

**3<sup>rd</sup> Cuneo City ImmunoTherapy Conference (CCITC)**

# Immunotherapy in Hematological Malignancies **2023**

CUNEO  
May 18-20, 2023  
Spazio incontri Fondazione CRC

Organized by Prof. Massimo Massaia, SC Ematologia AO S.Croce e Carle, Cuneo, Italy  
and Centro Interdipartimentale di Ricerca in Biologia Molecolare (CIRBM), Torino, Italy

## Immunotherapy in Hematological Malignancies 2023

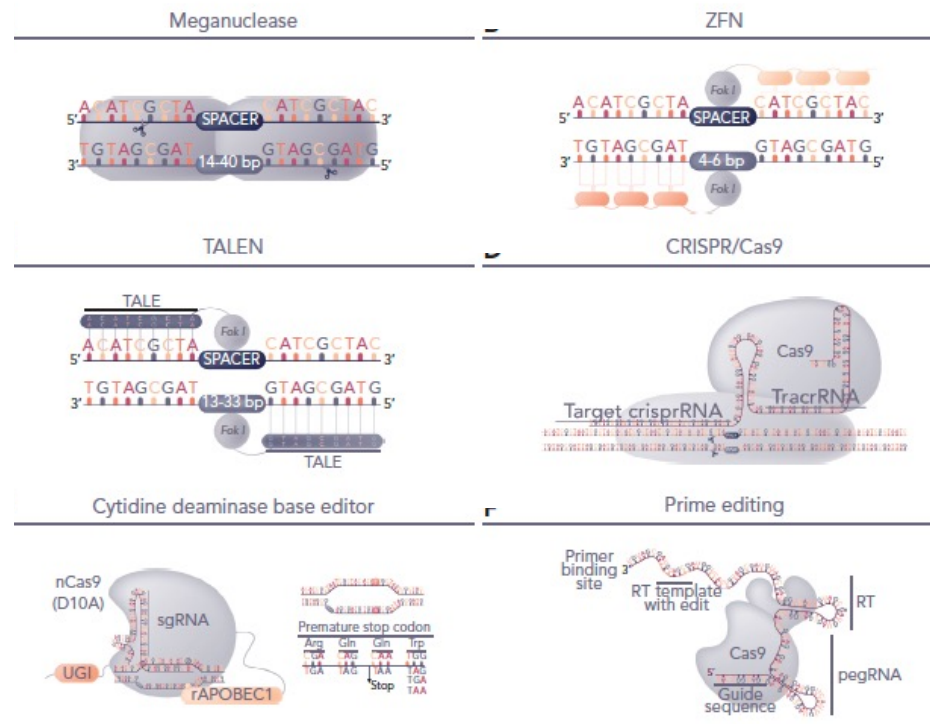
### David Teachey's Disclosures

Company name	Research support	Employee	Consultant	Stockholder	Speakers bureau	Advisory board	Other
BEAM Therapeutics	X					X	
Sobi						X	
Janssen	X						
NeolImmune Tech	X						
Jazz	X					X	
Servier	X						
Ichnos	X						

## Introduction

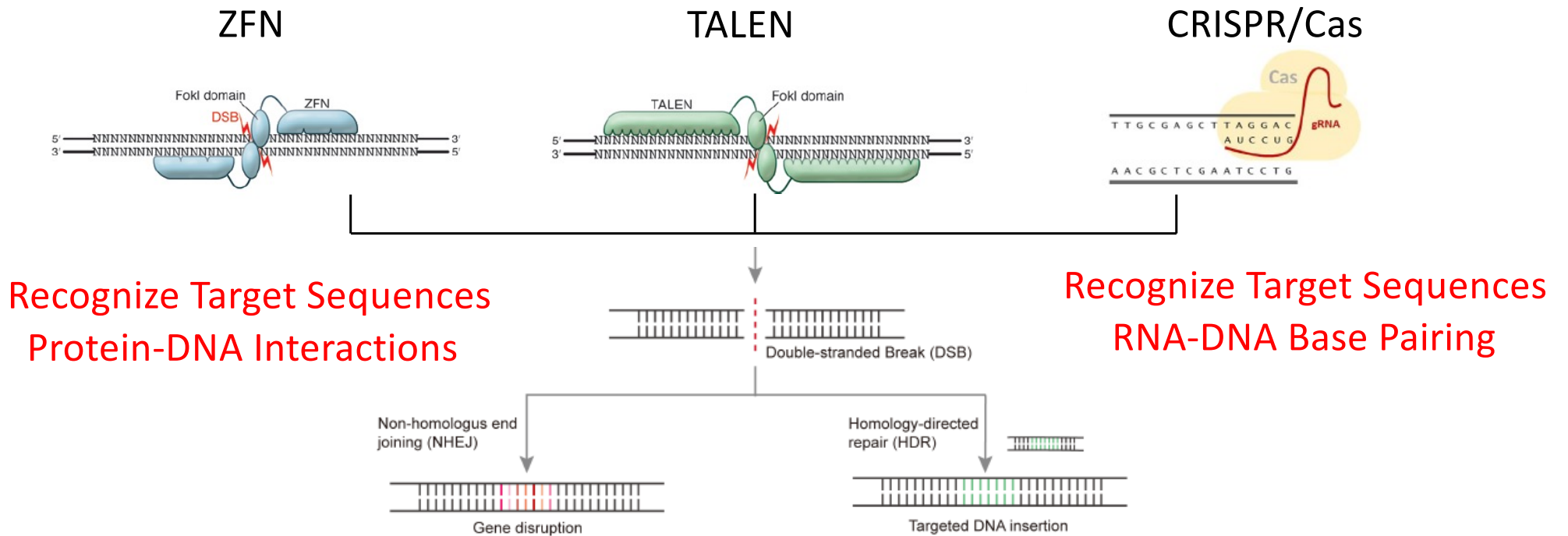
- Cytosine Base Editing Overview
- Current Landscape in T-cell Acute Lymphoblastic Leukemia (T-ALL) and Lymphoblastic Lymphoma T-LL with Focus on Immunotherapy
- Cytosine Base Edited CAR-T for T-ALL/T-LL

# Gene Editing Technologies



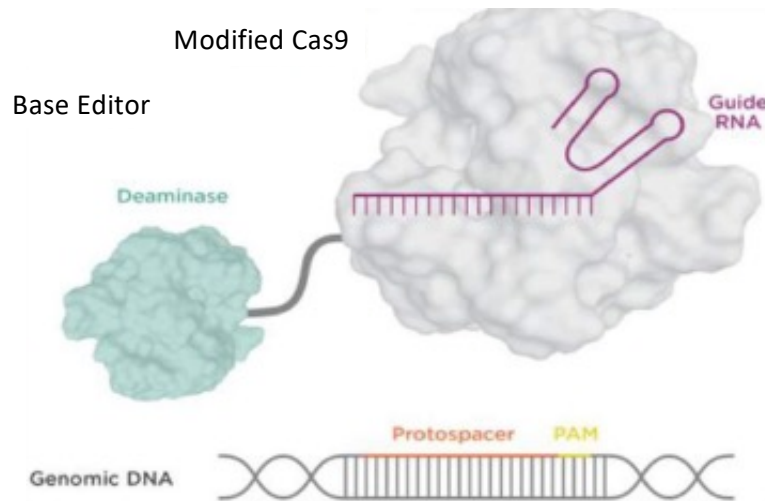
Quasim, et. al. Blood 2022

# Editing Through DSBs



Liu, et. al., Front. Immunol. 2023; Ottaviano, et. al., Heme Onc Clin North Am. 2022; Gupta, et. al. JCI 2014

## Cytosine Base Editors (CBEs)

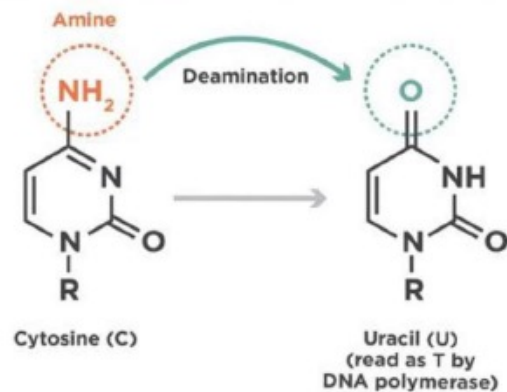


### • 4 Main Components

- **Modified Cas9 protein**
  - Binds DNA target with help guide RNA
  - Opens 4-5 base single strand segment
  - Engineered to nick unedited DNA instead of DSB
- **Cytosine Deaminase**
  - Modifies Cytosine to Uracil Intermediate
- **Two Uracil Glycosylase Inhibitor (UGI) Domains**
  - Suppress Uracil Excision

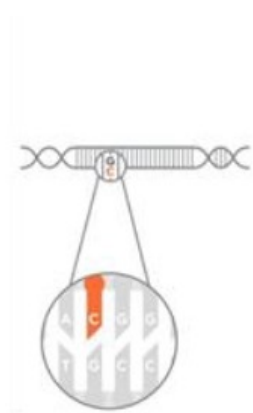
## Cytosine Base Editors (CBEs)

Cytosine Base Editor Converts C→T



- Uracil Intermediate Read by DNA polymerase as Thymine
- Leads to C-to-T change

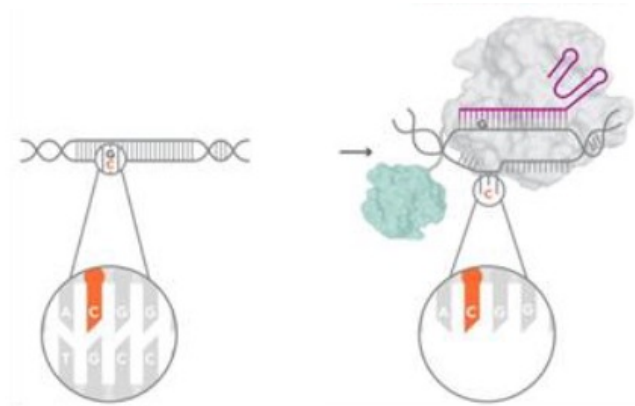
## Cytosine Base Editors (CBEs)



Starting Sequence with Target  
Point mutation Base Pair (C:G)

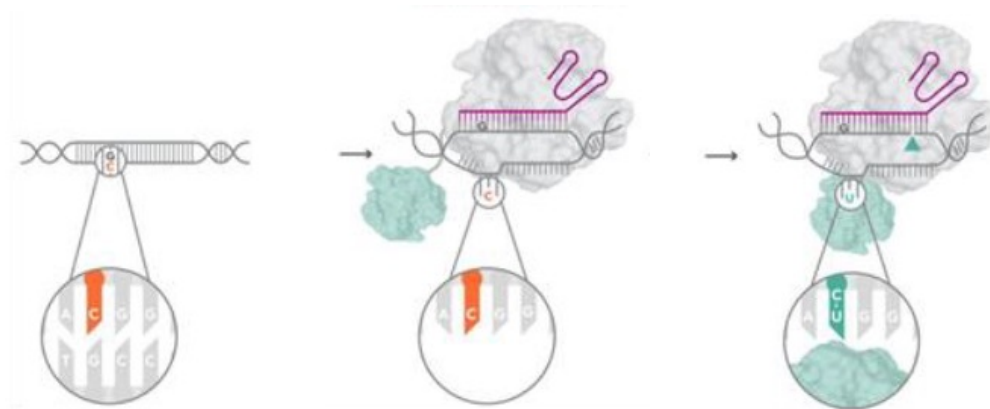


## Cytosine Base Editors (CBEs)



Base Editor Binds to Target DNA Sequence  
Exposes Editing Window

## Cytosine Base Editors (CBEs)

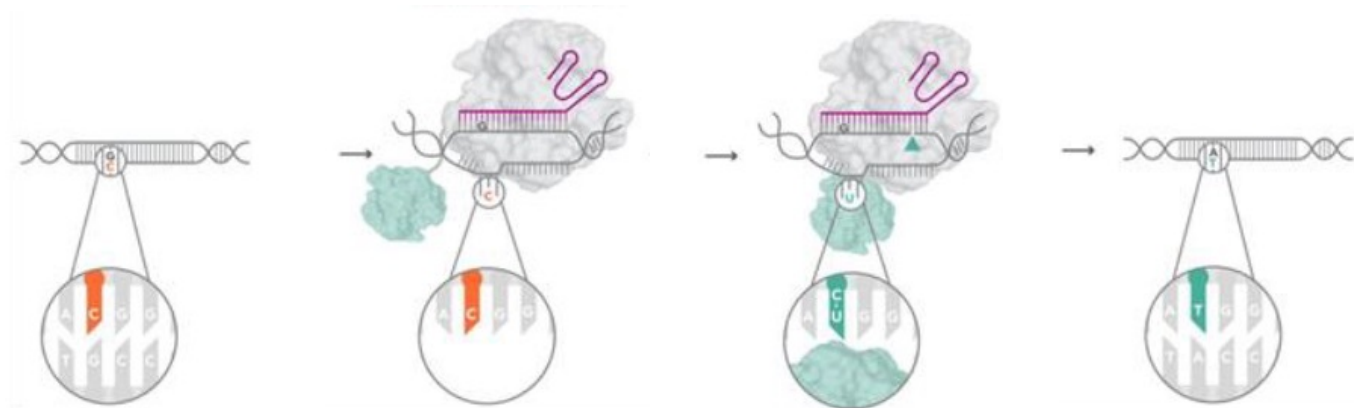


Deaminase Converts Target Base (C → U)

Uracil Glycosylase Inhibitor (UGI) Domains Prevent Uracil Excision

Modified Cas9 Nicks Opposite Strand

## Cytosine Base Editors (CBEs)



Cell Repairs Nicked Strand (G → A)

Cell Completes Correction of Target Point Mutation (U → T)

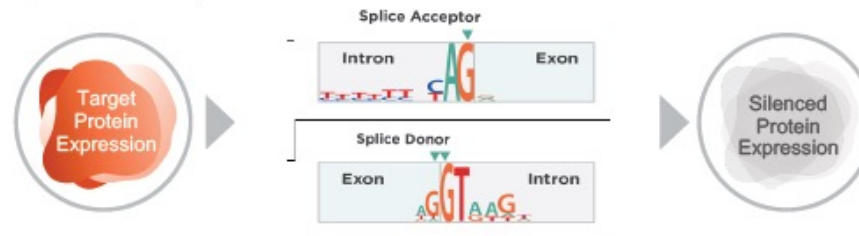
# Cytosine Base Editors (CBEs)

## Two strategies for silencing with base editors

### Create a stop codon with CBE



### Splice disruption with CBE



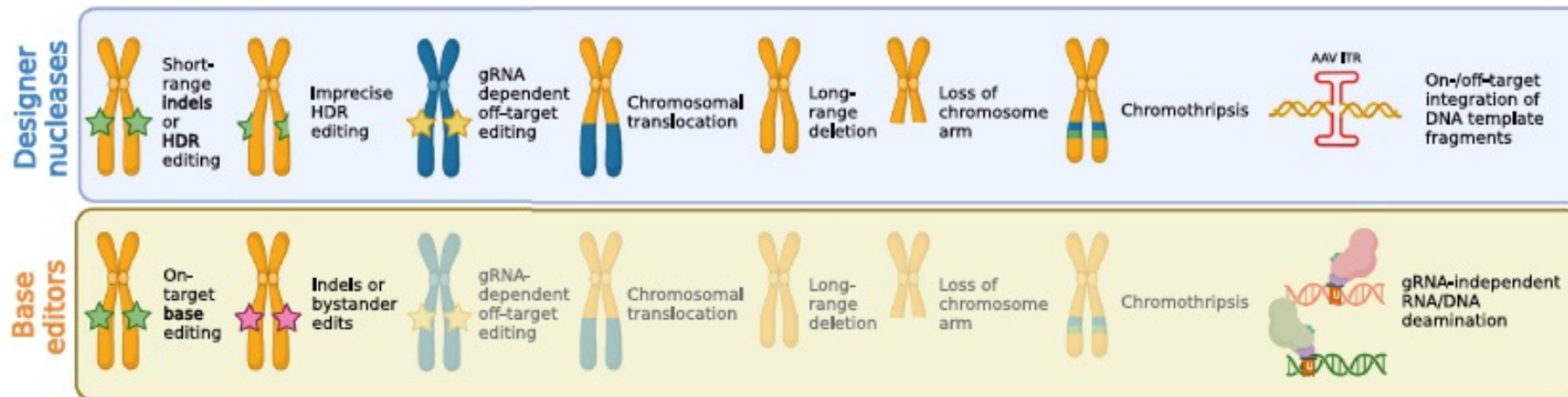
## Designer Nucleases vs Base Editors: Pros and Cons

"Traditional" CRISPR/Cas9	Base Editors
Pro: Can make large edits	Pro: Single base change with each edit
Con: Higher-risk of off-target and on-target error	Pro: More precise editing
Con: Multiple edits to different genes complicated	Pro: Easier to make multiple edits to different genes
	Pro: Less off-target and on-target error
	Con: Less targetable sites
	Con: Only can make specific base pair conversions

# Designer Nucleases and Base Editors

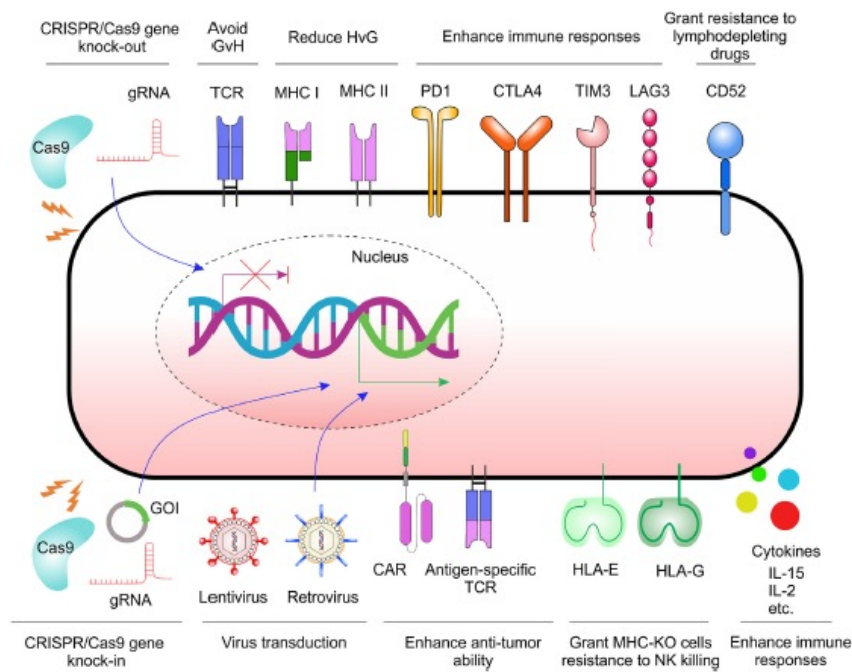
Intended Edit

Unintended Edit



Ferrari, et. al. Cell Stem Cell 2023

# What to Edit When Making CAR-T



## Increase Antitumor Efficacy

### Overexpression

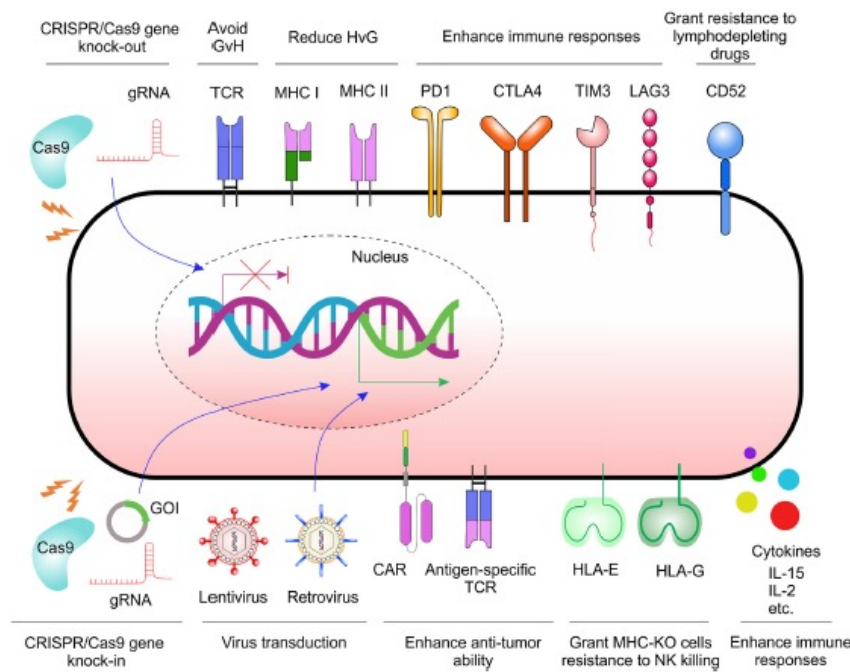
- IL-2
- IL-7
- IL-12
- IL-15
- IL-21

### Knock-out

- PD-1
- CTLA-4
- LAG-3
- TIM-3
- NKG2A
- EZH1
- DNTM3A
- CD16

Li, et. al., Cell Stem Cell 2023; Chen, et. al. Front. Med. 2023

# What to Edit When Making CAR-T



Prevent GVHD

Knock-out  
Modify  
TCR

Prevent Fratricide

Knock-out  
Modify  
Target of the CAR-T

Reduce Allojection

Knock-out  
HLA  
CD52  
Overexpress  
Inhibitory Ligands

Improve Safety

Overexpress  
Suicide Genes

Li, et. al., Cell Stem Cell 2023; Chen, et. al. Front. Med. 2023

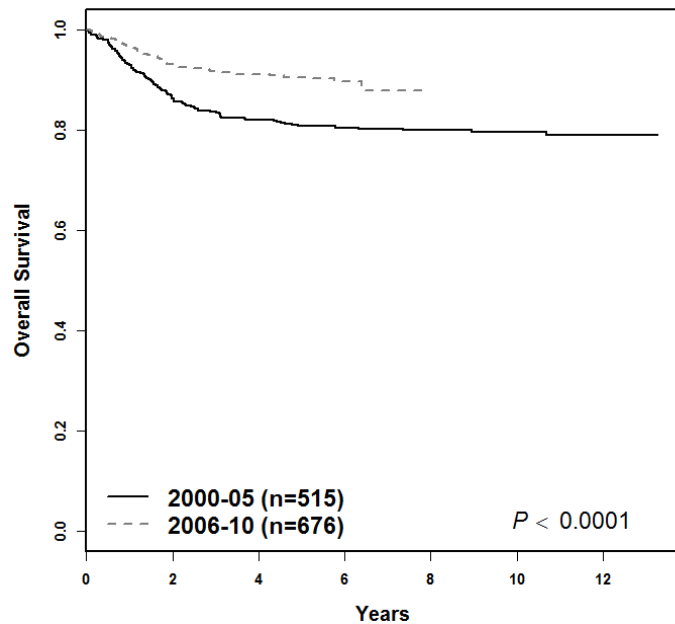


## Clinical Trials with Genome-edited T cells with Published Data

Study	Investigational product	Modifications	Indication	Lymphodepletion	Number	Toxicity	Outcomes
Great Ormond Street Hospital Special's License	UCART19	LV-CAR19 TALEN ko of TRAC & CD52	B-ALL	F,C, AntiCD52	2	Grade2 GVHD	CR 100% <sup>79</sup>
Service/Allogene NCT02808442 NCT02746952	UCART19	LV-CAR19 TALEN ko of TRAC & CD52	B-ALL	F,C, ± AntiCD52	21	Grade 3+ CRS 15%; Grade 3+ infections 39%	CR 67% <sup>80</sup>
Allogene NCT04416984 NCT03939026	ALLO-501A ALLO-501	LV-CAR19 TALEN ko of TRAC & CD52	LBCL	F,C, AntiCD52	47	Grade 3+ CRS: 2%; Grade 3+ infections 24%	CR 50% <sup>113</sup>
Allogene NCT04093596	Allo-715	LV-anti BCMA TALEN ko of TRAC & CD52	MM	F,C, AntiCD52	26 (DL3,4)	Grade 3 infections 13%	ORR 61% <sup>114</sup>
Collectis NCT04150497	UCART22	LV-CAR22 TALEN ko of TRAC & CD52	B-ALL	F,C, ± AntiCD52	9	No Grade 3 CRS or infection	No CR <sup>115</sup>
Precision Bio NCT03666000	PBCAR0191	Arcus ko TRAC AAV site specific inserted CAR19	B-ALL NHL	F, C	27	Grade 3+ CRS 6%; Grade 3+ infections 31%-80%	CR/CRi 62%-80% <sup>72,73</sup>
CRISPR Tx NCT04035434	CTX110	CRISPR/Cas9 TRAC & B2m AAV site specific inserted CAR19	LBCL	F,C	24 DL2+	Grade 3+ infections 9%; Grade 3+ ICANS 4%	CR 38% <sup>74</sup>
Great Ormond Street Hospital NCT04557436	TT52CAR19	CRISPR/Cas9 TRAC, CD52 LV CAR19	B-ALL	F,C, AntiCD52	6	NCT04557436, TT52CAR19 <sup>*</sup> Grade 3+ ICANS 17%	CR/CRi 66% <sup>70</sup>
Beijing Chinese PLA General Hospital NCT03166878	U-Car	CRISPR/Cas9 TRAC, B2m LV CAR19	DLBCL	F,C	2		No CR <sup>71</sup>
Zhejiang University Nanjing Bioheng Biotech	CTA 101	CRISPR/Cas9 TRAC,CD52 LV CAR19/22	B-ALL	F,C, AntiCD52	6	Grade 3 CRS 16% Grade 3 infections 50%	CR/CRi 83% <sup>116</sup>
Gracell ChiCTR1900025311	GCD27	CRISPR TRAC CD7 LV-CAR7	T-ALL	F,C ± Mel	6	Grade 3 CRS 100% Grade 3 infections 50%	CR/CRi 83% <sup>114</sup>
CRISPR Tx NCT04502446	CTX130	CRISPR/Cas9 TRAC, B2m, CD70 AAV site specific	TCL	F,C	15	Grade 3+ infections 7%;	CR 29% <sup>75</sup>

Quasim, et. al. Blood 2022

## Dramatic Improvements in *De Novo* T-ALL/T-LL Survival



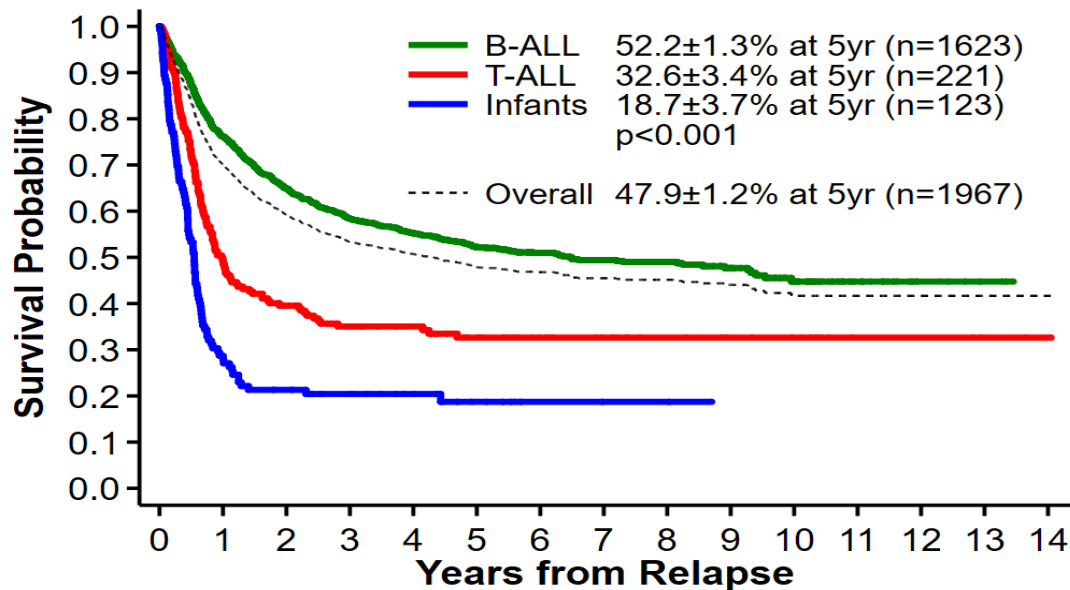
Era	# Pts	5-yr OS±SE
2000-05 B-ALL	6631	91.0±0.4%
2006-10 B-ALL	7397	91.7±0.4%
2000-05 T-ALL	515	80.8±1.9%
2006-10 T-ALL	676	90.6±1.7%

**T-LL 5-year OS: 89.0±2.0%**

Raetz et al., ASPHO 2018; Hayashi, et. al. JCO 2019

## Immunotherapy in Hematological Malignancies 2023

### Outcomes for Relapsed T-ALL/T-LL Remain Poor



At Risk	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14
B-ALL	1623	1184	962	807	681	540	375	259	185	108	55	23	8	3	0
T-ALL	221	101	74	55	45	35	26	21	15	12	10	4	4	2	1
Infants	123	35	25	20	15	8	3	2	2	0	0	0	0	0	0
Overall	1967	1320	1061	882	741	583	404	282	202	120	65	27	12	5	1

### 5-year OS post relapse

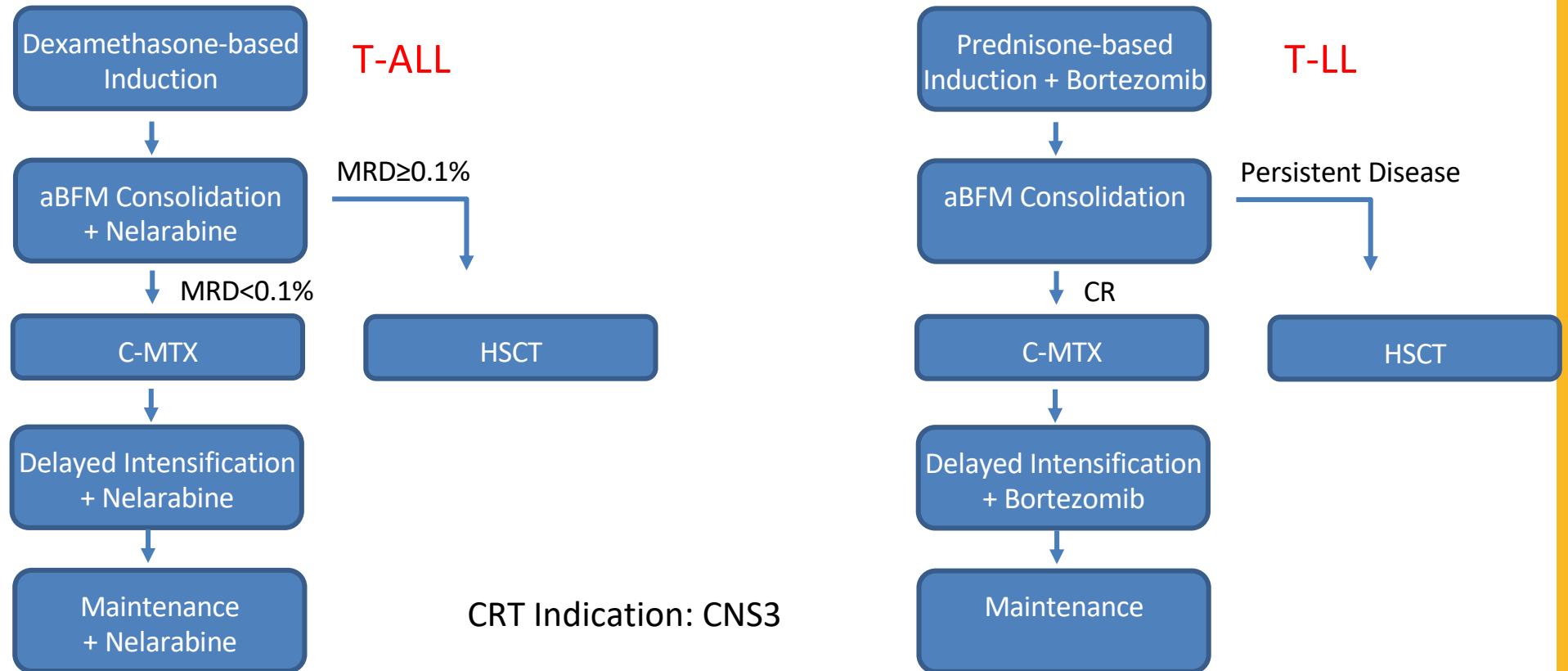
	Current	2008
B-ALL	52%	37%
T-ALL	33%	23%
Infant	19%	20%

T-LL 5-year OS <15%

Rheingold, et al. ASCO 2019

## Immunotherapy in Hematological Malignancies 2023

### Current Approach: *De Novo* T-ALL and T-LL



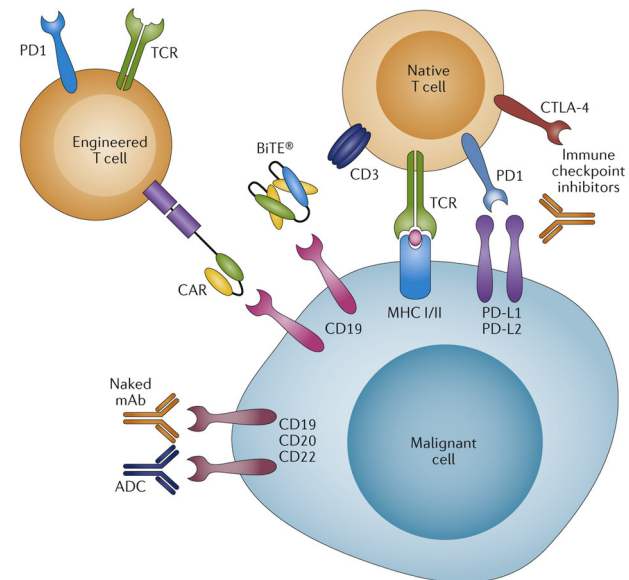
## Immunotherapy for T-ALL/T-LL

### B-ALL/B-LL

- Naked Monoclonals
  - Rituximab
  - Epratuzumab
- Conjugated Monoclonals
  - Inotuzumab
  - Moxetumomab
- BiTEs
  - Blinatumomab
- CARs

### T-ALL/T-LL

- UM.....



Nature Reviews | Clinical Oncology

## Immunotherapy for T-ALL/T-LL

### B-ALL/B-LL

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- BiTEs
  - Blinatumomab
- CARs
- Combination with Check-point Blockade

### T-ALL/T-LL

- T-cell Aplasia and Toxicity

## Immunotherapy for T-ALL/T-LL

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### T-ALL/T-LL

- T-cell Aplasia and Toxicity
- **Fratricide**

## Immunotherapy for T-ALL/T-LL

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### T-ALL/T-LL

- T-cell Aplasia and Toxicity
- Fratricide
- **Eliminating Blasts from Product**



## Immunotherapy for T-ALL/T-LL

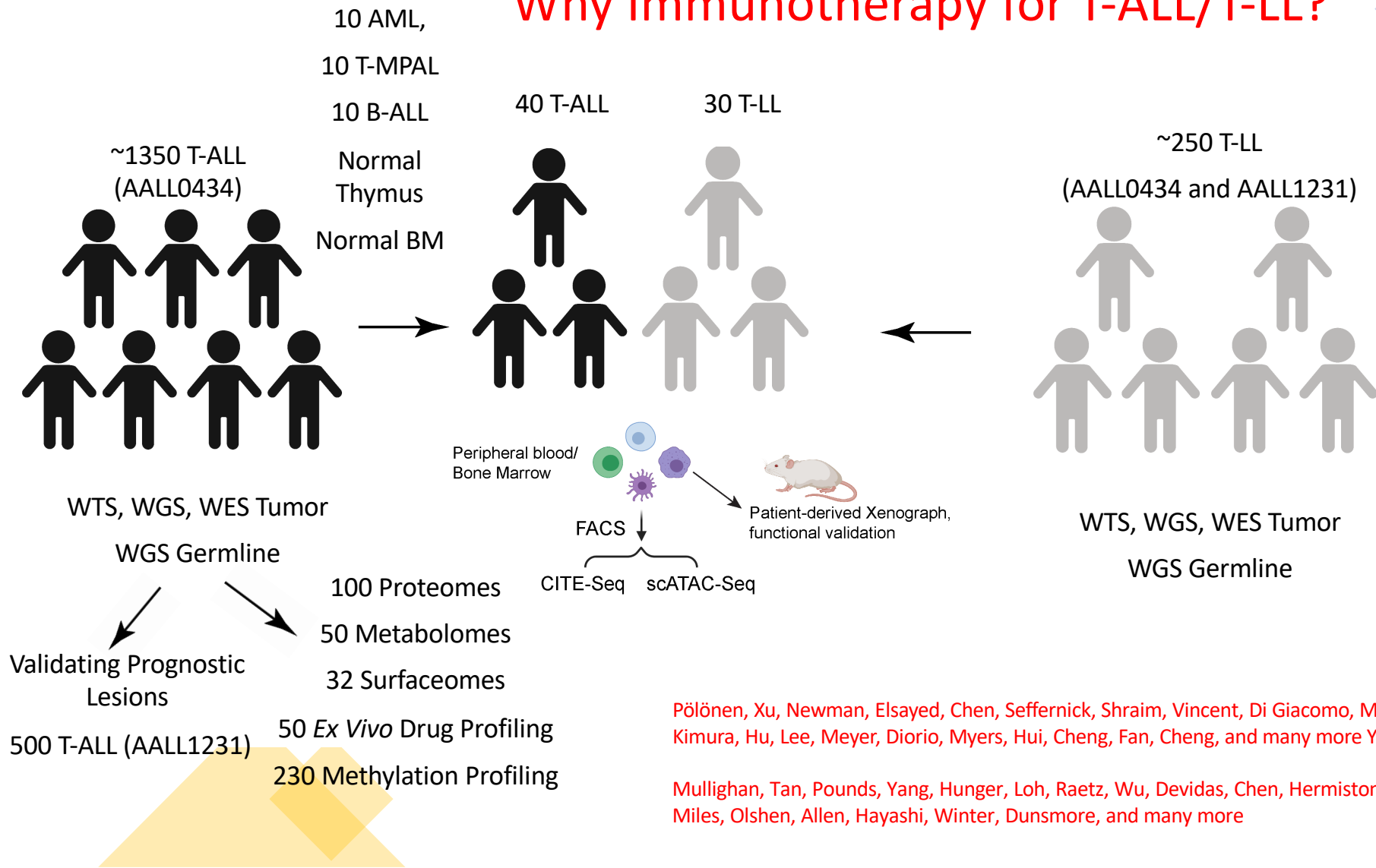
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### T-ALL/T-LL

- T-cell Aplasia and Toxicity
- Fratricide
- Eliminating Blasts from Product
- Host Issues that Impair Manufacturing
- Patients Can Progress Rapidly

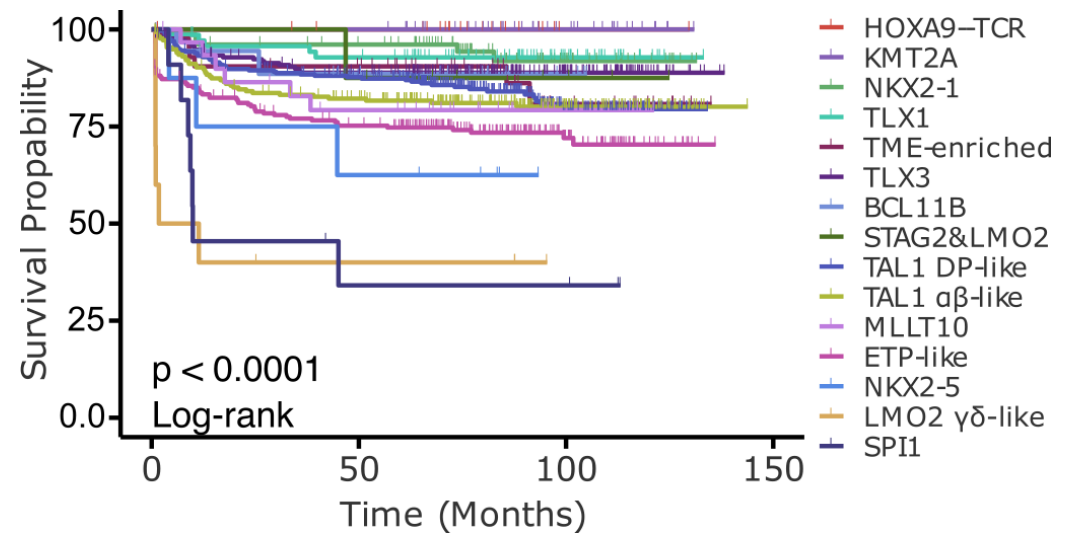
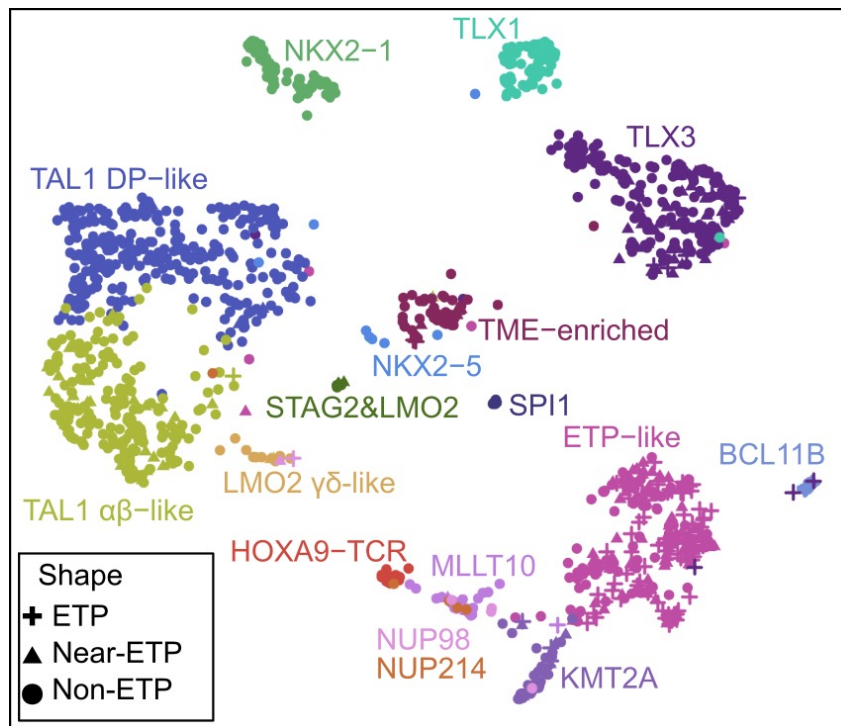
# Why Immunotherapy for T-ALL/T-LL?



Pölänen, Xu, Newman, Elsayed, Chen, Seffernick, Shraim, Vincent, Di Giacomo, Montefiore, Kimura, Hu, Lee, Meyer, Diorio, Myers, Hui, Cheng, Fan, Cheng, and many more YIs

Mullighan, Tan, Pounds, Yang, Hunger, Loh, Raetz, Wu, Devidas, Chen, Hermiston, Horton, Wood, Miles, Olshen, Allen, Hayashi, Winter, Dunsmore, and many more

## Why Immunotherapy for T-ALL/T-LL?

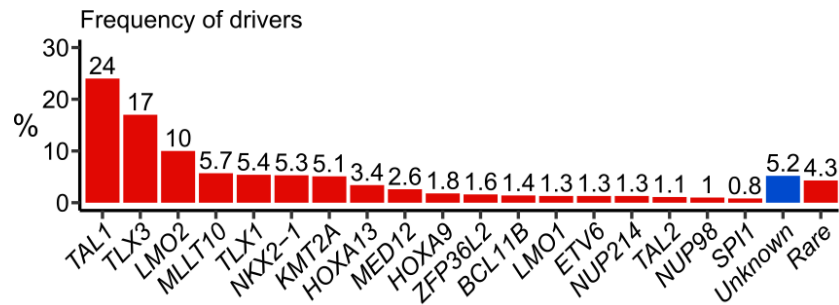


Pölönen, et. al. EHA 2022

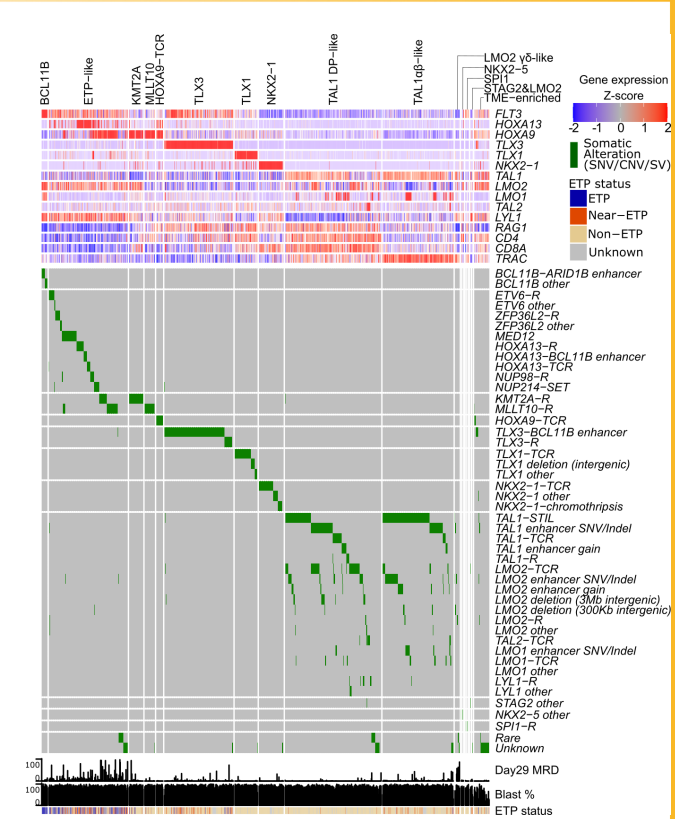
Pölönen, et. al. ASH 2022

# Immunotherapy in Hematological Malignancies 2023

## Why Immunotherapy for T-ALL/T-LL?



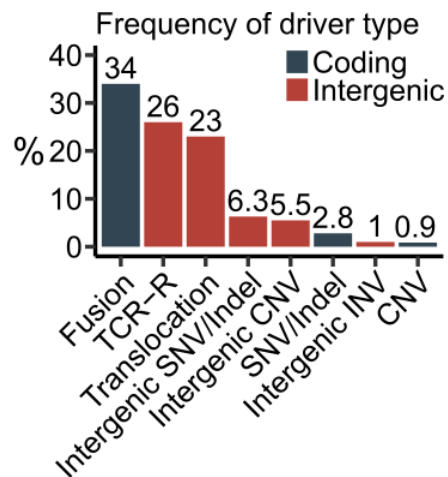
**T-ALL classifying drivers (Clonal, subtype defining) identified in 96.5% of the samples using combination of WGS/WES/RNA**



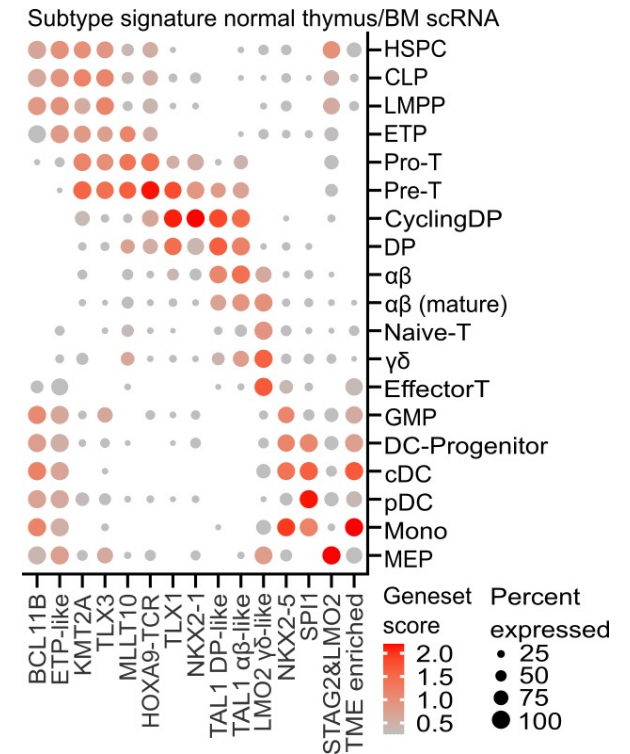
Pölonen, et. al. EHA 2022  
Pölonen, et. al. ASH 2022

## Immunotherapy in Hematological Malignancies 2023

### Why Immunotherapy for T-ALL/T-LL?



- **59%** of the patients have alteration in non-coding regions
- 28% detectable only by WGS
- 16% of cases have novel alterations

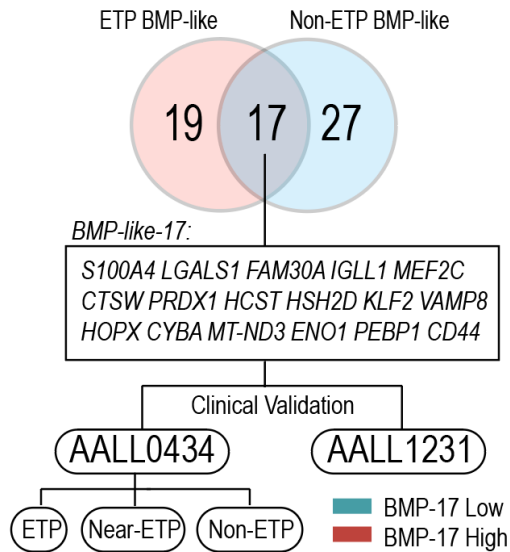


Pölönen, et. al. EHA 2022

Pölönen, et. al. ASH 2022

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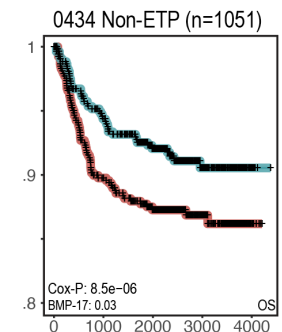
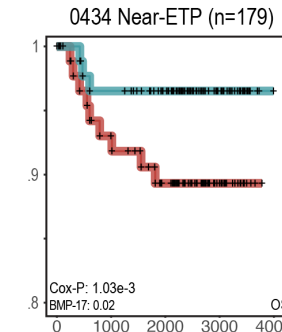
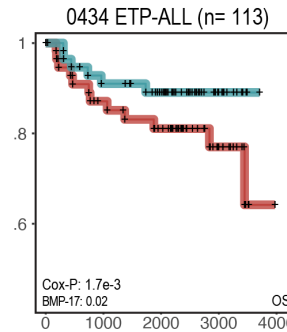
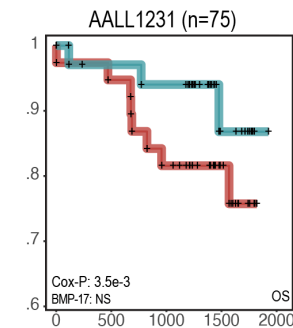
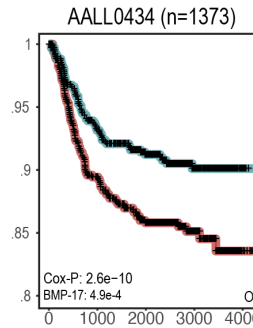
## Why Immunotherapy for T-ALL/T-LL?



Clinical Validation



■ BMP-17 Low  
 ■ BMP-17 High



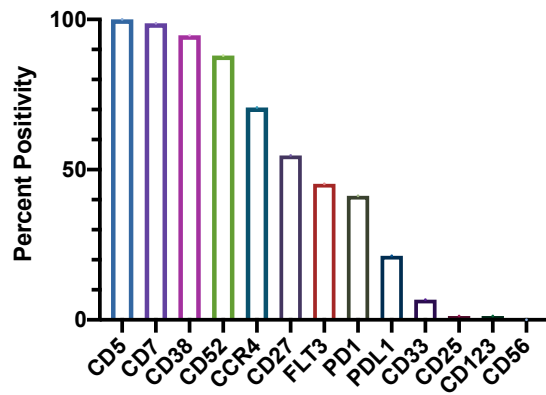
Xu, Chen ASH 2022

## Why Immunotherapy for T-ALL/T-LL?

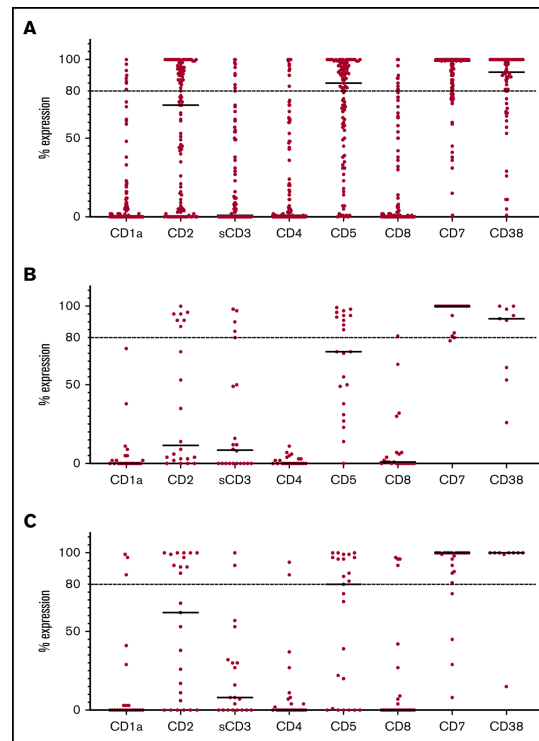
- Prognosis
  - Survival in Relapsed and Refractory Disease is Low (<40% OS)
  - Adults with *De Novo* T-ALL/T-LL Need New Therapies (<50% OS)
- Biology of T-ALL
  - Significant Biologic Heterogeneity (Intra- and Inter-patient)
  - Many Leukemic Drivers Currently Not Targetable

# Immunotherapy in Hematological Malignancies 2023

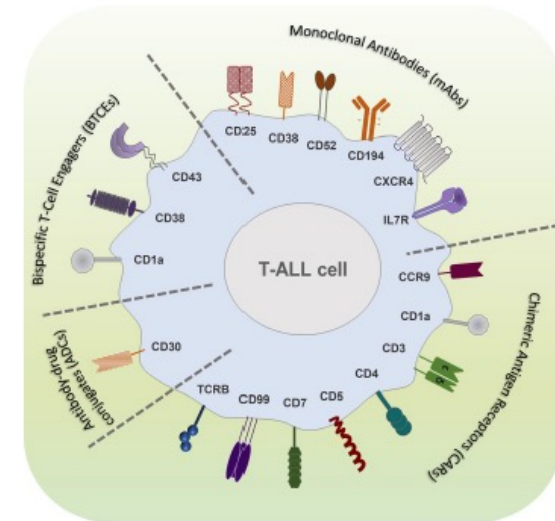
## What to Target in T-ALL?



Diorio, Vincent



Leong, et. al., Blood Advances 2020



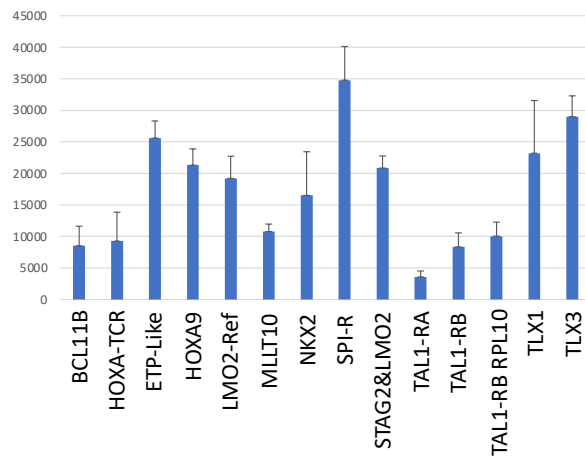
Caracciolo, et. al. Exp. Hematol 2023



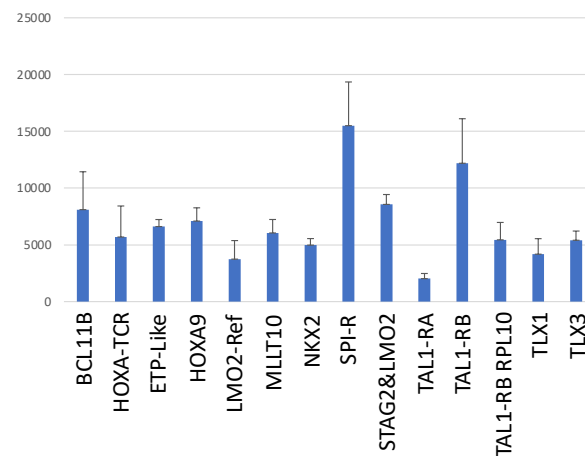
# Immunotherapy in Hematological Malignancies 2023

## What to Target in T-ALL?

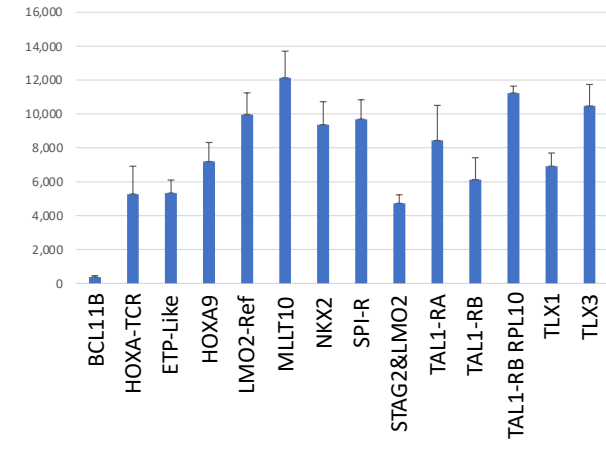
CD7 MFI By Omics



CD38 MFI By Omics

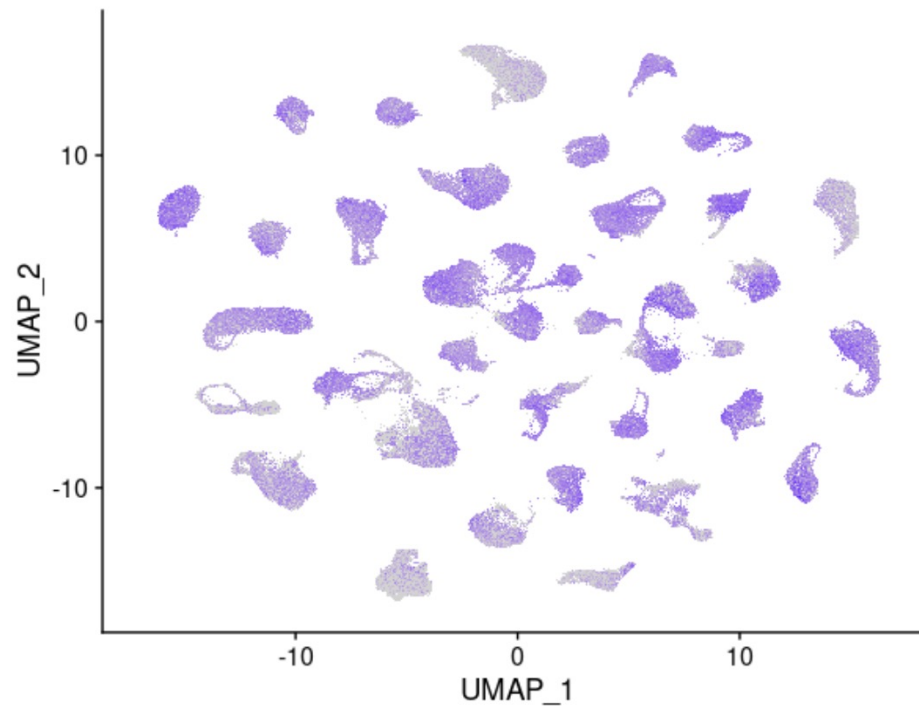


CD5 MFI By Omics



Diorio, Mullighan, Pölönen, Newman, Shraim, Vincent

## What to Target in T-ALL?

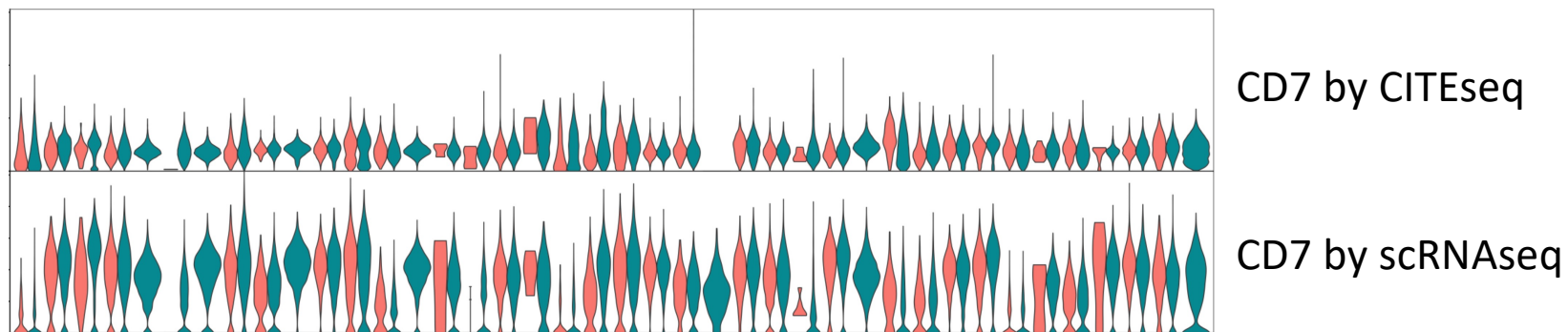
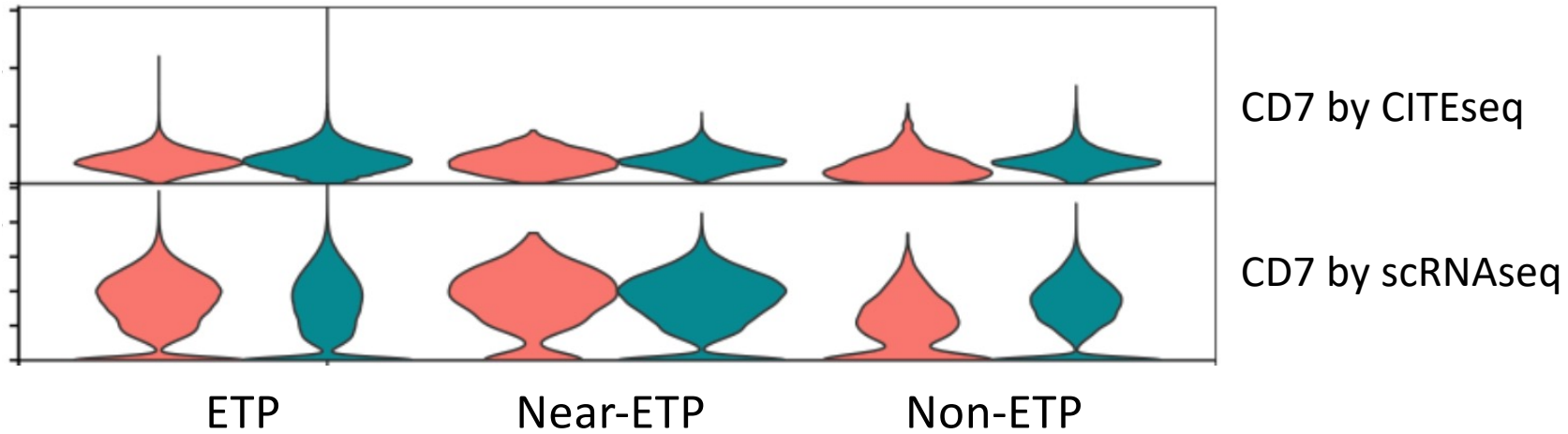


CD7 Expression  
Single Cell

Xu, Chen, Tan

# What to Target in T-ALL?

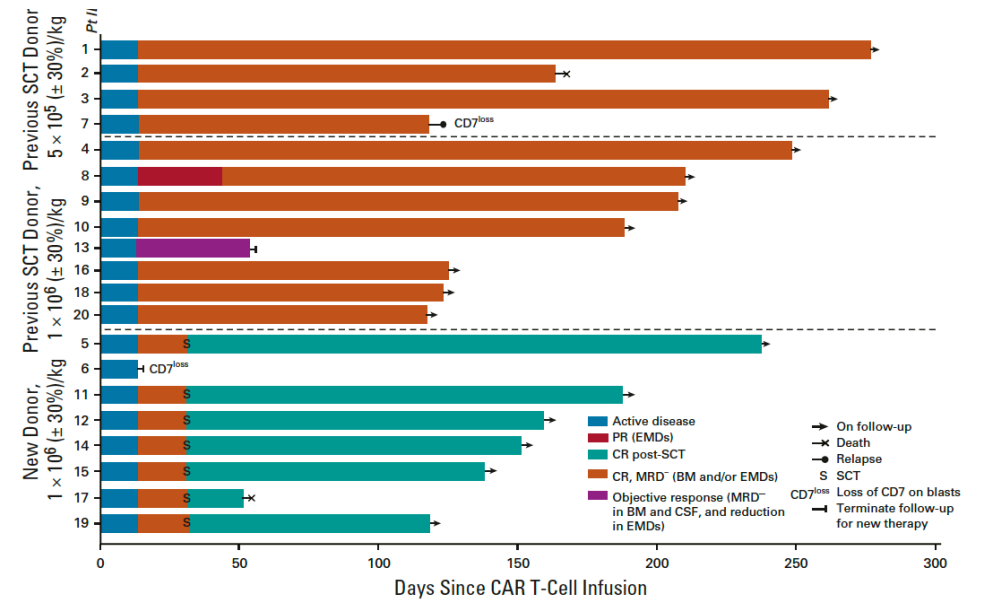
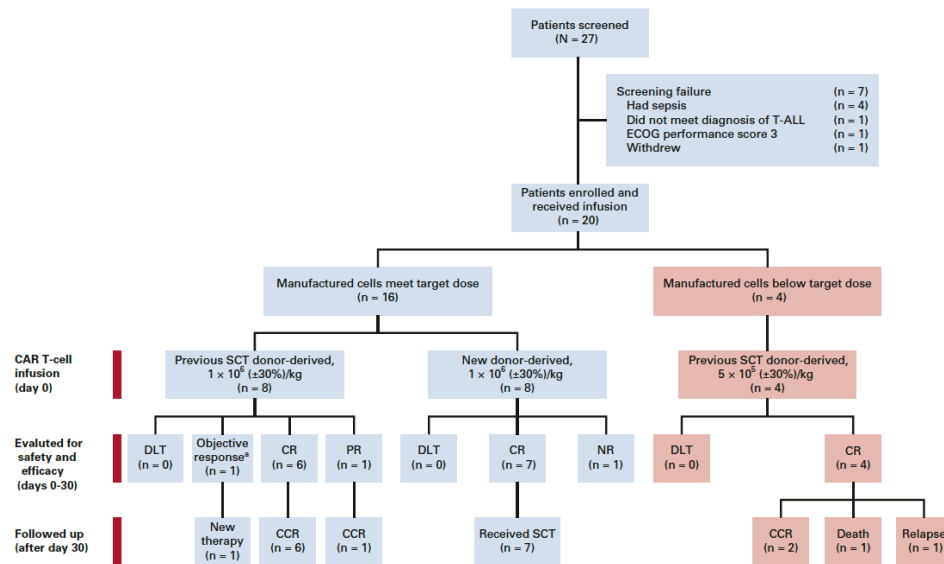
■ BMP-like  
■ T-Lineage



Xu, Chen, Tan

# Immunotherapy in Hematological Malignancies 2023

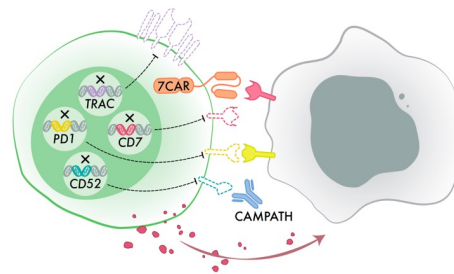
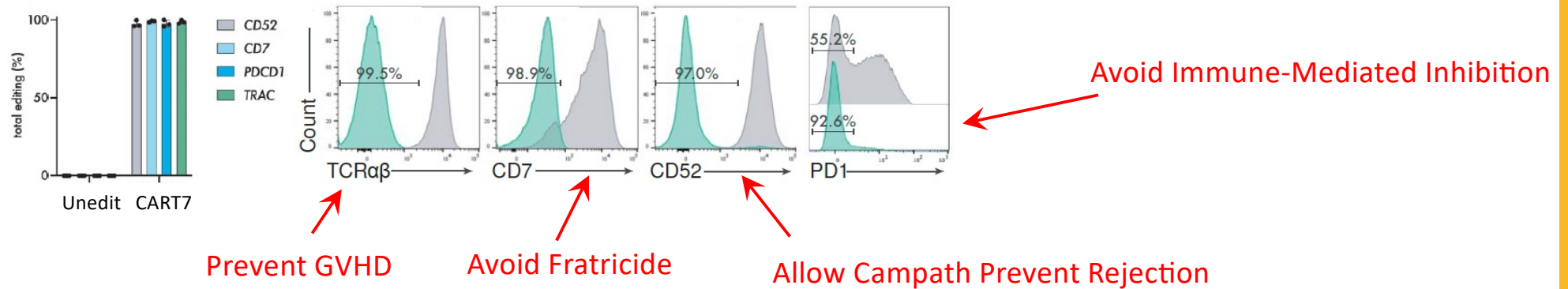
## CD7 CAR-T for T-ALL



Pan, et. al. JCO 2021

# Immunotherapy in Hematological Malignancies 2023

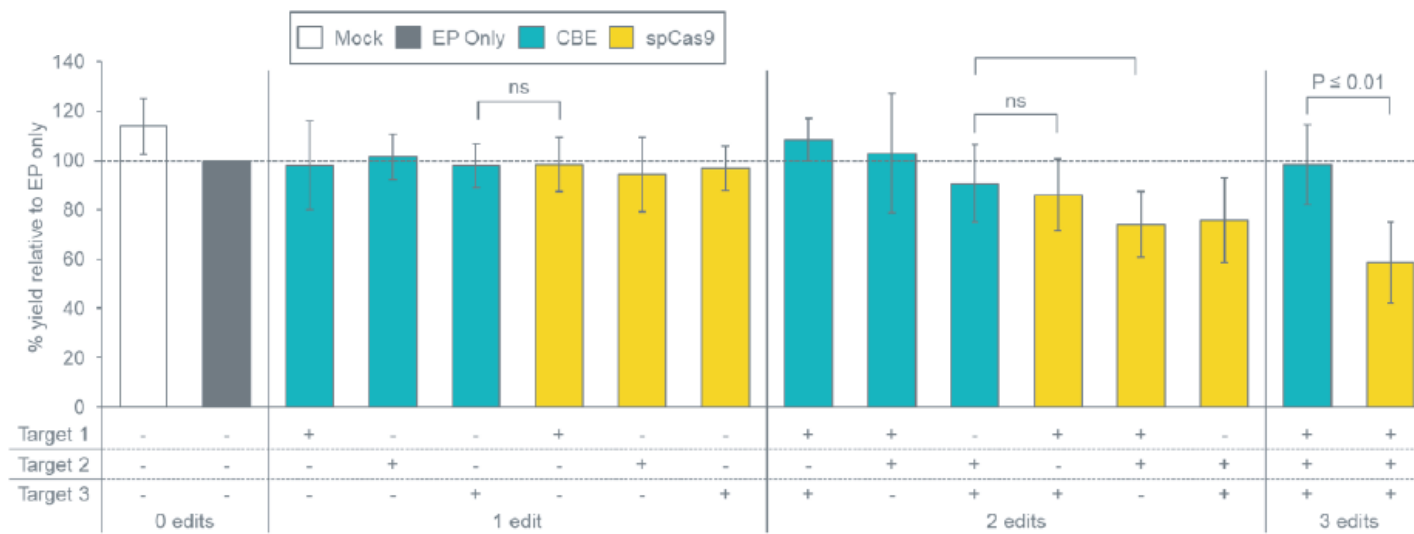
## Cytosine Base Editing for Off-the-Shelf CAR-T



Anti-CD7 CAR-T with CD28 co-stimulatory domain

Diorio, Gehrke, et. al, Blood 2022

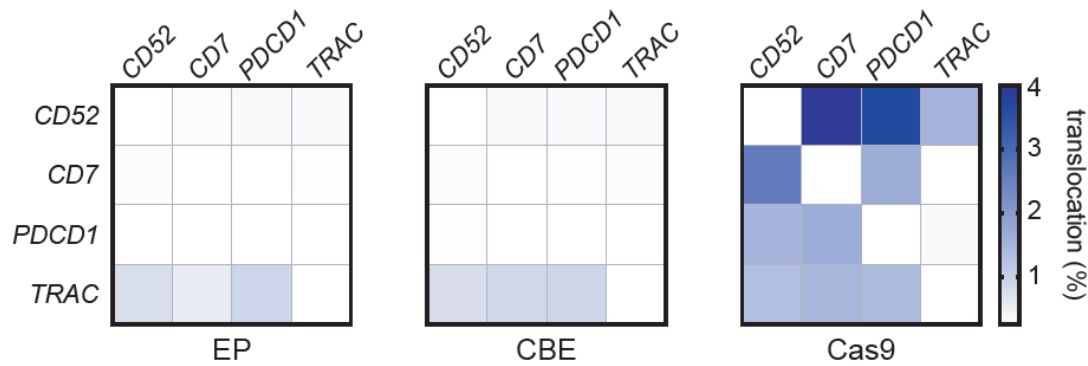
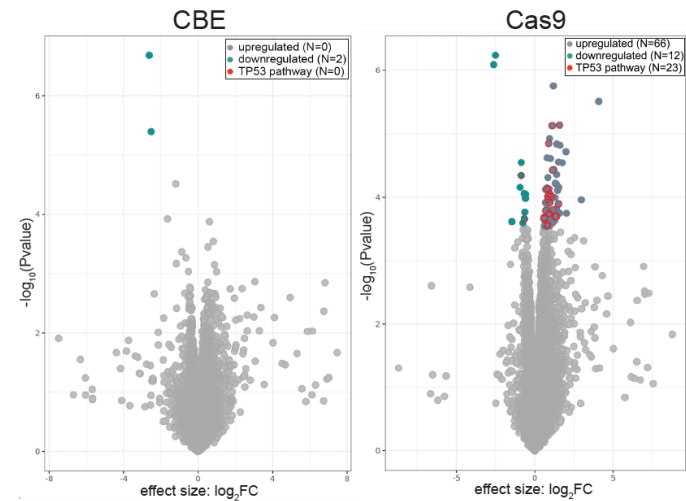
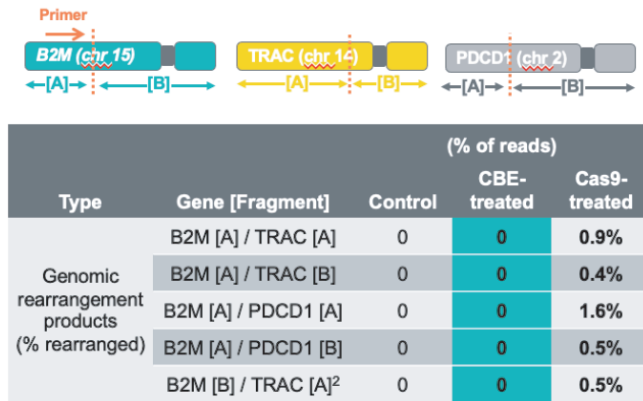
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Diorio, Gehrke, et. al, Blood 2022

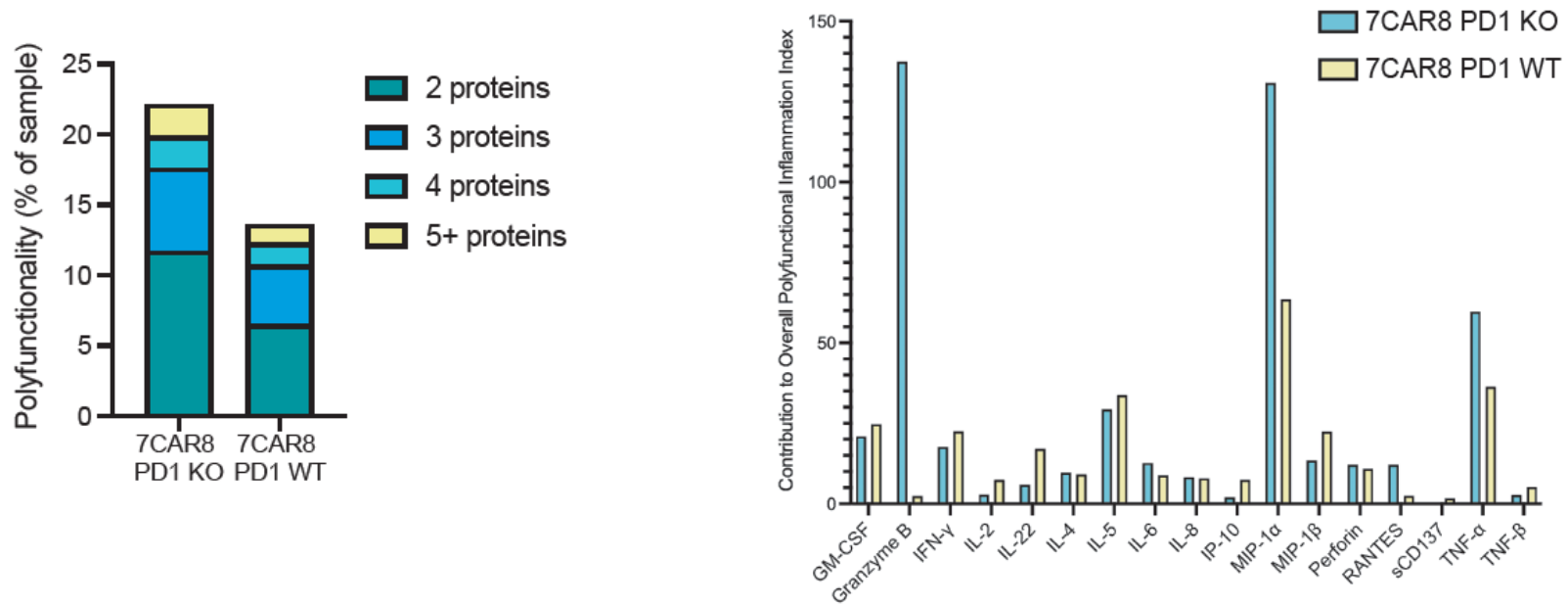
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## Cytosine Base Editing for Off-the-Shelf CAR-T



Diorio, Gehrke, et. al, Blood 2022

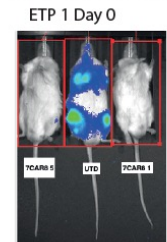
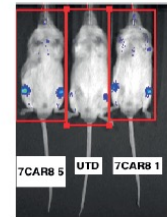
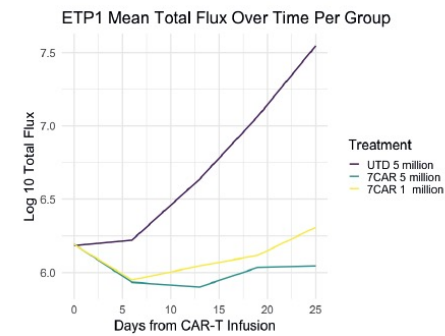
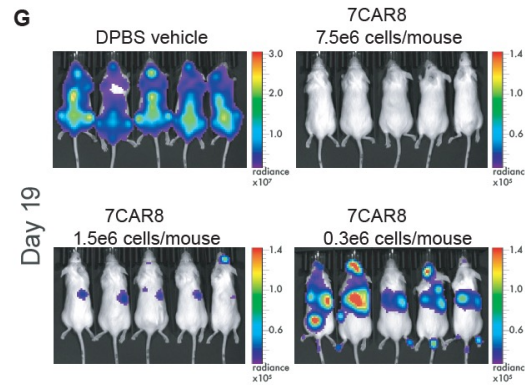
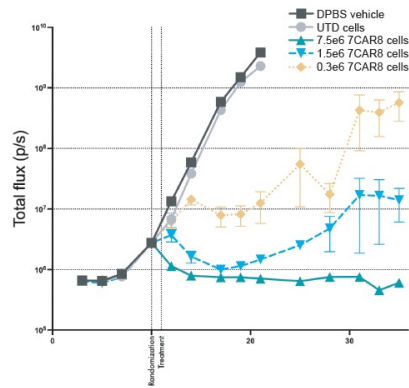
## Cytosine Base Editing for Off-the-Shelf CAR-T



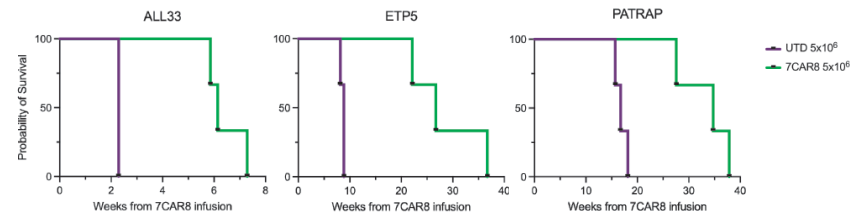
Diorio, Gehrke, et. al, Blood 2022



# Cytosine Base Editing for Off-the-Shelf CAR-T

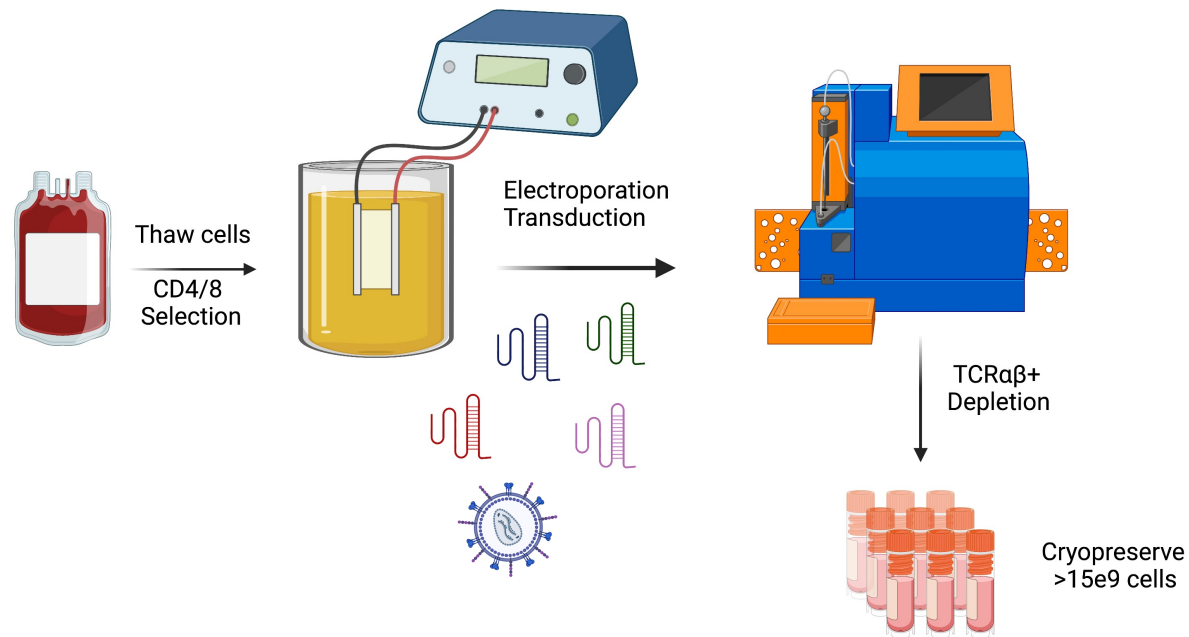


ETP 1 Day 28



Diorio, Gehrke, et. al, Blood 2022

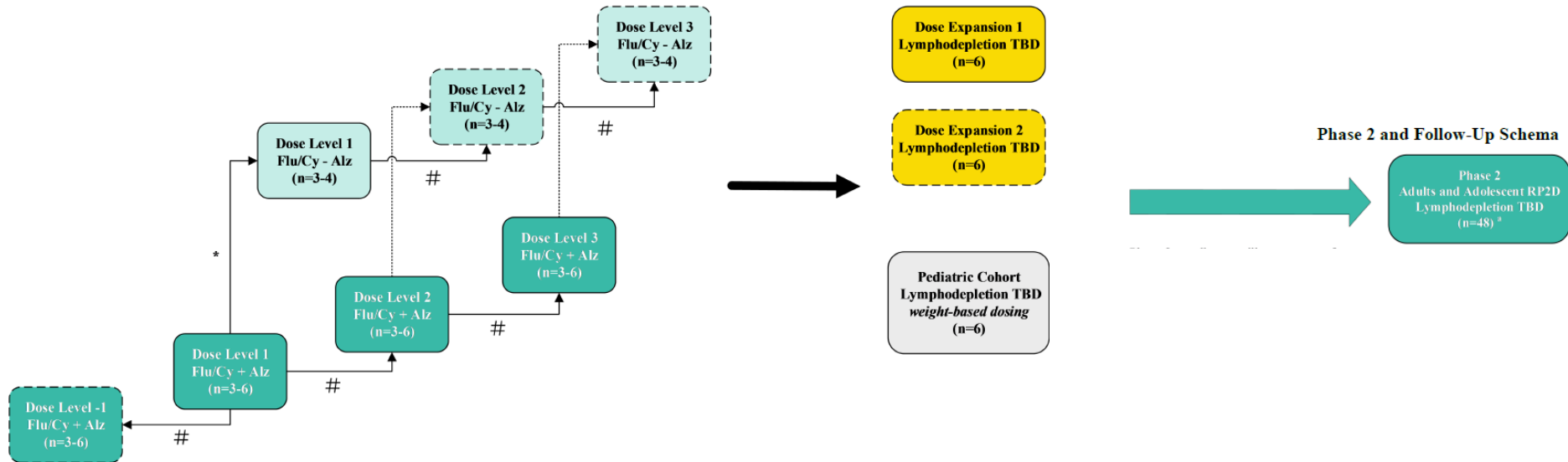
## Product Manufacturing



# Immunotherapy in Hematological Malignancies 2023

## Phase 1 / 2 Trial Open May 2023

### Dose Exploration into Dose Expansion Schema



## Conclusions

- Cytosine Base Editing (CBE) is a Powerful Tool to Make Multiple Edits with Limited Off-Target Effects
- Outcomes for R/R T-ALL are poor and biology is heterogeneous
- Difficulties with Autologous CAR-T for T-ALL
- CBE Allogenic CAR-T for T-ALL/T-LL is a Potential Strategy to Improve Outcomes

# Teachey Lab



Rawan  
Shraim



Tiffany  
Vincent



Haley  
Newman



Caroline  
Diorio



Andrew  
Hughes



Ohaila  
Mustafa



Alexander  
Li



John  
Kim



Junior  
Hall



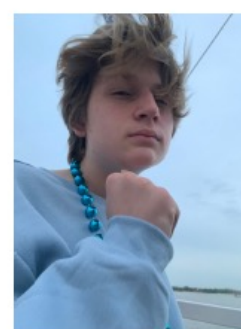
Jessica  
Lee



Tori  
Fuller



Zachary  
Martinez



Logan  
Teachey



Theresa  
Ryan



Viet  
Tran



Lahari  
Uppuluri

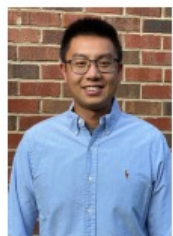
Former Lab Members Who Contributed to T-ALL/T-LL work:

Cecelia Sheen, Im (Lucy) Soo, Mahadevan Devidas, Abanoub Gad, Karen Bride, Sydney D'Ammadio, Harsha Krishnan

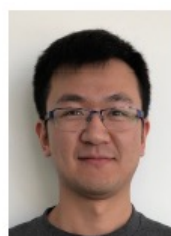
# CHOP/UPENN Leukemia/Lymphoma Collaborators



Kai Tan



Jason Xu



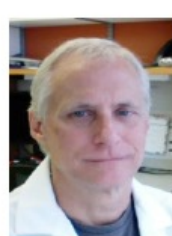
Changya Chen



Steve Grupp



Jess Perrazzeli



Ted Hofmann



Sarah Tasian



Tina Glisovec-Aplenc  
Richard Aplenc



Steve Hunger



Suzi McClory



Kathrin Bernt



Regina Myers



Mike Hogarty



Allie Leahy



Sharon Diskin



John Maris



Alix Seif



Shannon Maude



Carolyn Felix



Yimei Li



Stephan Kadauke



Carl June



Saar Gill



Warren Pear



Marco Ruella



Babak Faryabi



Martin Carroll



Kojo Johnson



Meghan Lim



Mike Milone



Ed Stautmayer



Simon Lacey

# ALL/LL Collaborators



Charles Mullighan



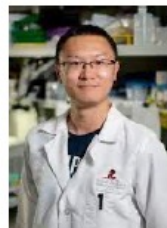
Petri Polonen



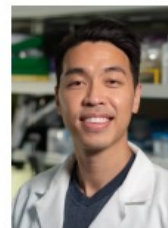
Lindsey Montefiori



Jun Yang



Jianzong Hu



Shawn Lee



Bill Evans



Mary Relling



Stan Pounds



Abdel Elsayed



Gang Wu



Mignon Loh



Bill Carroll



Elizabeth Raetz



Mini Devidas



Michelle Hermiston



Terzah Horton



Brent Wood



Steve Kornblau



Malcolm Smith



Kira Bona



Lia Gore



Ching Hon Pui



Karen Rabin



Dave Barrett



Rodney Miles



Stuart Winter



Kim Dunsmore



Bob Hayashi



Iannis Aifantis



Eric Schafer



Keith August



Maria Luisa Sulis

# ALL/LL Collaborators



Ajay  
Vora



Wendy  
Stock



Lewis  
Silverman



David  
O'Connor



Mitch  
Cairo



Adam  
Olshen



Lauren  
Meyer



Bruce  
Chen



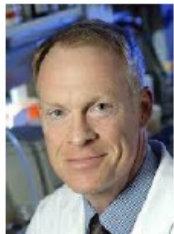
Lingyun  
Ji



John  
Kairalla



Nathan  
Gossai



Pat  
Brown



Sumit  
Gupta



Carl  
Allen



Naomi  
Winick



Mats  
Heyman



Laura  
Hogan



Michel  
Zwann



Richard  
Lock



Rachel  
Rau



Lena  
Winestone



Jen  
McNeer



Elizabeth  
MacIntyre



John  
Moppett



Steph  
Si



Maureen  
O'Brien



Jean-Pierre  
Bourquin



Andy  
Place



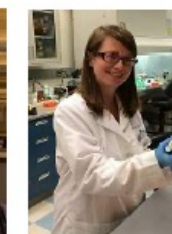
Patrick  
Zweidler-Mckay.



Gabriele  
Escherich



Mike  
Borowitz



Stacy  
Cooper



Teena  
Bhatla



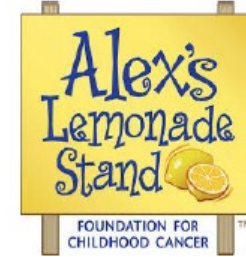


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