Allogeneic “off the shelf” IO approaches for MDS/AML
2nd Annual Cell Coast Conference

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Disclosures

I disclose the following financial relationship(s):

- Agios - Advisory Board or Consultant
- Avencell - Advisory Board or Consultant
- BlueBird Bio - Advisory Board or Consultant
- Dark Blue Therpeutics - Advisory Board or Consultant
- Servier - Advisory Board or Consultant
- Jazz - Grants/Research Support
- Novartis - Advisory Board or Consultant
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- Molecular Partners - Advisory Board or Consultant
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Challenges for novel IO CART Development in Myeloid Malignancies

- Escape Variants: Complex Clonal Architecture of AML, resulting in Heterogeneous expression profile Inter- and Intraindividually of target antigens
  - Multi-Antigen-Targeting by novel CART Designs or engineered molecular Shielding of HSCs
  - Intracellular Antigen Targets – presented in the context of defined HLA Molecules
- Small Therapeutic Window: On-Target-Off-Leukemia Toxicity; Possible Impact on CRS Occurrence, necessity for salvage allo HSCT; key timing of when to treat (? MRD)
- Antigen Sink: Ubiquitous Expression of Internalizing Target Antigens like CD33, CD123, CLL-1, e.g. relevant in the context of adapter CART
- T-cell Dysfunction: Chronic stimulation through continous antigen exposure within the (healthy) myeloid compartment
  - Healthy Donor T cells
  - Transcriptional Reprogramming of Effector Cells
- **CHAT AMT – Interplay of AML Cells, Microenvironment and T Cells**
  - Combinatorial Approaches with approved AML Drugs
  - Combinatorial Approaches with other Stimulators / Immune modulators of the Immune System

Courtesy of Subklewe M

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IMPACT-AML: International Multi-center Partnership in CAR T Cell Therapy for AML

- Workshops convened to discuss Challenges faced and discuss Solutions
- Included 21 participating Institutions for both adults and pediatrics
- Manuscript of Recommendations accepted
  - Defining minimal Reporting Standards
  - Harmonizing AML Definitions, Response and Toxicity Criteria
  - Suggesting relevant correlative Studies to maximize Outputs
  - Translational Collaborations Around Cell Expansion and Cytokine Profiling amongst others.

Led by Naik S, Velasquez P and Gill S; Manuscript accepted to *Blood Advances*

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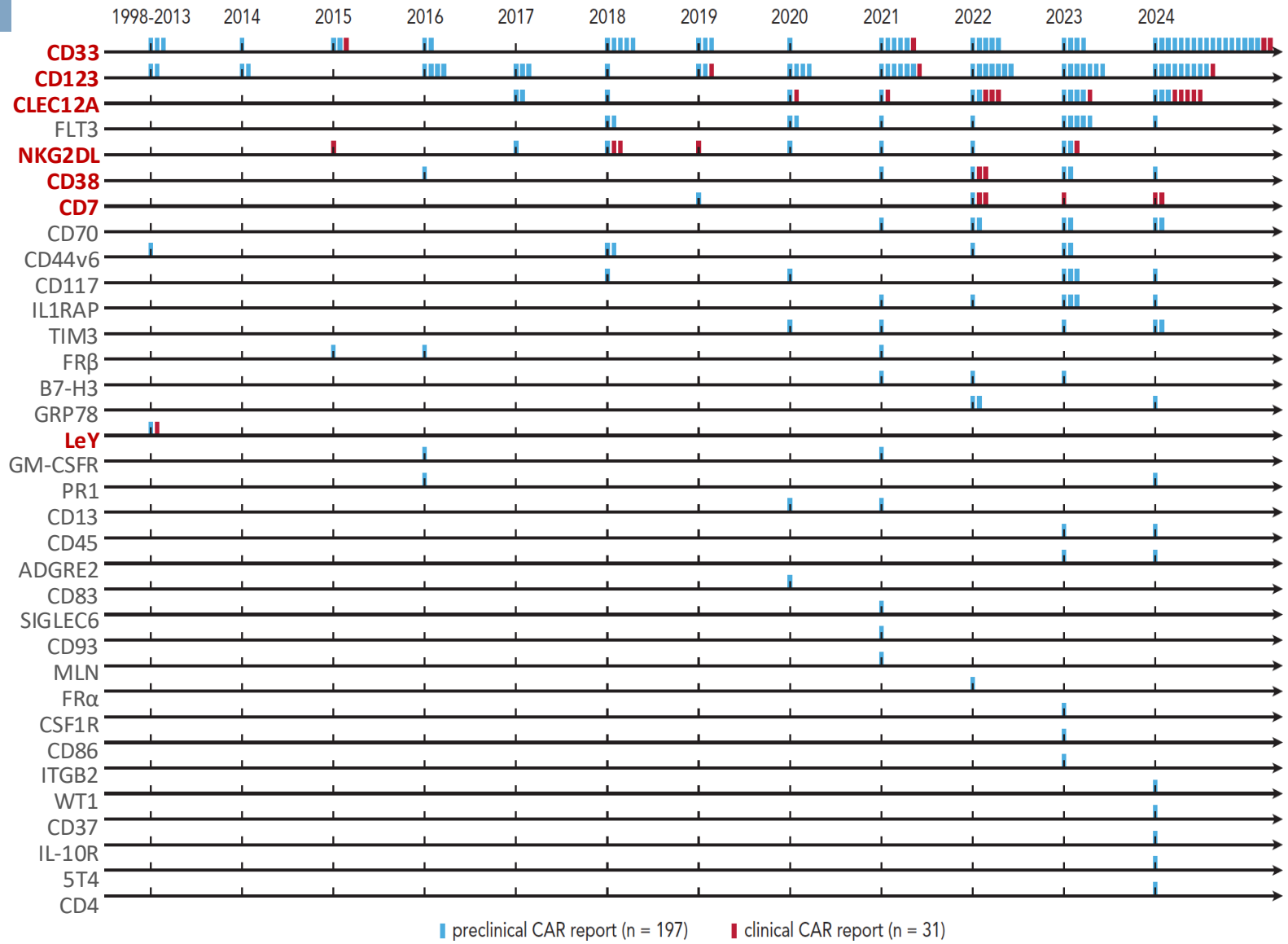
CAR Targeting in Myeloid Malignancies



- Single CAR-T Against Myeloid Targets (CD33, CD123, CLEC12A/CLL-1) has been underwhelming based on durability of responses
- Challenges of T-cell function with autologous products.
- ? Bridge to allo-SCT

Haubner, Subklewe, Sadelain, *Blood* 2025

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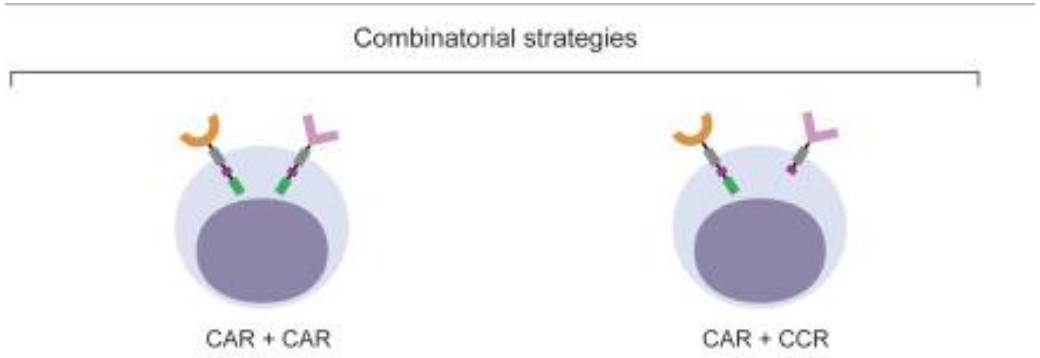
Combinatorial CAR Strategies

- For example, CD33+CD70 stained >97% of cells in AML samples, while stained <5% of normal HSCs and T cells
- Combinatorial CAR: co-expression of two CARs (CAR + CAR)
  - T cells eliminate any cells expressing at least one of the two targets, thereby reducing the chance of antigen escape.
- Co-expression of a CAR and a chimeric costimulatory receptor (CAR + CCR)
  - T cells only eliminate cells that co-express both targets, thereby limiting cytotoxicity to double-positive tumor cells and relatively sparing single-positive normal tissue



Perna F et al., 2017 *Cancer Cell*

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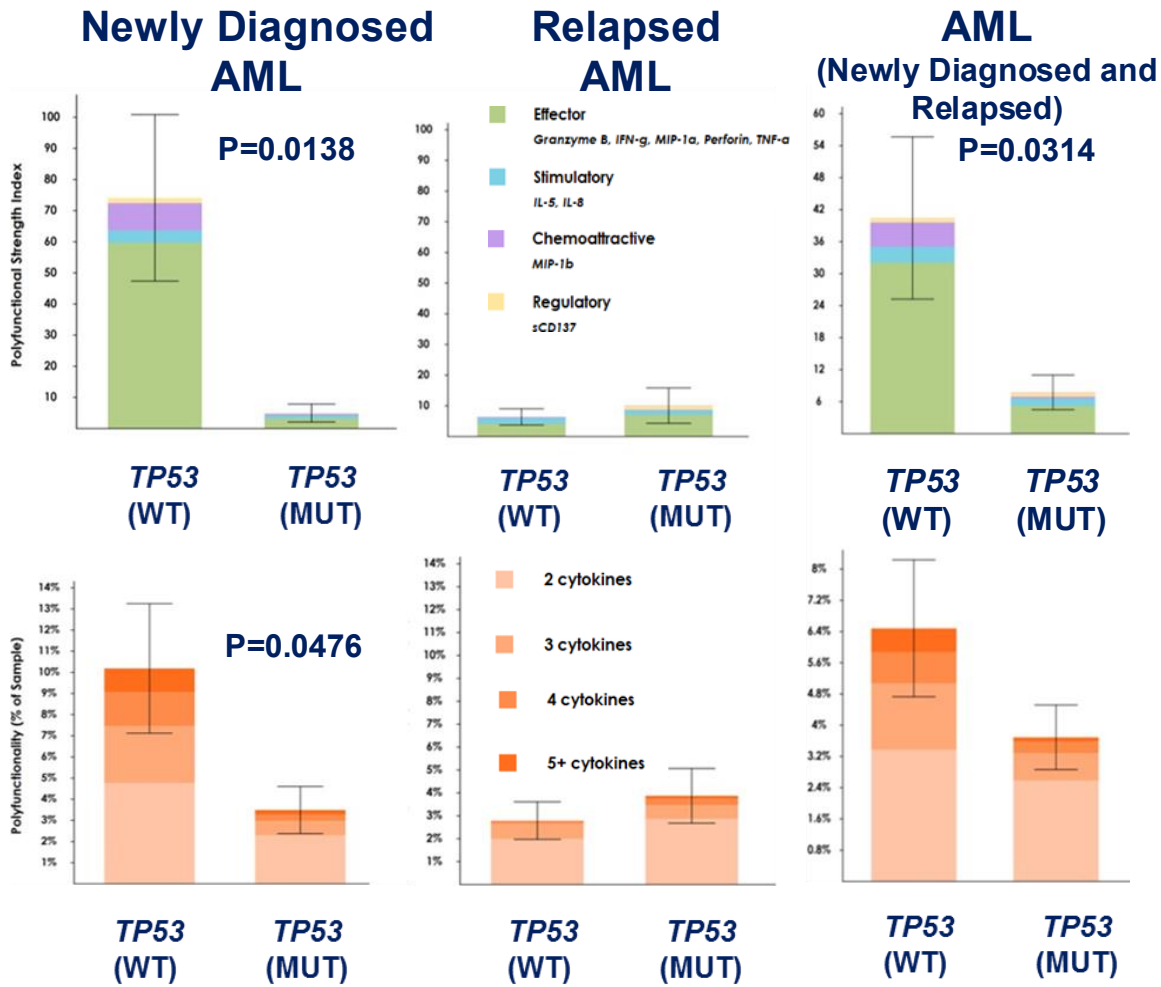
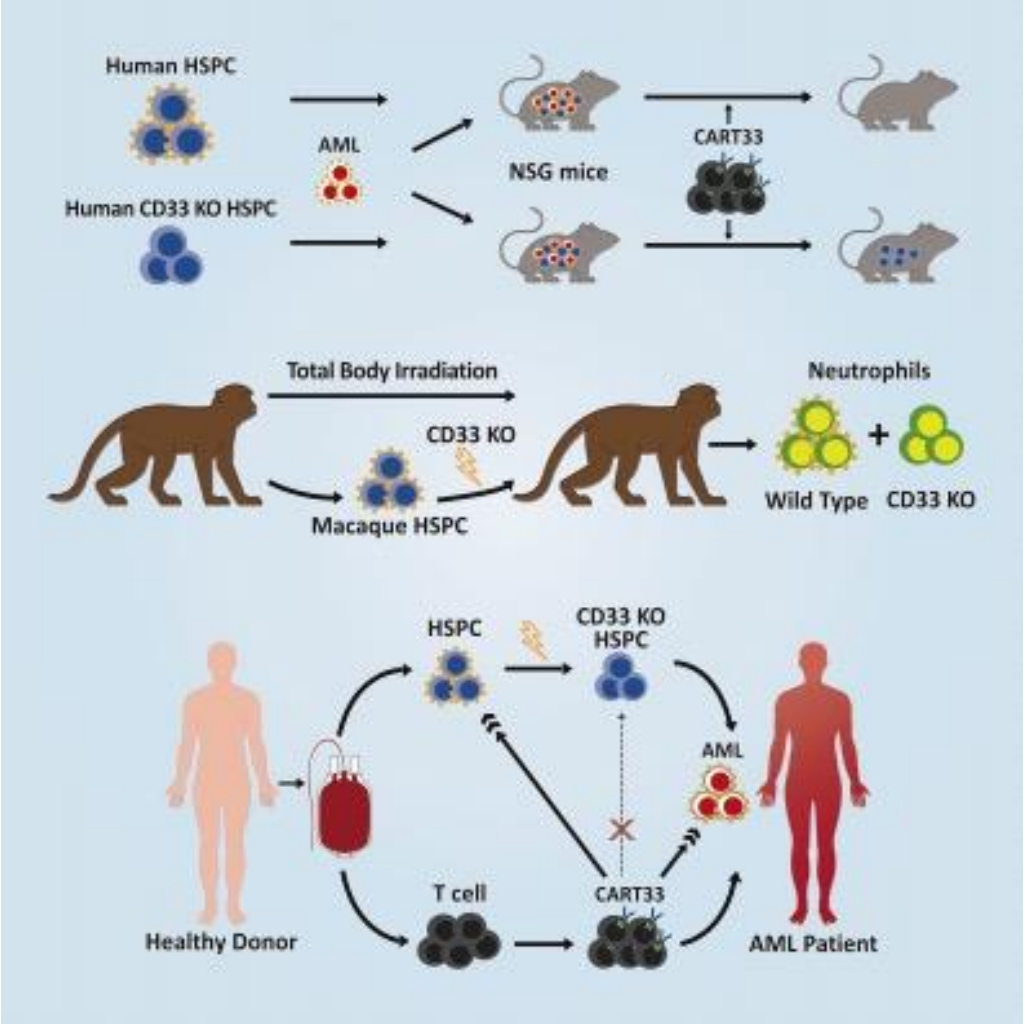
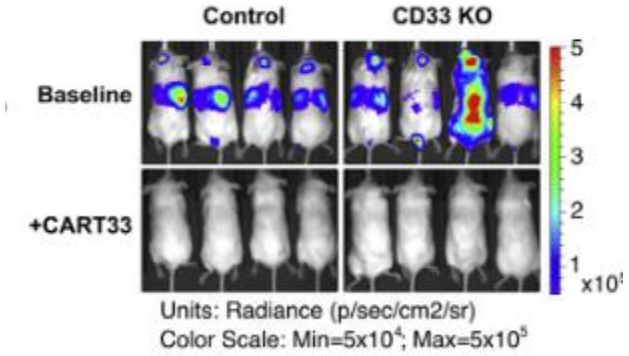
TP53 mutant patients have significant defect in polyfunctionality vs wild-type patients at diagnosis



Abbas H.…Daver, N. et al., *Blood Advances* 2021

CD33 KO in Stem Cell Product – Leukemia Specific Antigen





CD33-CLL1 Multiplex Deletion similar Data *in vitro*

Kim & Yu | Cell 2018; Xavier-Ferrucio J et al., EHA 2022

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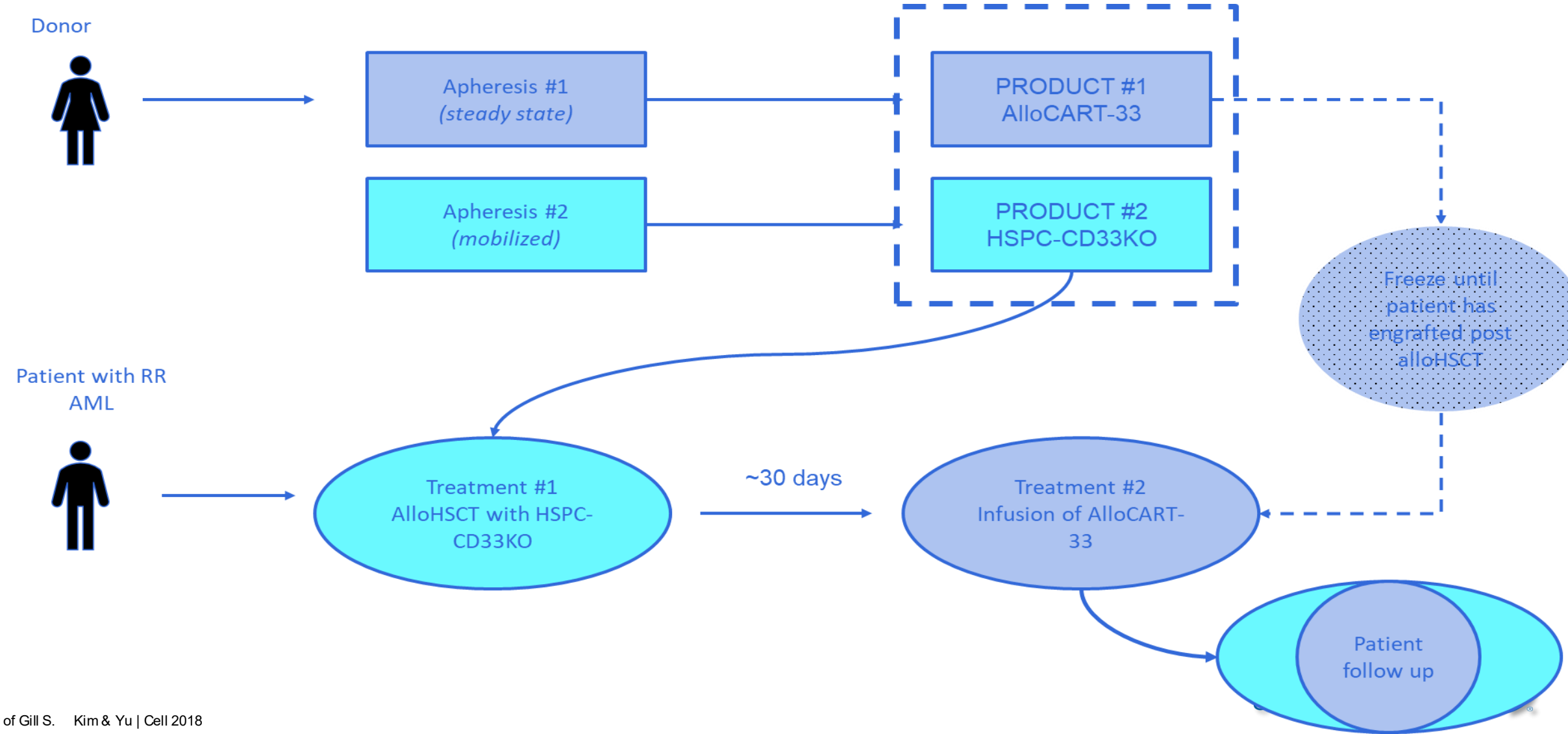
CD33 KO in Stem Cell Product – Leukemia Specific Antigen



Courtesy of Gill S. Kim & Yu | Cell 2018

Tremcell (CD33KO stem cell product) with Successful Engraftment

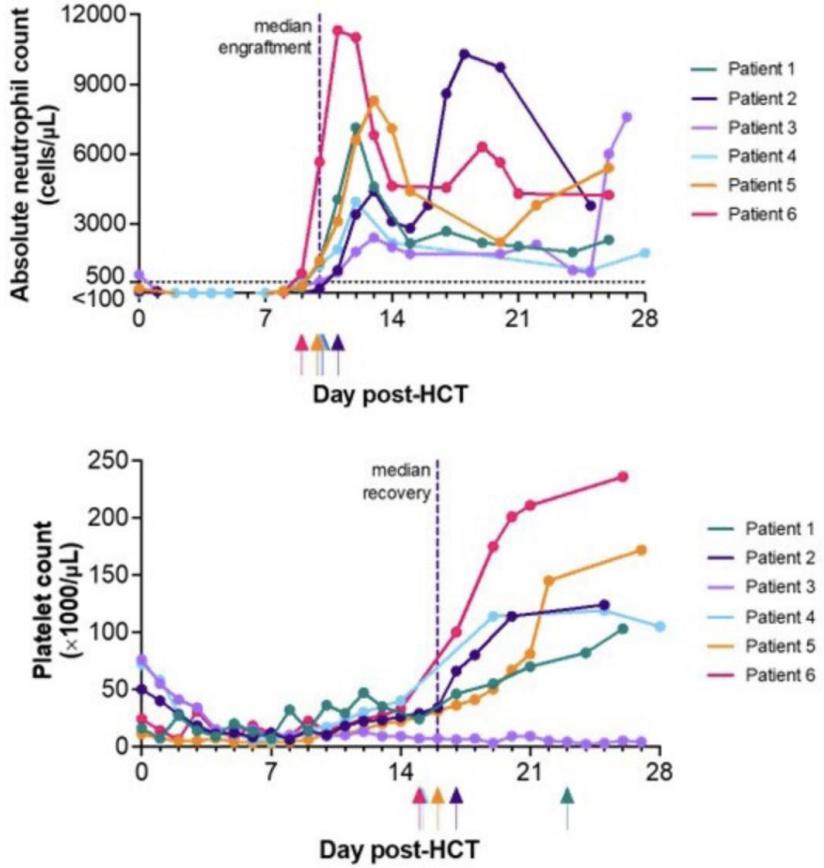


Figure 1. Kinetics of neutrophil engraftment and platelet recovery (n=6). Arrows denote time of individual patient neutrophil engraftment and platelet recovery.

DiPersio J et al., ASH 2024 (update EHA 2025); company has closed study

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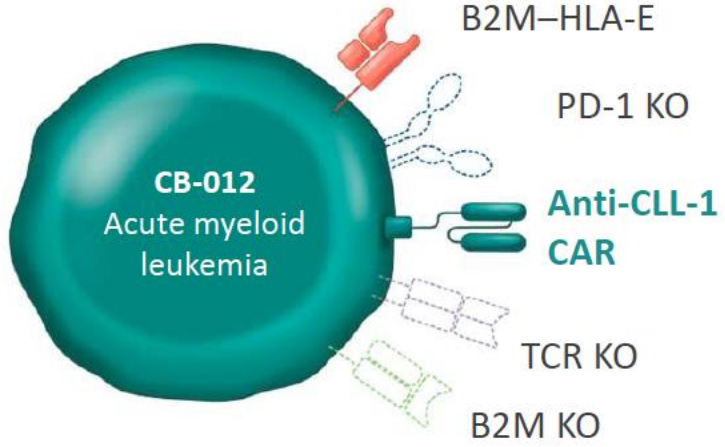
CB-012 CLL1 Allo CART for r/r AML

Key attributes	CB-012	Other allogenic CAR-Ts for AML
Cas12a chRDNA editing for enhanced genomic integrity • Reduced off-target editing and enhanced insertion rates	✓	✗
1 ***TRAC* gene knockout (KO)** • Eliminates TCR expression, reduces GvHD risk	✓	Varies
2 **Human anti-CLL-1 CAR site-specifically inserted into *TRAC* gene** • Eliminates random integration, targets tumor antigen	✓	Varies
3 ***B2M* gene KO** • Reduces HLA class I presentation and T cell-mediated rejection	✓	✗
4 **B2M–HLA-E-peptide fusion site-specifically inserted into *B2M* gene** • Blunts NK cell-mediated rejection	✓	✗
5 **PD-1 KO for enhanced antitumor activity** Potentially better therapeutic index via initial tumor debulking	✓	✗

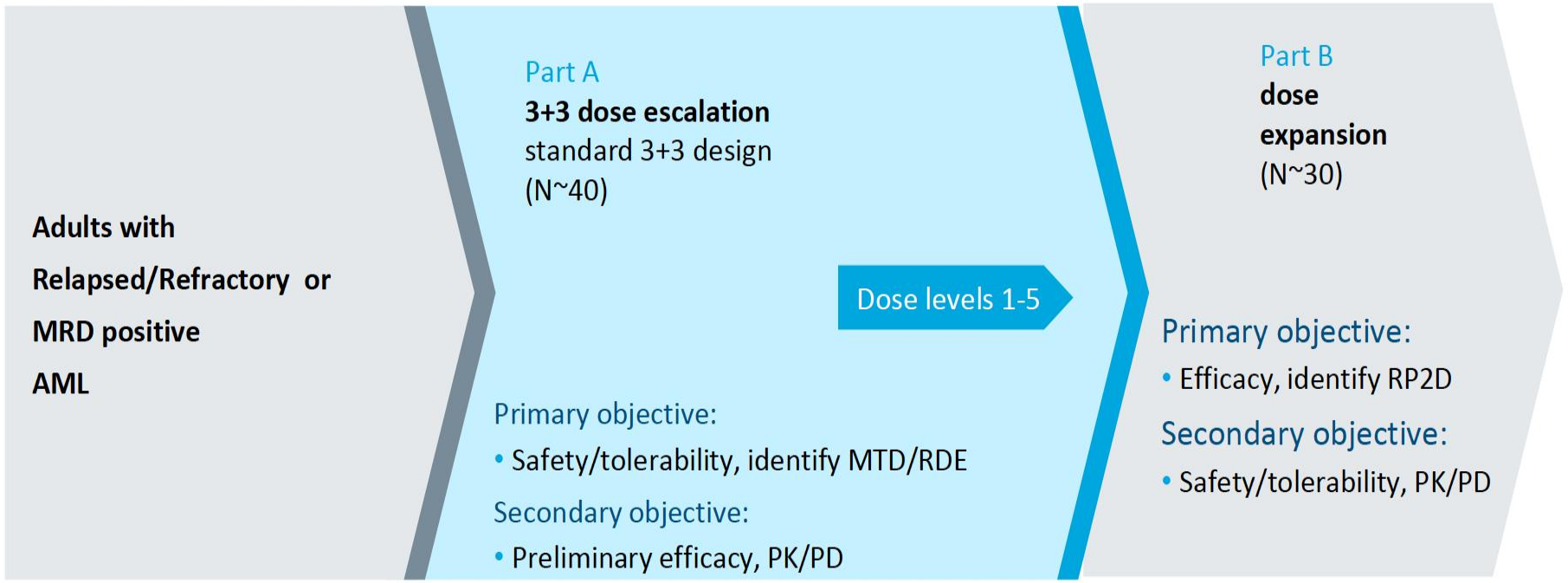
CB-012 uses a potent, fully human anti-CLL-1 scFv[1] with a CD28 costimulatory domain

5 Edits



1st allogeneic CAR-T cell therapy with both **checkpoint disruption** and **immune cloaking**

CB-012 r/r AML Study Design



Lymphodepletion (LD):

- Fludarabine 30 mg/m^2/d and cyclophosphamide 750 mg/m^2/d for 3 consecutive days (Days -5 to -3)

Each patient receives a single flat-fixed dose:

- Dose level 1 (Cohort 1): 25 × 10^6 viable CAR$^+$ cells
- Dose level 2 (Cohort 2): 75 × 10^6 viable CAR$^+$ cells
- Dose level 3 (Cohort 3): 150 × 10^6 viable CAR$^+$ cells
- Dose level 4 (Cohort 4): 300 × 10^6 viable CAR$^+$ cells
- Dose level 5 (Cohort 5): 400 × 10^6 viable CAR$^+$ cells

SPONSOR CLOSED STUDY BASED ON PRIORITIES

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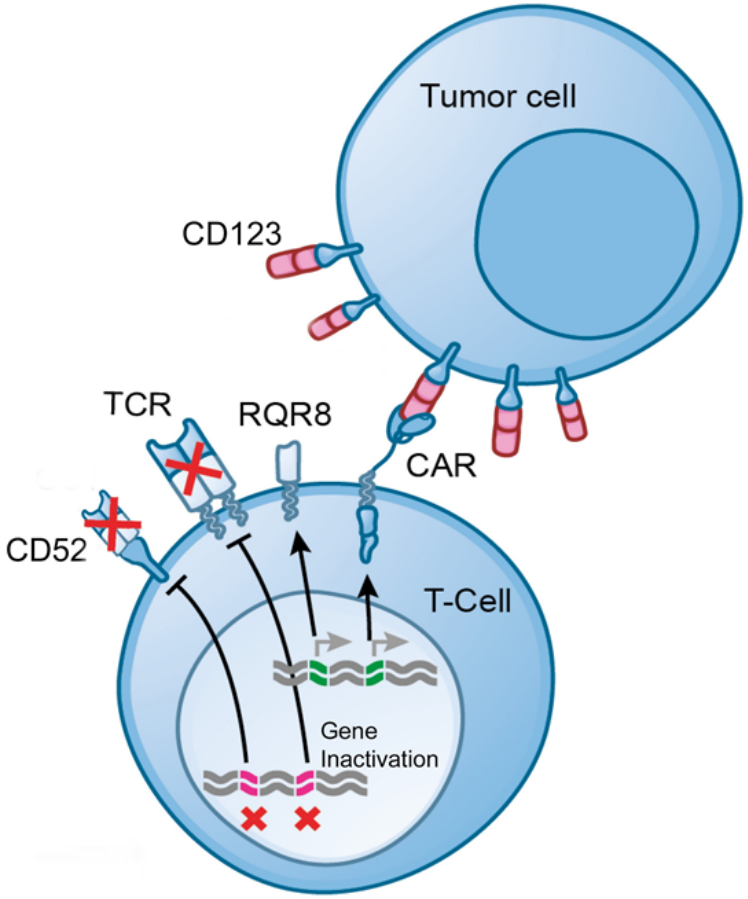
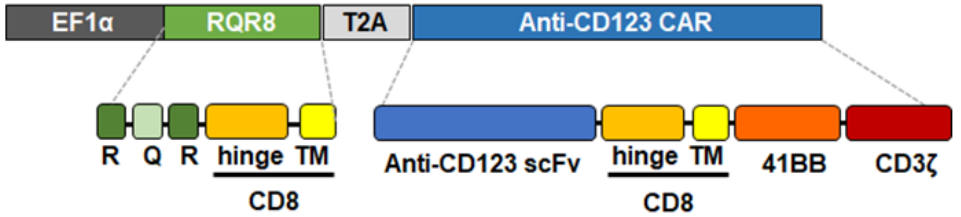
UCART123: Allogeneic "off-the-shelf" T cell product





R=CD20 mimetope (rituximab)
Q= CD34 epitope (Qben10)

UCART123:

- ✓ Second-generation CAR targeting CD123
- ✓ Mouse-derived scFv
- ✓ Derived from healthy donor T cells
- ✓ Reduces risk of GvHD (TCR K/O and TCRαβ-purification)
- ✓ CD20 mimotope for rituximab "safety switch"
- ✓ Alemtuzumab resistance (CD52 K/O)
- ✓ Available "off the shelf"
- ✓ Manufactured at large scale

CAR, chimeric antigen receptor; GvHD, graft-versus-host disease; K/O, knock-out; scFv, single-chain variable antibody fragment; TCR, T-cell receptor.

Roboz G, DeAngelo D, Sallman D et al., ASH 2020

UCART123v1.2 - Serious TEAEs (All Cause – FC + FCA)

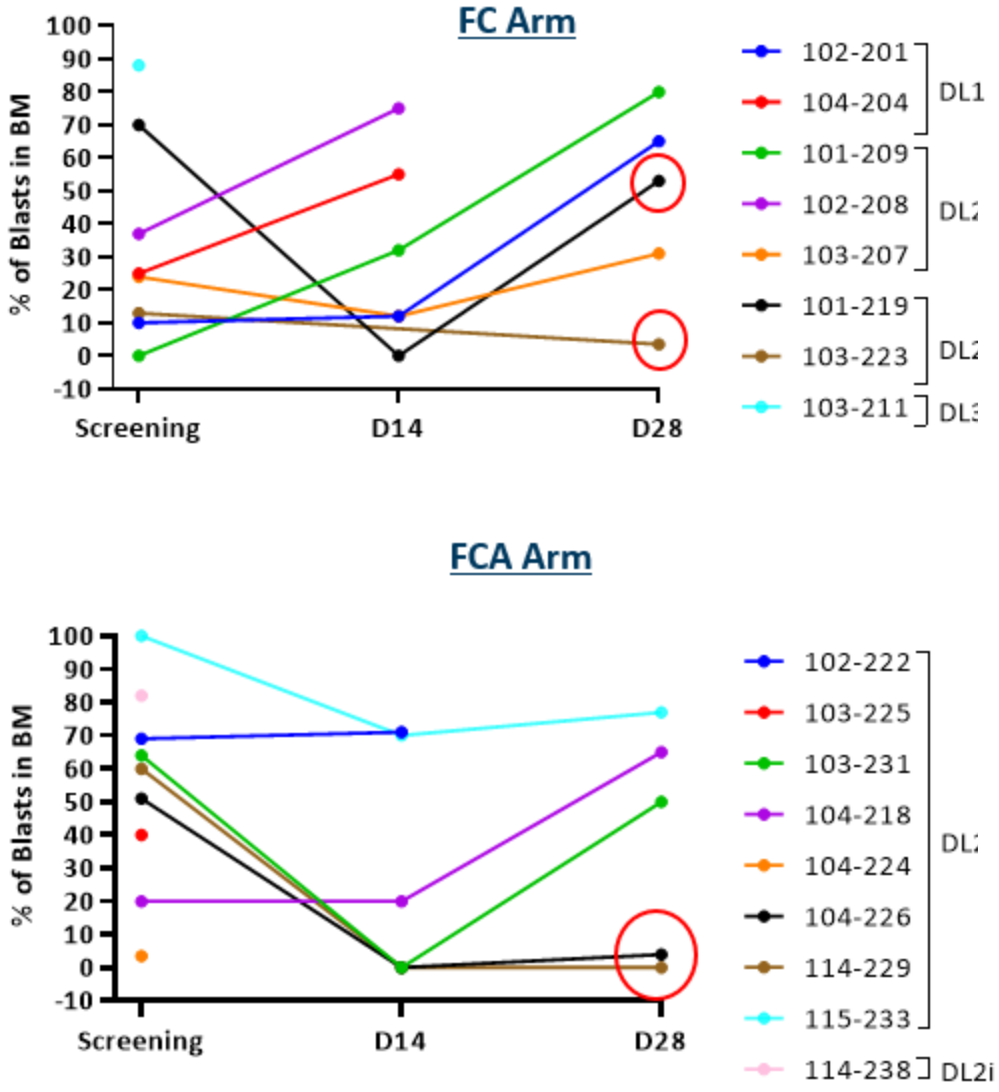
Serious TEAE, n (%)	FC		FCA		FC + FCA	
	FC Total [n=8] DL1=2; DL2=3; DL2i=2; DL3=1		FCA Total [n=9] DL2=8; DL2i=1		Total patients N=17*	
	Any grade	Gr ≥3	Any grade	Gr ≥3	Any grade	Gr ≥3
CRS	3	2	2	2 ✿	**5**	**4**
ICANS	1	1	0	0	**1**	**1**
Pneumonia	1	1	1	1	**2**	**2**
Pneumonia fungal	2	2	0	0	**2**	**2**
Febrile neutropenia	0	0	1	1	**1**	**1**
Fungemia	0	0	1	1	**1**	**1**
Hemorrhage intracranial	0	0	1	1	**1**	**1**
Large intestinal hemorrhage	1	1	0	0	**1**	**1**
Pericardial effusion	1	1	0	0	**1**	**1**
Septic shock	1	1	0	0	**1**	**1**

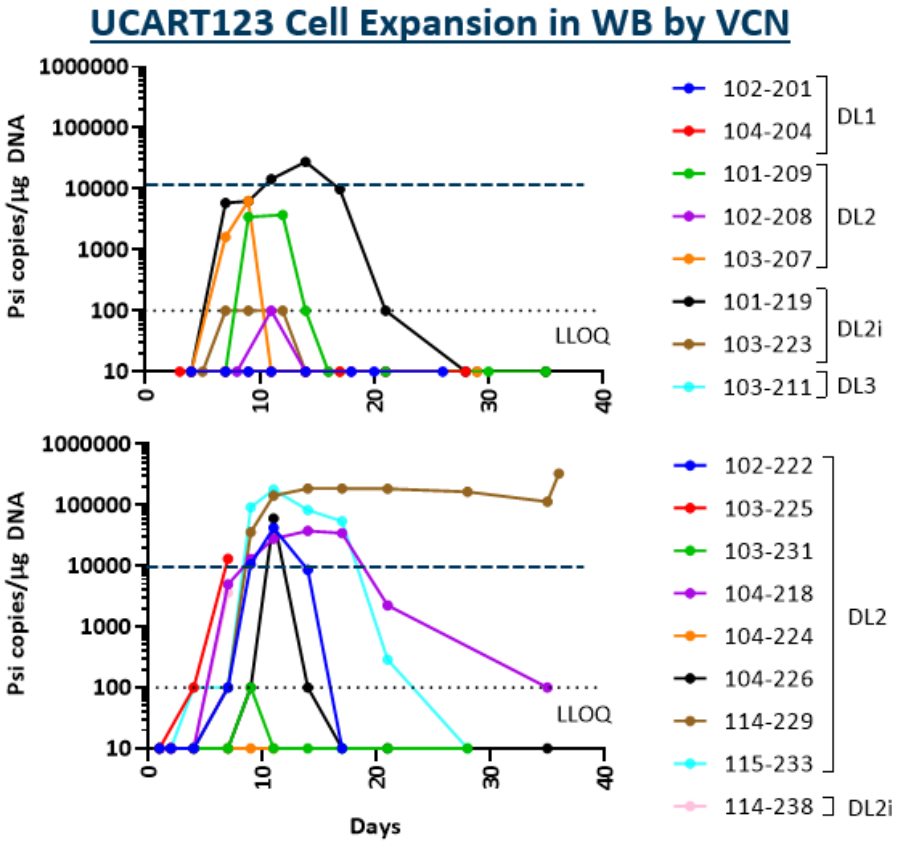
DL, dose level; FC, fludarabine + cyclophosphamide; FCA, FC + alemtuzumab; TEAE, treatment-emergent adverse event; CRS, cytokine release syndrome; ICANS, immune effector cell associated neurotoxicity syndrome

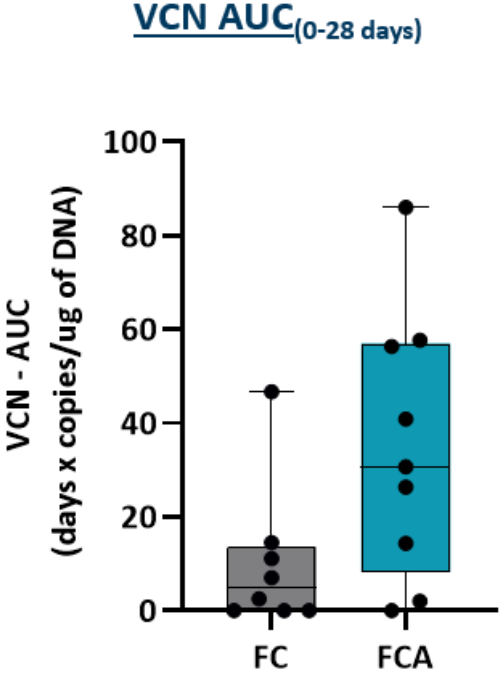
* As of Oct. 10, 2022, 18 patients received LD, 17 received UCART123v1.2
✿ 2 Grade 5 events related to CRS

Sallman D et al., ASH 2022

Efficacy and Kinetics







Sallman D et al., ASH 2022

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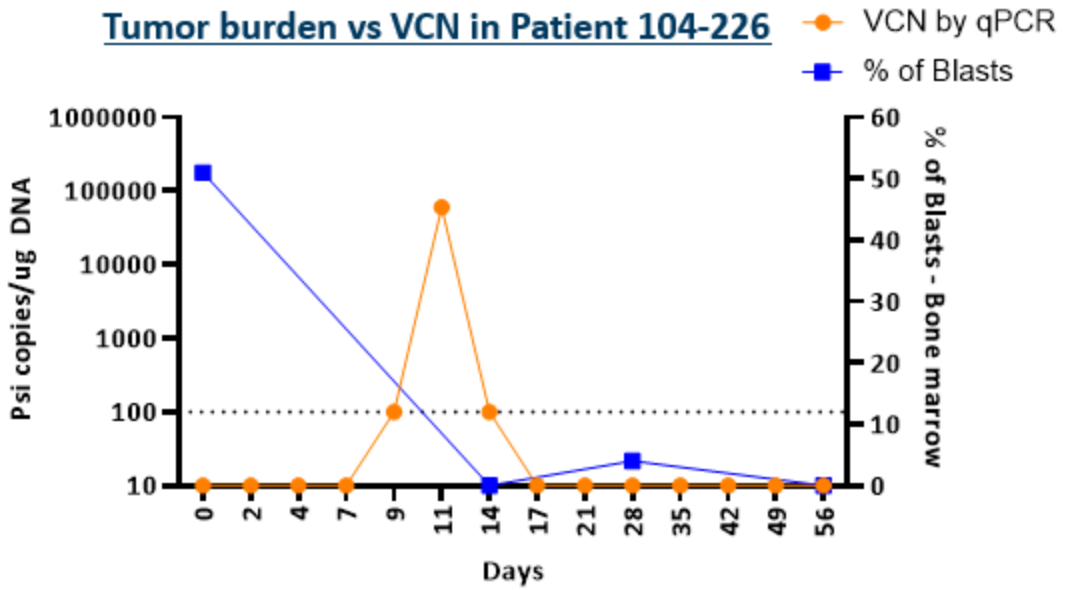
Patient Achieved a Durable MRD Negative CR without DLI/2nd allo

Clinical Characteristics	
Age, Race, Sex	64 year old white female
ECOG	1
ELN 2017 Classification; WHO Classification	Adverse risk; AML with myelodysplasia-related changes
Cytogenetic and Molecular Abnormalities	45,XX,-7,t(10;12)(q24;p13)[5]; IDH1, EZH2
Number of prior treatments	5 - including allogeneic HSCT 2016
Past Medical History	MDS, 2011; Focal nodular hyperplasia of the liver, 2016

Response Summary	BM Biopsy Blast %	BM Aspirate Blast %	MRD	ELN Response
Screening Day -14	51%	Not done		
Day 14	0%	Not done		
Day 28	3.8%	4%	Pos 0.6%	CRi
Day 56	2.8%	0%	Neg	CR
Day 84	0%	0%	Neg	CR
FU 1, Day 181	2%	0%	Neg	CR
FU 2, Day 270	1%	0%	Neg	CR
FU 3, Day 365	0%	0%	Neg	CR



MDS myelodysplastic syndrome; HSCT Hemopoietic stem cell transplant; MRD minimal residual disease; CRi compete response with incomplete hematologic recovery; CR complete response

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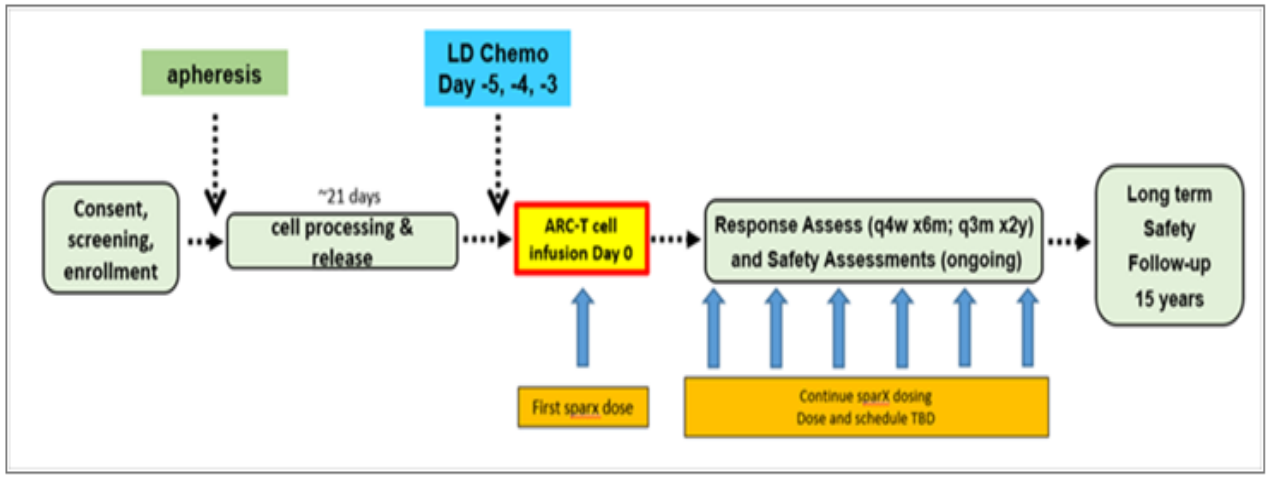
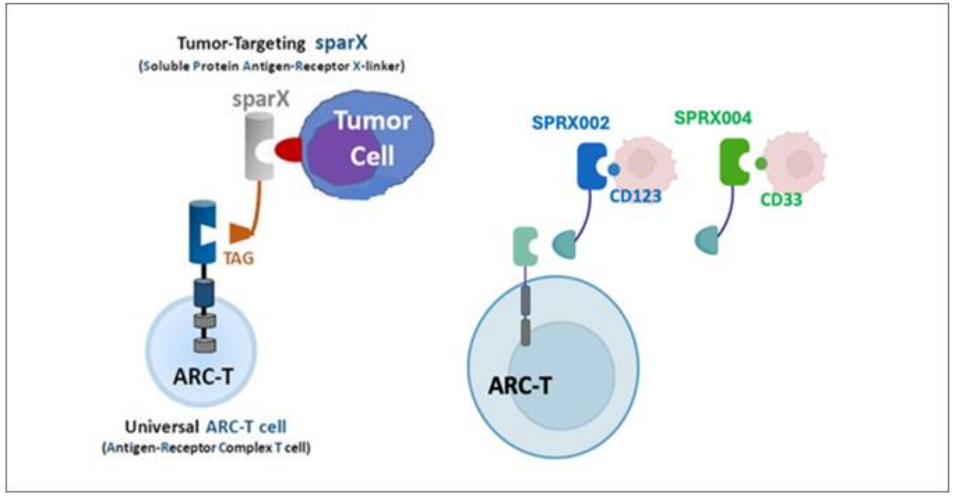
Sallman D et al., ASH 2022

ARC-221 Study Design (Auto-Universal CAR Platform)

FIH study to characterize the safety and clinical activity of **ACLX-004** in RR AML or HR MDS

- Dual Targets: **CD123 (SPRX002) and CD33 (SPRX004)**. No required minimum level of CD123 or CD33 expression.





- **AML Cohorts:**
  - **Cohort #1** relapsed disease with **higher-disease burden** (bone marrow blasts ≥30%) **or refractory disease**
  - **Cohort #2** relapsed disease with **lower-disease burden** (bone marrow blasts ≥5% and <30%) or persistent **MRD positivity** following hematopoietic stem cell transplant (or following any treatment for those with adverse risk genetic abnormalities)
- **MDS Cohort:** A diagnosis of MDS and ≥5% bone marrow blasts with indication of high-risk disease defined as those having resistant or refractory disease to at least one course of therapy

Trial to activate end of 2025.

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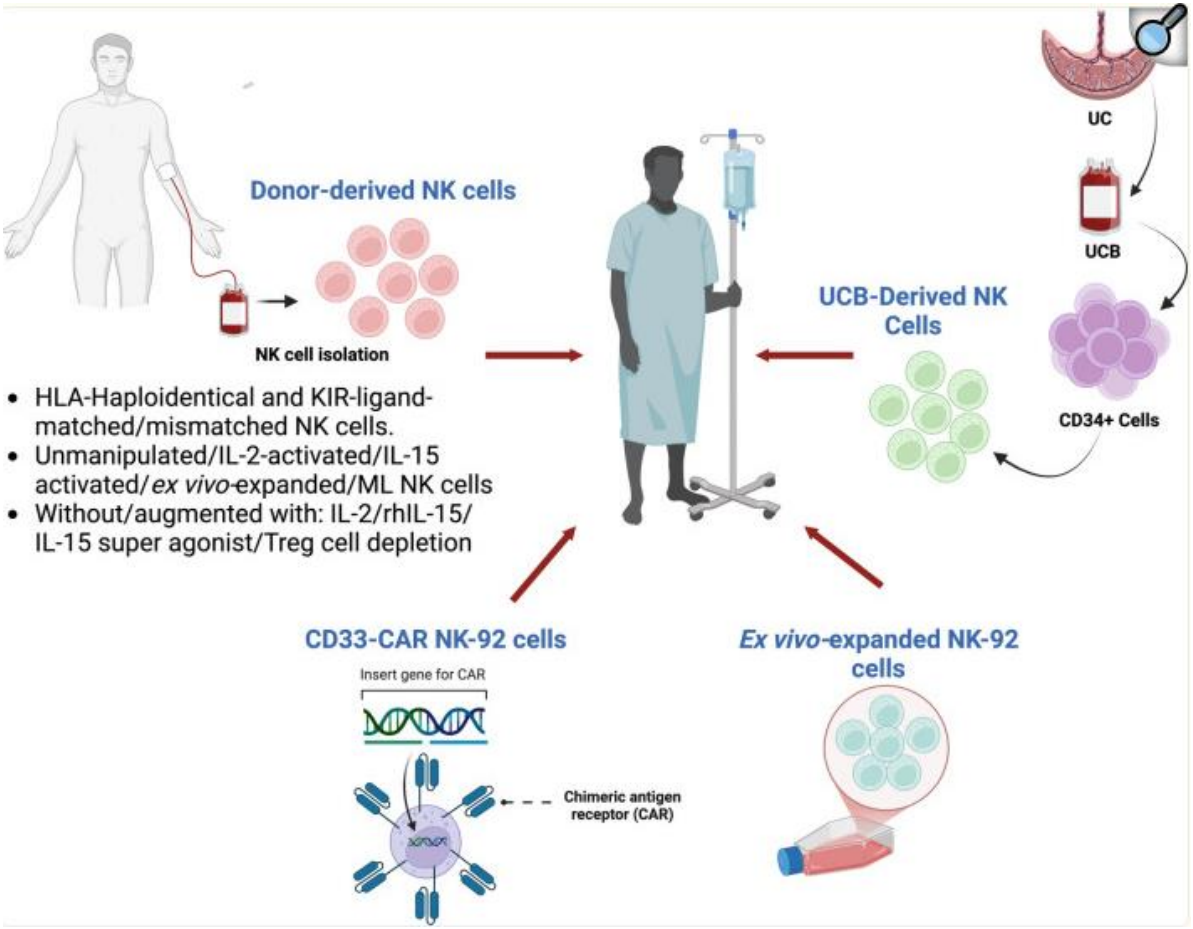
NK Cell Therapy Data

- ~48 AML trials conducted to date, overall well tolerated with low grade CRS toxicity and some level of efficacy across study.
- Some activity of FT516 and FT538 (iPSC NK) with early cohorts although appears not being developed currently per corporate press release
- NKARTA (CAR-NK) response in low blast patients, 22% CR/Cri (4/18, 1 new response reported at update 2023). Short duration outside of transplant
- Several additional companies with phase 1 studies
- Potential augmentation of NK cell therapies by targeting mitochondrial readiness (Pan R et al., Cell 2022) as well as cytokine support approaches



Bakhtiyaridoovvombaygi et al., BMC Cancer 2025; Rady M et al., Clin Exp med 2025.

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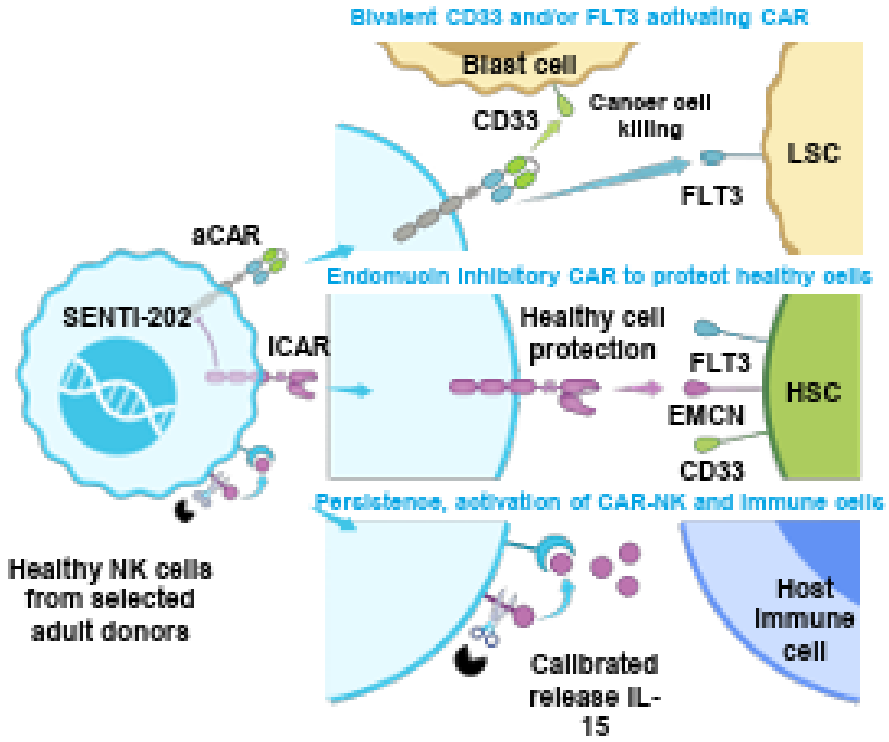
SENTI-202 – Off the Shelf CD33 or FLT3 CAR NK with enhanced safety and efficacy modifications



SENTI-202 Gene Circuit Design

- **OR Logic Gate "Kills"** leukemia blasts and LSCs via CD33 OR FLT3 activating CAR (aCAR)
  - CD33 and/or FLT3 expressed in ~95% of AML patients with CD33 being predominantly expressed on bulk blasts and FLT3 on LSCs
- **NOT Logic Gate "Protects"** healthy HSC/HSPCs from 'off-tumor, on-target' effects
  - Protection of HSC/HSPCs via NOT Endomucin (EMCN) inhibitory CAR (iCAR) even when they express CD33 and/or FLT3
  - EMCN found predominantly on healthy HSC/HSPC surface, rarely on AML blasts
- **Calibrated release IL-15 "Enhances"** SENTI-202 and host immune cell activity and persistence

Strickland S et al., AACR 2025

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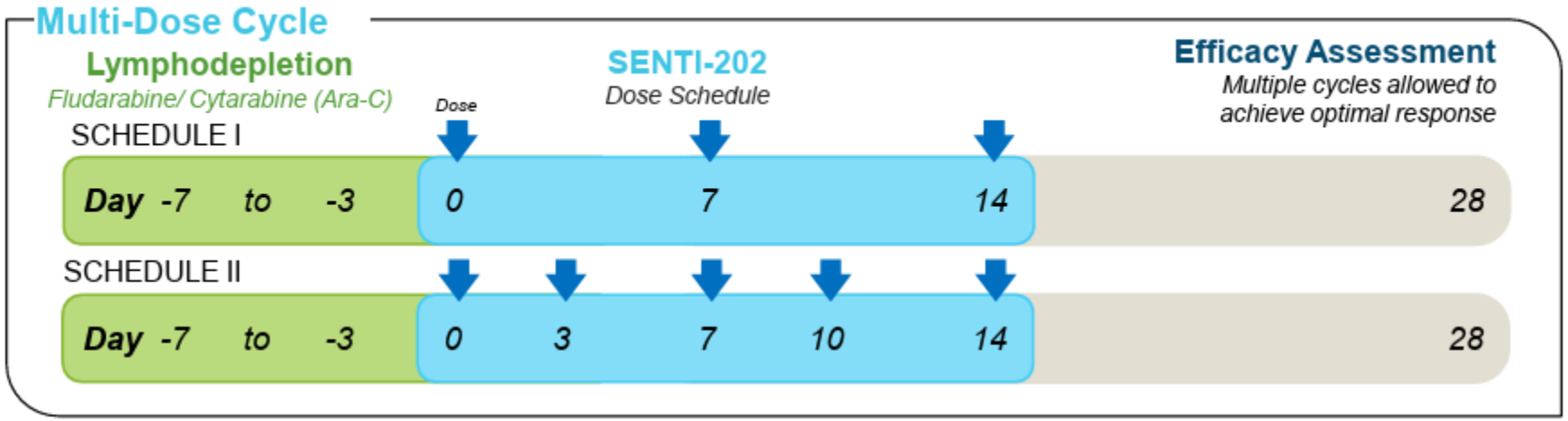
SENTI-202 – Dosing Schema

SENTI-202 Dose Levels

Dose Level	CAR+ NK Cells/Dose
1	1 x 10^9
2	1.5 x 10^9



Opening Dose Cohort was Anticipated to be Biologically Active
Schedule I Dose Level 1, N = 3
No DLTs

Schedule I Dose Level 2, N = 3
No DLTs
Identified as preliminary RP2D based on totality of clinical data

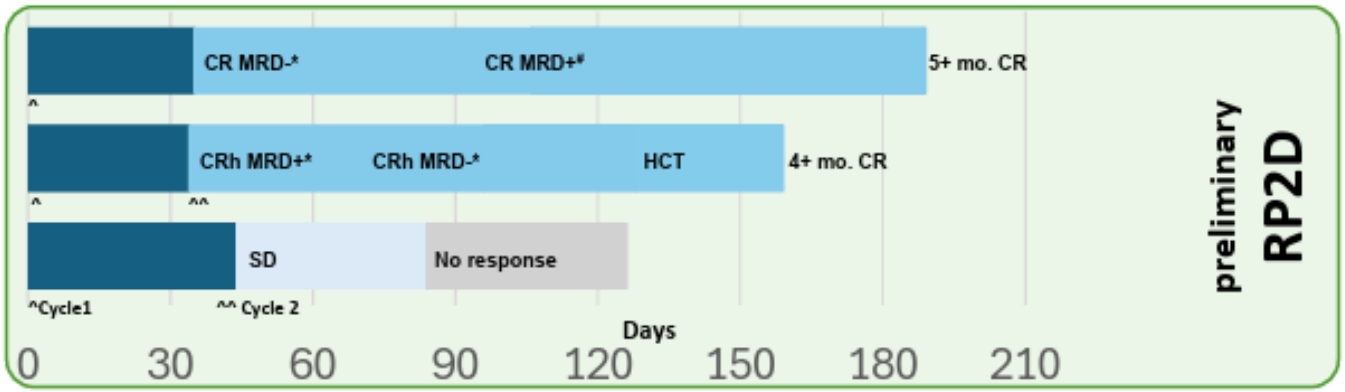
Schedule II Dose Level 1, N = 3
No DLTs

Strickland S et al., AACR 2025

SENTI-202 – Promising Early Efficacy

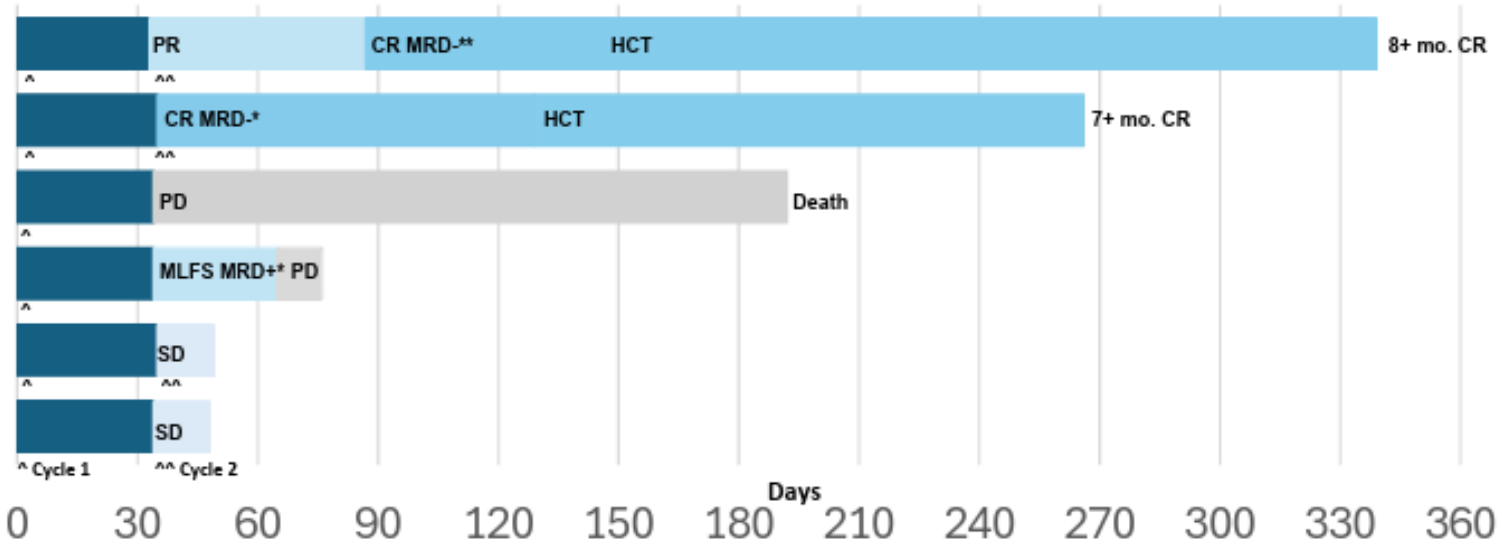
Pt	I⁰ Ref	Adv. Risk	FA Exp	FA Ref
Pt4	Yes	Yes	Yes-both	Yes-both
Pt5	No	Yes	Yes-both	No
Pt6	Yes	Yes	Yes	Yes

Pt	I⁰ Ref	Adv. Risk	FA Exp	FA Ref
Pt1	No	Yes	Yes	No
Pt2	No	No	Yes	No
Pt3	Yes	Yes	Yes	Yes
Pt7	Yes	No	Yes-both	Yes-both
Pt8	Yes	Yes	Yes	Yes
Pt9	Unk	Yes	Yes-both	Unk



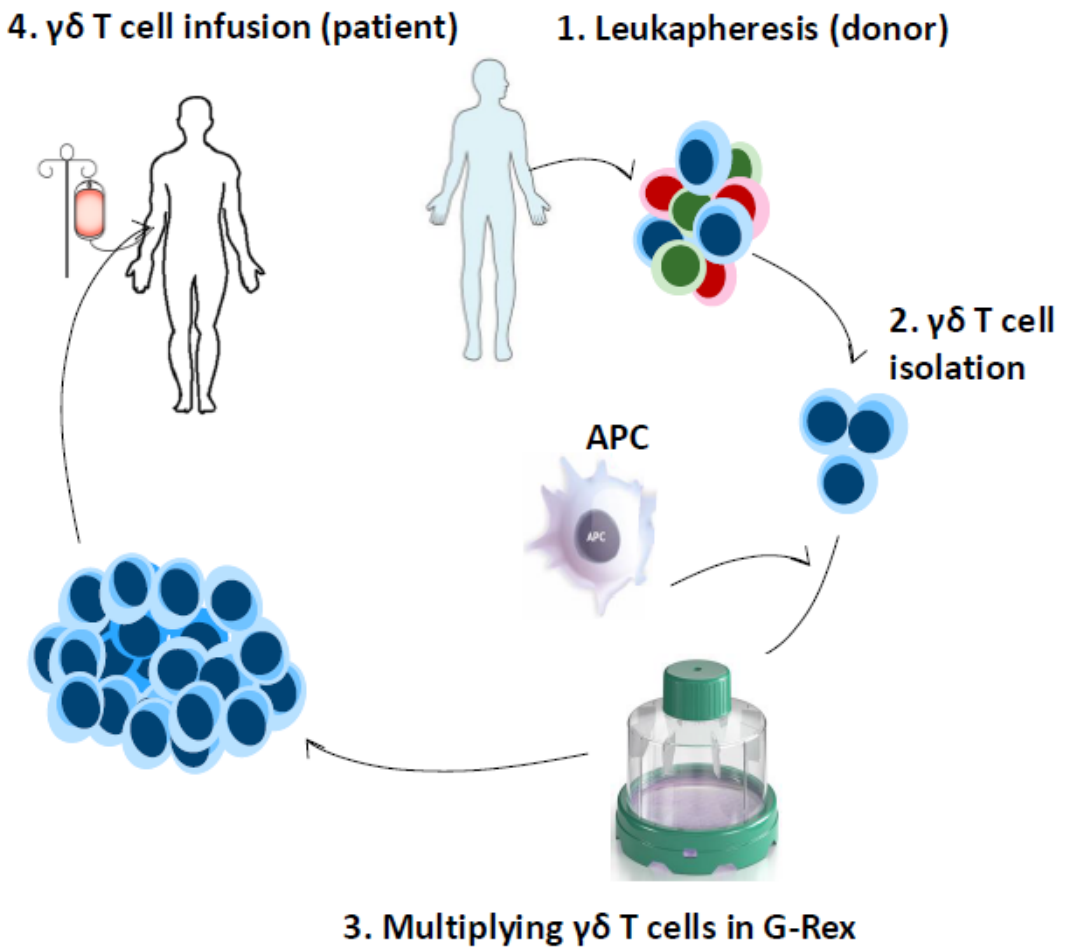
*MRD by multi-parametric flow (sensitivity ≤ $1/10^{-4}$),

**MRD by NGS (sensitivity ≤ $1/10^{-2}$)

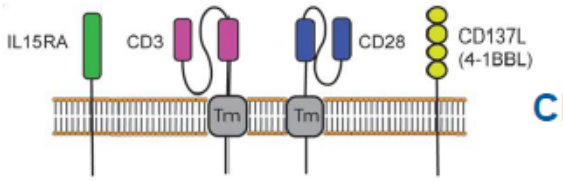


Strickland S et al., AACR 2025

γδ T Cell Isolation and *Ex Vivo* Expansion with APCs

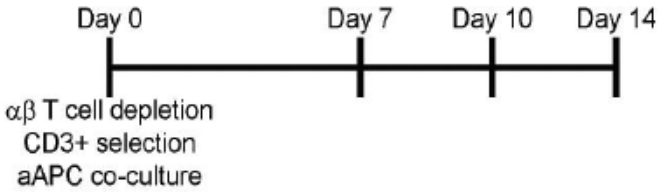


Boucher et al. J Immunother 2022

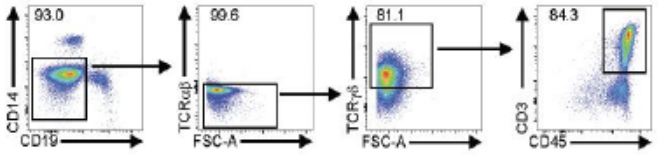


Schema of K-562 CD3scFv/CD137L/CD28scFv/IL15RA aAPC

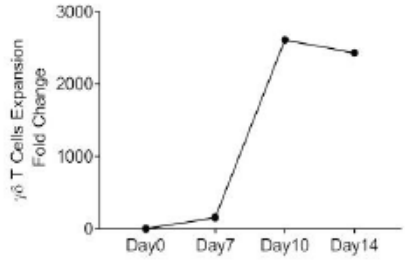
A Experimental timeline.



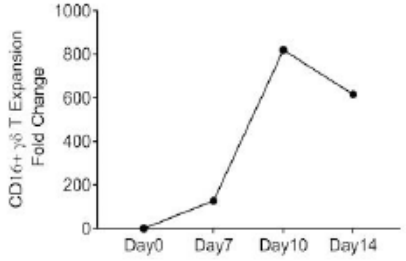
B Gating strategy for γδ T cells



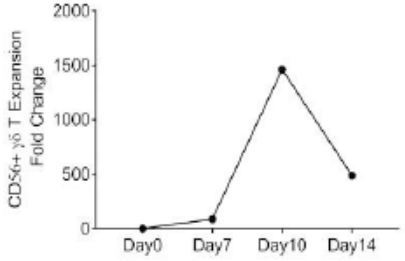
C γδ T fold expansion



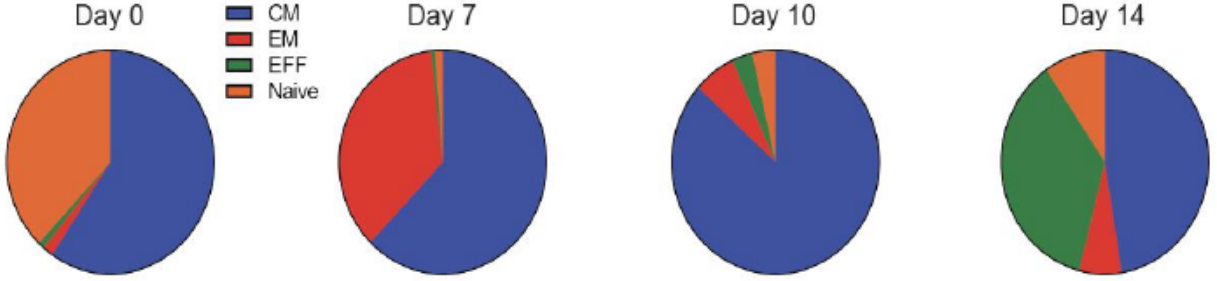
D CD16+ γδ T cell count



E CD56+ γδ T cell count

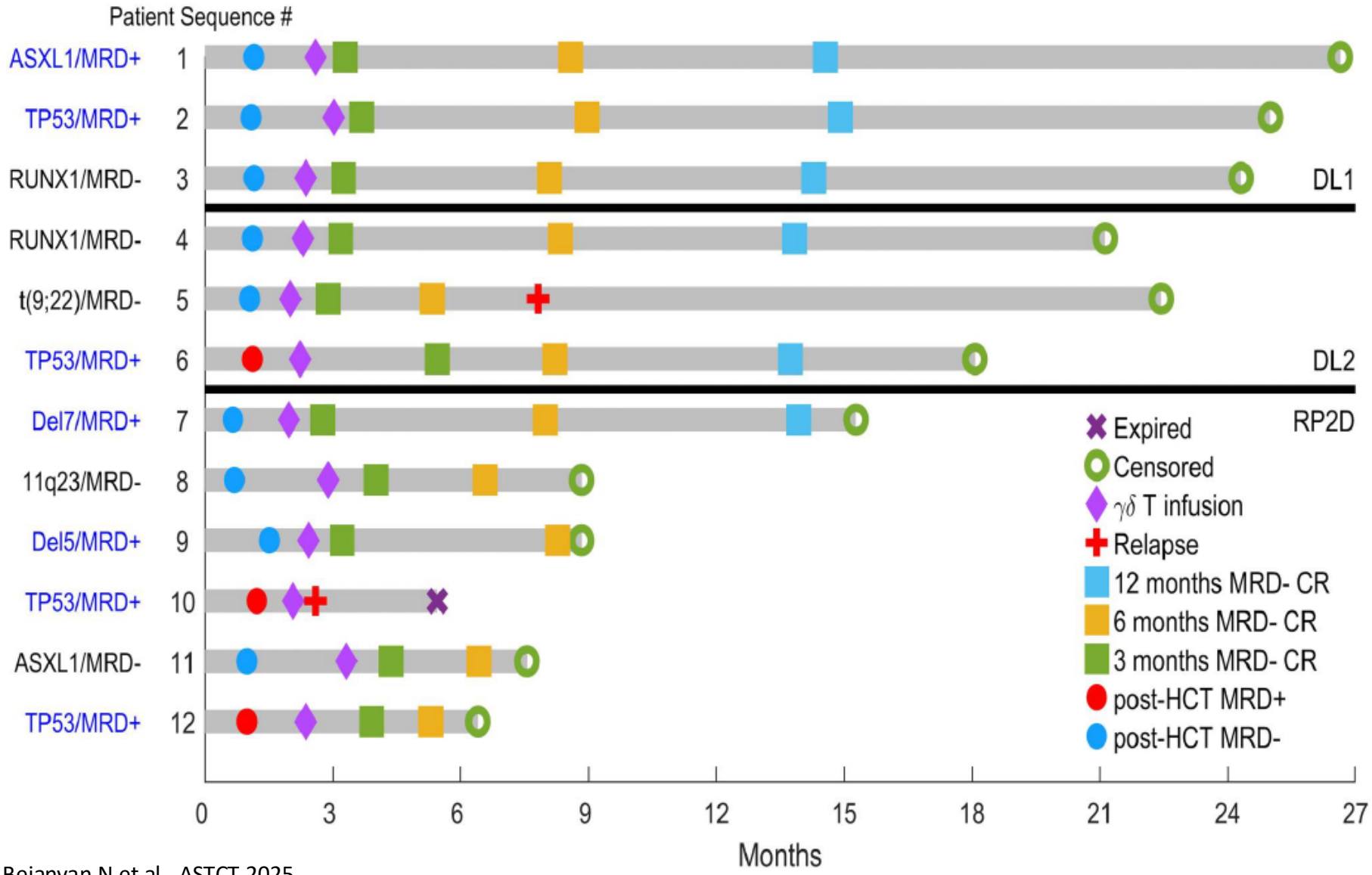


F Percentages of γδ T cell memory phenotypes



Courtesy of Bejanyan N

MRD Negative CR in Most Patients Treated with GDT Cells



- **17%** (2 of 12) relapse in these patients with **adverse risk AML** receiving RIC/NMA HCT.
- **85%** (6 of 7) of **pre-HCT MRD^{pos}** patients achieved **MRD^{neg} CR**.
- **75%** (3 of 4) of **TP53+/pre-HCT MRD^{pos}** patients achieved **MRD^{neg} CR**.
- **67%** (2 of 3) of **pre-HCT MRD^{pos}/TP53+** patients achieved **MRD^{neg} CR**.

Bejanyan N et al., ASTCT 2025

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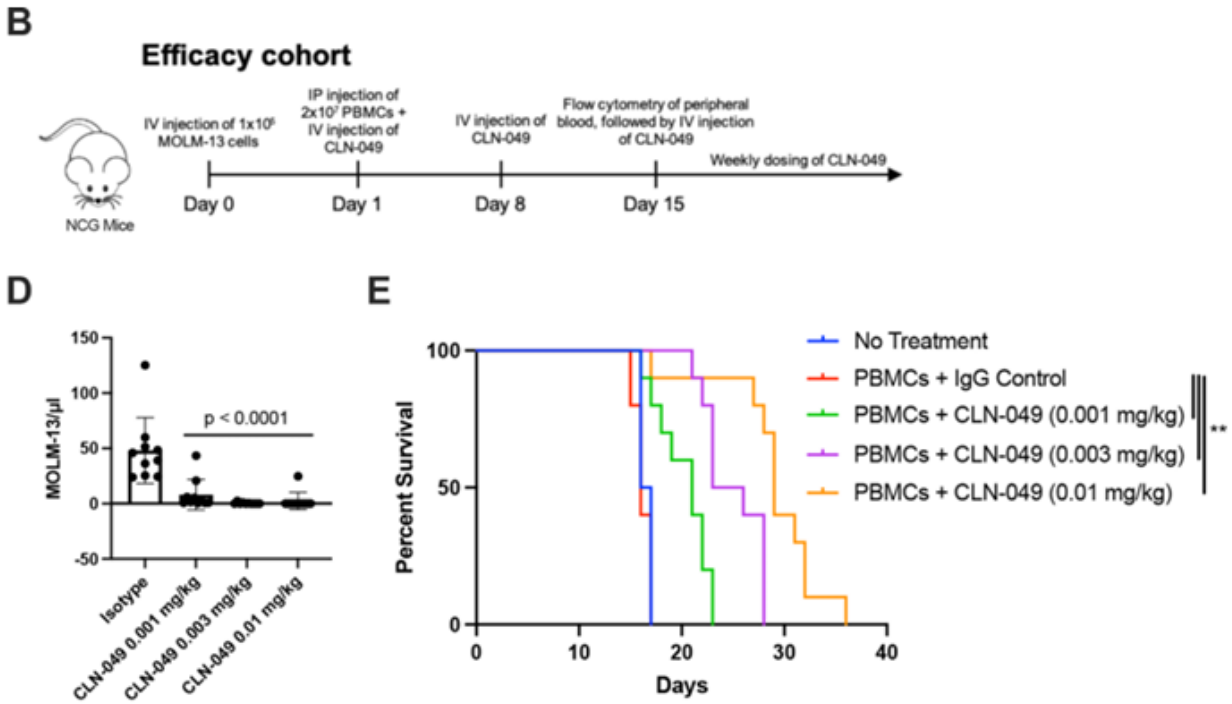
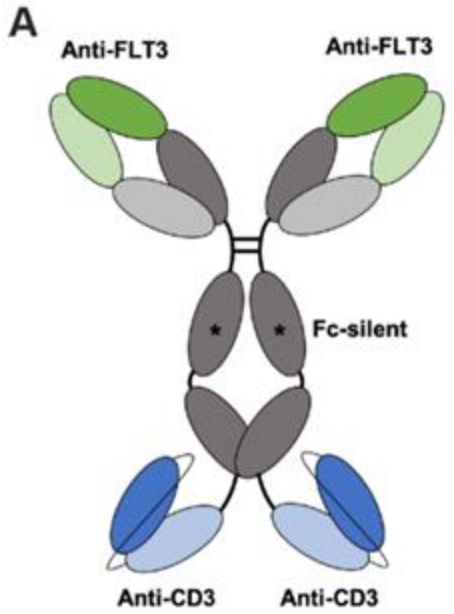
Novel Targets: FLT3 Receptor via Bispecific

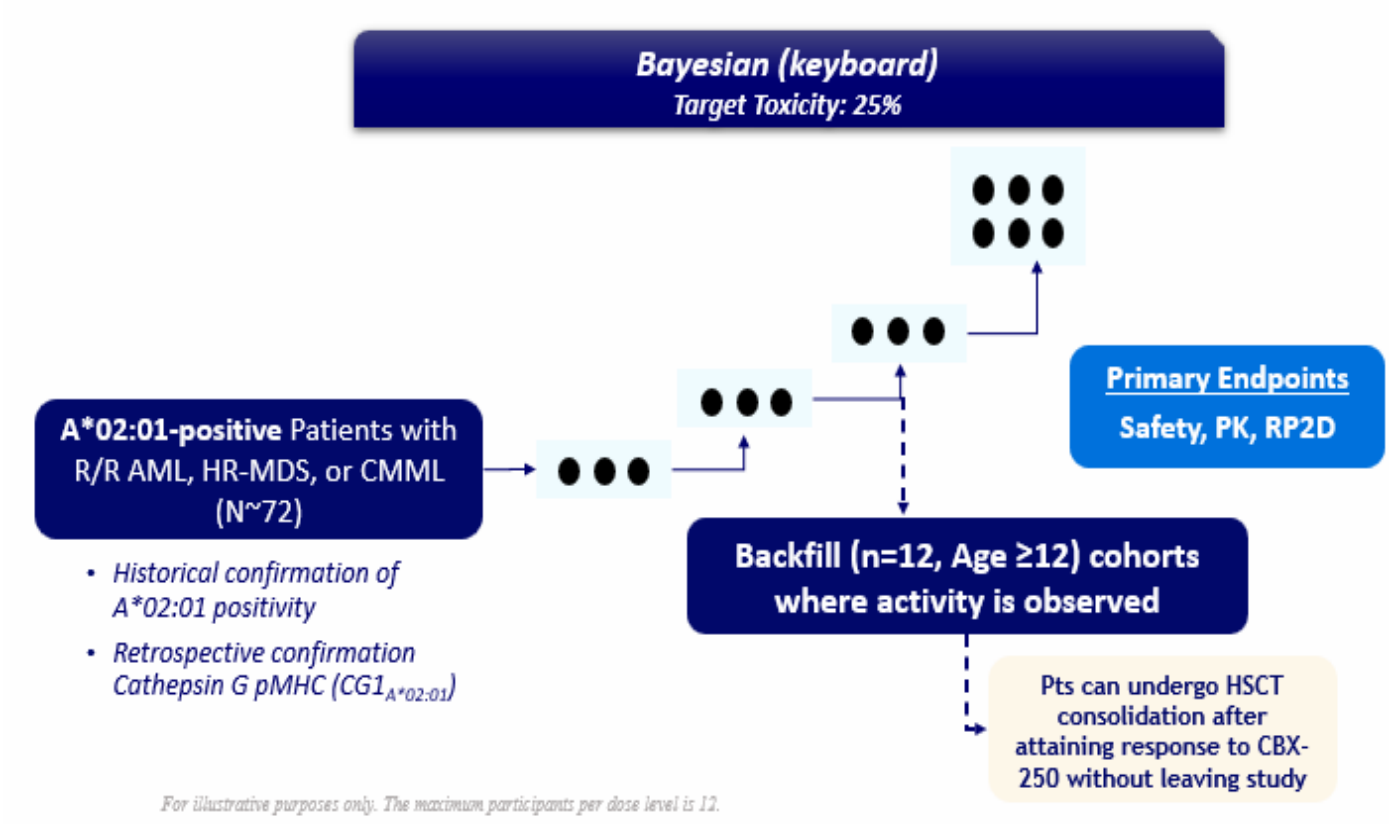


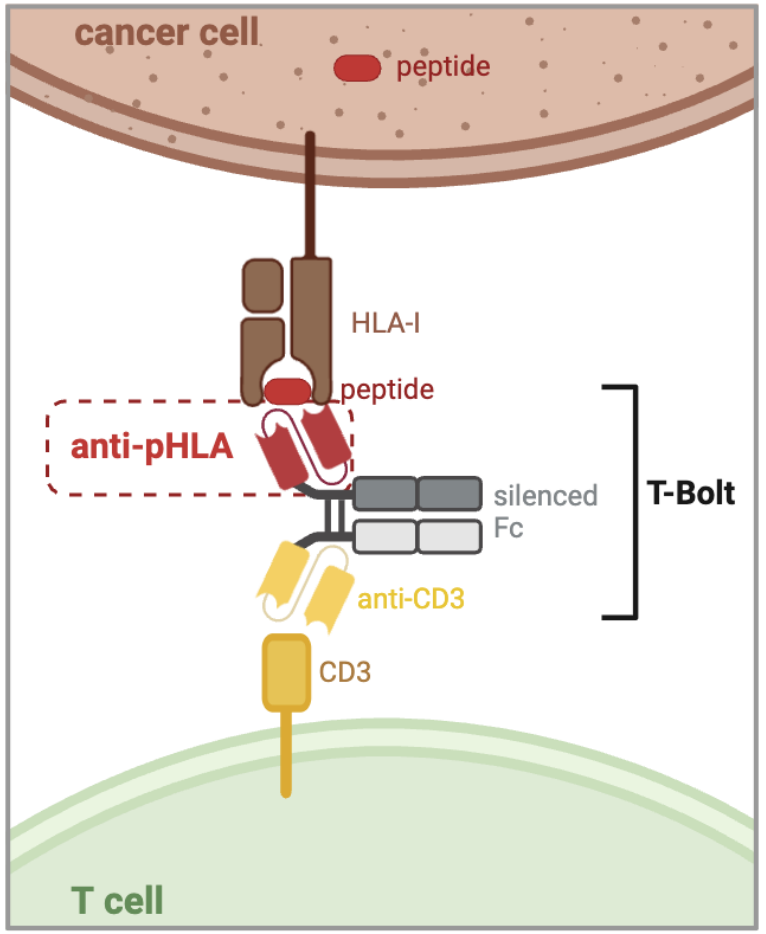


Phase 1 Clinical Trial in R/R AML is Ongoing (Data to be presented at ASH)

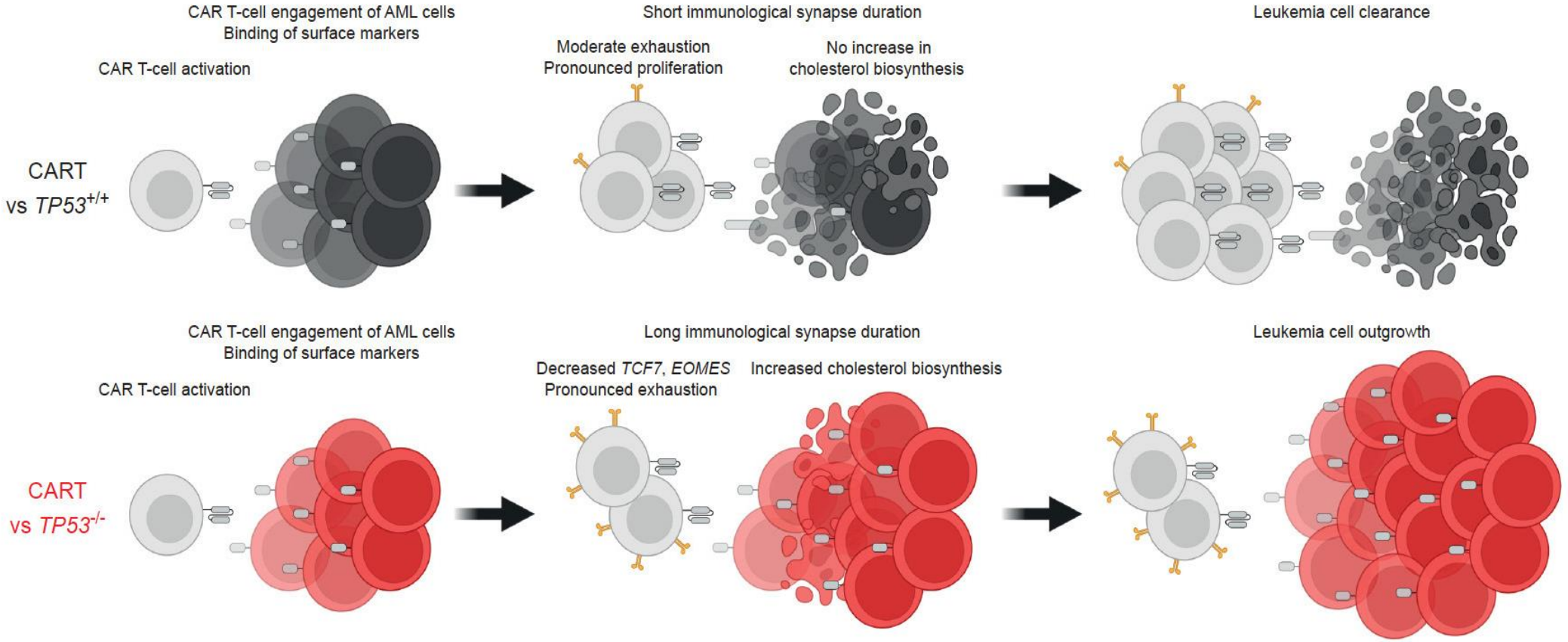
Mehta N, et al. *JITC*. 2021;

Novel Targets: T-cell Engager for Cathepsin G





TP53 Deficiency in AML Confers Resistance to CAR T-Cells



Cholesterol pathway identified as a potential therapeutic vulnerability of *TP53*-deficient AML

Mueller J et al., EMBO Mol Med, 2024

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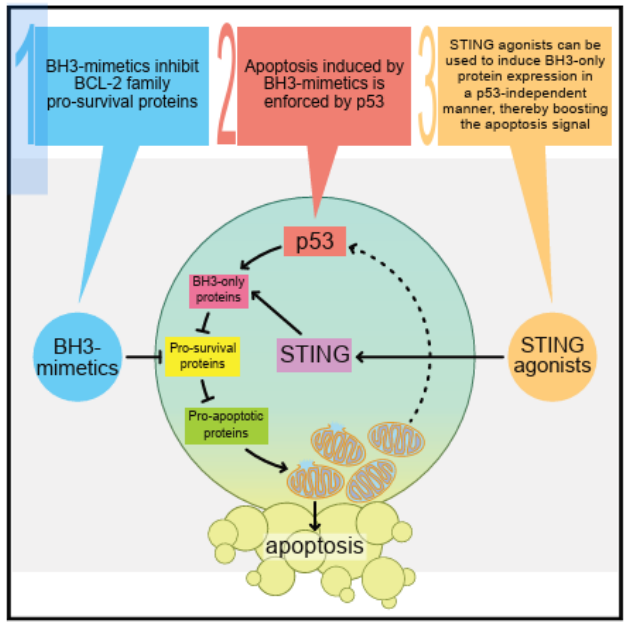
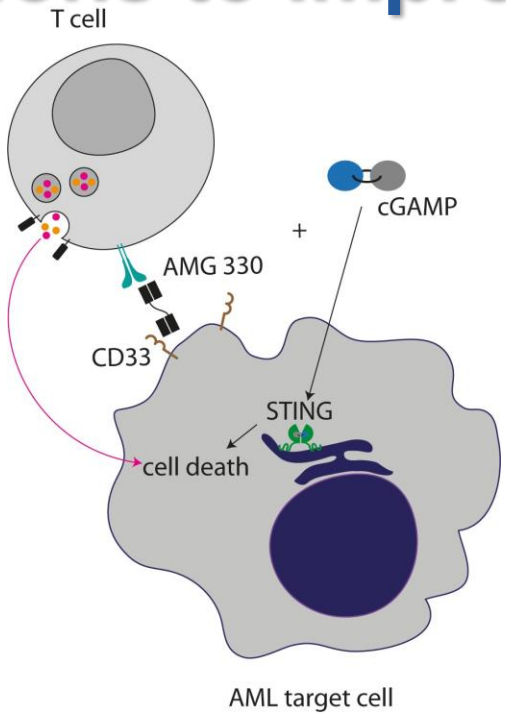
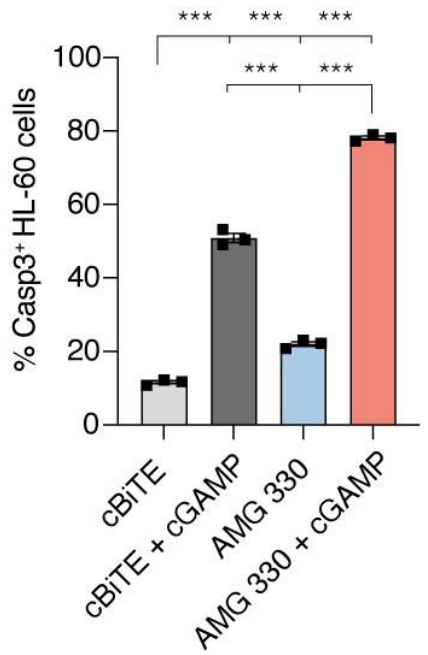
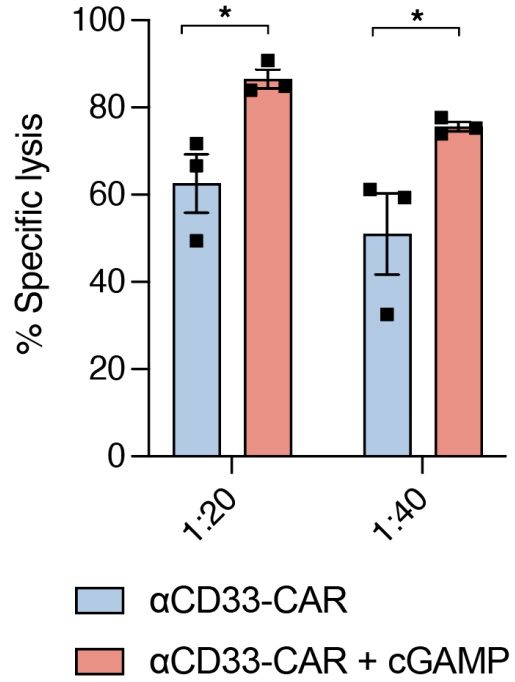
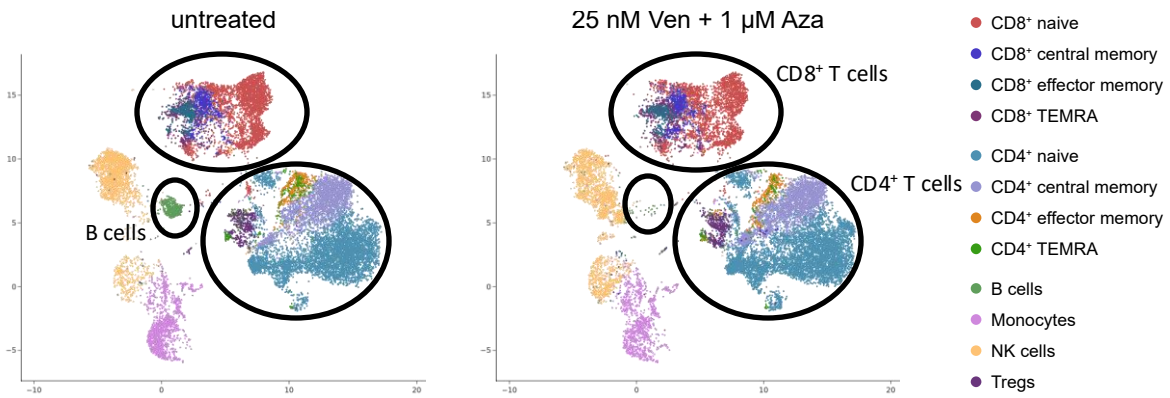
Combinatorial Options to Improve IO Therapy?

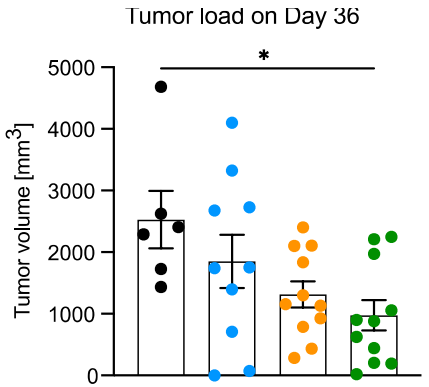


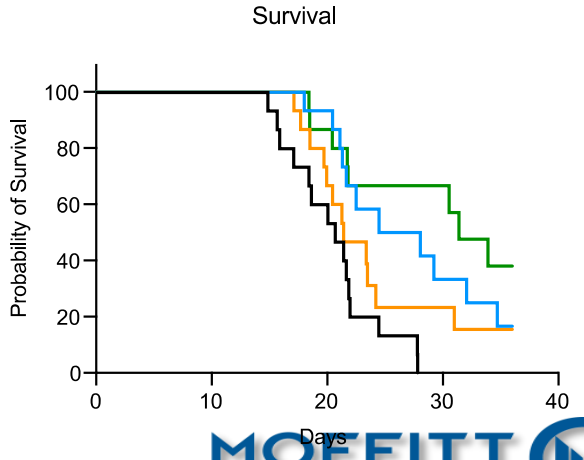












Diepstraten S, et al. *Cancer Cell*. 2024; Linder et al., Subklewe M. *Blood* 2025; Haenel et al. Subklewe, Leukemia 2024

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