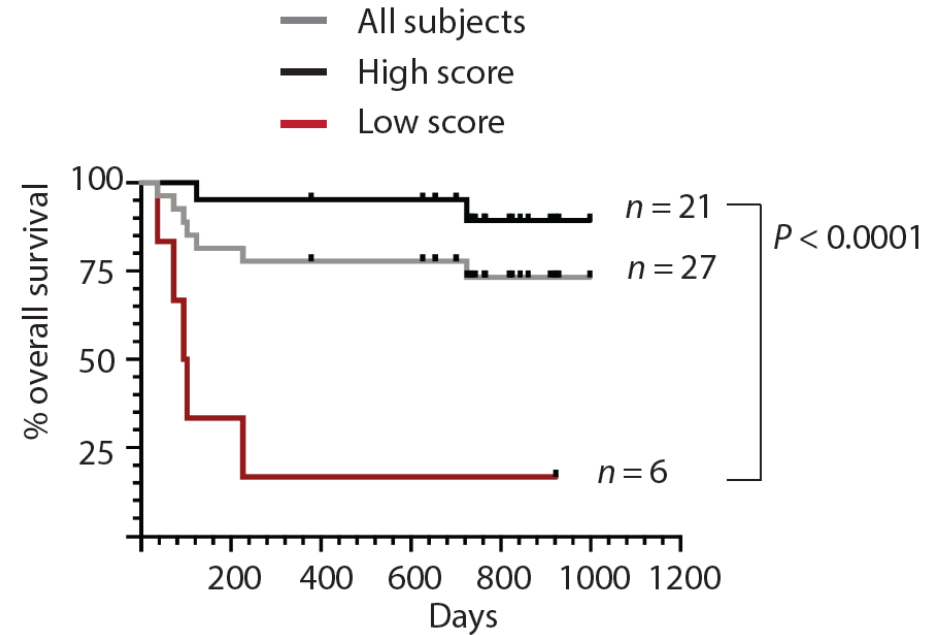
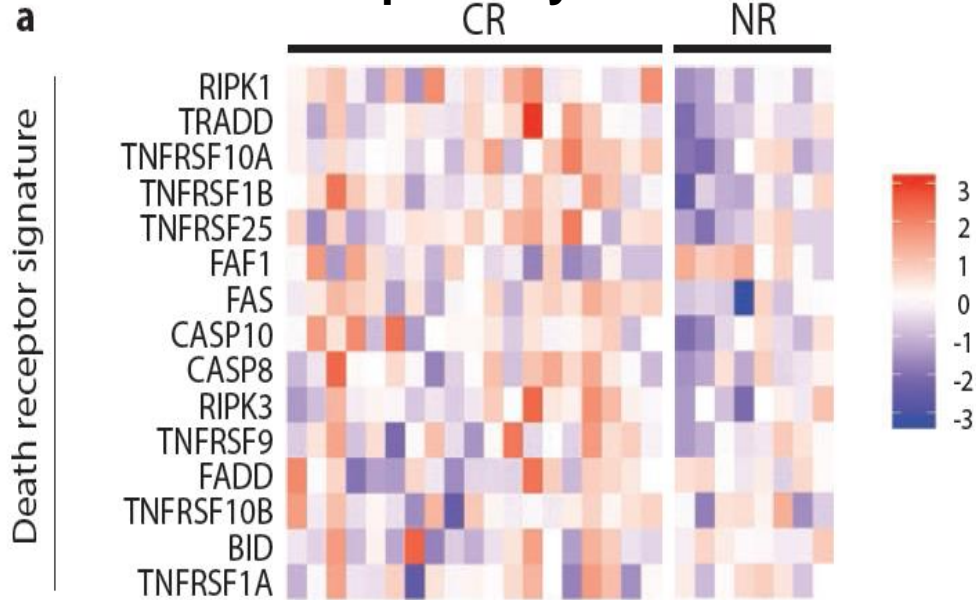
MDSC



# Extrinsic apoptosis and CART outcomes in ALL

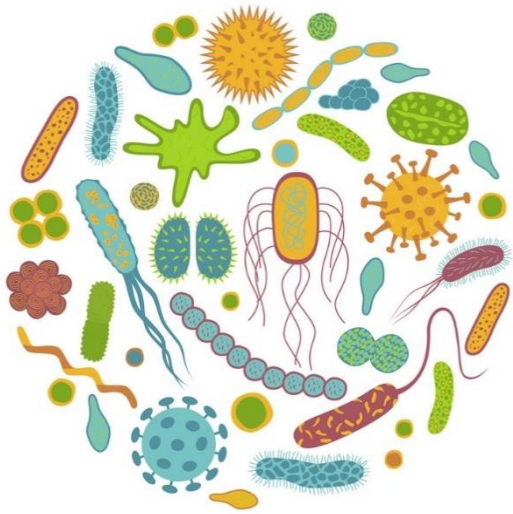
## Pediatric B-ALL trial (ELIANA)

mRNA expression of positive regulators of the extrinsic apoptotic pathway



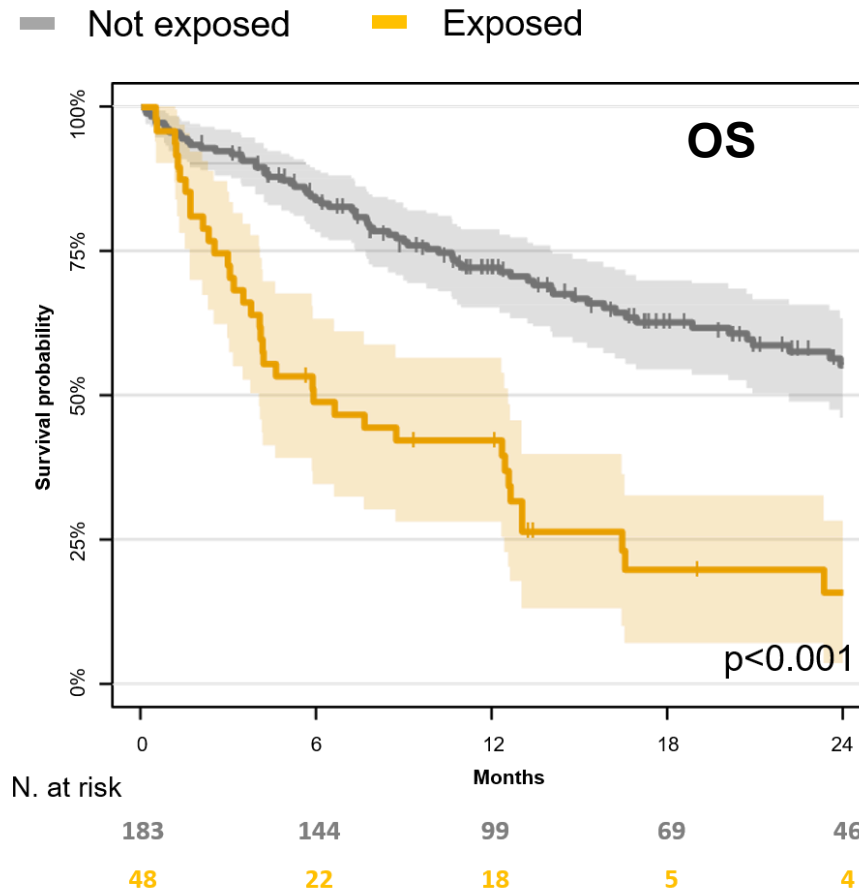


# Impact of the **gut microbiota** on **CART** outcomes

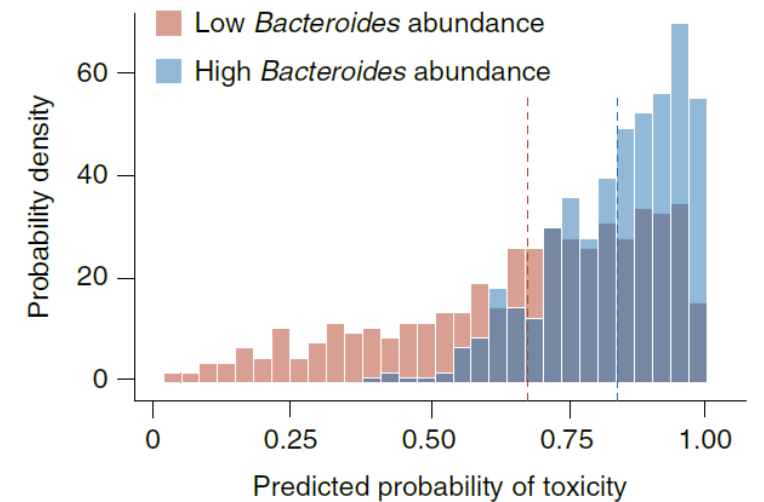
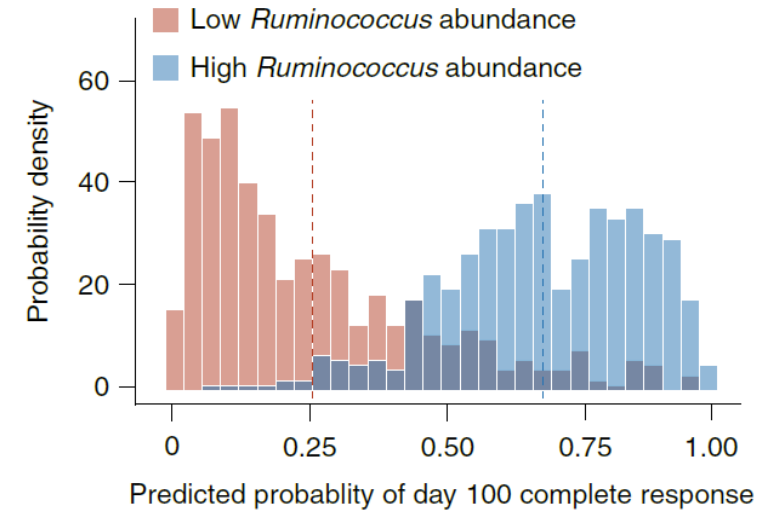


Pre-CART exposure to:

- Piperacillin/tazobactam
- Imipenem/cilastatin
- Meropenem



Stool microbiome sequencing:



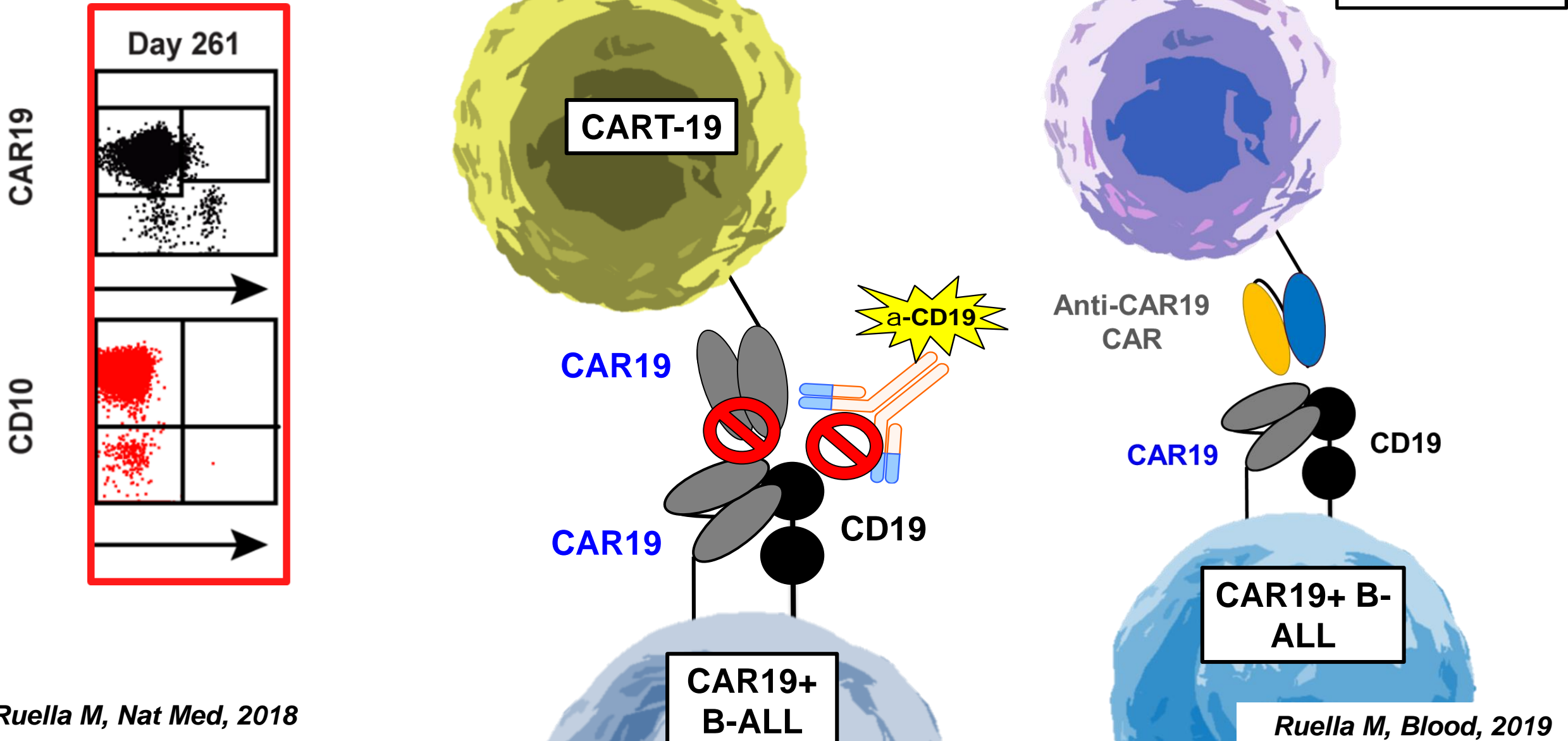
# CD19-Negative Escape after CART19 in B-ALL

Trial	Phase	Population	CD19 CAR Construct	Relapse Rate, % (n/N)	CD19-Negative Relapse Rate, % (n/N)	
Children's Hospital of Philadelphia	I	Pediatric	FMC63-4-1BB-ζ	36 (20/55)	24 (13/55)	<b>65%</b>
ELIANA	II	Pediatric	FMC63-4-1BB-ζ	33 (20/61)	25 (15/61)	<b>75%</b>
Seattle Children's	I	Pediatric	FMC63-CD28-ζ	45 (18/40)	18 (7/40)	<b>39%</b>
NCI	I	Pediatric	FMC63-4-1BB-ζ	29 (8/28)	18 (5/28)	<b>63%</b>
MSKCC	I	Adult	SJ25C1-CD28-ζ	57 (25/44)	9 (4/44)	<b>16%</b>
FHCRC	I	Adult	FMC63-4-1BB-ζ	31 (9/29)	7 (2/29)	<b>22%</b>
ZUMA-3	II	Adult	FMC63-CD28-ζ		3/6	<b>33%</b>

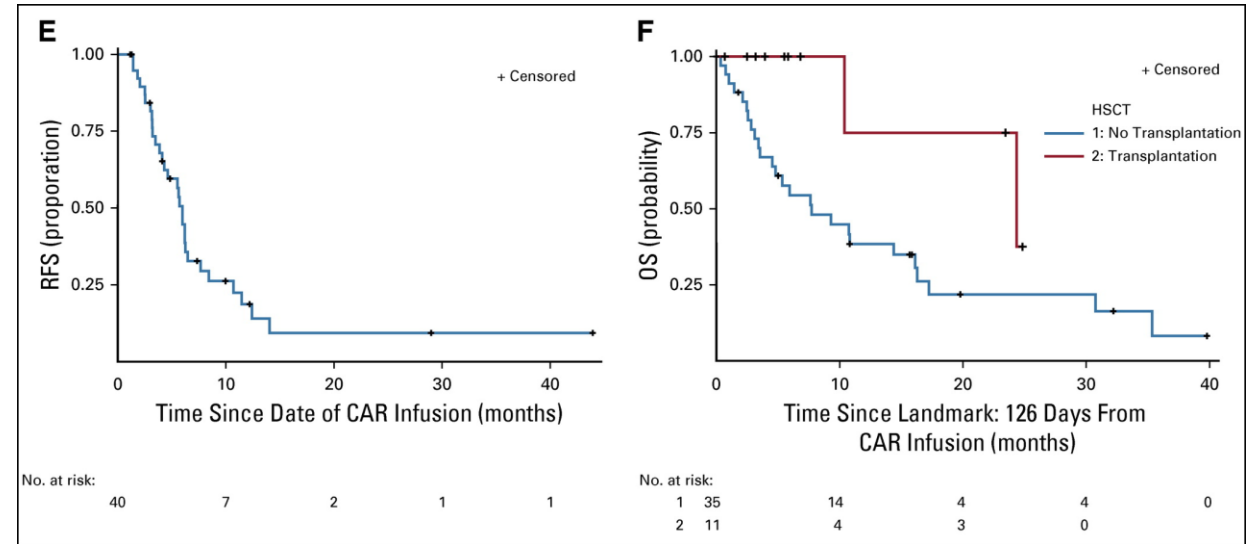
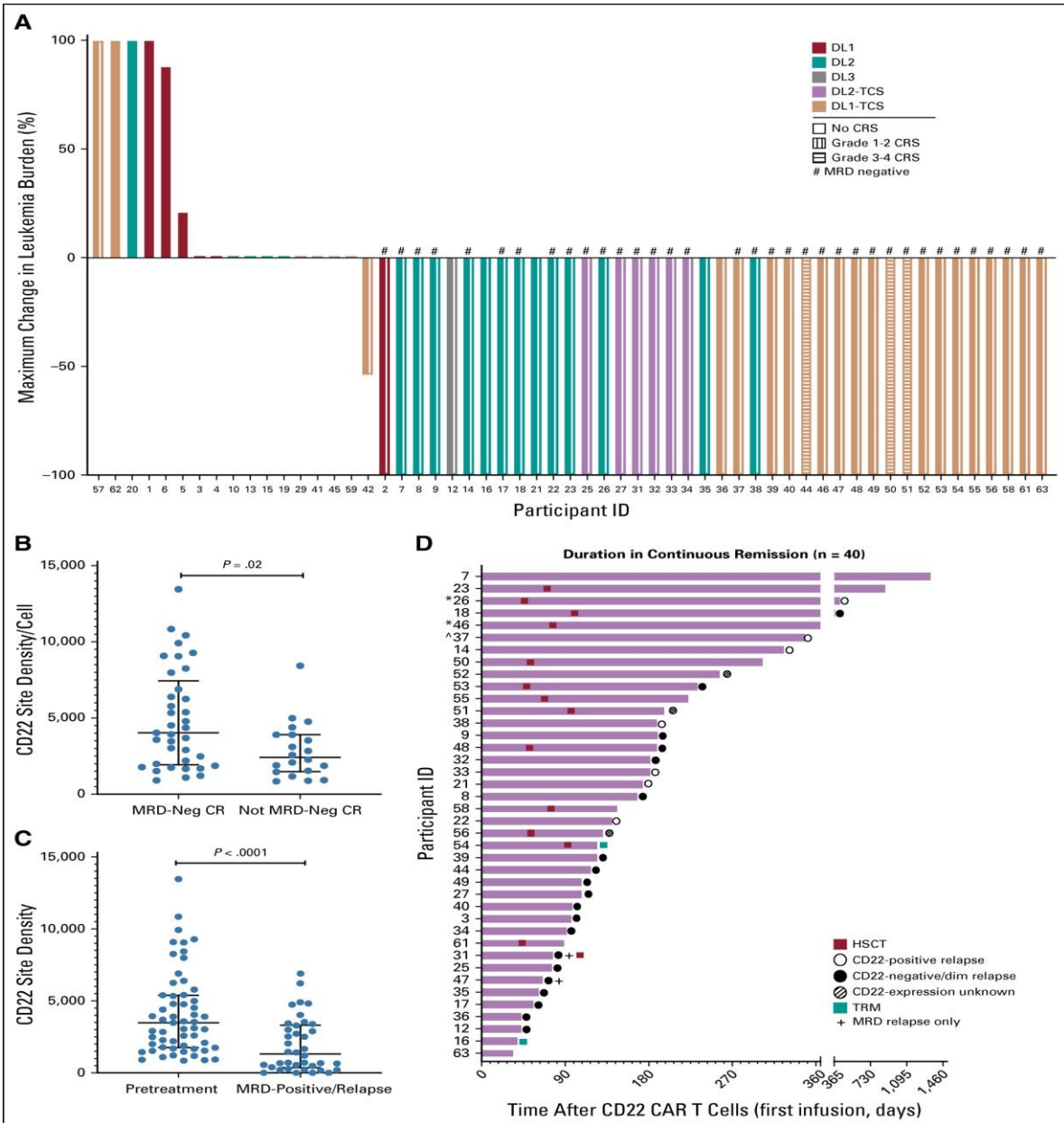
- Long-term outcomes confounded across trials by differing HCT use and other unique practices following CAR T-cell therapy
- True incidence of CD19+ and CD19- relapse unknown



# CAR19+ B-ALL: relapse by **epitope-masking**



# NCI CART22 trial



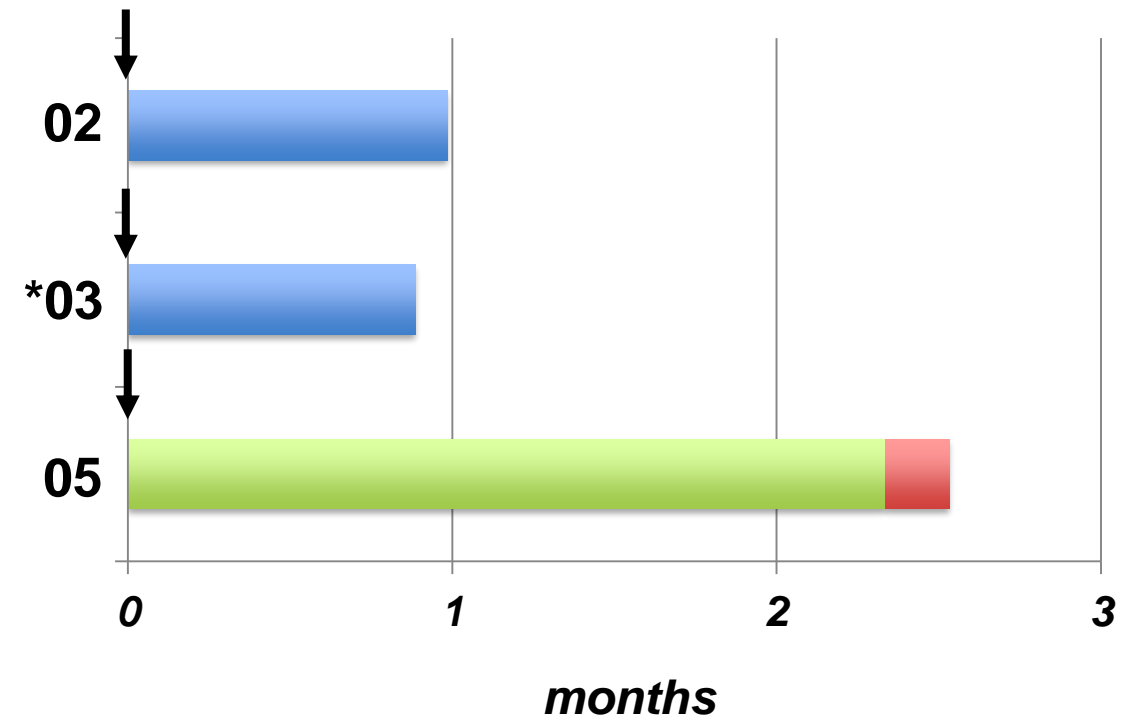
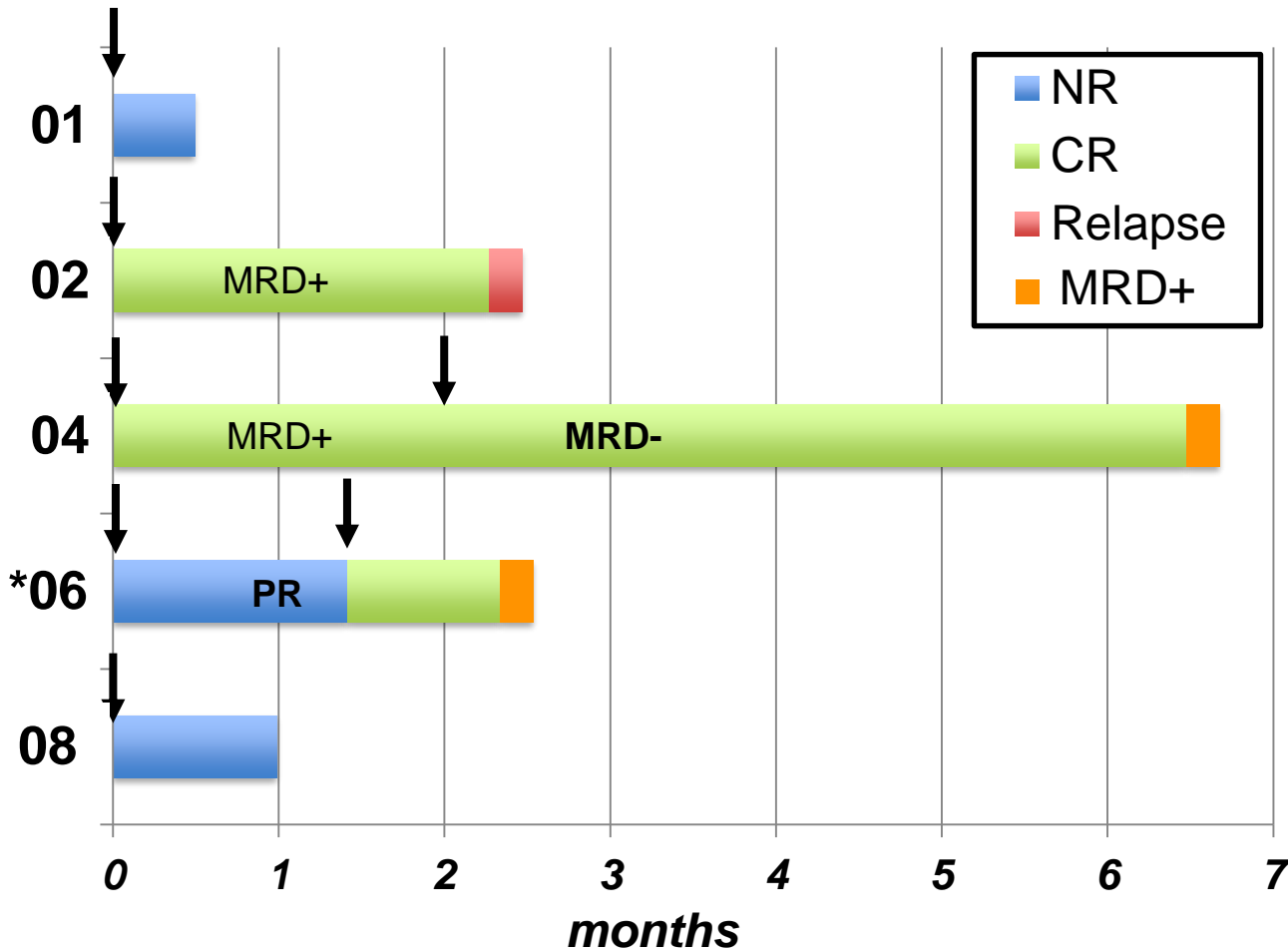
Fry T., Nat Med, 2018, updated in Shah NN JCO 2020

# CART22 Clinical Trial in B-ALL (PI: Frey and Grupp)

## Pediatric

At month 2, 4/8 (50%)  
of patients were in CR

## Adult



↓ = CART22 infusion  
\* = dose not reached

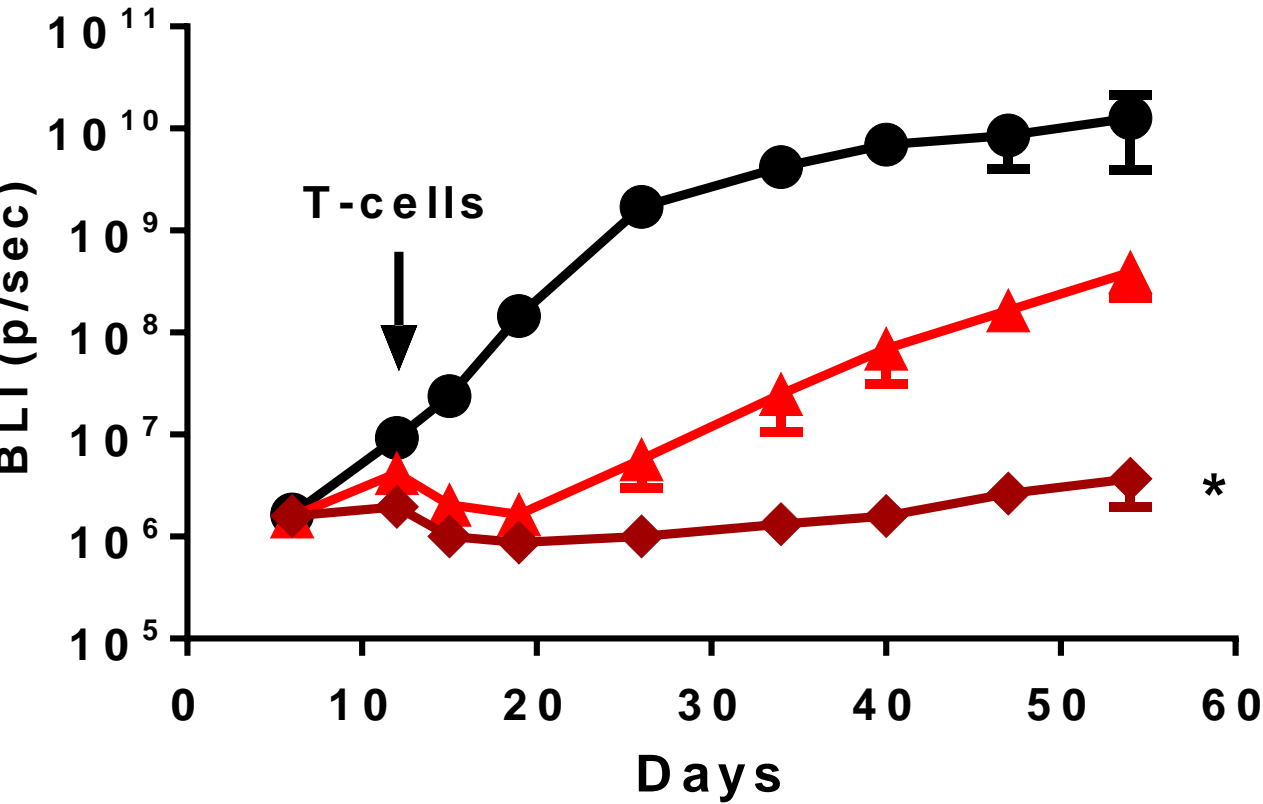
# Short CART22 (65s) is superior than long CART22 in vivo

## Primary B-ALL model

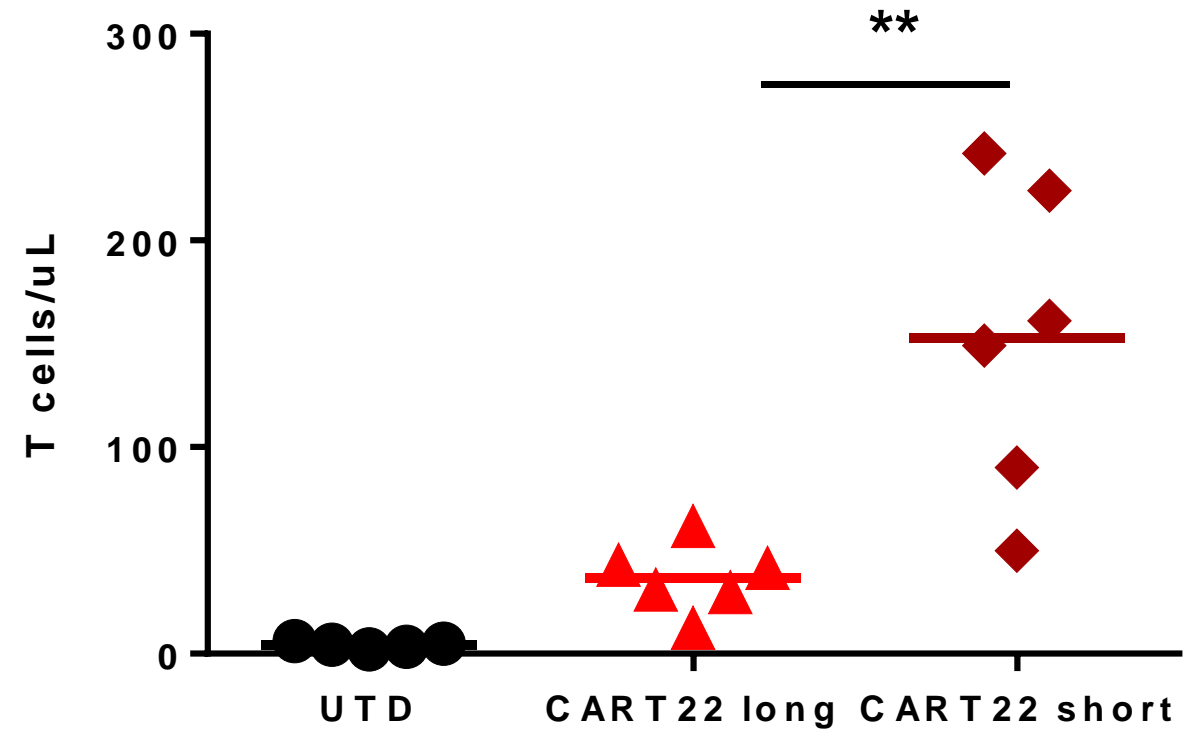
- UTD
- ▲ CART22 long
- ◆ CART22 short

Tumor burden  
(luminescence)

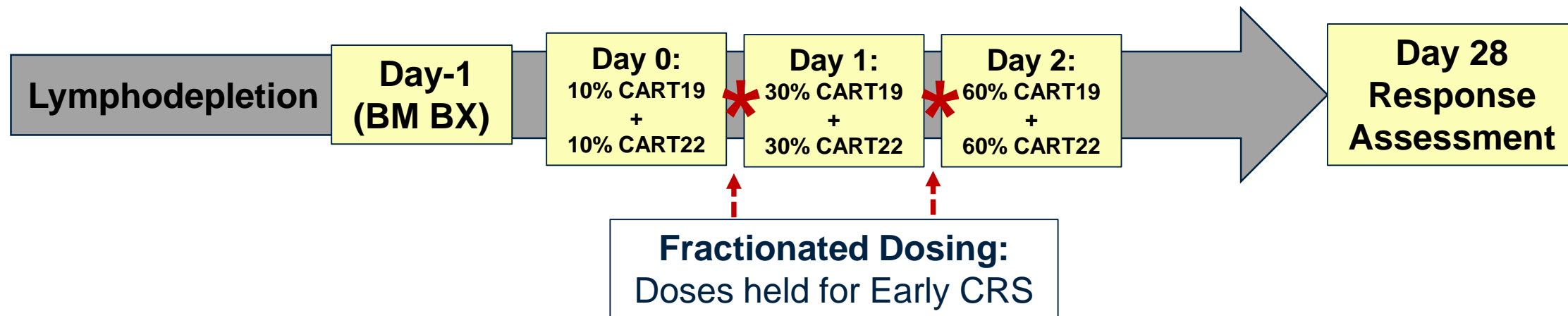
T-cells



T cell expansion in the PB  
(flow cytometry)



# huCART19 + CART22-65s for Adults with Rel/Ref ALL:



Patient characteristics	N=13
Age median (range)	46 (28-72)
Median prior lines of therapy (range)	4 (2-7)
Prior inotuzumab	8/13 (62%)
Prior blinatumomab	8/13 (62%)
Prior CART19	2/13 (15%)
Prior allogeneic SCT	10/13 (77%)
Philadelphia Chromosome +	3/13 (23%)

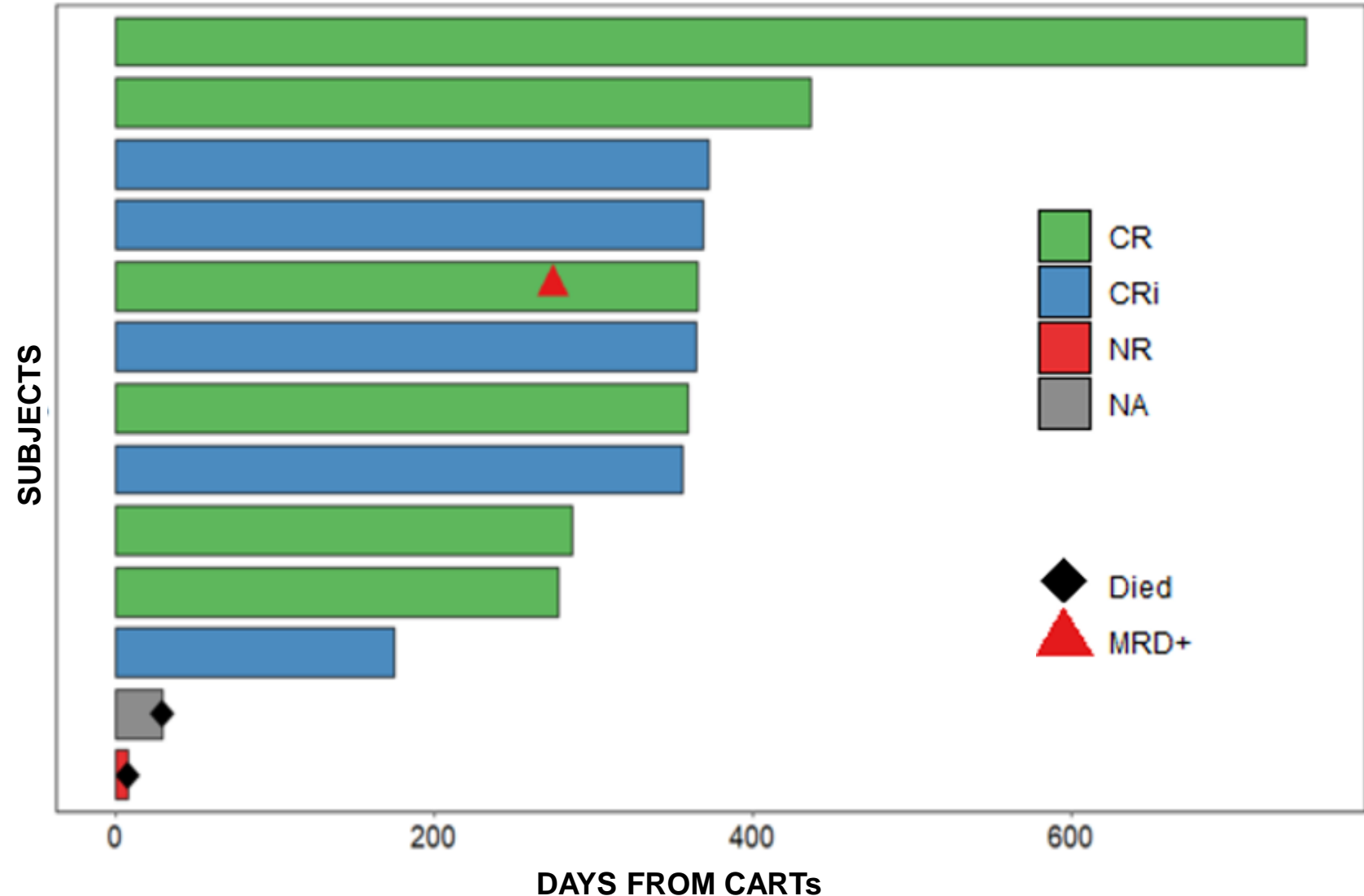
# Pooled CART19 + CART22: responses

CART19 and CART22: (N=13)

- 13 pts infused
- 11 pts evaluable D28
- 11 CR/CRi (MRD - )  
**85%**

**Med follow up 11.8 mo:**

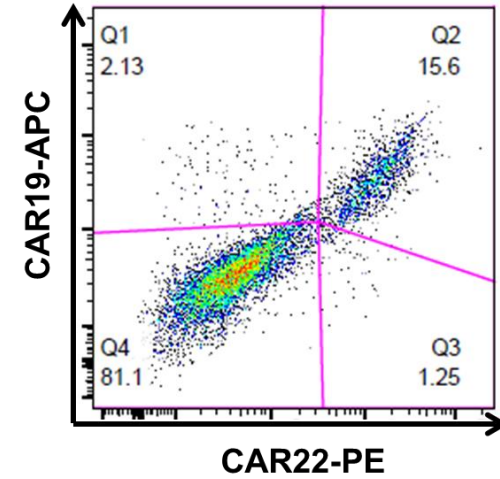
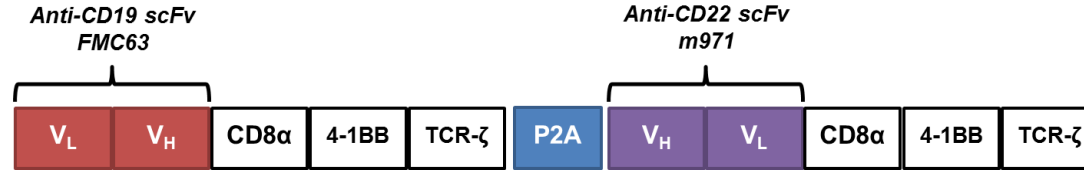
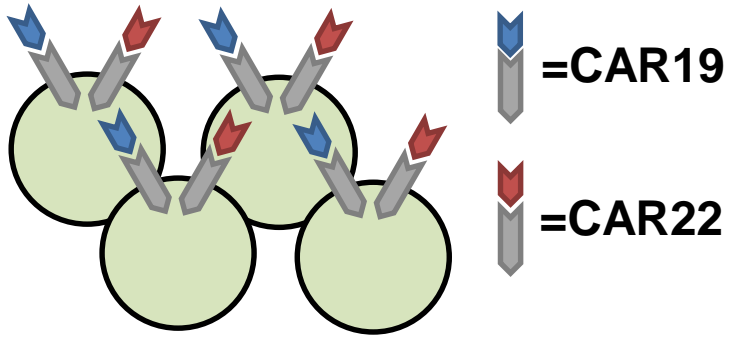
- One pt with molecular recurrence
- 10 with ongoing CR/CRi



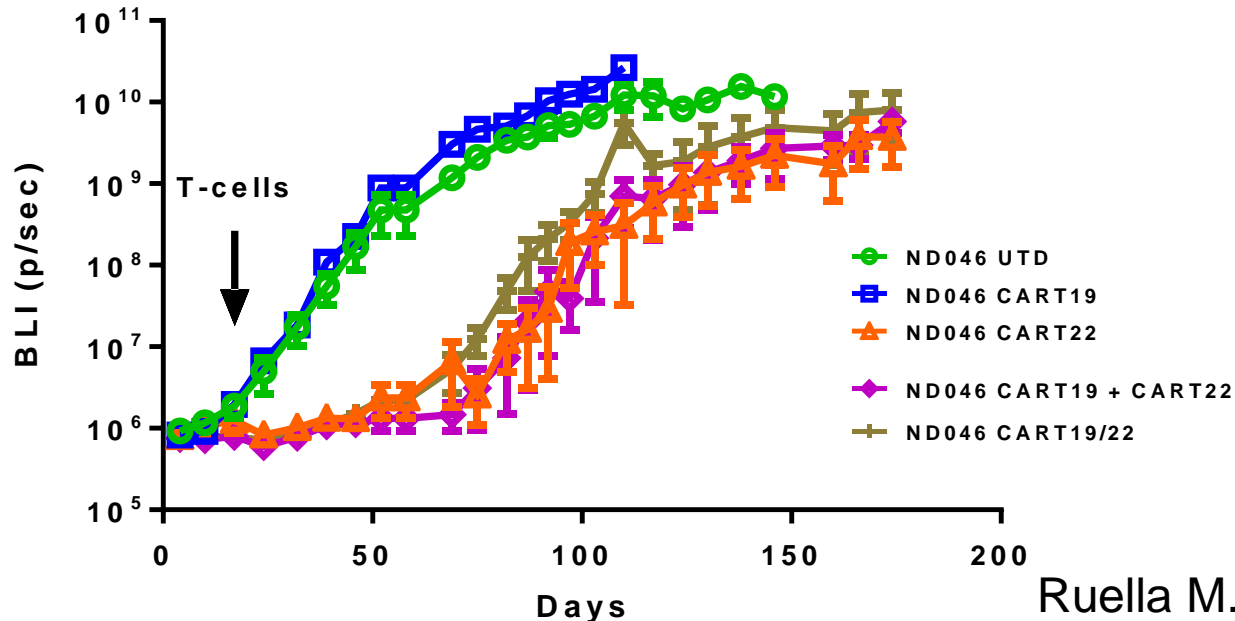


# Dual CART19/22

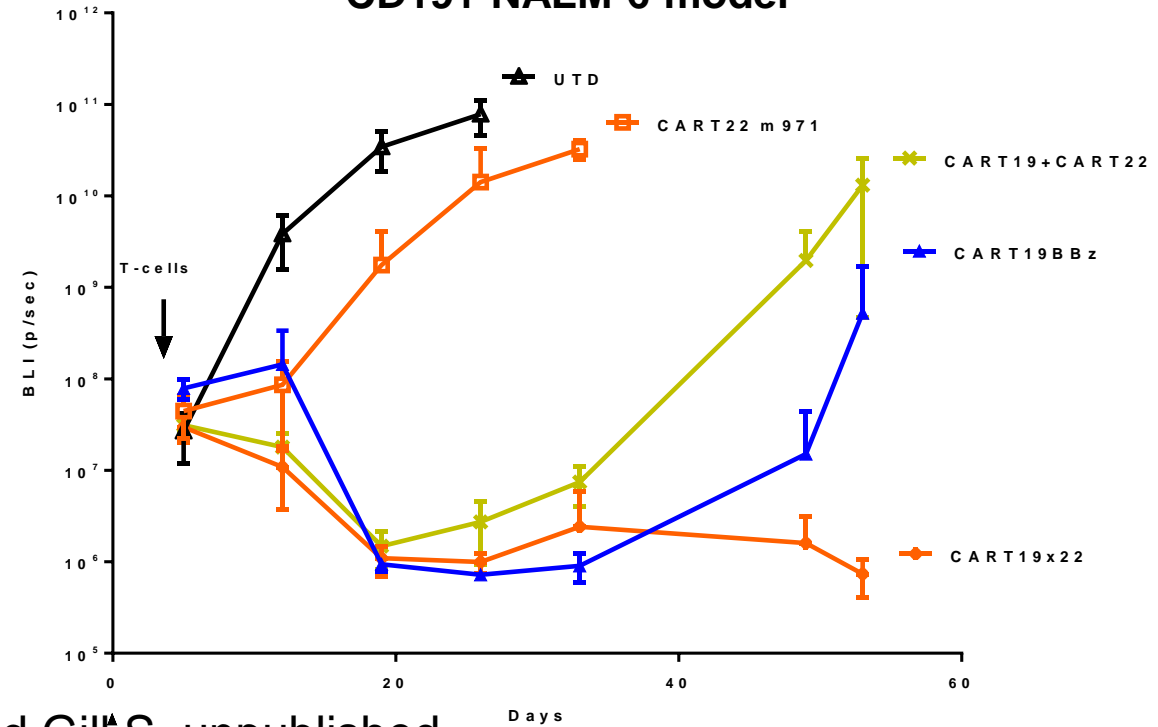
## Dual (or bi-) CART



## Primary CD19-neg B-ALL model



## CD19+ NALM-6 model



# Acknowledgments

## Ruella Lab

Patrizia Porazzi  
Raymone Pajarillo  
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## Correlatives and manufacturing

## CTT and Leukemia Program

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**Noelle Frey**  
**Elizabeth Hexner**

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Brink**

## Novartis

Jenn Brogdon  
Boris Engels  
Carla Guimares

And all  
collaborators!!!

Patients and their  
families





Looking for  
*post-docs*  
*senior*  
*scientists*

[mruella@upenn.edu](mailto:mruella@upenn.edu)

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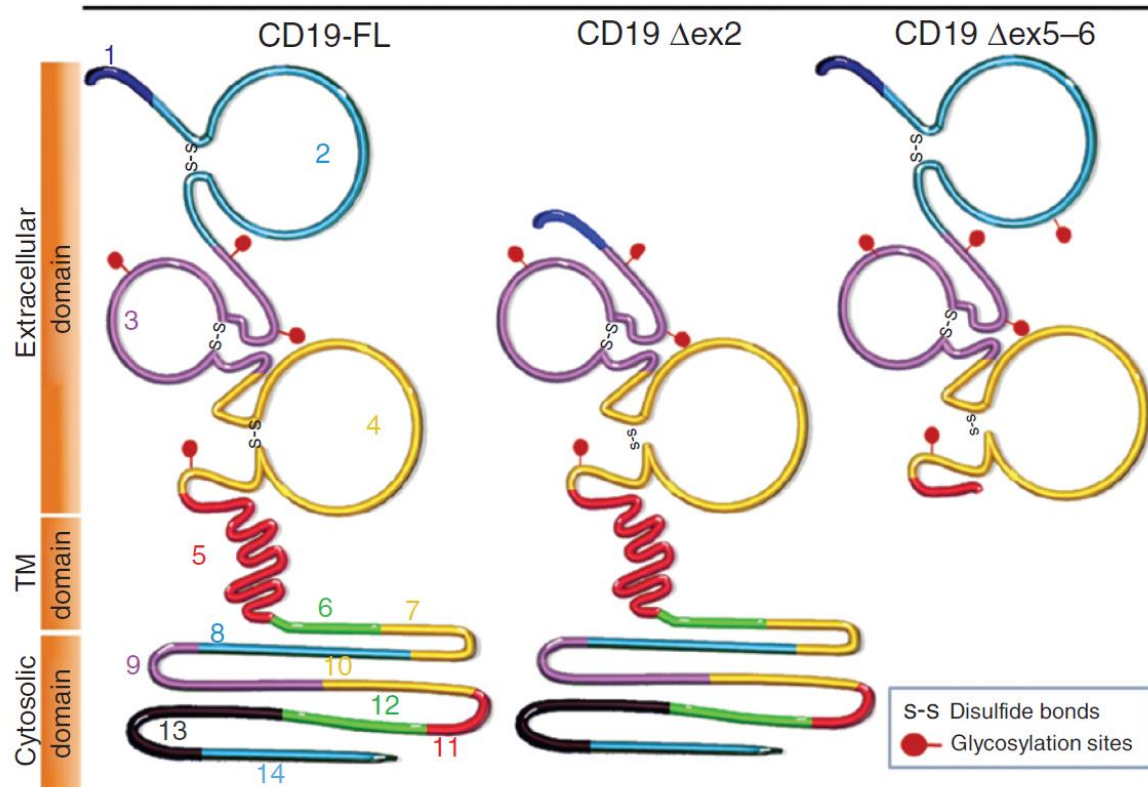
backup

# Mechanisms of CD19-targeted therapy resistance

## Convergence of acquired mutations and alternative splicing of CD19

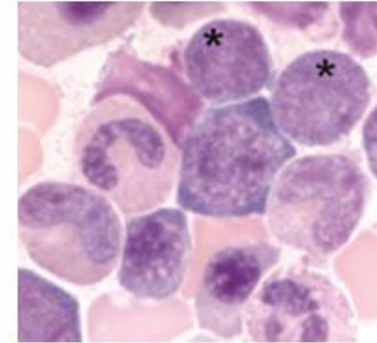
(Sotillo, 2015; Orlando EJ, Nat Med, 2018)

Predicted protein products for CD19 isoforms

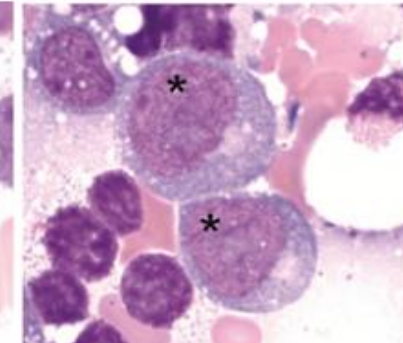


## Transdifferentiation

Lymphoid blasts



Myeloid blasts



(Gardner, 2016, Oberley Mj, 2018)

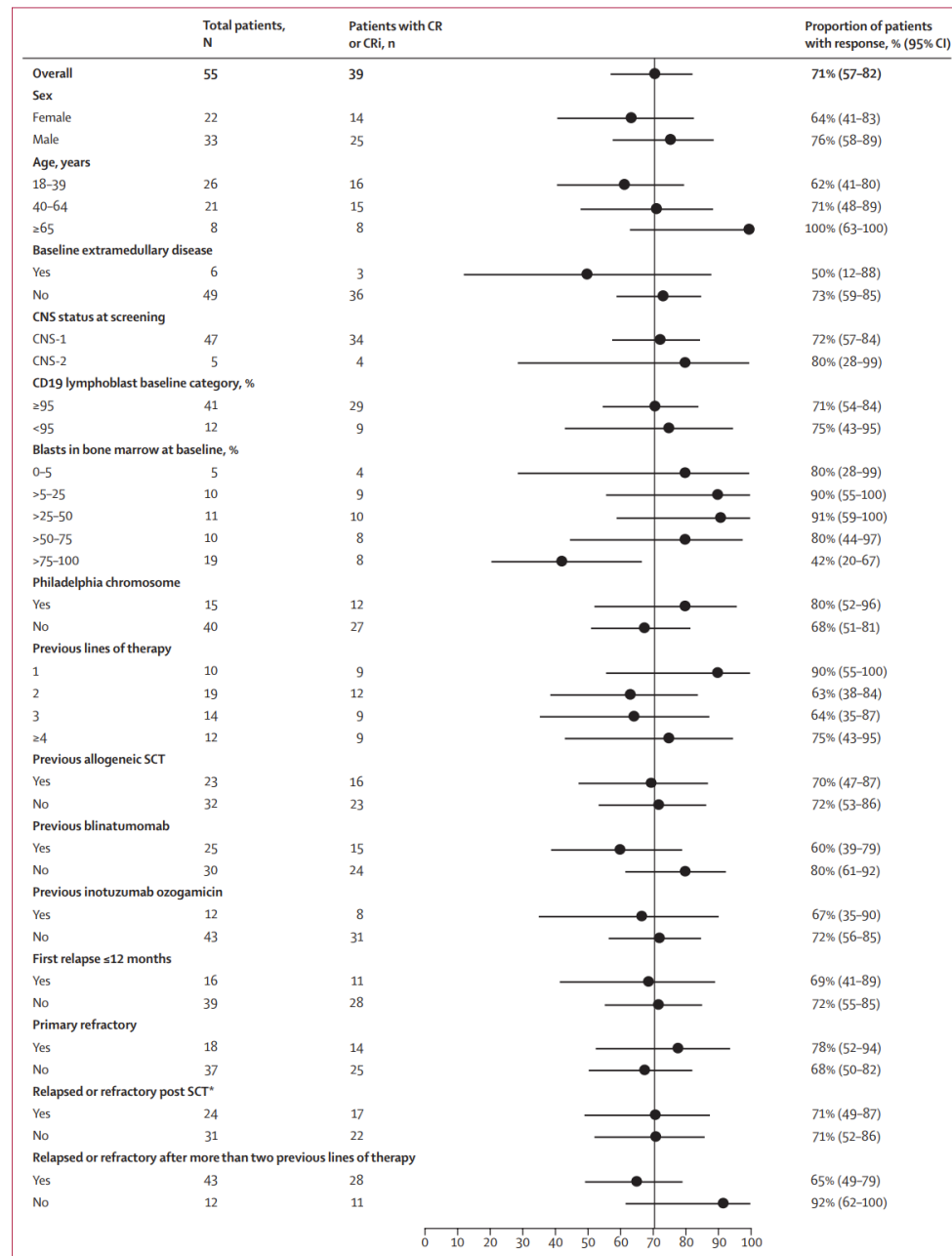
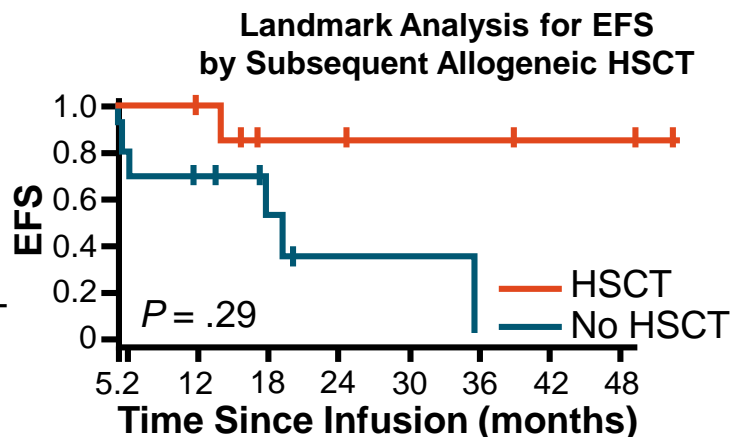
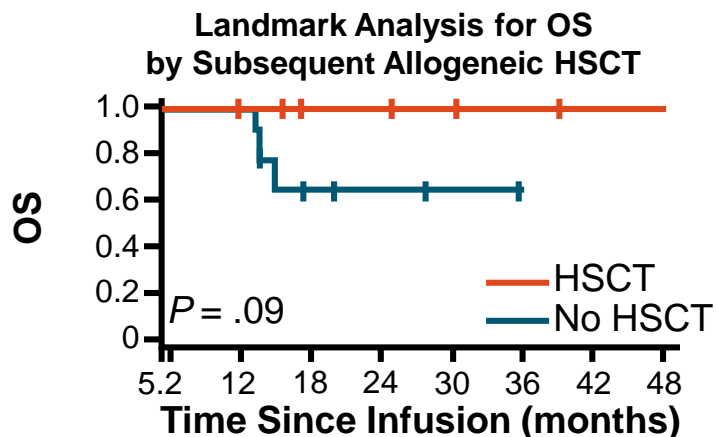


Figure 2: Subgroup analyses of overall CR or CRi rate for baseline and clinical covariates based on central assessment

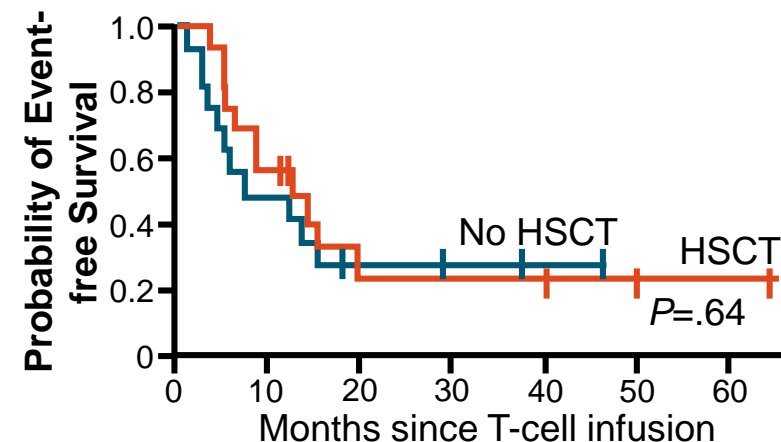
# Post-CAR HSCT in Adult ALL

## CTL019 in Adult ALL at UPenn (N = 35)

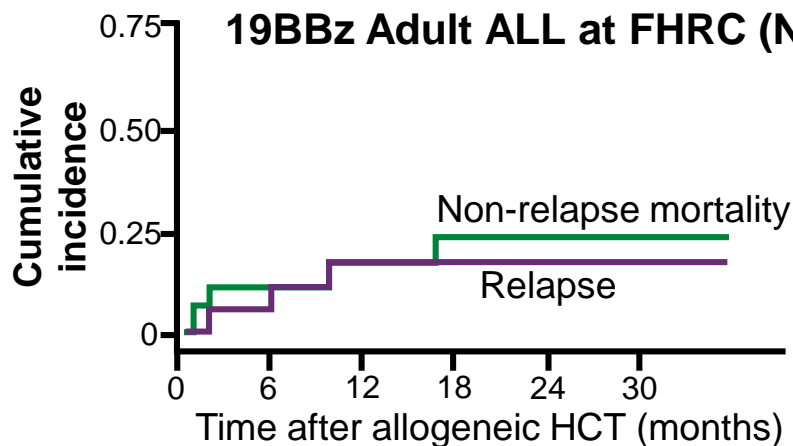


38% of responding pts proceeded to alloHSCT

## 1928z adult ALL at MSK (N = 53)



## 19BBz Adult ALL at FHRC (N = 53)

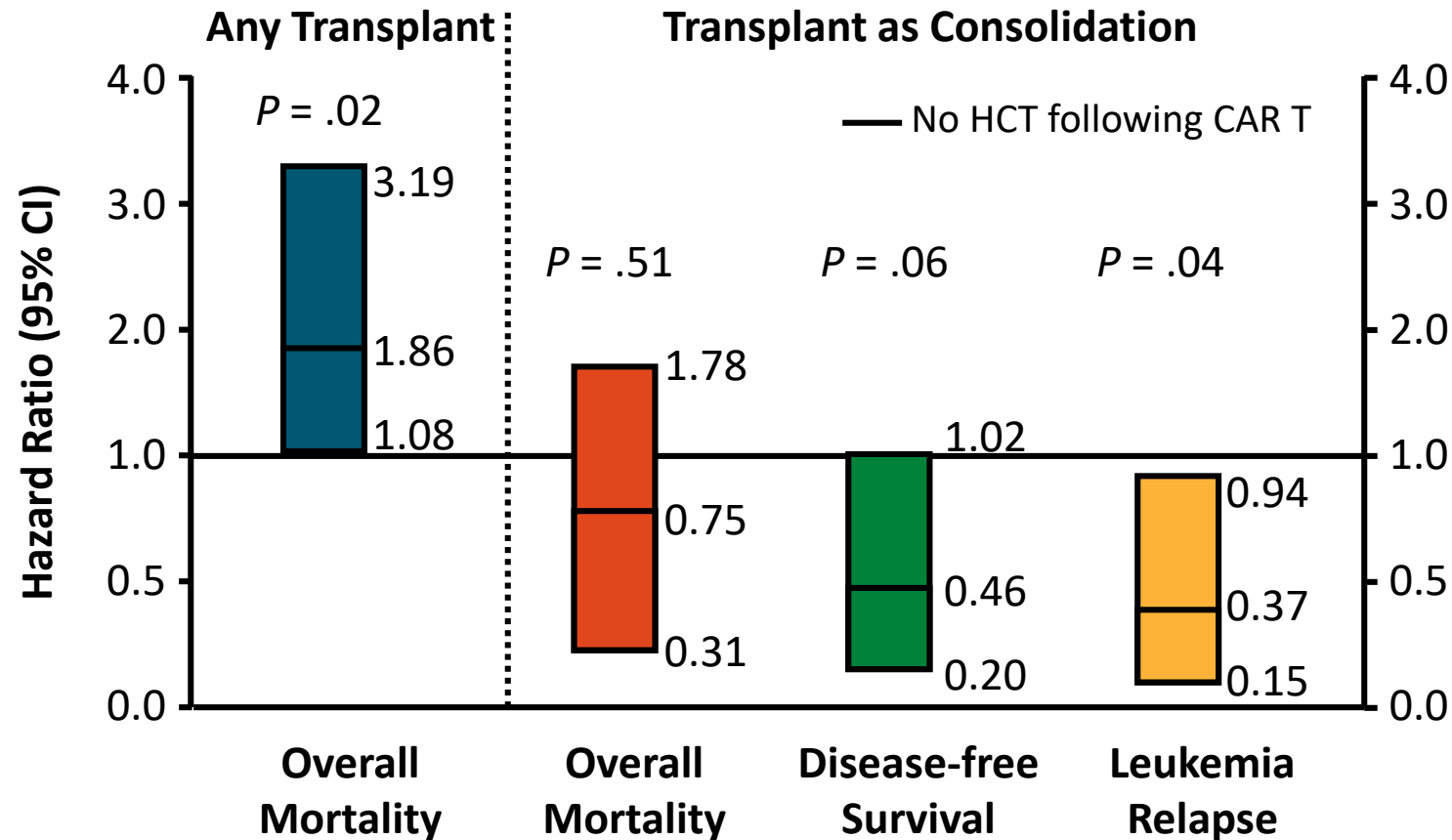


~40% of responding pts proceeded to allo-HSCT

Variable	Multivariable Analysis		
	HR	95% CI	P
LDH prelymphodepletion (per 100 U/L increment)	1.39	1.11-1.73	.004
Platelets prelymphodepletion (per 50,000/ $\mu$ L increment)	0.74	0.53-1.03	.069
Fludarabine added to lymphodepletion	0.25	0.15-0.78	.003
HCT after CAR T-cell therapy	0.39	0.13-1.15	.088

# ASH 2021: Consolidation Allogeneic HCT Following CART Therapy

- Retrospective analysis of outcomes in patients who received consolidation allogeneic hematopoietic cell transplantation post-CD19 CAR T-cell therapy (N = 347)





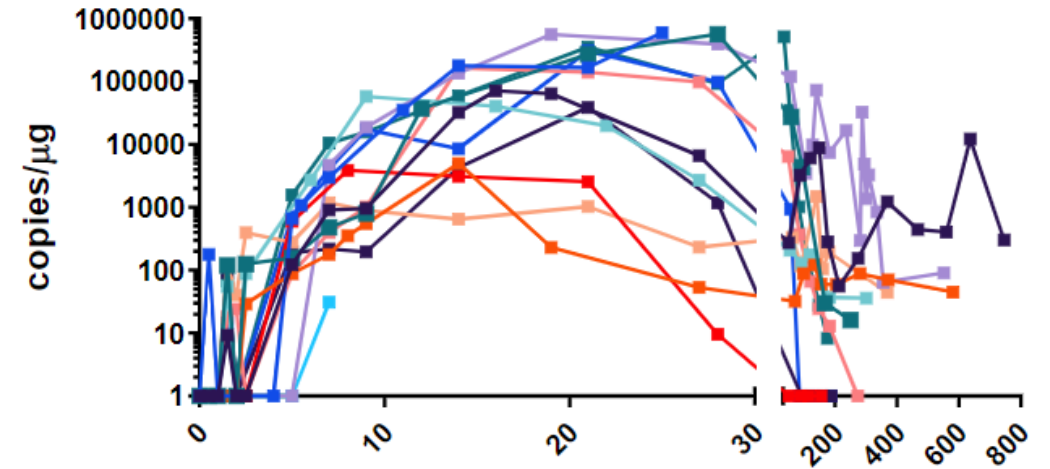
# Toxicity and CART dynamics in the blood

N=13	Peak Grade (ASTCT SCALE)			
	1	2	3	4
CRS	8	3	0	0
ICANS	0	1	0	1

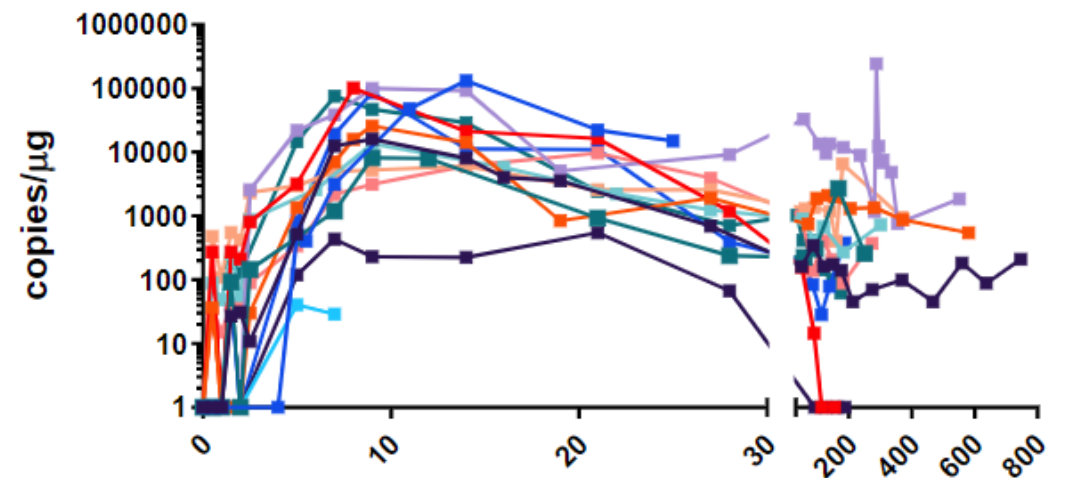
- 4 patients received tocilizumab
- 3 patients received corticosteroids

	Median (range) Days to Peak Expansion
CART22-65s	16 (7-28)
huCART19	9 (5-21)

CART22-65s persistence

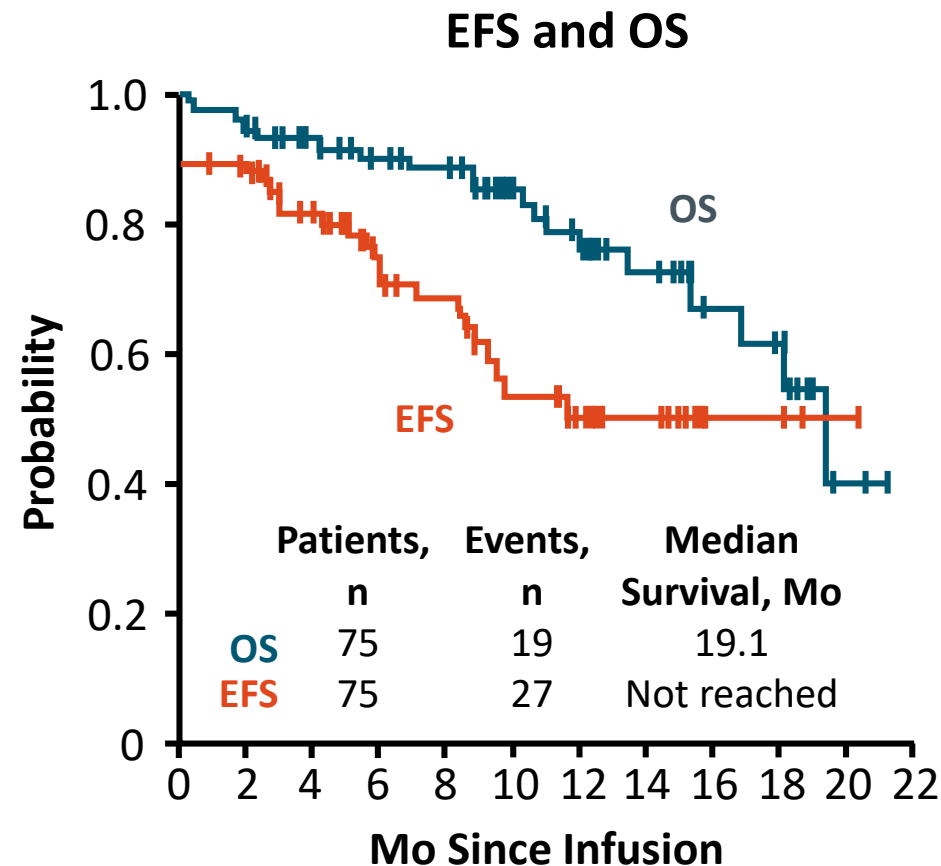


huCART19 persistence



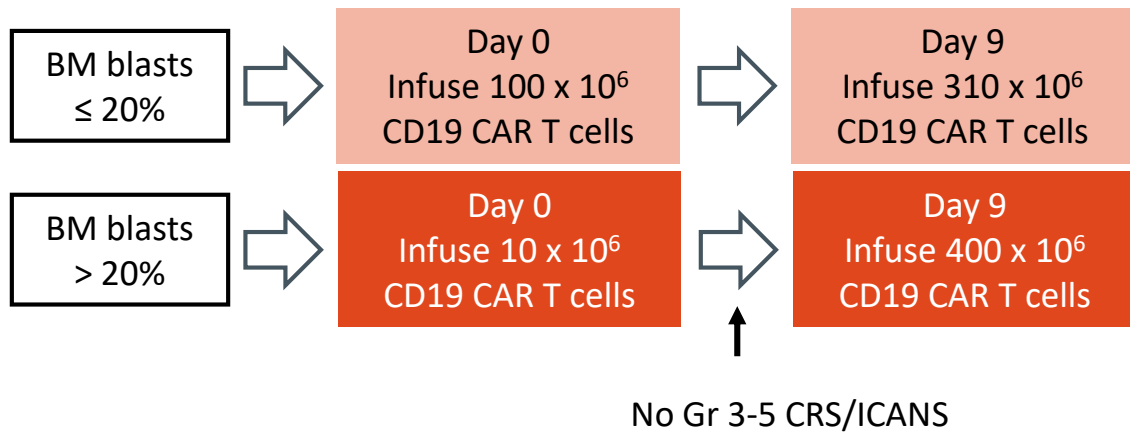
# ELIANA: Tisagenlecleucel in Children and Young Adults With R/R B-ALL

- International, open-label, single-arm phase II study (N = 92)
  - Patients aged 3-21 yr with relapsed or refractory B-cell ALL
  - Patients underwent lymphodepletion with fludarabine + cyclophosphamide followed by single-dose tisagenlecleucel
  - At baseline: median number of prior therapies, 3; prior allogeneic SCT, 46%; median BM blast count at time of treatment, 74%
- ORR at 3 mo: 81%



Outcome, %	Mo 6	Mo 12
OS	90	76
Event-free survival	73	50

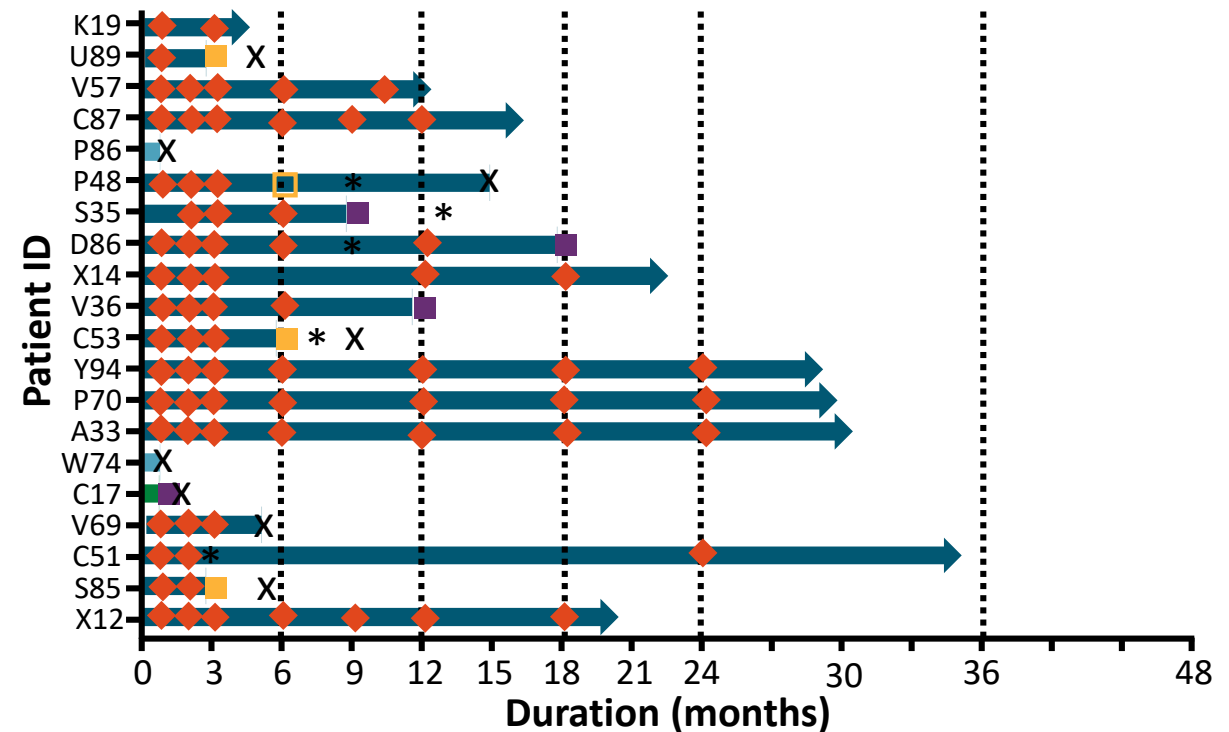
# CD19-Targeted CAR (AUTO1) for R/R Adult B-ALL



75% >20% blasts received 410 x 10<sup>6</sup> cells; 25% received 10 x 10<sup>6</sup> cells due to ongoing grade 1 CRS at D10.

Parameter, %	Patients (N = 20)
Prior blinatumomab	25
Prior inotuzumab	50
Prior HSCT	65
BM blasts before LD chemo	
<5% blasts	35
5-49% blasts	20
≥50% at T cell infusion	45

Roddie. JCO. 2021;39:3352.



- Complete response
- Not evaluable
- MRD-negative CR (PCR or flow)
- Ongoing disease
- CD19-negative relapse
- CD19-negative relapse
- CD19-negative MRD level relapse
- \* Allo-SCT
- X Death

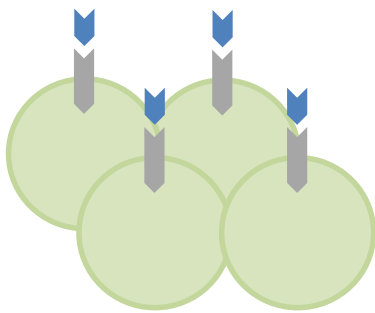
- 13% of responders proceeded to alloHSCT
- EFS at 6 and 12 mo: 68% and 48%



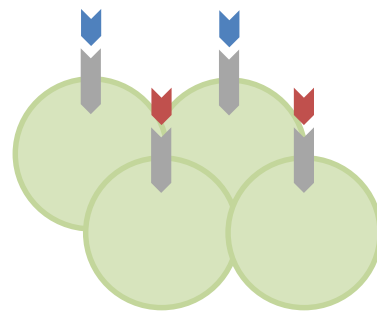
Slide credit: clinicaloptions.com

# Strategies to overcome antigen-escape

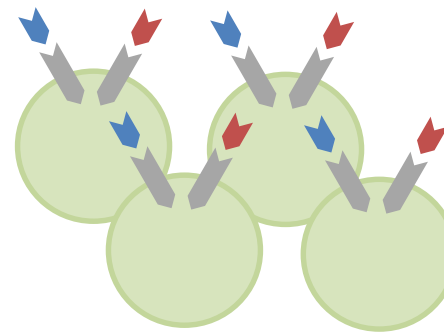
## Single CART



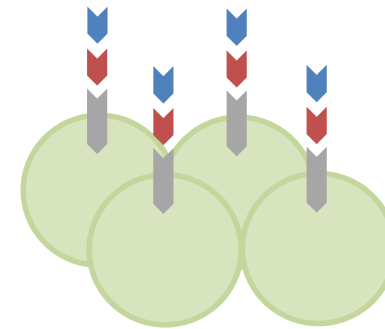
## Pooled CART



## Dual CART



## Tandem CART

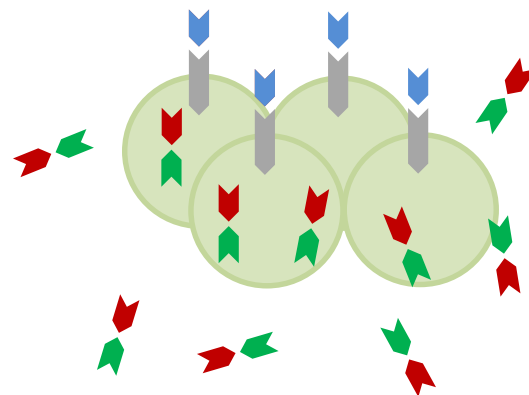


♣ = anti-X scFV

♣ = anti-Y scFV

♣ = anti-CD3 scFV

## 4<sup>th</sup> GEN CART



## Combinations

