Cellular Therapies for Myeloid Malignancies
2024 Cell Coast Conference

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Disclosures

I disclose the following financial relationship(s):

- Agios, Advisory Board or Panel, Speaker's Bureau
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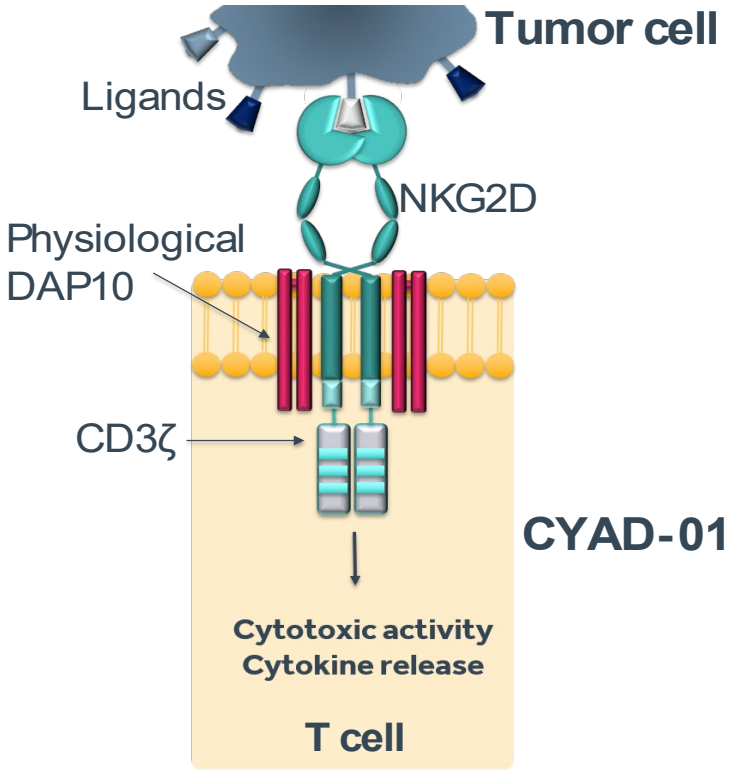
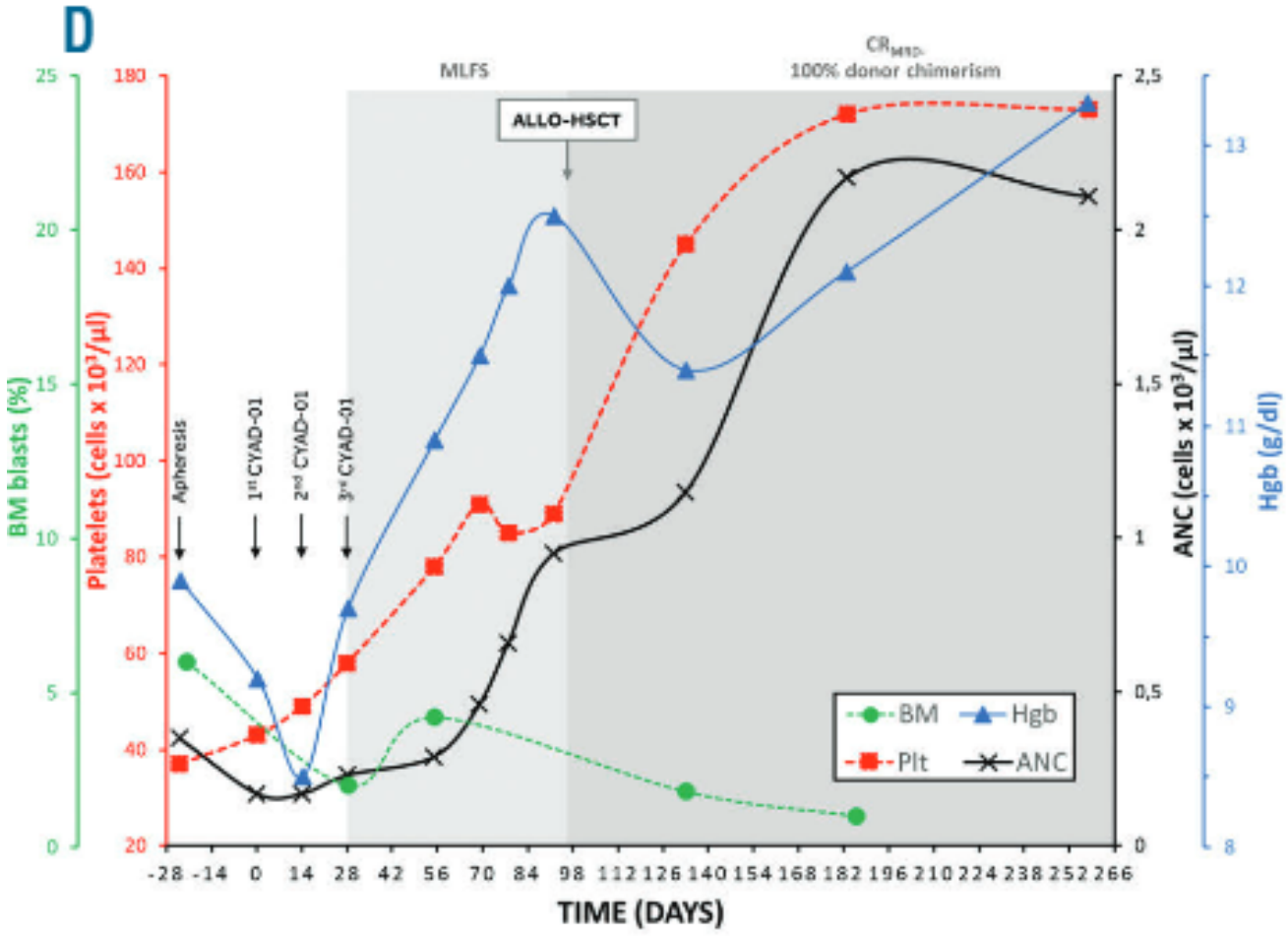
Case Report – CYAD-01 NKG2D CAR





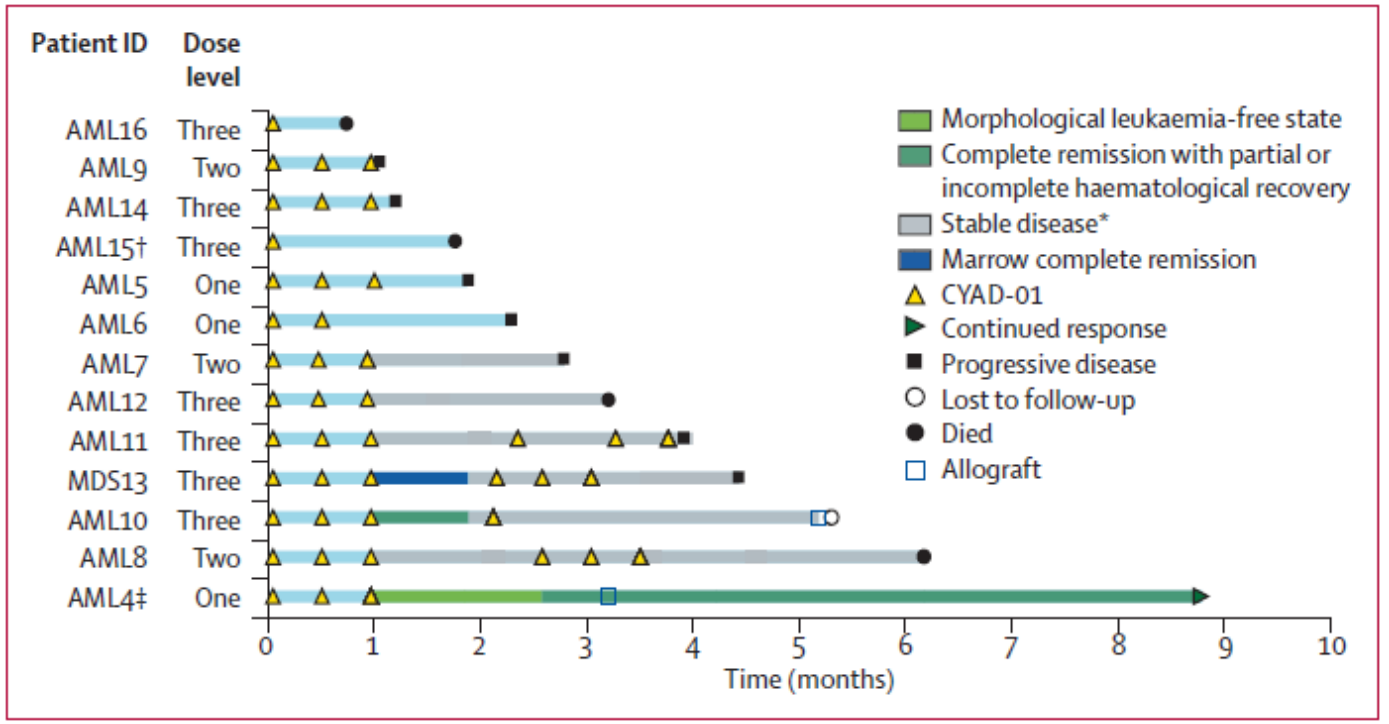
Sallman D et al., Haematologica 2018

Efficacy in AML/MDS Patients

	Dose level one (n=3)	Dose level two (n=3)	Dose level three (n=7)
Complete remission with partial or incomplete haematological recovery or marrow complete remission	1 (33%)	0	2 (29%)
Stable disease	0	2 (67%)	2 (29%)
Progressive disease	2 (67%)	1 (33%)	2 (29%)
Non-evaluable*	0	0	1 (14%)

Data are n (%). *Patient at dose level three with a dose-limiting toxicity upon first CYAD-01 administration was withdrawn from study treatment and did not proceed to any disease assessment.

Table 3: **Best tumour response according to dose level in the acute myeloid leukaemia or myelodysplastic syndrome population**



CRS occurred in 7 out of 15 pts patients (2 G3 and 1 G4); no neurotoxicity

Sallman D et al., Lancet Haematology 2023

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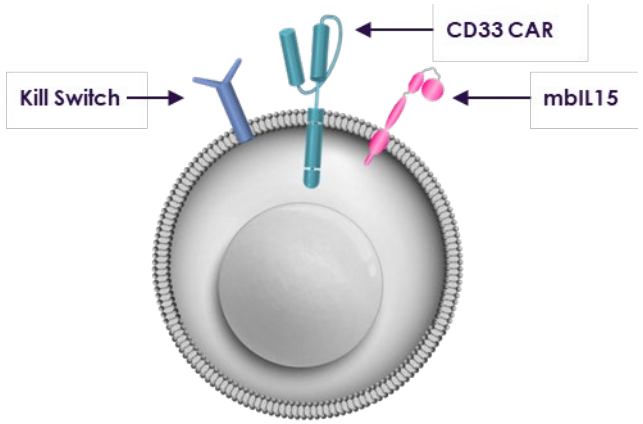
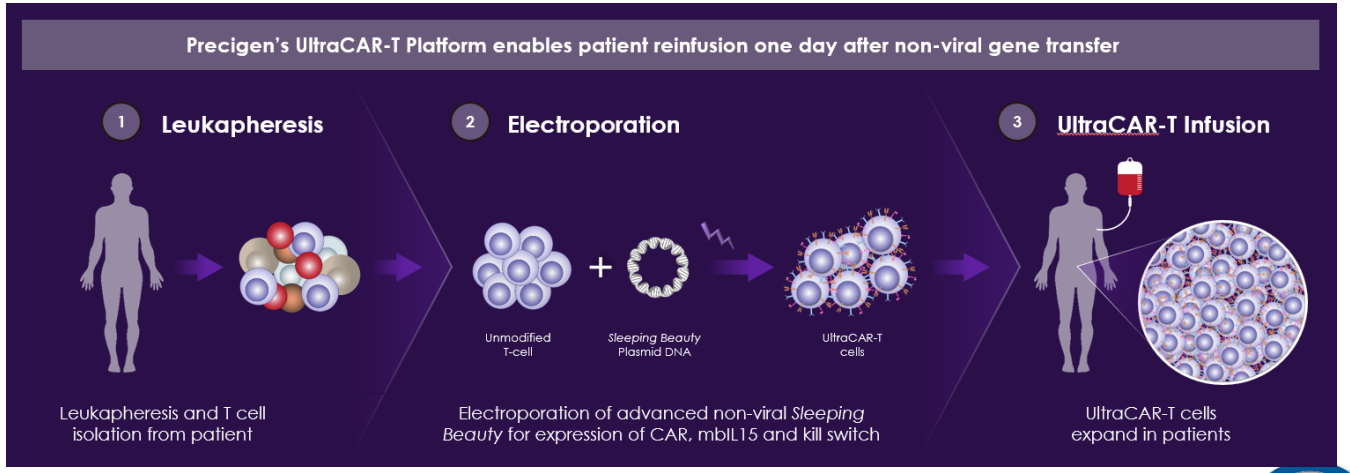
PRGN-3006 UltraCAR-T: CD33 CAR Approach

- Non-viral gene delivery via electroporation of *Sleeping Beauty* plasmid
- Multigenic CAR-T product with expression of CAR, kill switch and membrane bound IL-15 (mbIL15)
- Overnight UltraCAR-T manufacturing process eliminates the long wait times associated with conventional autologous CAR-T





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PRGN-3006 UltraCAR-T Phase 1 Dose Escalation Clinical Trial Design

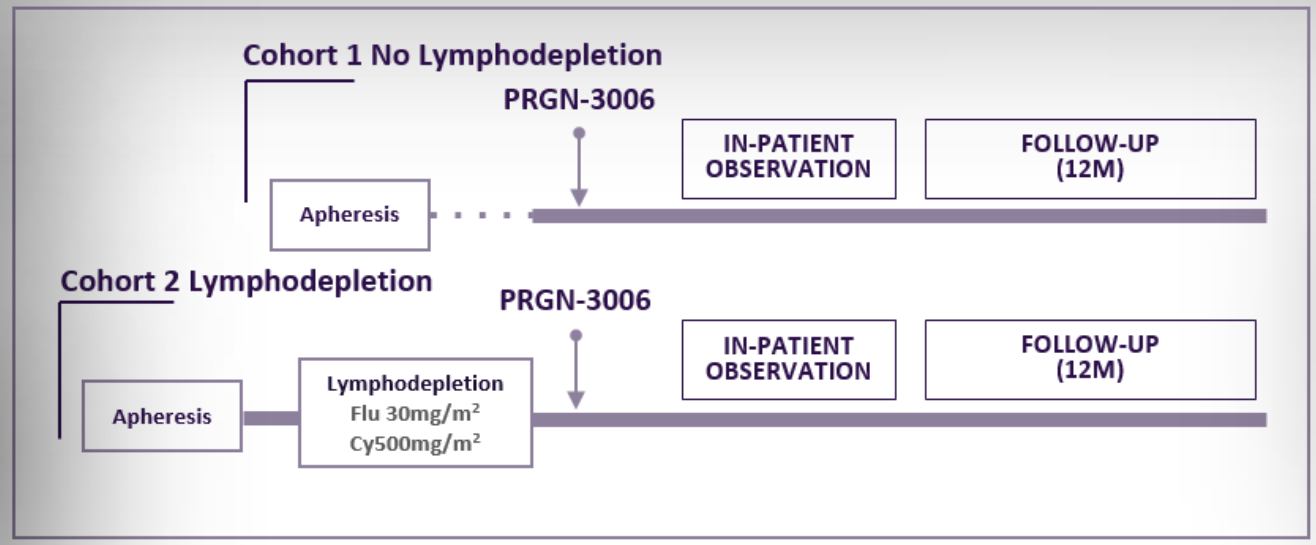
FIRST-IN-HUMAN, DOSE ESCALATION STUDY EVALUATING SAFETY AND EFFICACY OF A SINGLE DOSE OF PRGN-3006

ELIGIBILITY

- r/r CD33+ AML, high risk MDS or with >5% blasts
- ALC > 0.2 k/µL
- Prior HSCT allowed

STUDY OBJECTIVES

- PRIMARY: Evaluate the safety and determine the MTD of PRGN-3006 delivered intravenous (IV) infusion either with or without lymphodepletion
- SECONDARY : Evaluate in vivo persistence and anti-tumor activity of PRGN-3006



SAFETY MONITORING

- Management via ASTCT

DISEASE RESPONSE

- ELN Criteria (AML)
- IWG 2006 criteria (MDS)

CORRELATIVES

- PRGN-3006 expansion
- Immune phenotype
- Biomarkers

Dose Level (DL)	Dose Level (Cells/kg)
DL1	$>3x10^4$ to $\leq 1x10^5$
DL2	$>1x10^5$ to $\leq 3x10^5$
DL3	$>3x10^5$ to $\leq 1x10^6$

Sallman D et al., ASH 2022

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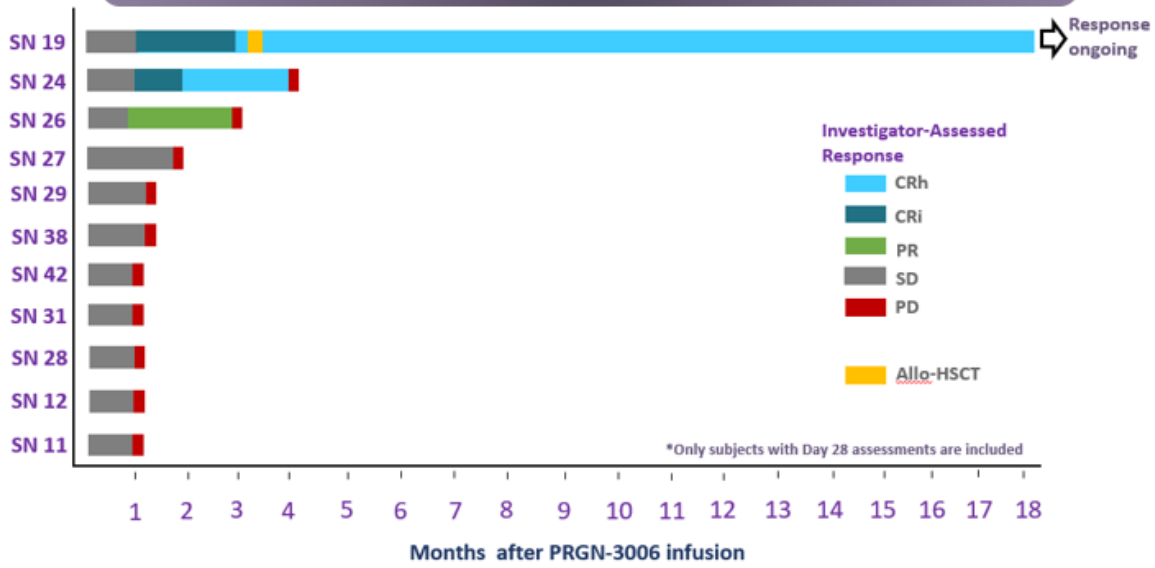
A Single Infusion of PRGN-3006 Leads to Objective Responses in AML Patients; No Response in MDS/CMML

PRGN-3006 Infusion resulted in Objective Response Rate of 27% in AML patients



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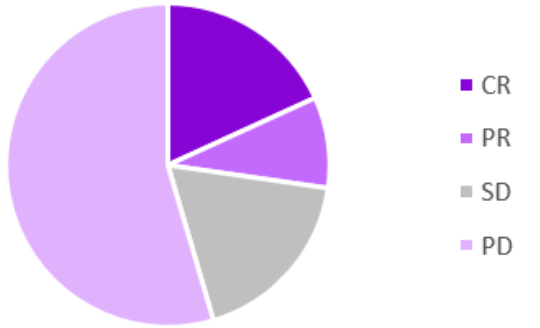

No Objective responses were observed in patients with MDS, which were treated at the highest dose level

	AML	MDS	CMML
Disease Control Rate (at D28)	45% (5/11)	3/3 (100%)	0/1
Objective Response Rate (ORR)	27% (3/11)	0/3	0/1

Sallman D et al., ASH 2022

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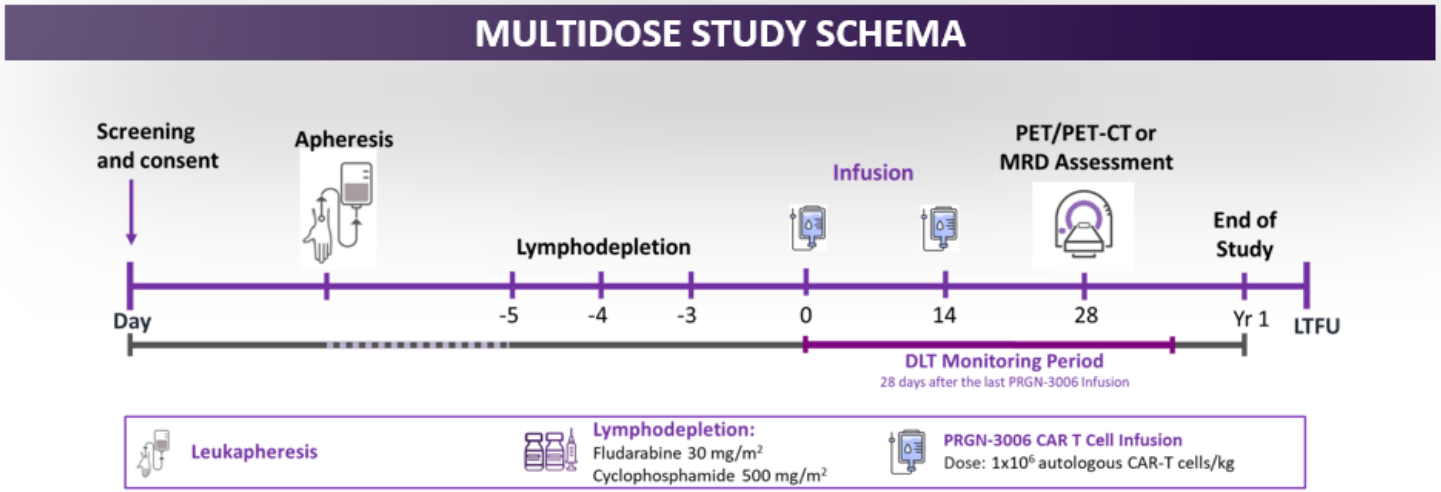
PRGN-3006 UltraCAR-T Dose Expansion Clinical Trial Design

- PRGN-3006 was **well-tolerated with no DLTs reported to date** and minimal neurotoxicity observed.
- A single infusion of PRGN-3006 cells at doses as low as 1 x 10^5 cells/kg resulted in objective responses in patients infused following lymphodepletion
- PRGN-3006 cells were able to expand and persist in patient peripheral blood and bone marrow even in the absence of lymphodepletion
- **1 x 10^6 cells/kg PRGN-3006 CAR-T cells** with lymphodepletion is currently being evaluated in the dose expansion phase
  - Patients can receive two infusions of PRGN-3006 at the discretion of investigators
  - There is no requirement for additional lymphodepletion in repeat dose patients due to the demonstrated ability of PRGN-3006 to expand in the absence of lymphodepletion



Sallman D et al., ASH 2022

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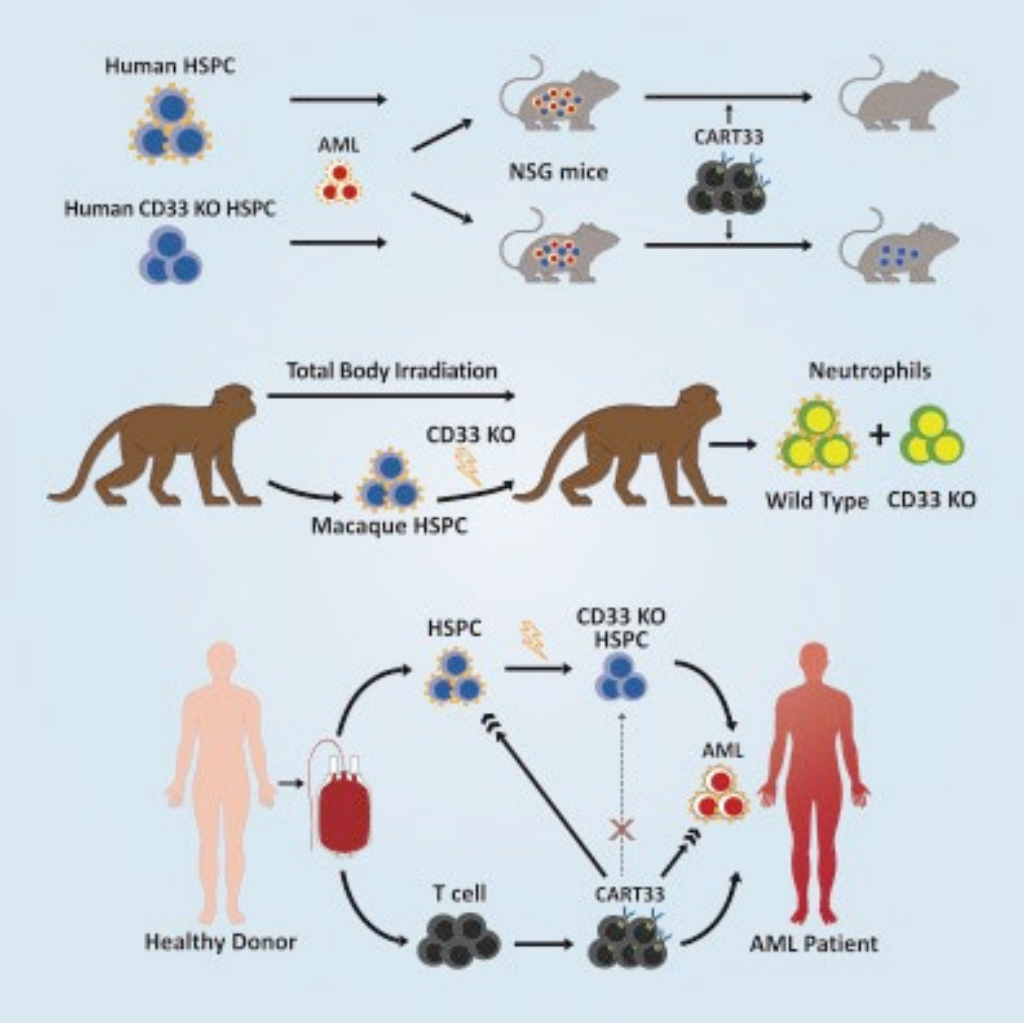
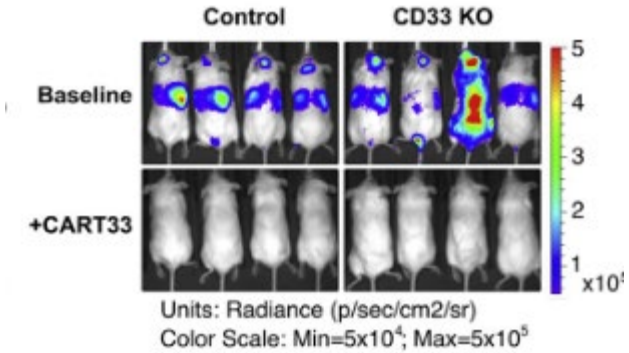
CD33 KO in Stem Cell Product – Leukemia Specific Antigen





CD33-CLL1 Multiplex Deletion similar Data *in vitro*

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

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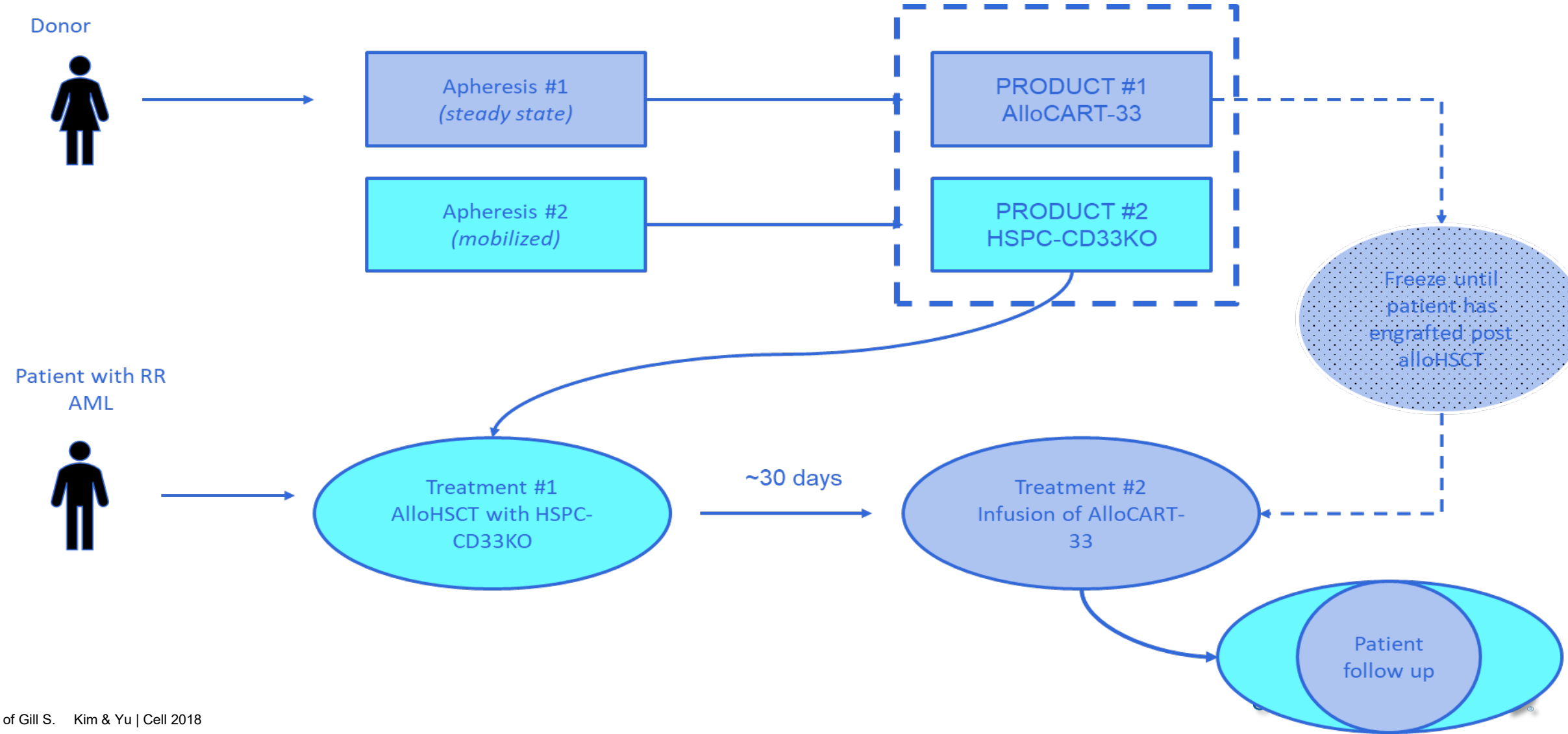
CD33 KO in Stem Cell Product – Leukemia Specific Antigen



Courtesy of Gill S. Kim & Yu | Cell 2018

Tremcell (CD33KO stem cell product) with Successful Engraftment

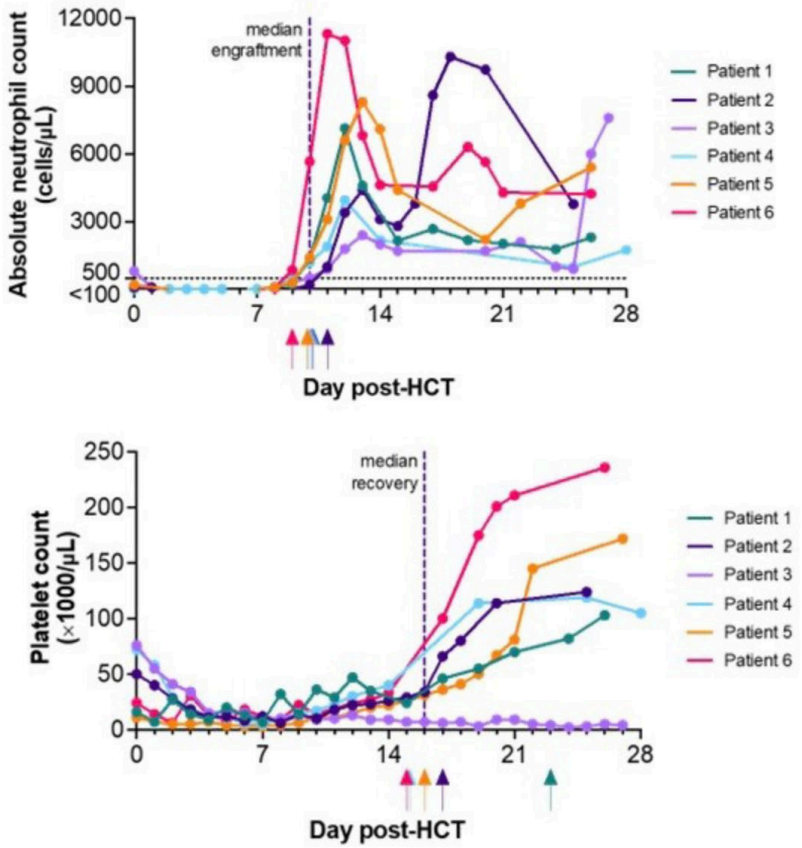


Figure 1. Kinetics of neutrophil engraftment and platelet recovery (n=6). Arrows denote time of individual patient neutrophil engraftment and platelet recovery.

- Current study follows with GO (low dose)
- Allo CART for CD33 underway, no data presented to date.
- Ultimate goal likely above followed by allocart, potentially for high risk vs MRD+ patients.

DiPersio J et al., ASH 2023

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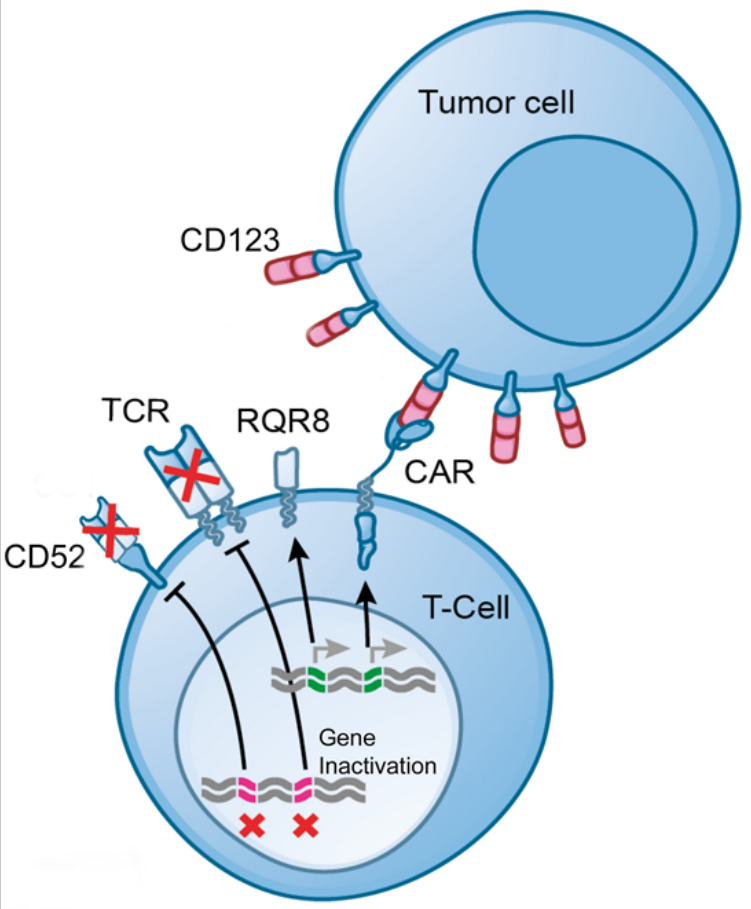
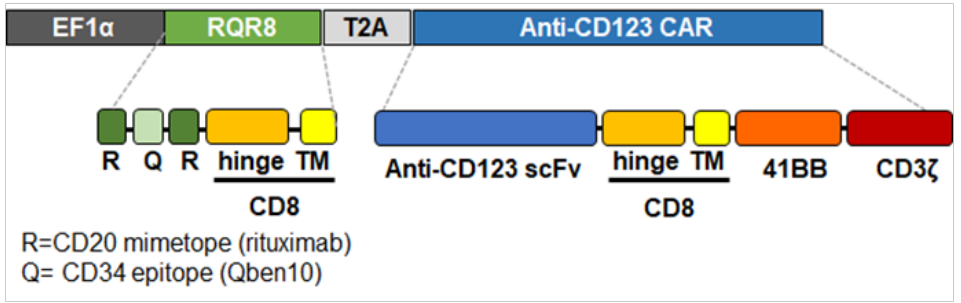
UCART123: Allogeneic “off-the-shelf” T cell product





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

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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

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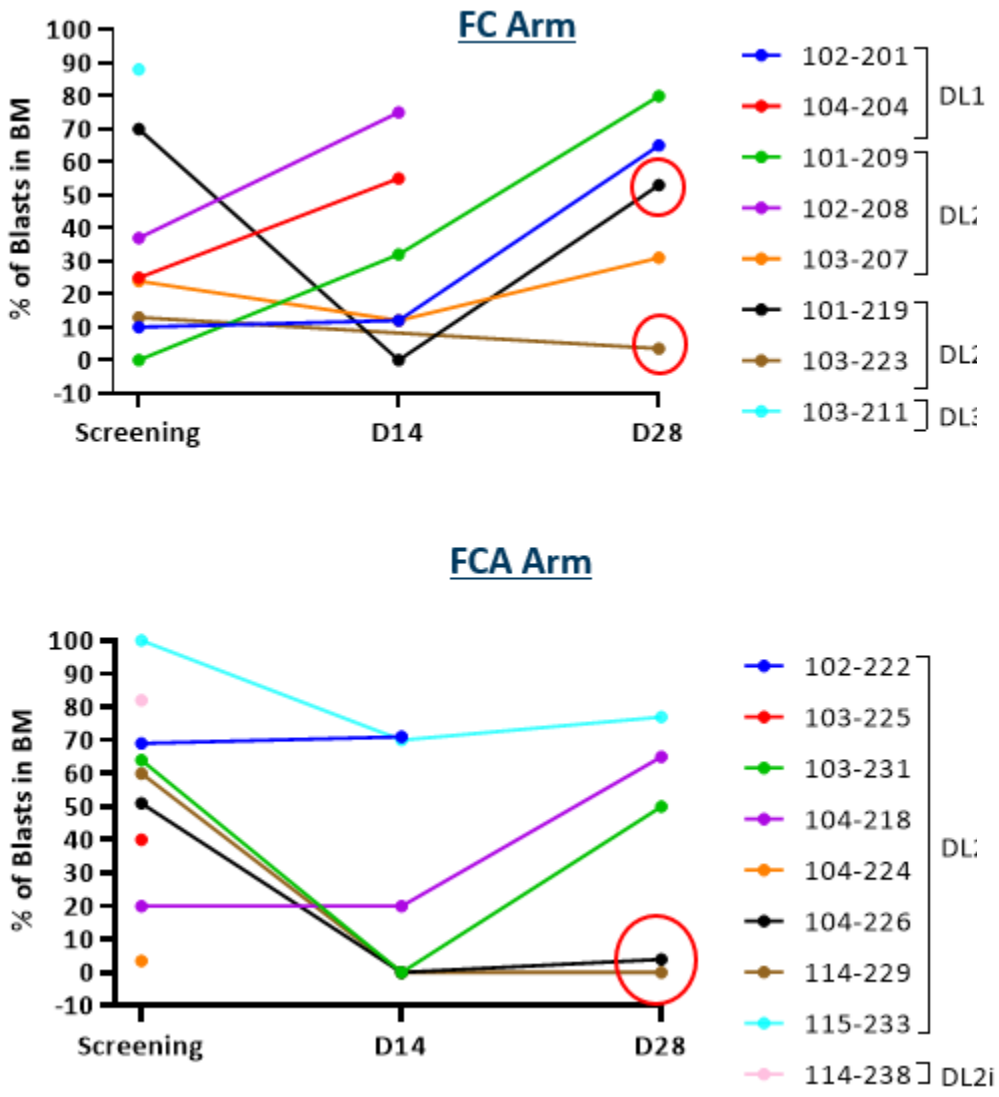
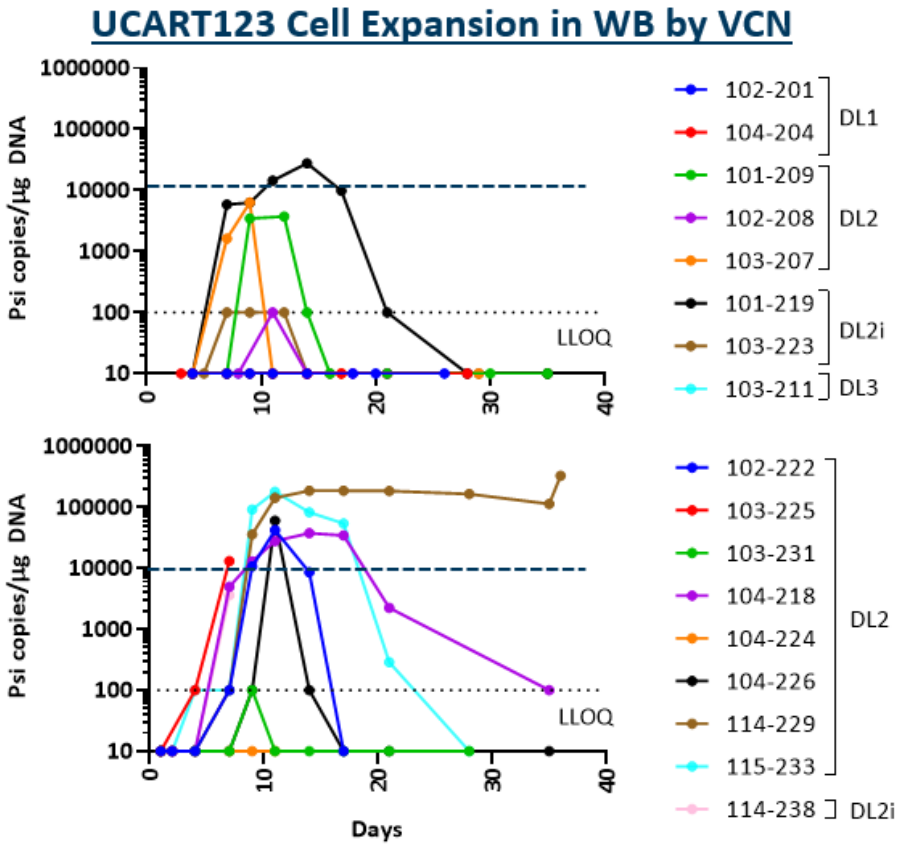
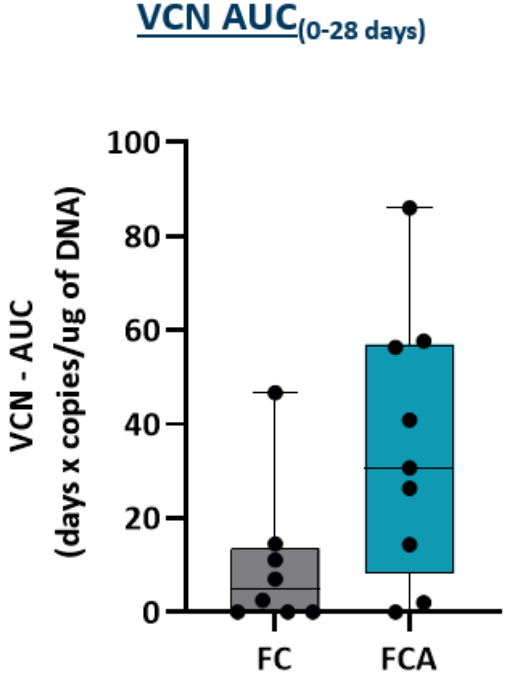
Efficacy and Kinetics







Sallman D et al., ASH 2022

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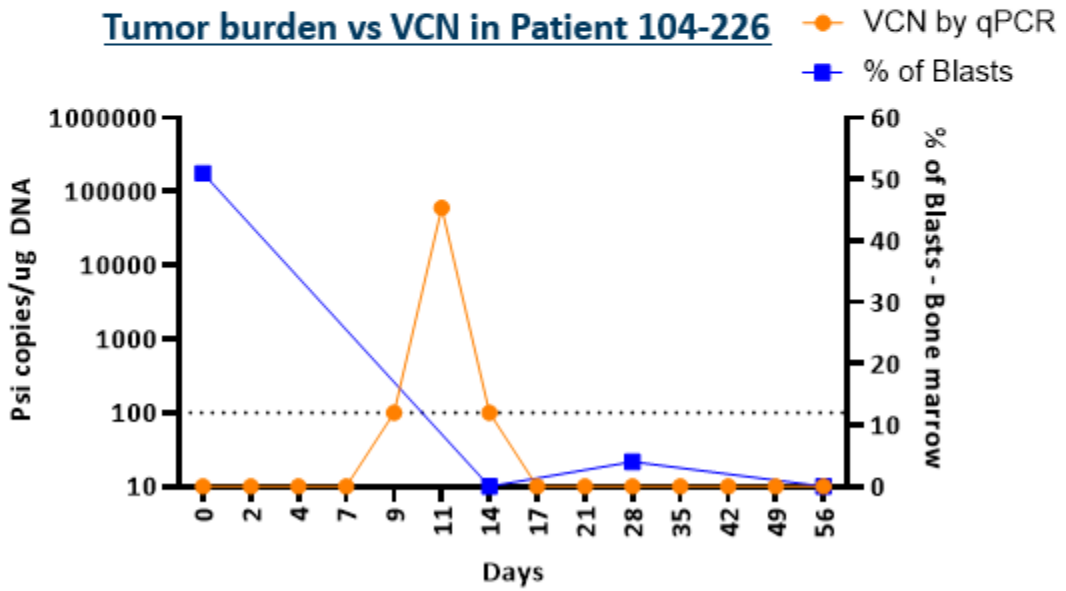
Patient Achieved a Durable MRD Negative CR without DLI/2nd allo

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

Sallman D et al., ASH 2022

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Other Studies Targeting CD123 in Relapsed/Refractory AML

- Auto CD123 CAR with optimized IgG4 CH2CH3 linker a CD28 co-stimulatory domain, and a CD3 zeta
  - 1/6 Cri (bridged to 2nd allo day day +70).
- Unicar-T CD123 in R/R AML
  - Modular, universal CAR-T treatment, consisting of a universal CAR-T cell with CD28 costimulatory domain (UniCAR-T) and a CD123 targeting module (TM).
  - TM binds to the CAR-T cell via a peptide tag derived from the human La protein.
  - 14 pts treated, CRS 12/14 (1G3); 1 G2 CRES. 1 DLT (reversible with stopping TM). Treatment has been safe through 500M CAR-T Cells
  - 2 CRi responses (14%), 10 pts with blast reduction. DL not associated with response
- Allo-CD123 CART entering clinic by 2025

Budde L et al, ASH 2017; Ehninger G et al., ASH 2022

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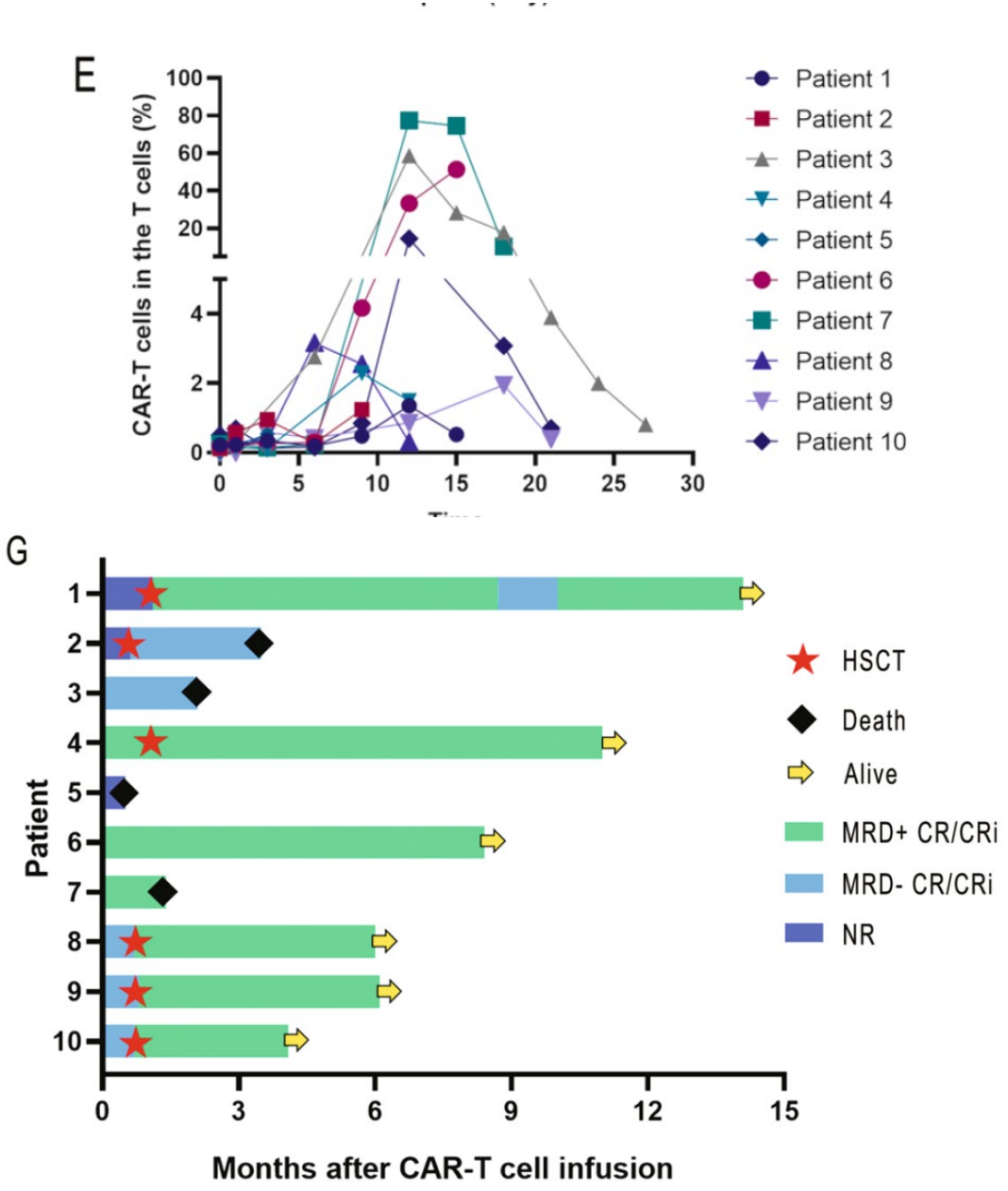
CLL1 CAR-T in Adults

- LD with cyclophosphamide (500 mg/m^2) and fludarabine (30 mg/m^2)
- 1–2 × 106 CAR-T cells/kg targeting CLL-1 with 41BB co-stim
- 100% CRS, 6/10 high grade, no CRES
- Severe cytopenias all pts, 7/10 with CR/CRi although unclear if ablated, only 1 pt alive and in remission without transplant.



Xin J et al., *Journal of Hematology & Oncology*; 2022

Safety and Efficacy of CD33-CLL1 Compound CART

- Autologous CART in heavily pretreated R/R AML
- Flu/Cy at 30mg/m^2 and 300mg/m^2, respectively
- Cell dose 1-3x10^6/kg
- 7/9 with MRD negative response by multiparameter flow cytometry
- 1 of 2 non-responding pts were CLL1 negative
- 6 pts to allo-HSCT

	Age/sex Dx	Prior treatment	BM Blast%	CD33/CLL1 expression	Cytogenetic /molecular	Origin of car-t cells	CAR-T Dose	response s
P1	44/m AML	4 chemo	47%	CD33$^+$/CLL1$^+$	ASXL1,TP53	auto	0.7x10^6/kg	MRD$^-$
P2	6/f JMML-AML	5 chemo	81%	CD33$^+$/CLL1$^+$	Complex FLT3-ITD	auto	2x10^6/kg	MRD$^-$
P3	23/F CML AP	3 TKIs for 5 years	1.63%	CD33$^+$/CLL1$^+$	t(9;22) T315mut	auto	1.1x10^6/kg	MRD$^-$
P4	43/F M2	3 chemo	42%	CD33$^+$/CLL1$^+$	NK FLT3-ITD	auto	2.8x10^6/kg	MRD$^-$
P5	32/F AML	3 chemo	19%	CD33$^+$/CLL1$^+$	NK MLL	auto	2x10^6/kg	MRD$^-$
P6	48/F AML	5 chemo	94%	CD33$^+$/CLL1$^+$	t(8;21) AML1/ETO CKIT	auto	1.3x10^6/kg	MRD$^-$
P7	23/F AML	4 chemo	74%	CD33$^+$/CLL1$^+$	t(8;21) AML1/ETO CKIT	auto	1x10^6/kg	NR
P8	27/F AML	5 chemo	93%	CD33$^+$/CLL1$^-$	NA MLL AF9	auto	2.3x10^6/kg	NR
P9	42/f AML	2 chemo	7%	CD33$^+$/CLL1+	T(3;3) RUNX1	MSD donor	3.7x10^6/kg	MRD$^-$

Liu, F et al., EHA 2020

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Kite-222 Dose Escalation

Table 2. Dose Cohorts

Dose Cohort	Number of Anti-CLL-1 CAR T Cells[a,b]	
	<50 kg	≥50 kg
Dose-escalation Cohort 1	2×10^7	3×10^7
Dose-escalation Cohort 2	7×10^7	1×10^8
Dose-escalation Cohort 3	2×10^8	3×10^8
Expansion Cohort	The optimal dose for the expansion cohort will be determined by the SRT	

Recruitment Status	Actual Primary Completion Date	Actual Study Completion Date
Terminated	2024-05-18	2024-05-18

TERMINATED

Study was terminated due to futility

Clinicaltrials.gov

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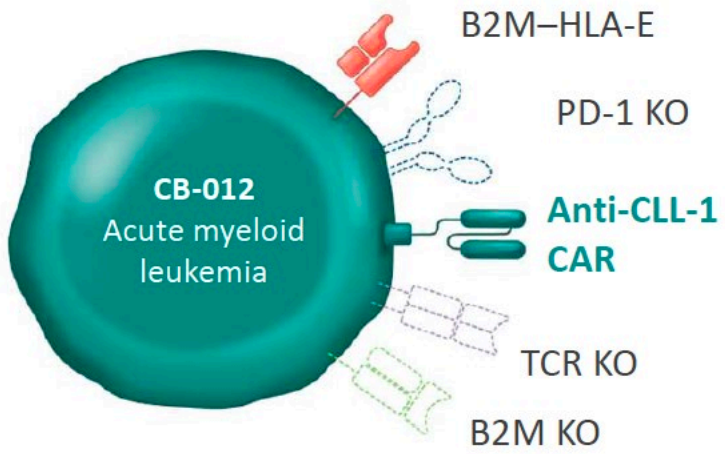
CB-012 CLL1 Allo CART for r/r AML

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	✓	✗

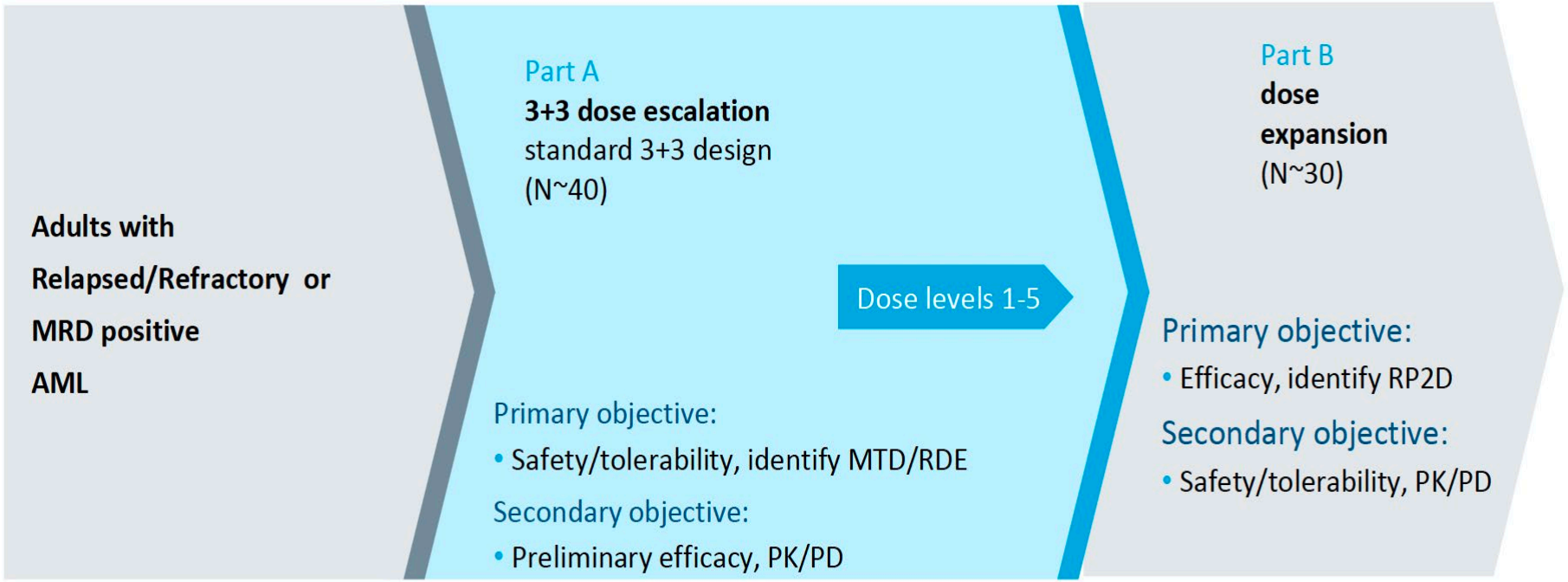
CB-012 uses a potent, fully human anti-CLL-1 scFv[1] with a CD28 costimulatory domain

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

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CLEC12A/CLL1 Expression in CMML

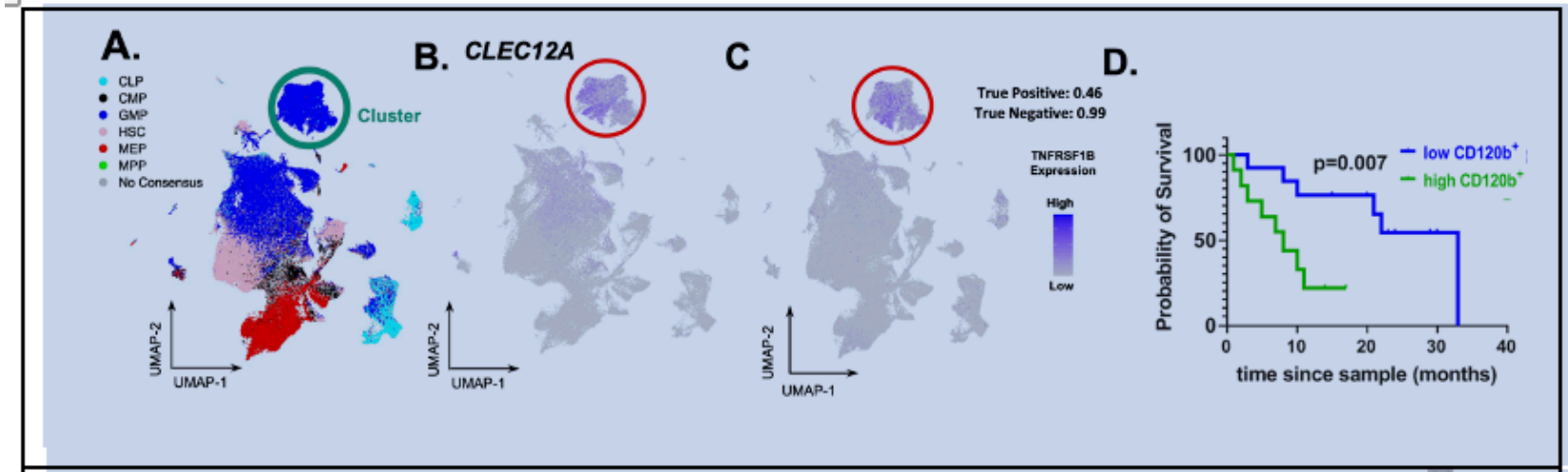

Figure 2. Expression of *CLEC12A* in scRNASeq of CMML patients. (A) **(A)** SingleR[20] used to determine cell type assignment using published bulk RNA sequencing references from sorted cells. Clus2 was enriched for GMP cell type assignment. **(B)** Expression of CLEC12A (CLL1) in Clus 2 cells. **(C)** COMET[21] analysis identified CD120b (TNFRSF1B) as the optimal flow marker of Clus2. **(D)** Overall survival in validation cohort of CMML patients with high CD120b^{+} GMP versus low. See Ferrall-Fairbanks, et all Blood Cancer Discovery 2022 for details.

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A Phase 2, Multicenter, Open-Label Study of CB-012, a CRISPR-Edited Allogeneic Anti-CLL-1 CAR-T Cell Therapy in Patients with Relapsed/Refractory Chronic Myelomonocytic Leukemia or Juvenile Myelomonocytic Leukemia.

- CMML patients will be treated at the RP2D determined by the ongoing CB12A trial in adults with AML
- Patients with JMML will be treated at the equivalent cell/kg-adapted dosing of the R2PD as is done in pediatric cell therapy trials

- Primary:
  To describe the efficacy of CB-012 in patients with relapsed/refractory CMML or relapsed JMML.

- Secondary:
  To characterize the pharmacokinetics and pharmacodynamics of CB-012 in r/r CMML and relapsed JMML.
  To further evaluate the safety and tolerability of CB-012 therapy in patients with r/r CMML and relapsed JMML.

- Exploratory:
  To further characterize the pharmacodynamics of CB-012 and biomarkers of immune function and their relationship with tumor activity and burden
  To determine whether replication competent AAV is present in patients who received CB-012
  To assess the immunogenicity of CB-012

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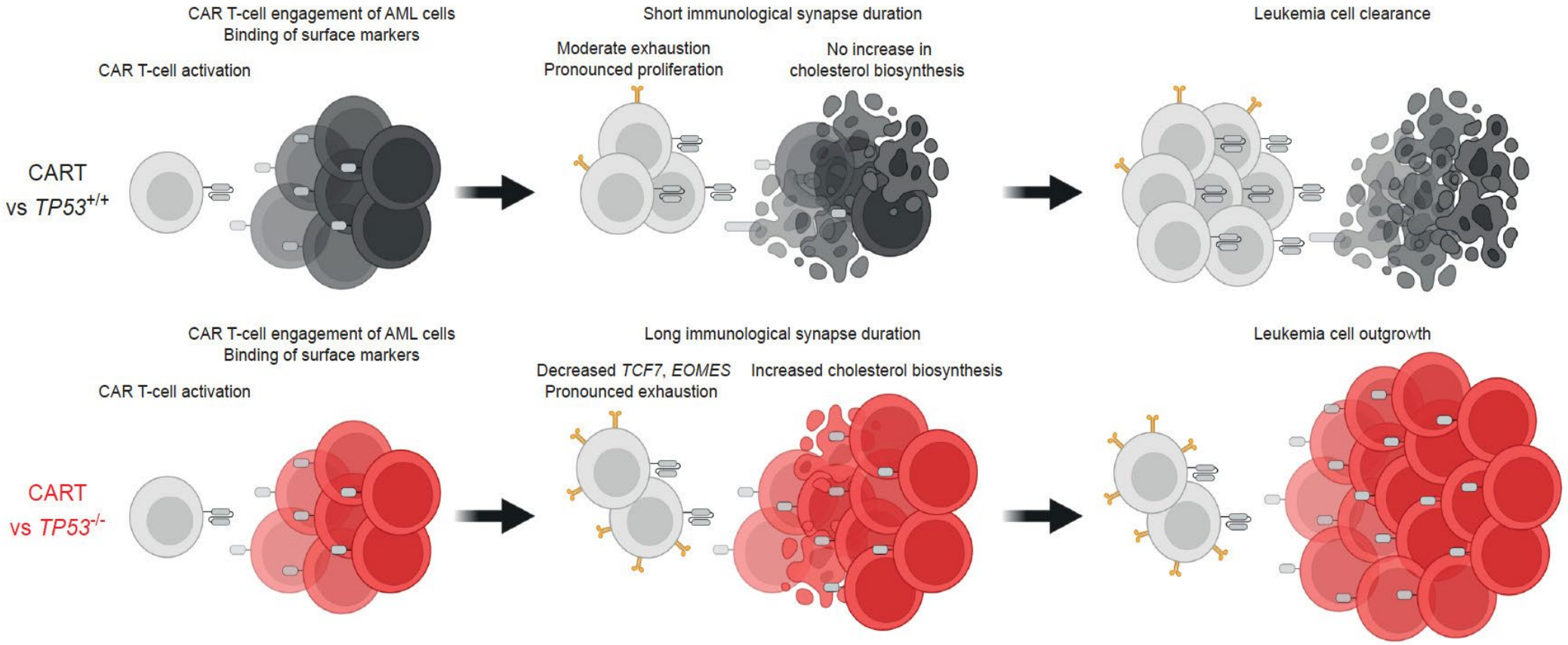
Challenges/Thoughts

- The rigorous FDA rules with staggered enrollments/etc.. Greatly slowing progress in field, taking years for phase 1 studies to be completed
- How do we more quickly evaluate CART with low burden disease patients, MRD positivity, CART production with more fit T-cells prior to multiple lines of therapy.
- What is optimal conditioning and bridging strategy
- Optimal endpoints

Questions to Address

- What is Target Expression on LSC, bulk BM bone marrow blasts for MDS, CMML and AML patients based on treatment?
  - HMA ven failure vs IC failure vs other
  - Are There differences based on molecular (e.g. TP53 vs other) and/or disease subsets (e.g. secondary AML, AML-MRC)
- What combination of surface antigen targets with CLL-1/CD33/other would cover 100% of LSCs and prevent antigen escape?
- At the single cell level in LSCs for patients with MRD + disease after either HMA + ven for Intensive therapy, what is CLL-1/CD33/other positivity and MFI and what combination with other targets would eradicate 100% MRD
- How do t-cell subset change based on MRD positivity vs past therapies
  - Important both from auto-CAR perspective and immunosuppressive microenvironment

TP53 Deficiency in AML Confers Resistance to CAR T-Cells



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

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