Editing of CD33 to facilitate anti-CD33 CAR T cell therapy for AML

Saar Gill, MD PhD
University of Pennsylvania

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Disclosures

Research Funding: Novartis, Tmunity Therapeutics, Carisma Therapeutics

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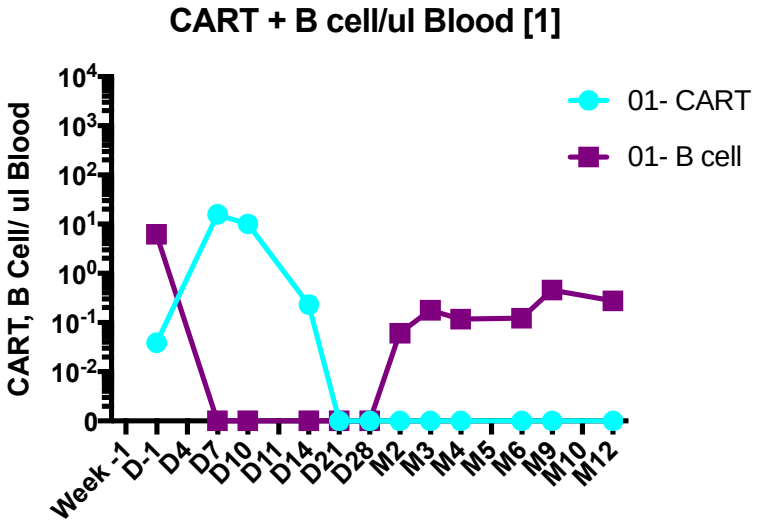
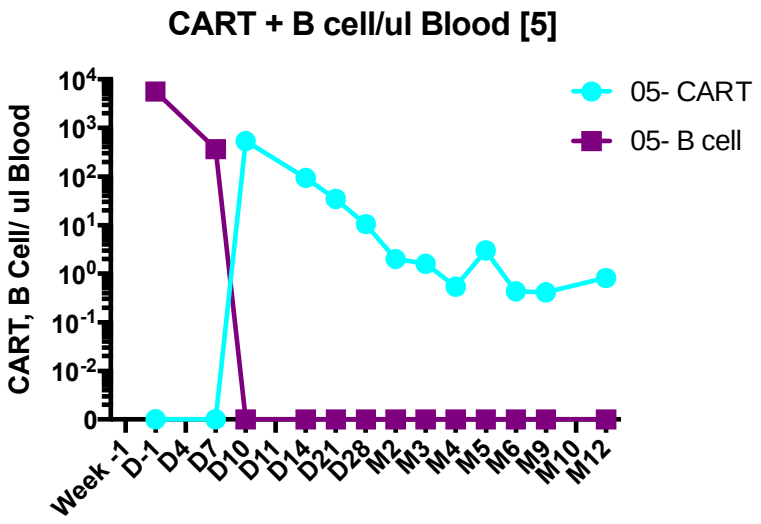
Consulting: Aileron, Amphivena, Aileron, Fate, Sensei

B cell aplasia is a surrogate for CART cell functional persistence





Gill et al, ASH 2018

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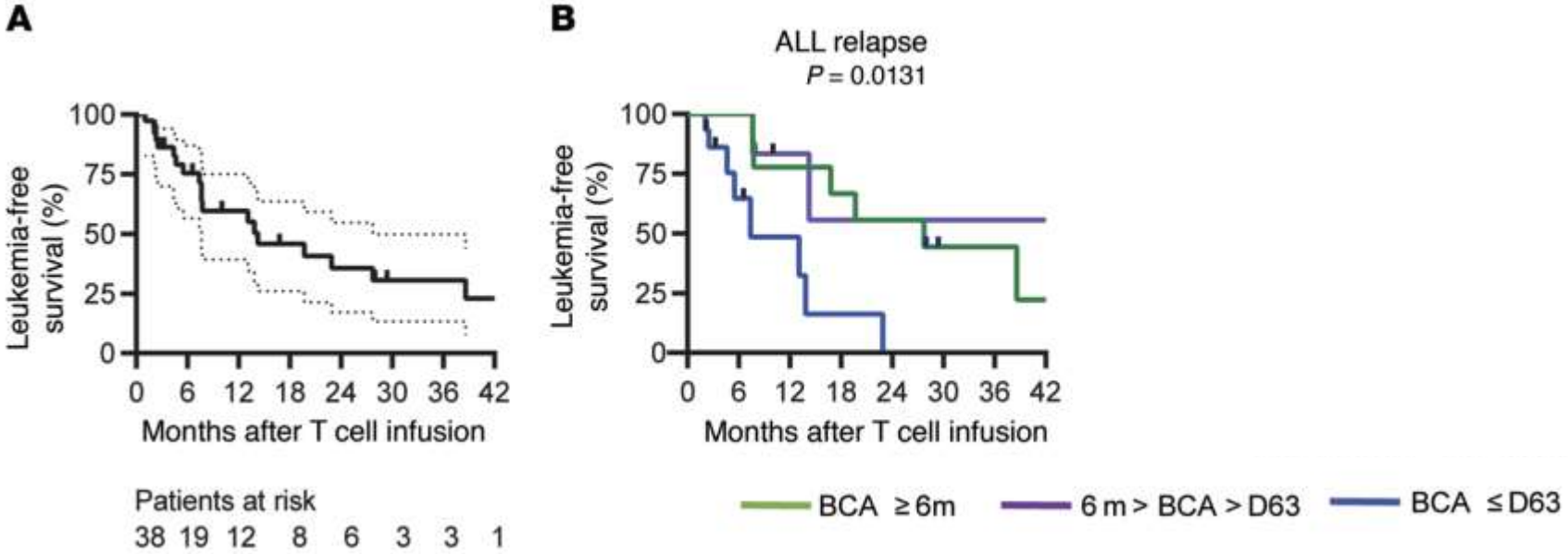
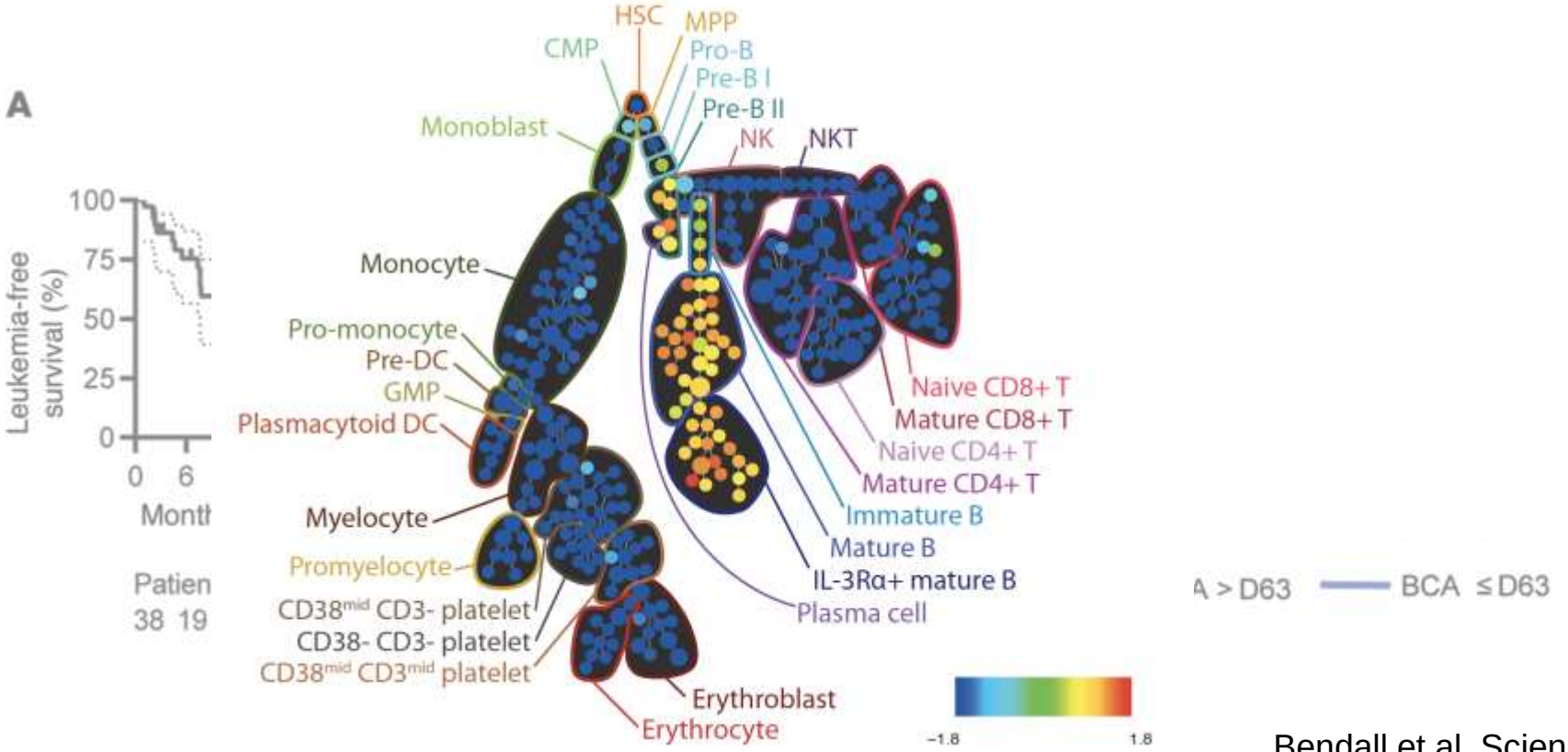


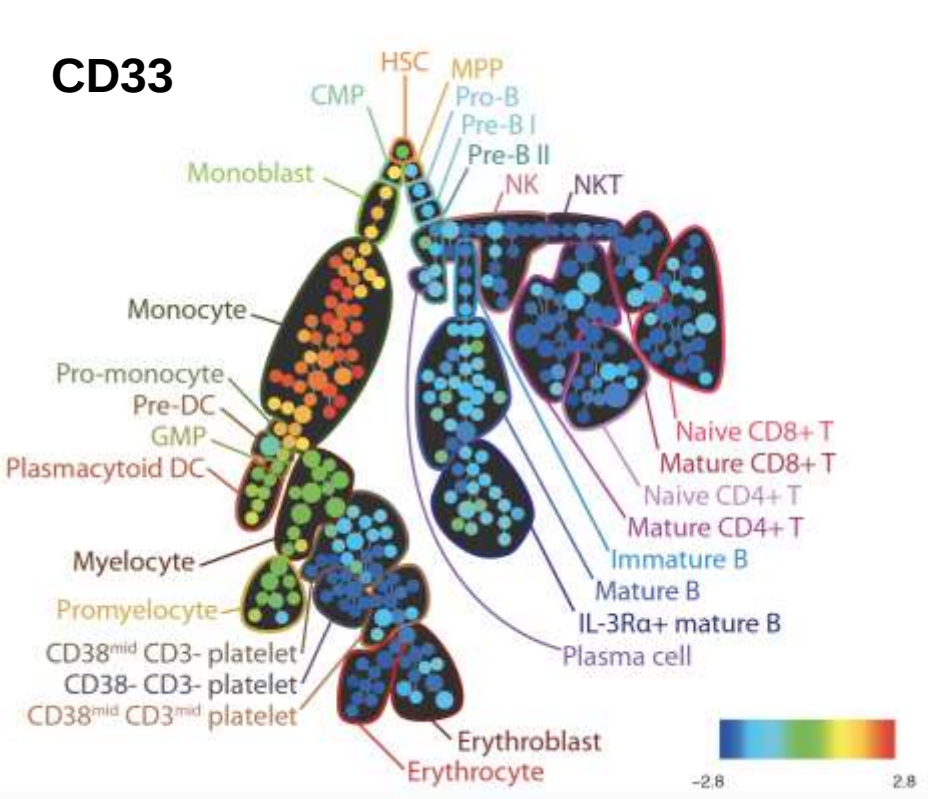
Finney et al, JCI 2019

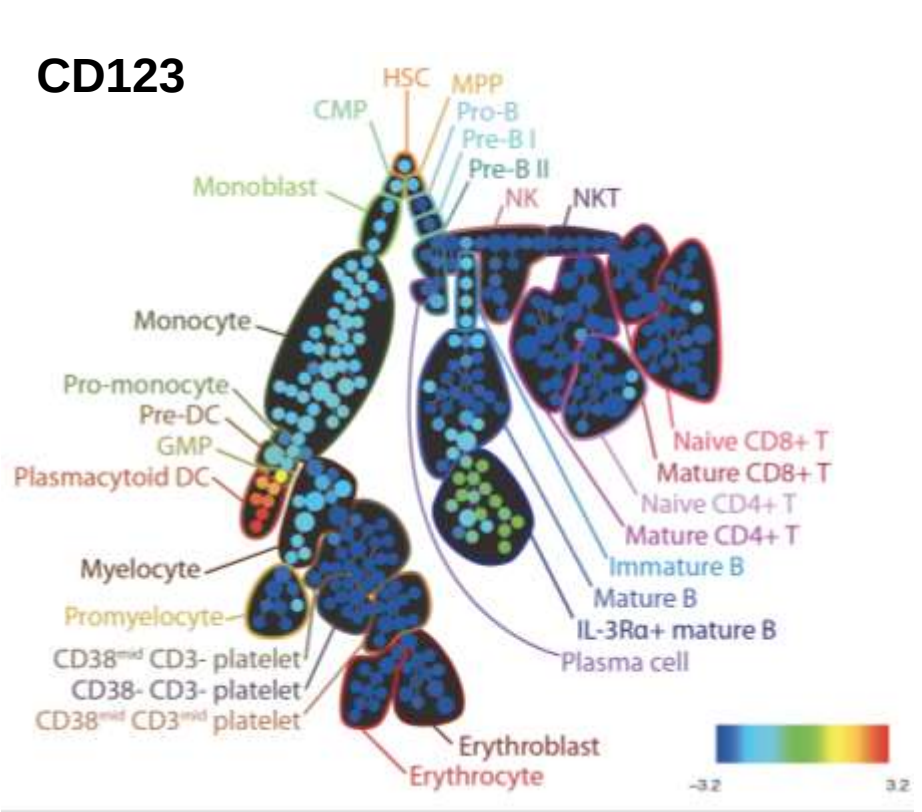
B cell aplasia is a surrogate for CART cell functional persistence



Bendall et al, Science 2011

CD33 and CD123 expression in healthy human marrow





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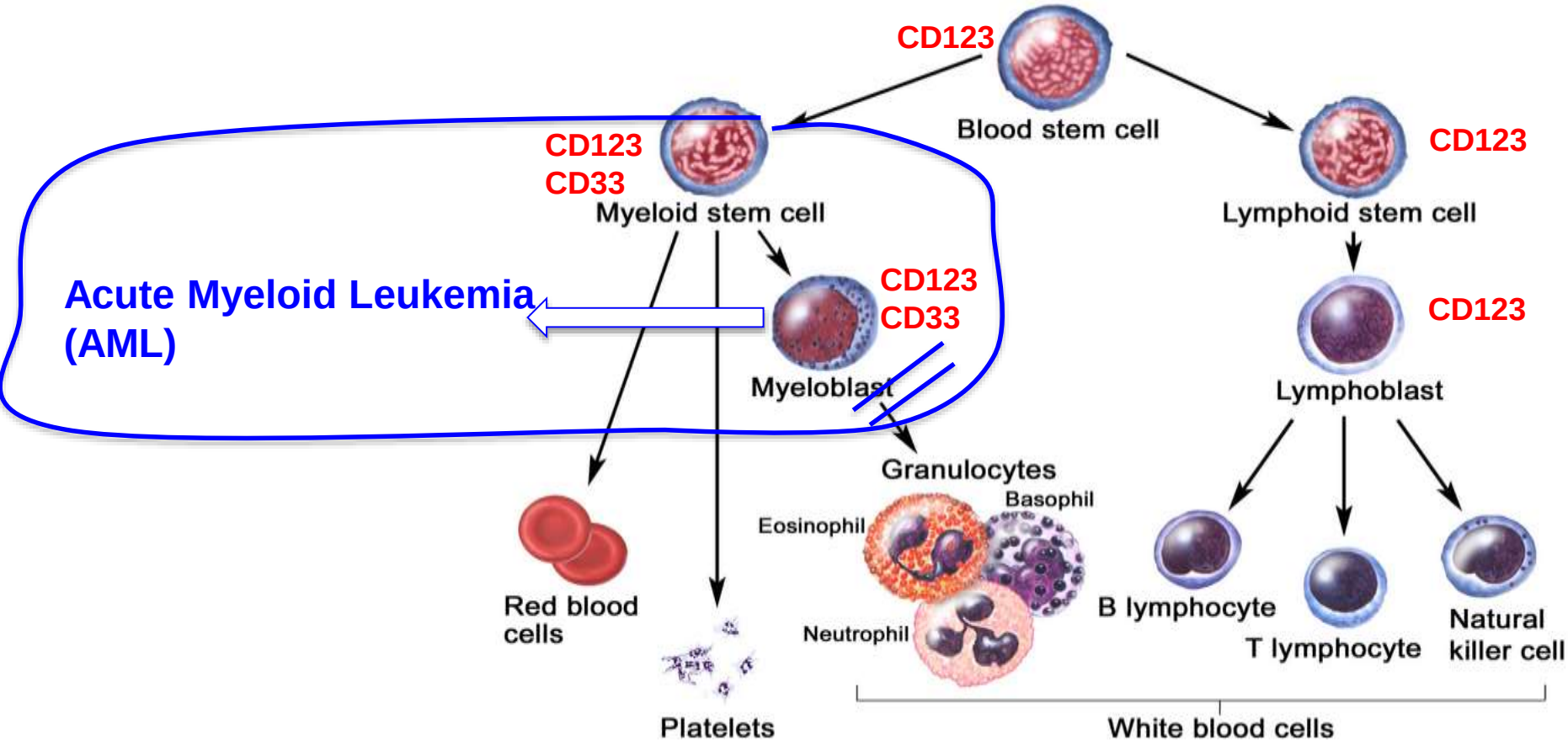
CAR T cell therapy of AML



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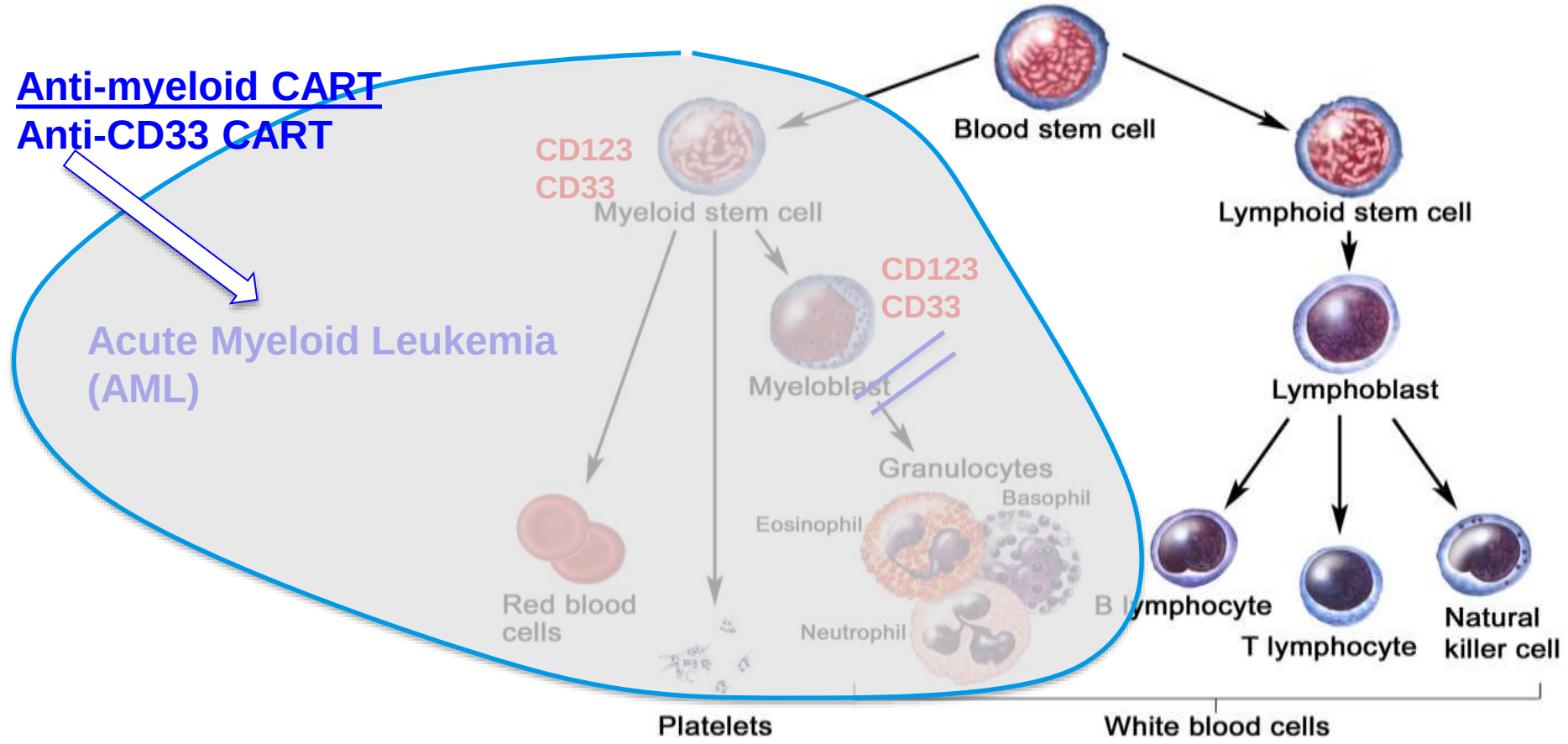
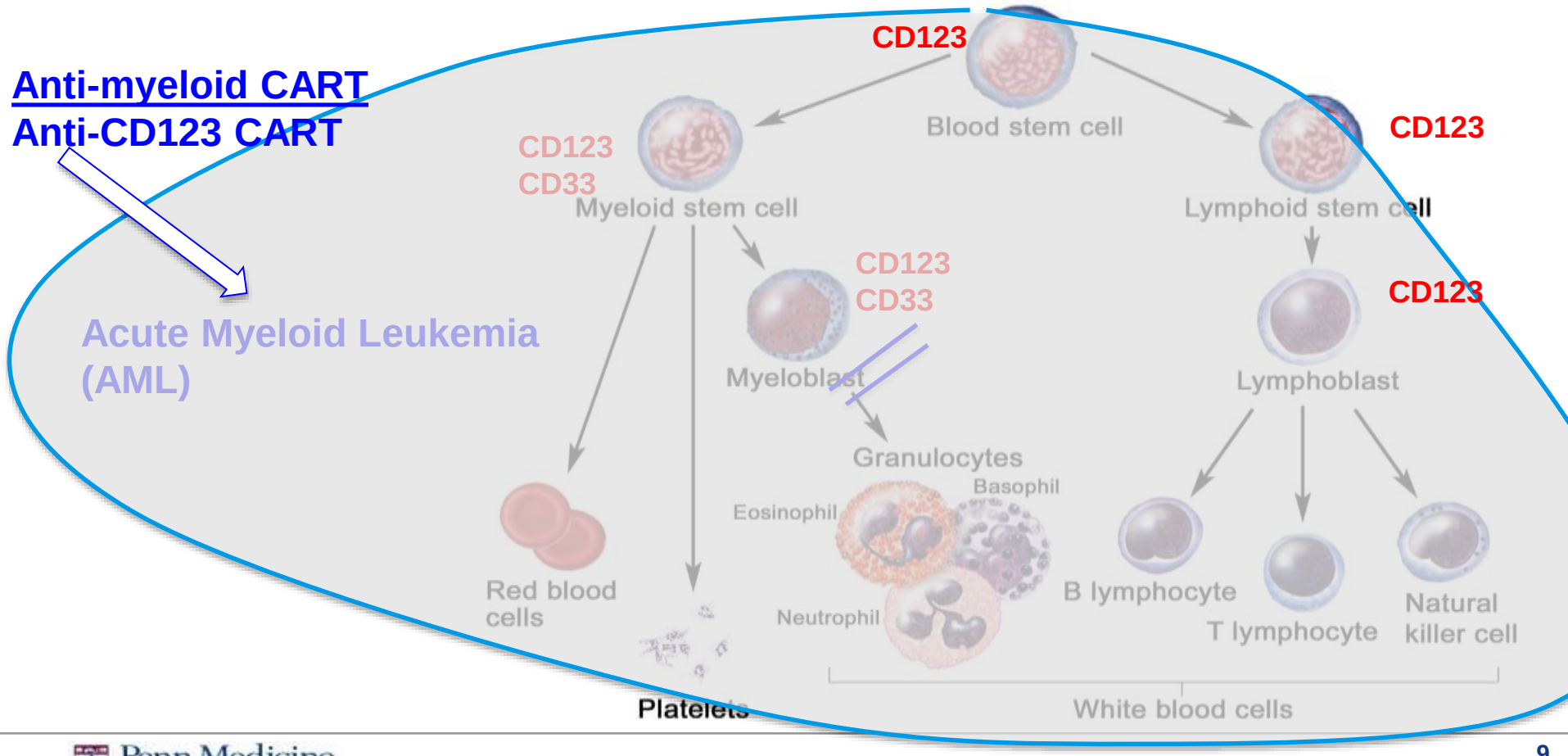
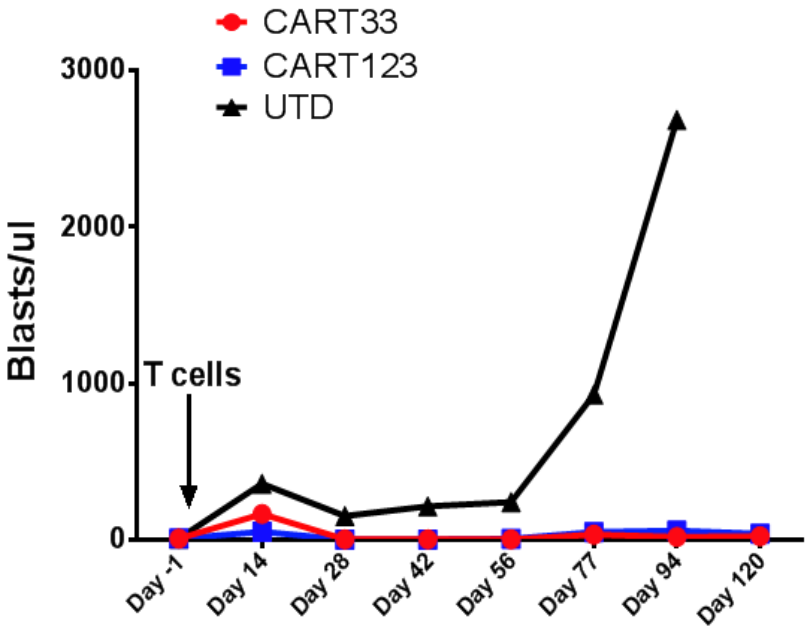


CAR T cell therapy of AML

Anti-myeloid CART
Anti-CD123 CART

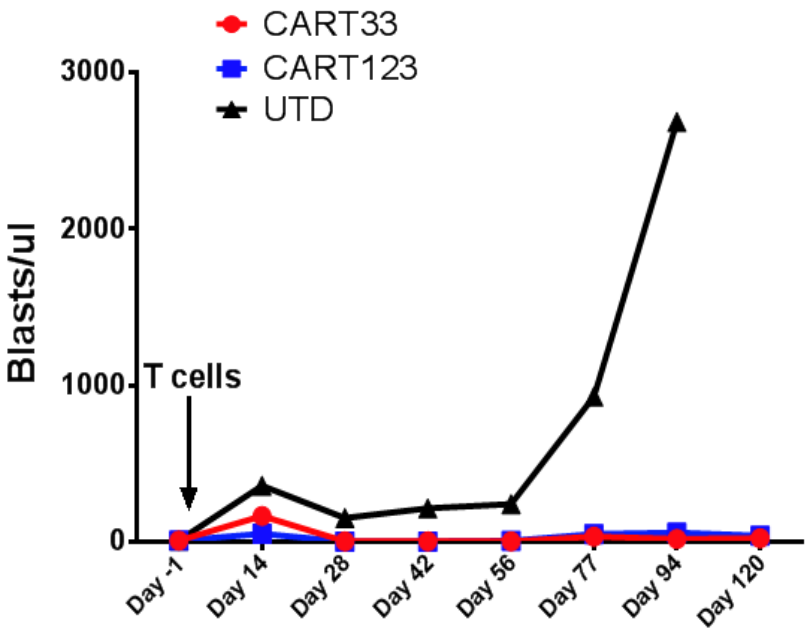


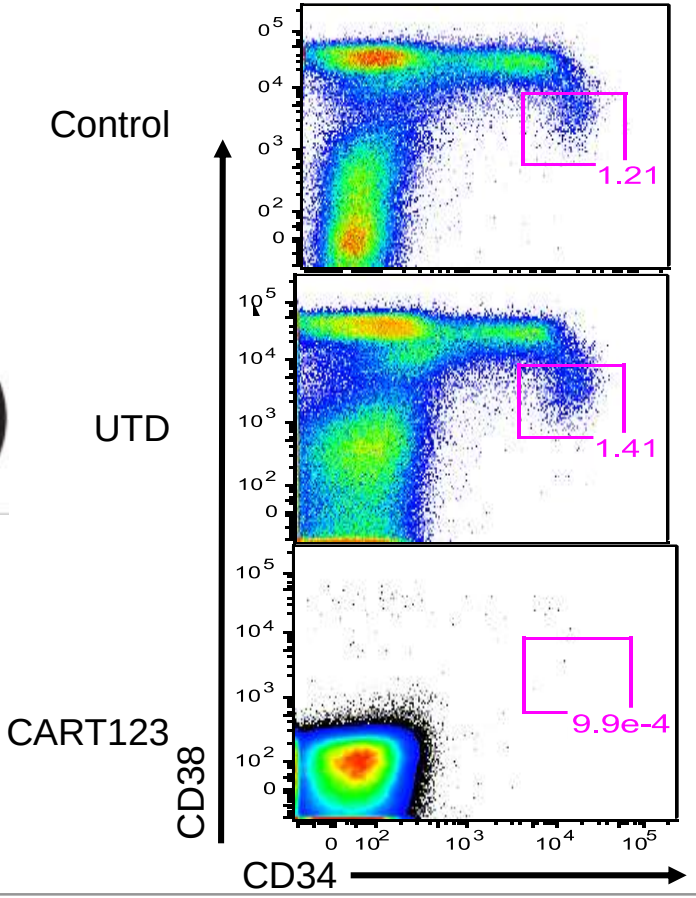
Anti-myeloid CART cells eradicate AML / hematopoiesis



Gill et al Blood 2014; Kenderian et al, Leukemia 2015

Anti-myeloid CART cells eradicate AML / hematopoiesis





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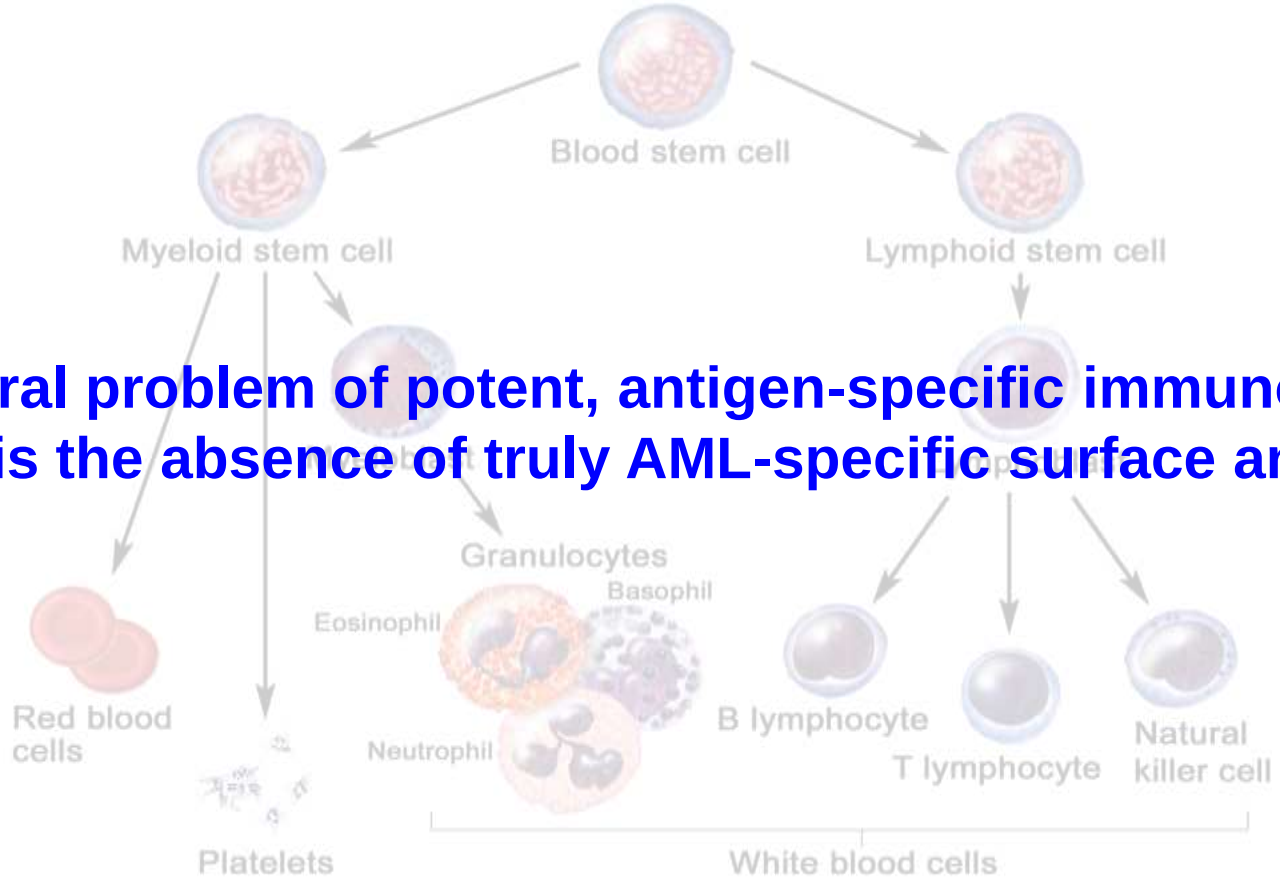
CAR T cell therapy of AML



The central problem of potent, antigen-specific immunotherapy for AML is the absence of truly AML-specific surface antigens

Solution 1: AML CART with attenuated activity

- Relies on differential expression of the target between normal and malignant cells[1]
  - This is not the case for most myeloid antigens
  - Opens the way to antigen-dim relapse[2]

1. Arcangeli et al, Molecular Therapy 2017

2. Fry et al, Nature Medicine 2018

Current clinical trials in AML

- Budde **CD123** 2017, 2018: 1 MRD+ CR, 1 CRi, 1 MLFS, 2 SD, 1 PD
- Ritchie **LeY** 2013 0/4 response
- Wang **CD33** 2014 case report
- Chang **CD123** 2015 case report
- Cummins **CD123** 2017 0/5 response
- Baumeister **NKG2DL** 2019 0/7 response

To date, anti-AML CAR have shown limited activity

➢ Strategies to *reduce activity* in order to enhance safety are not likely to work.

Solution 2: AML CART as a novel conditioning regimen

- Anti-myeloid CAR T cells
- Transient or depletable
- AlloHCT to reconstitute hematopoiesis

Transient or depletable CART cells

901 First-in-Human CLL1-CD33 Compound CAR T Cell Therapy Induces Complete Remission in Patients with Refractory Acute Myeloid Leukemia: Update on Phase 1 Clinical Trial

Program: Oral and Poster Abstracts

Type: Oral

Session: 616. Acute Myeloid Leukemia: Novel Therapy, excluding Transplantation: Immunotherapy

Hematology Disease Topics & Pathways:

Biological, Therapies, bone marrow, CAR-Ts, immunotherapy, Clinically relevant, transplantation, stem cells

Monday, December 3, 2018: 4:30 PM

Seaport Ballroom F (Manchester Grand Hyatt San Diego)

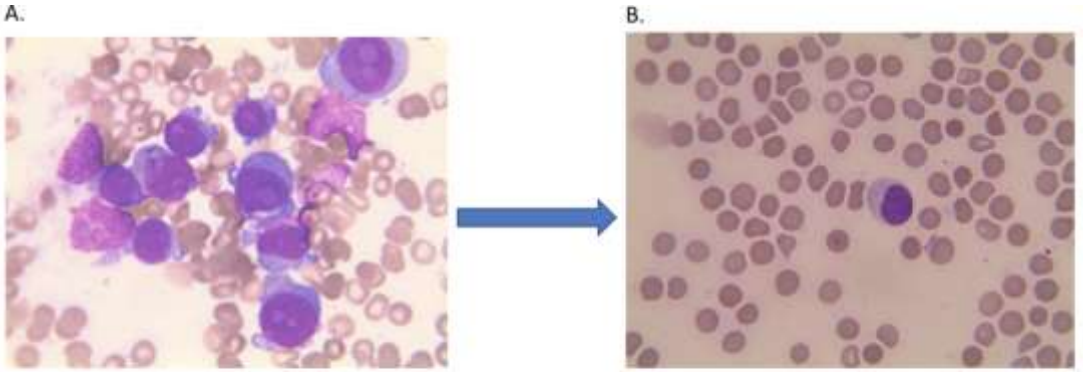


Figure 1. Patient treated with cCAR achieved complete remission. A. 12 days post cCAR infusion, leukemia blasts comprised 98% of the bone marrow. **B.** 19 days post cCAR infusion, total myeloid ablation had taken place in patient's bone marrow with only CAR T cells existing. Results were confirmed by flow cytometry showing the absence of blasts. Sternal bone marrow aspiration also showed similar findings.

- 2 pts with RR-AML, received FluCy → 2 infusions of $1x10^6$/kg CART-33/CLL1
- Both attained MRD-ve remission within 3 weeks, a/w myeloablation → rescue alloSCT

Liu et al, ASH 2018

Transient or depletable CART cells

Disease and key inclusion/ exclusion criteria	Location	Trial number	Intervention	Strategy to mitigate potential adverse events including myeloablation
RR AML - **>18 yo** - **alloHSCT eligible with stem cell source identified** - **if relapsed post prior alloHSCT, must be off immune suppression and have no active GvHD (>Gr II)**	The University of Pennsylvania, Pennsylvania, USA	NCT03766126	Autologous lentivirally transduced anti CD123 CAR T-cells (CD123CAR-41BB-CD3ζ)	- Fractionated dosing of CART-123 - Patient must have a suitably matched donor or stem cell source available, alloHSCT expected to be required in responding patients
RR AML or relapsed BPDCN - **>12 yo** - **alloHSCT eligible with stem cell source identified** - **if relapsed post prior alloHSCT, must be off immune suppression**	City of Hope Medical Center, California, USA	NCT02159495	Autologous lentivirally transduced anti CD123 CAR T-cells (CD123CAR-CD28-CD3ζ-EGFRt)	- EGFRt in CAR construct allows for in vivo eradication of CAR T-cell population if needed with anti-EGFR mAb - Patient must have a suitably matched donor or stem cell source available
RR AML or ELN adverse AML in up-front treatment - **18-65 yo** - **if relapsed post prior alloHSCT, must be off immune suppression for 6 wks, and have no evidence of GvHD** - **CD123(+) blasts by standard flow cytometry**	MD Anderson Cancer Center, Texas, USA	NCT03190278	Universal (TCR KO) allogeneic anti CD123 CAR T-cells (UCART123)	- TCR KO to reduce risk of GvHD from allogeneic CAR T-cells - Patient must have a suitably matched donor or stem cell source available
RR AML - **Pediatric 1-18yo** - **Adult >18-80yo** - **if relapsed post prior alloHSCT, must be at least 3mo post alloHSCT, be off immune suppression, and have no evidence of GvHD**	MD Anderson Cancer Center, Texas, USA	NCT03126864	Autologous lentivirally transduced anti CD33CAR T-cells	- Incremental dosing of CART-33 (starting dose in both cohorts is >1.5 x10^5/kg and <4.5 x10^5/kg)

Transient or depletable CART cells: *Loss of CART-mediated immunosurveillance*

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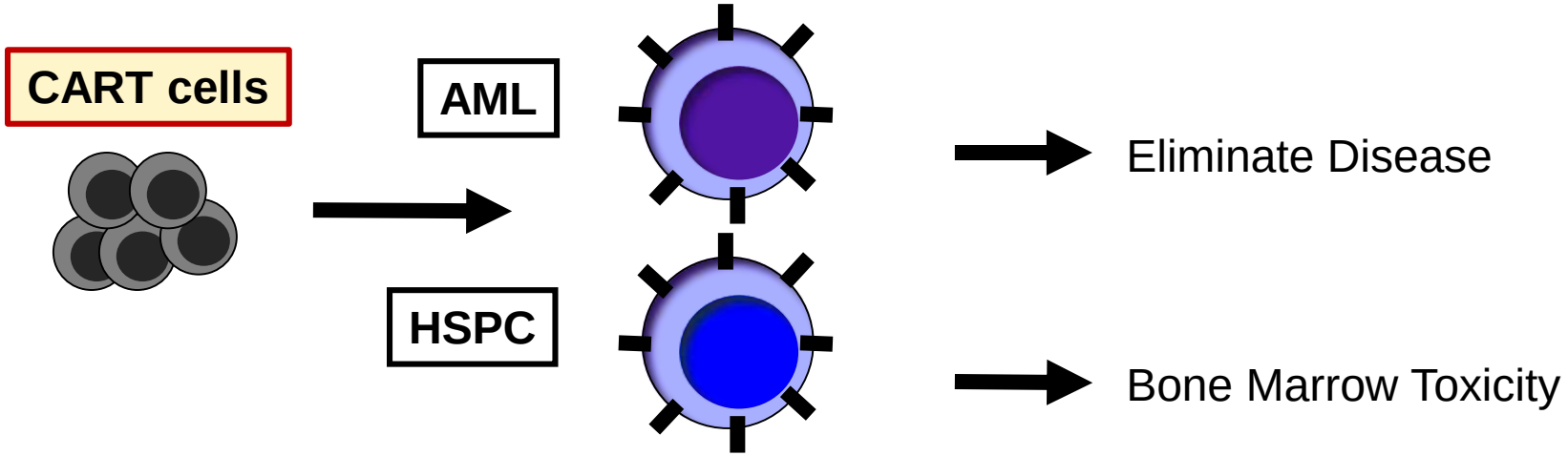
Solution 3:

- Anti-myeloid CART cells
- Make hematopoiesis resistant to CART
- (synthesize a leukemia-specific antigen)

Antigen-Specific Immunotherapy for AML



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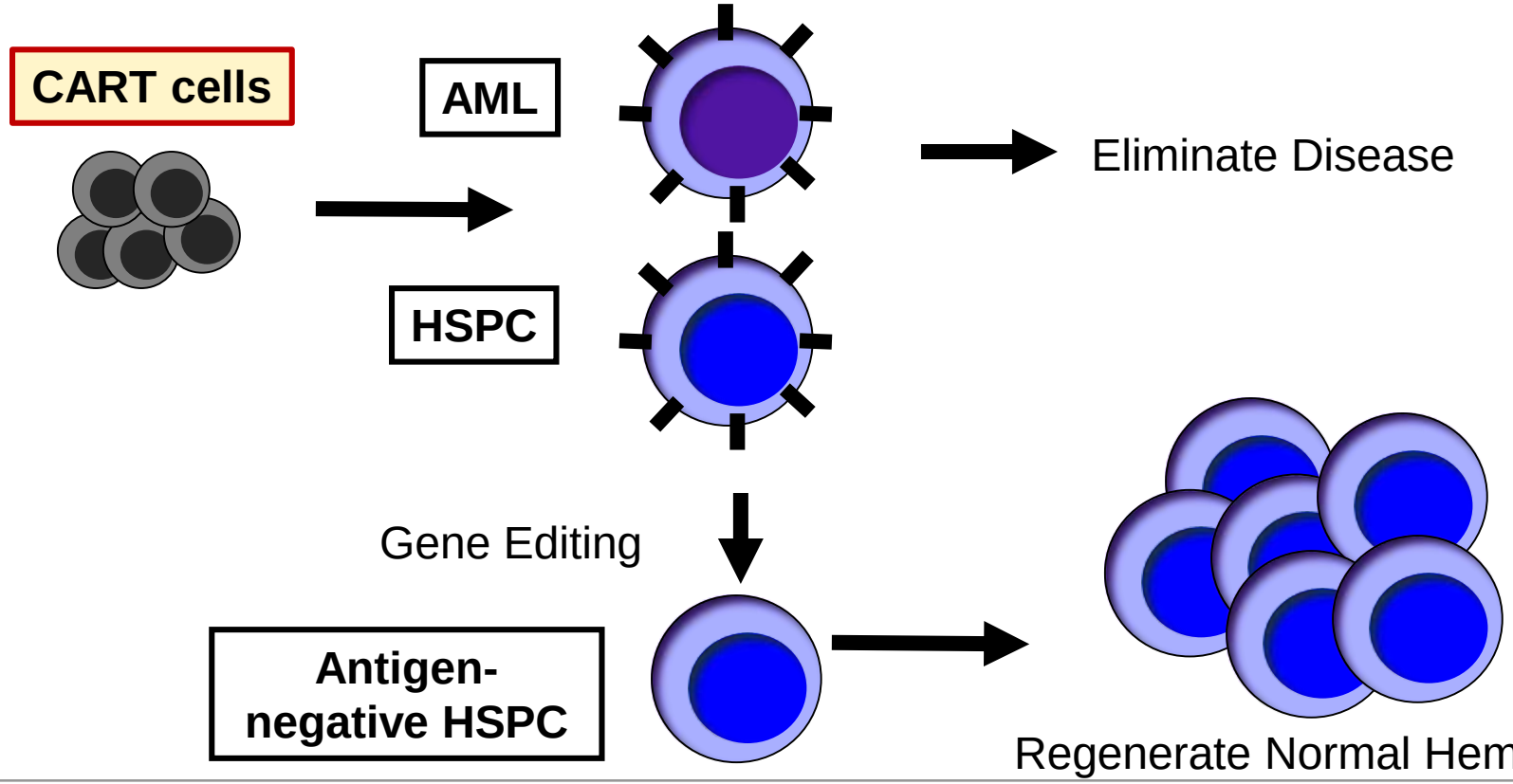

Questions

- Can we remove CD33 from normal HSPCs without impairing their ability to self-renew and differentiate?
- Will CD33 KO myeloid cells retain normal function?
- Will CD33 KO HSPCs be resistant to CD33-targeted therapy?

Kim, Yu et al, Cell 2018

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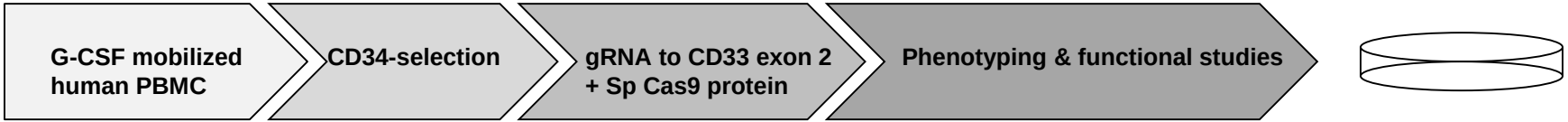
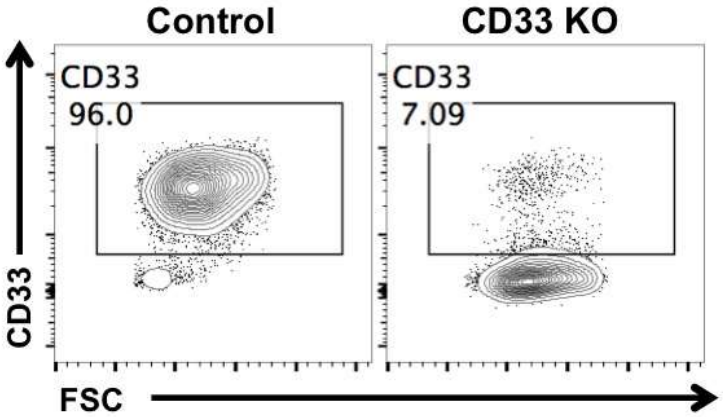
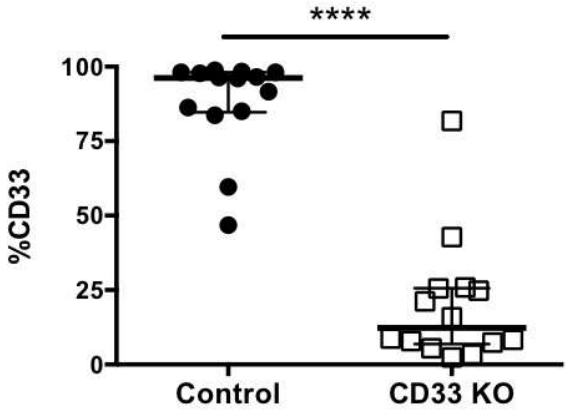
CD33 can be removed from human HSPCs without impairing growth or differentiation







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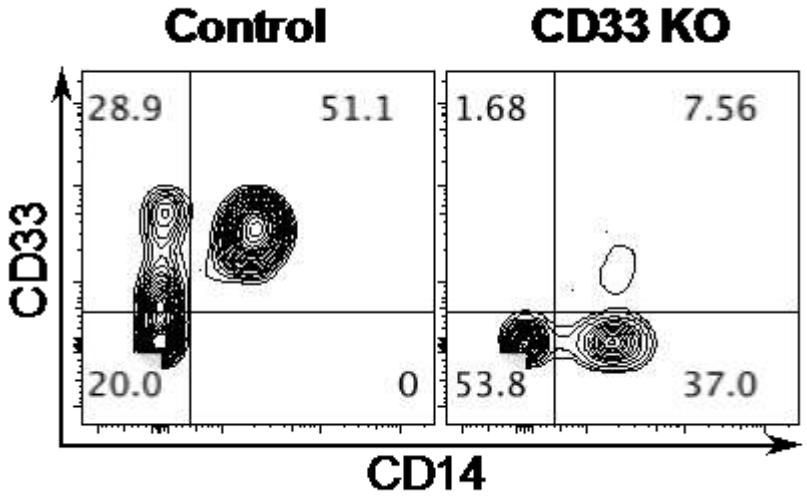

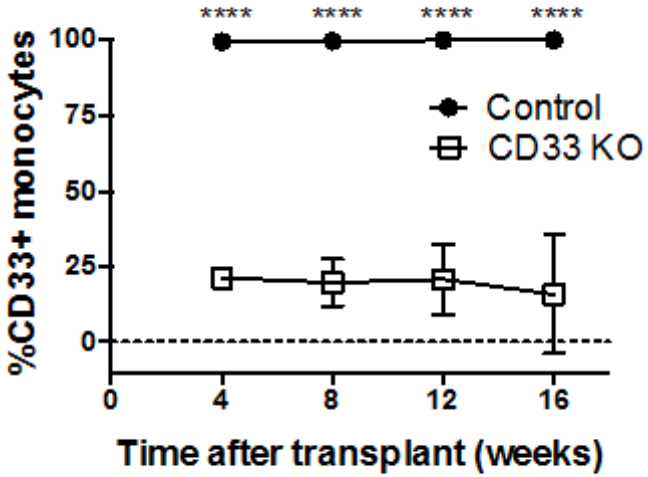

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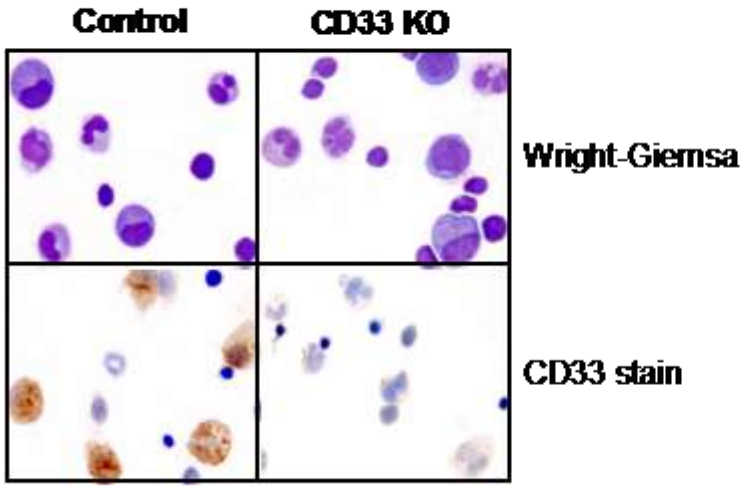
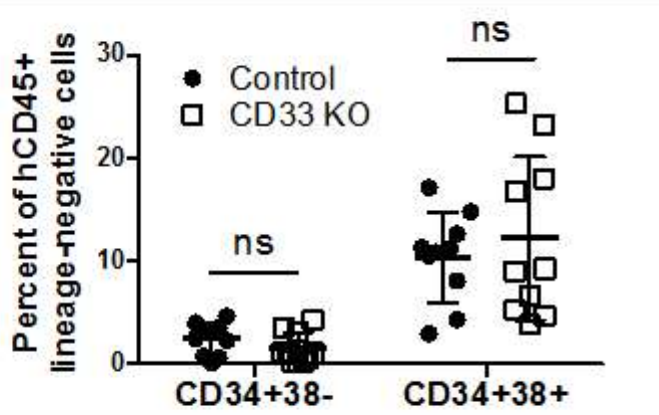
D.



E.



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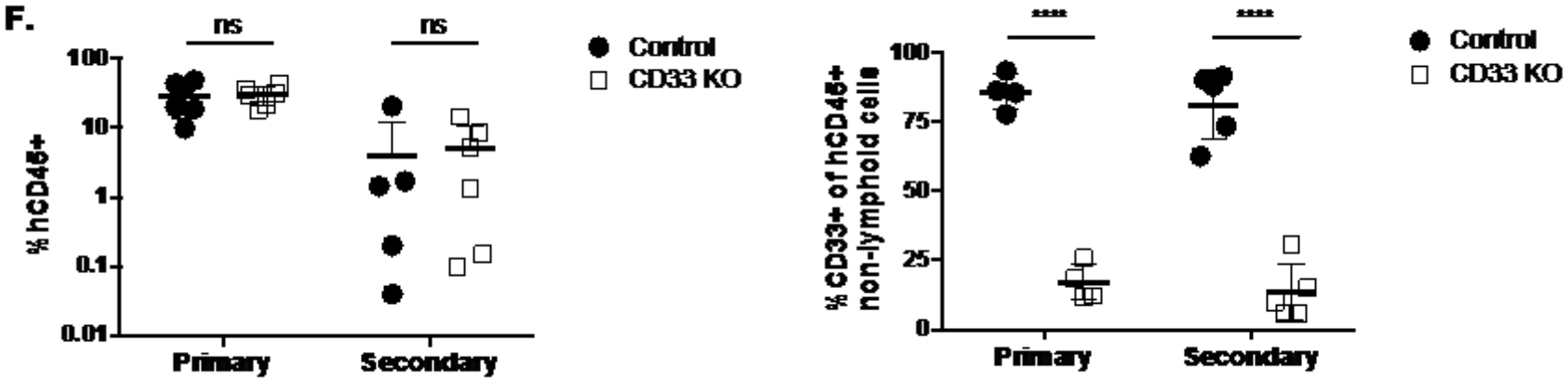

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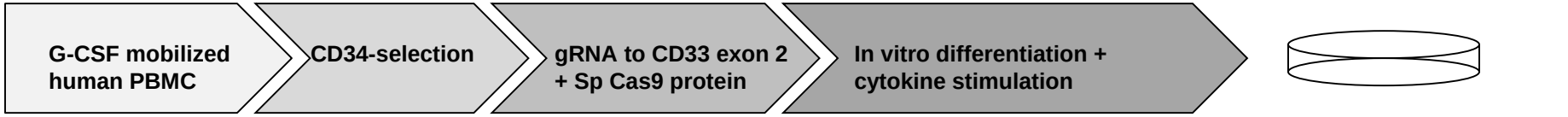
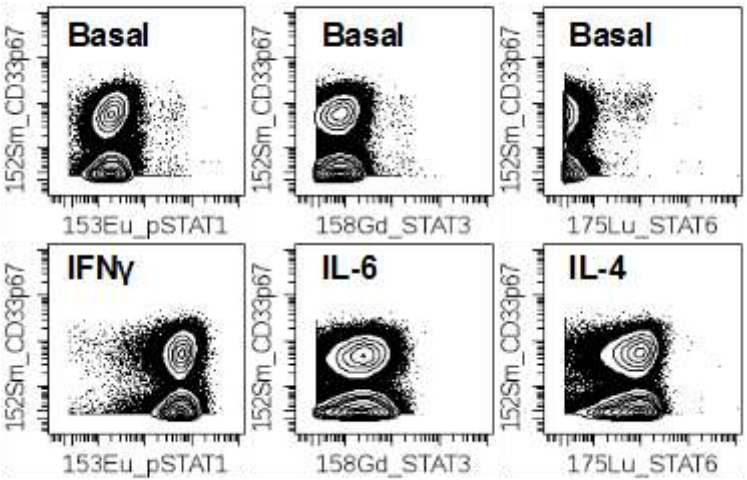
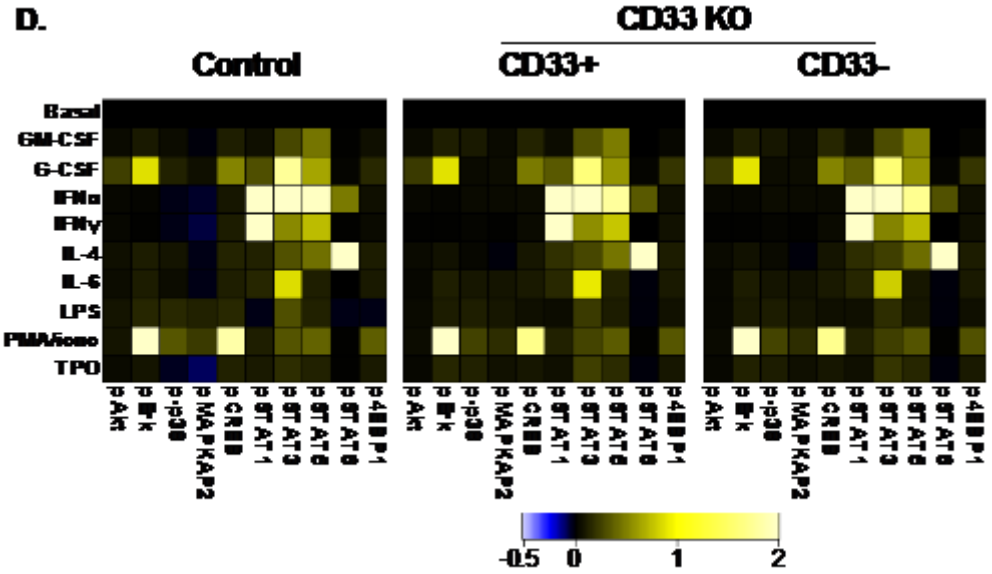
Loss of CD33 does not impair myeloid cell function: signaling



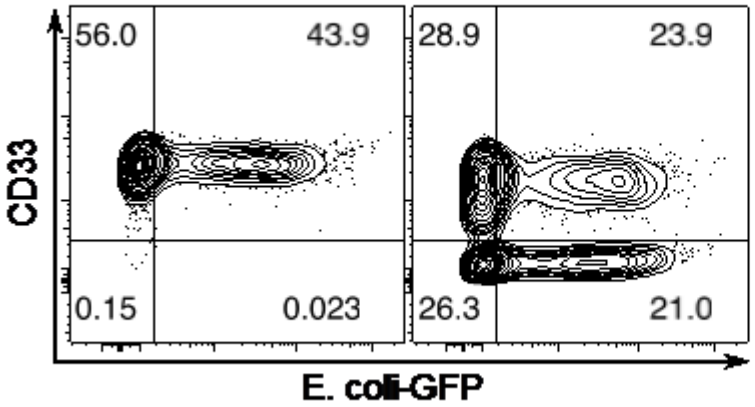


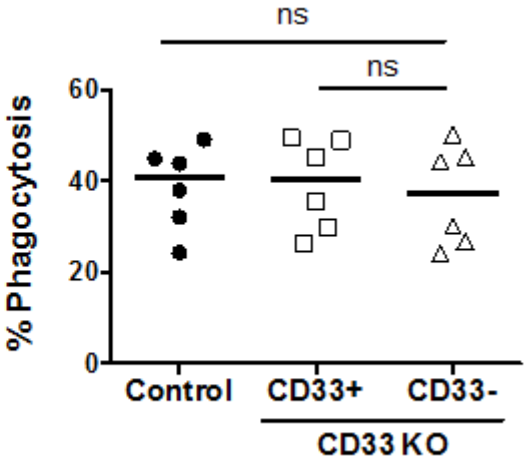


With G Behbehani, OSU

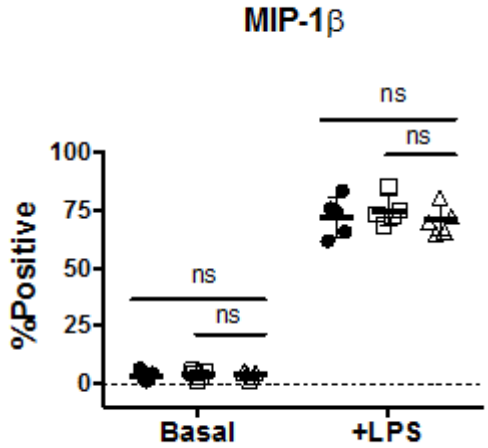
Loss of CD33 does not impair myeloid cell function: phagocytosis

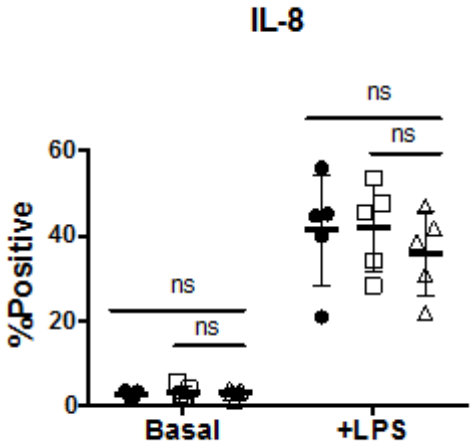
G-CSF mobilized human PBMC → CD34-selection → gRNA to CD33 exon 2 + Sp Cas9 protein → In vitro differentiation + phagocytosis

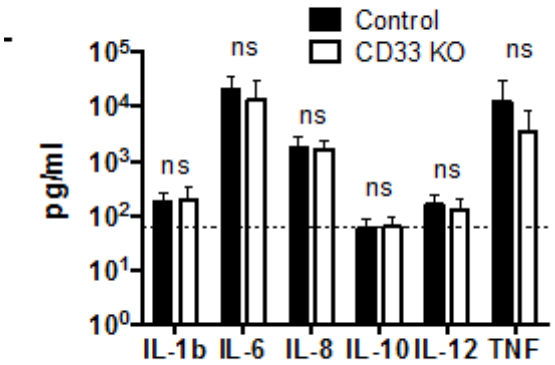




Loss of CD33 does not impair myeloid cell function: response to infection







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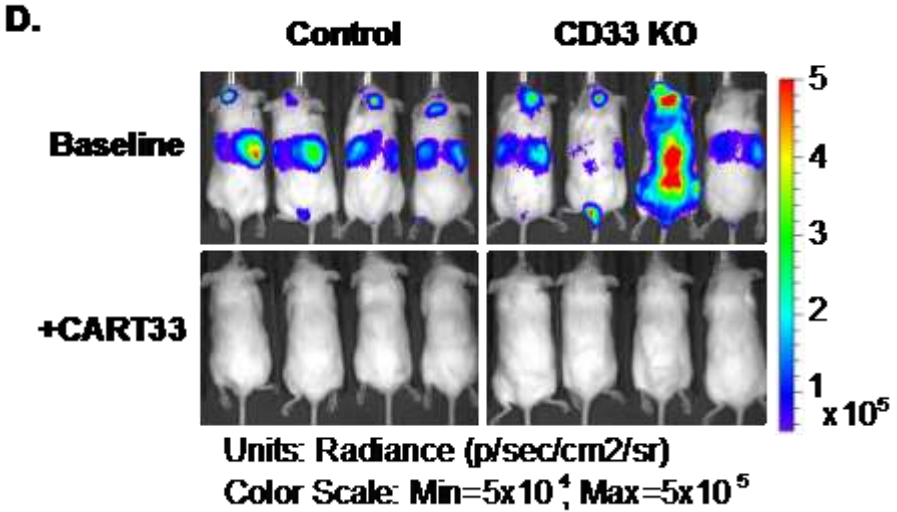
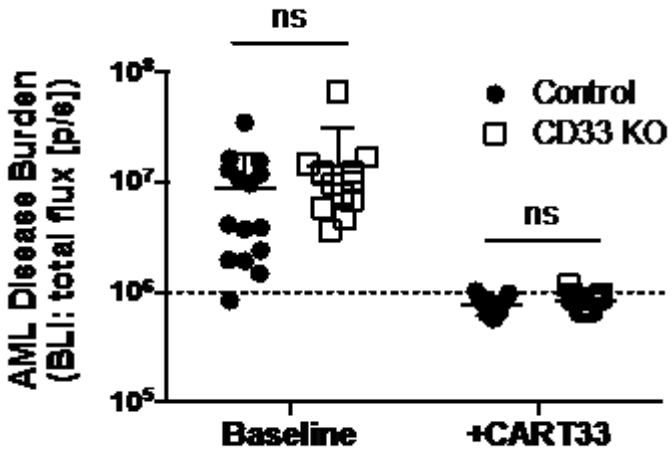
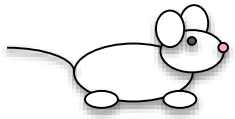
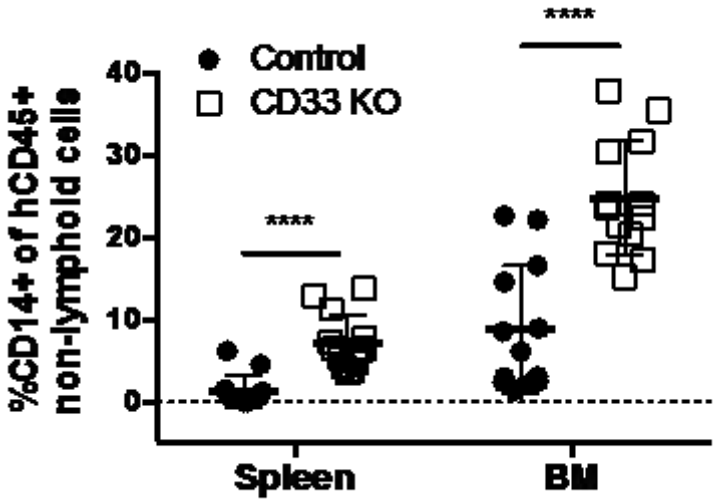


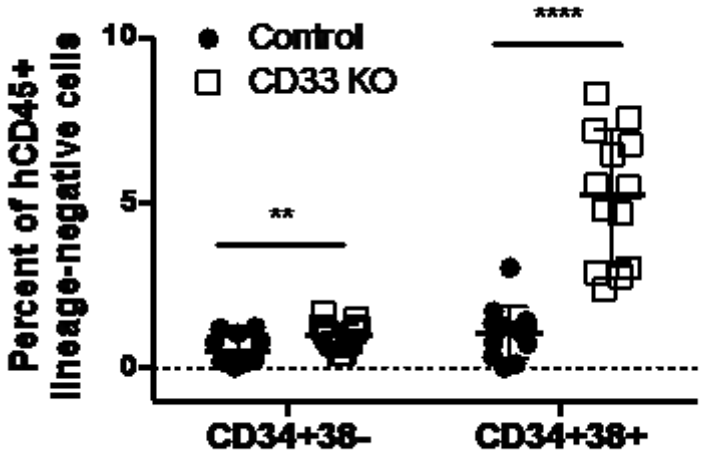


CD33KO HSPC are resistant to anti-CD33 CART cells

G-CSF mobilized human PBMC → CD34-selection → gRNA to CD33 exon 2 + Sp Cas9 protein → HSC Engraftment followed by AML engraftment, then: CART-33 challenge

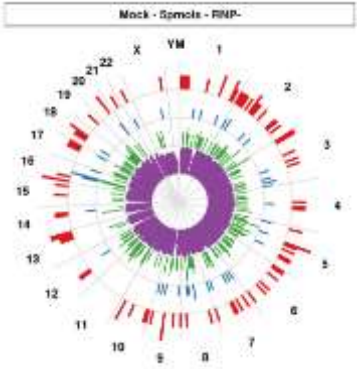






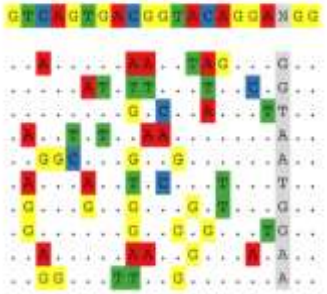
Unbiased off-target prediction in edited human CD34 (iGUIDEseq)

Mock
(RNP-)



Mock
(RNP-)

Mock - 10pmol - RNP-
gRNA: CD33



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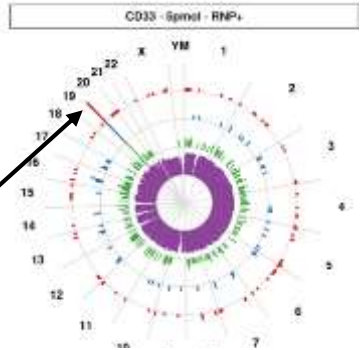
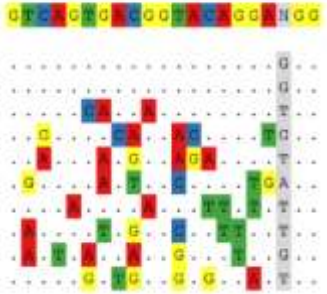
6	Off	2	0	CALCB
4	Off	1	0	RMND5B-
5	Off	1	0	SPAG17*
5	Off	1	0	MMEL1*
5	Off	1	0	ILDR2*
5	Off	1	0	HHAT*
5	Off	1	0	MIR4454
5	Off	1	0	AK2
5	Off	1	0	RSPN

CD33KO
(RNP+)



CD33KO
(RNP+)

CD33 - 10pmol - RNP+
gRNA: CD33



mismatch	target	aligns	MESL	gene_id
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0	On	318	94.3	MIR8074
3	Off	2	94.3	NCOA2*-
5	Off	2	30.2	PRR7*
6	Off	2	27.5	RBFOX1
6	Off	2	27.5	NUP35
5	Off	1	94.3	APC*-
6	Off	1	76.1	MIR8070
6	Off	1	76.1	CYP2F1
6	Off	1	76.1	AAK1*

All Align. Pileup Align. Flanking Pairs gRNA Matched

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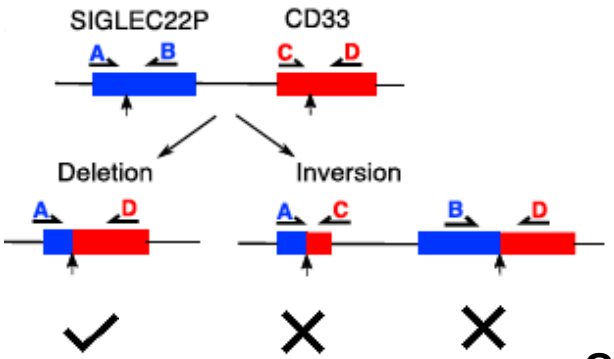
Unbiased off-target prediction in edited human CD34 (iGUIDEseq)



Mock
(RNP-)

CD33KO
(RNP+)

Mock - 10pmol - RNP-
gRNA: CD33

mismatch	target	aligns	MESL	gene_id
6	Off	3	0	TUBA1C
6	Off	2	0	CALCB
4	Off	1	0	RMND5B-
5	Off	1	0	SPAG17*
5	Off	1	0	MMEL1*
5	Off	1	0	ILDR2*
5	Off	1	0	HHAT*
5	Off	1	0	MIR4454
5	Off	1	0	AK2
5	Off	1	0	RSPN

CD33 - 10pmol - RNP+
gRNA: CD33

mismatch	target	aligns	MESL	gene_id
0	On	387	94.3	CD33* ⟸
0	On	318	94.3	MIR8074 ⟸
3	Off	2	94.3	NCOA2*-
5	Off	2	30.2	PRR7*
6	Off	2	27.5	RBFOX1
6	Off	2	27.5	NUP35
5	Off	1	94.3	APC*-
6	Off	1	76.1	MIR8070
6	Off	1	76.1	CYP2F1
6	Off	1	76.1	AAK1*

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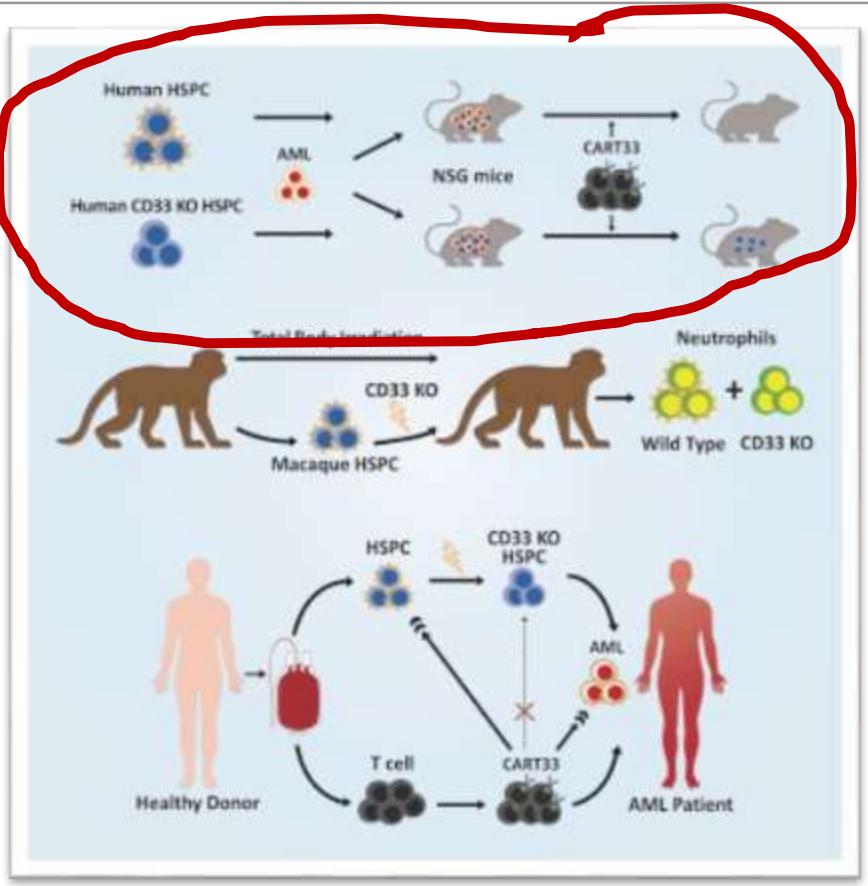
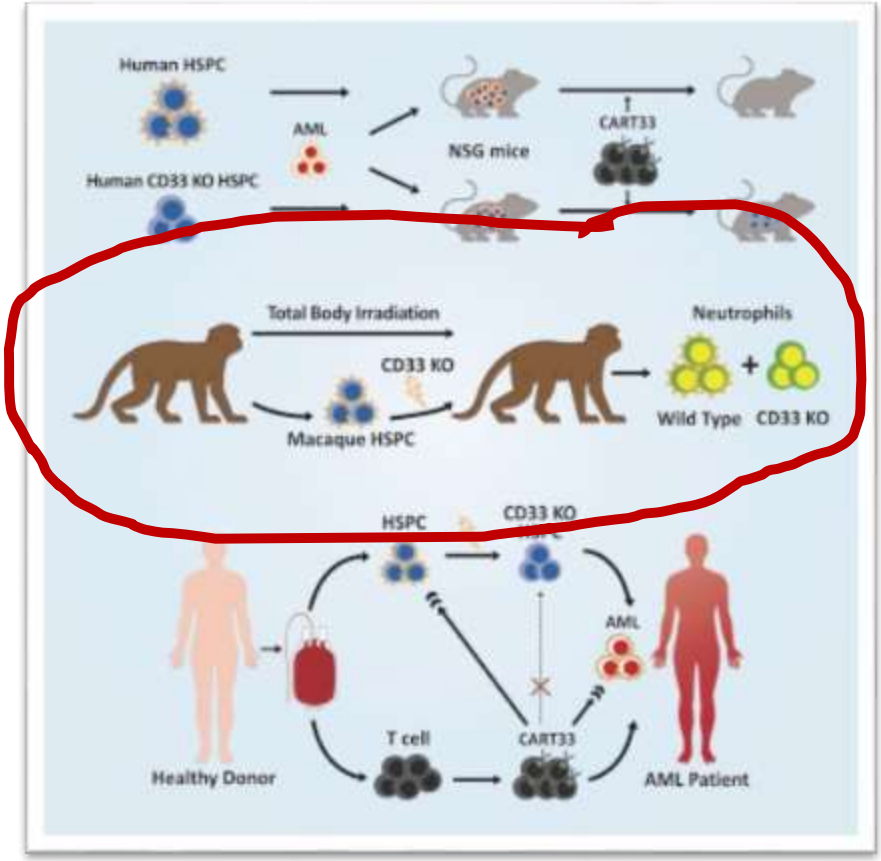
- Human CD34+ differentiate and function
- Human myeloid progeny function normally
- CD33KO myeloid cells are resistant to CART-33



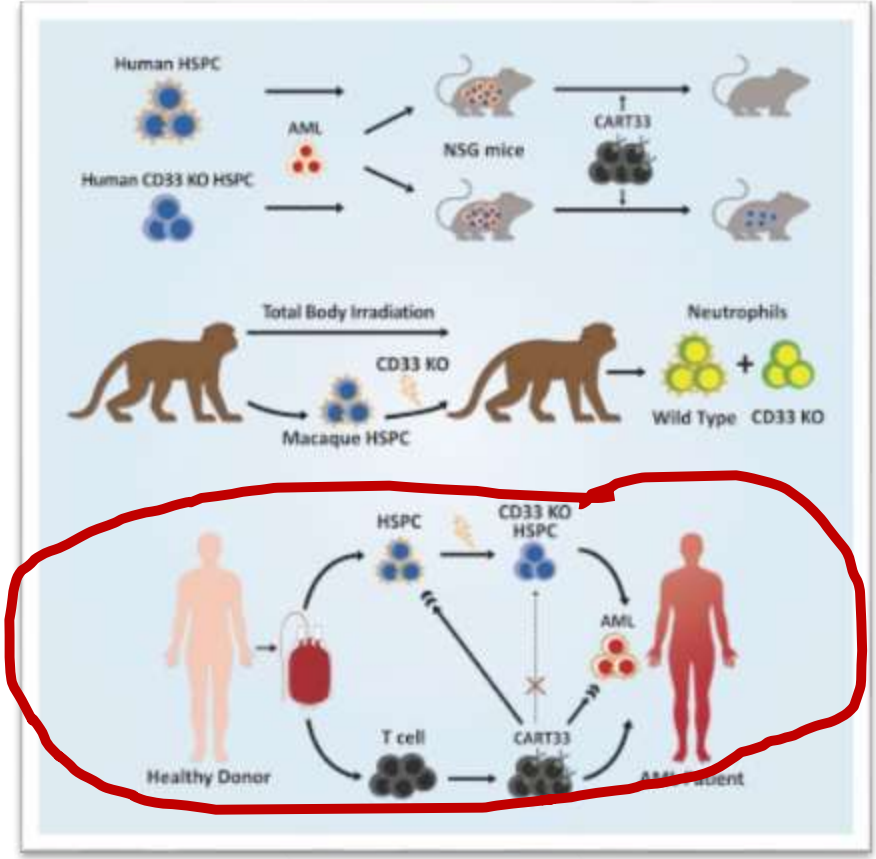
Kim, Yu et al, Cell 2018

- Rhesus macaque engraft normally after CD33KO autoSCT
- Follow-up of up to 2 years
- No leukemogenesis



Kim, Yu et al, Cell 2018

- Towards a clinical trial of allo CD34-selected CD33KO HSCT followed by CART-33 in pts with RR-AML



Kim, Yu et al, Cell 2018

Summary: how close are we to CART cell therapy for AML?

- **There is likely no AML- or LSC-specific surface marker**
- **There is no room for decreasing potency / activity of CART**
- **Myeloablation may be a consequence of potent anti-myeloid CART cell therapy**
- **Two feasible approaches (unless we are very lucky):**
  - Transient/depletable CART as part of pre-transplant conditioning
  - Gene-edited allogeneic *donor* hematopoiesis may allow safe, protracted anti-AML CART cell function (***synthesis of a truly leukemia-specific antigen)***

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