THE UNIVERSITY OF TEXAS
MD Anderson Cancer Center
Making Cancer History®

Next Generation Cellular Therapies for the Treatment of Cancer

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Dutch Hematology congress

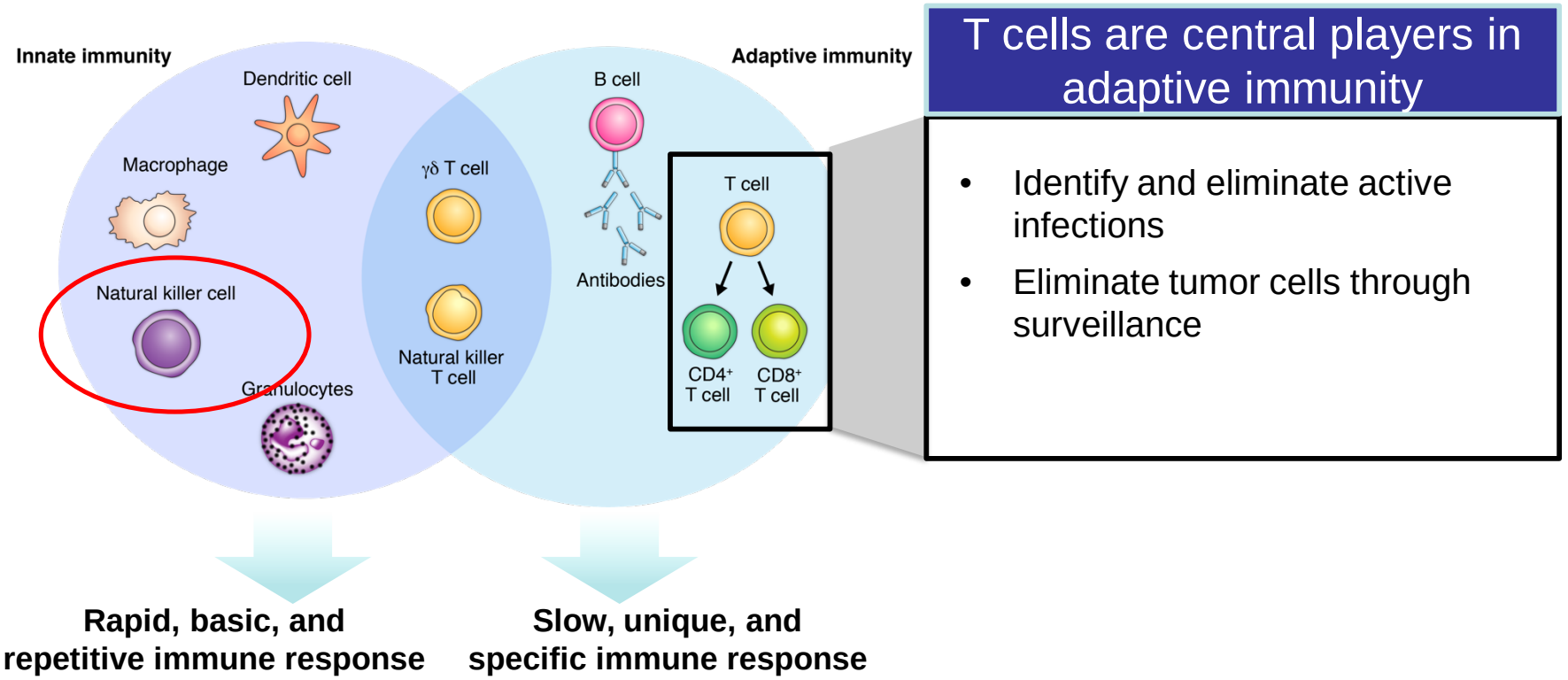
24th January 2019

Disclosures

- Educational grant:
  - Affimed; Pharmacyclics
- Consultancy/SAB
  - EMD Serono; Adicet Bio; Onkimmune; Formula Pharma;

Role of the Immune System in Cancer Control/Eradication

Two distinct forms of immunity[1]



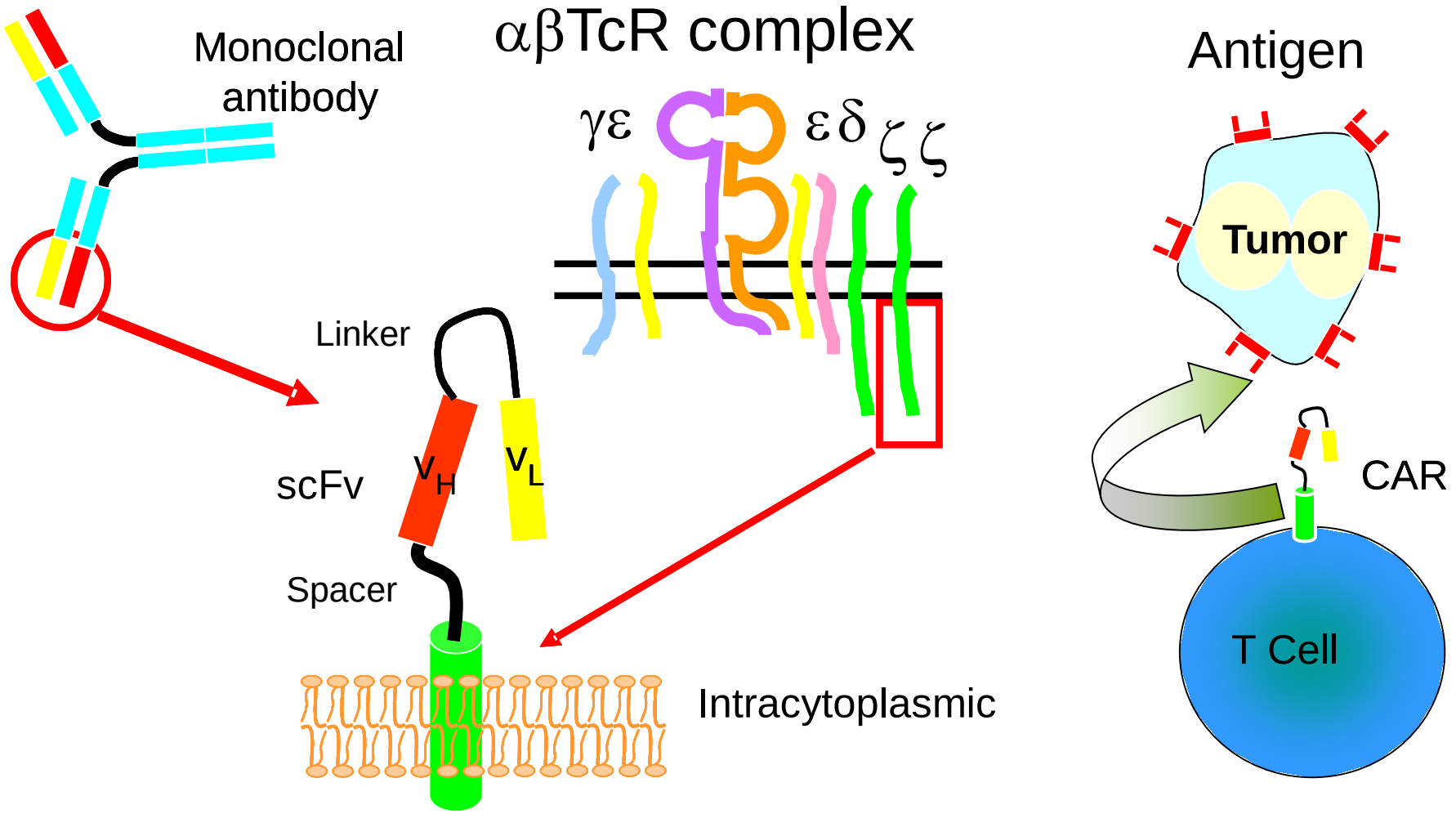
T cells are central players in adaptive immunity

- Identify and eliminate active infections
- Eliminate tumor cells through surveillance

- 1. Sharpe M, Mount N. *Dis Model Mech*. 2015;8:337-350.

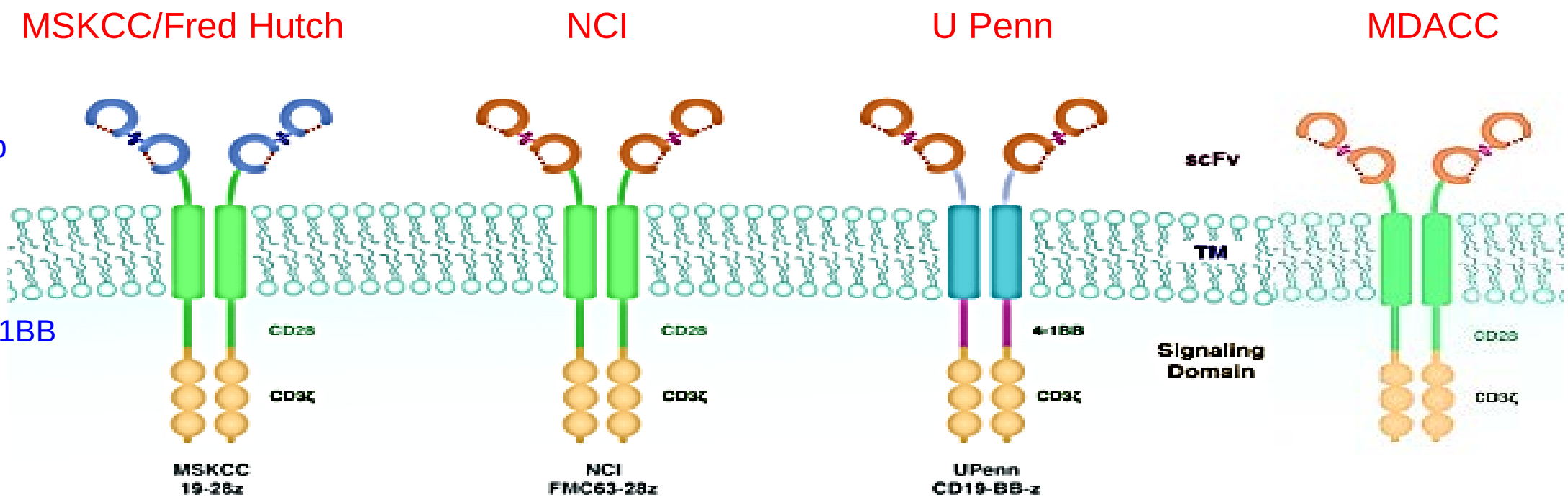
Gene Transfer of CARs

Eshhar et al; PNAS 1993



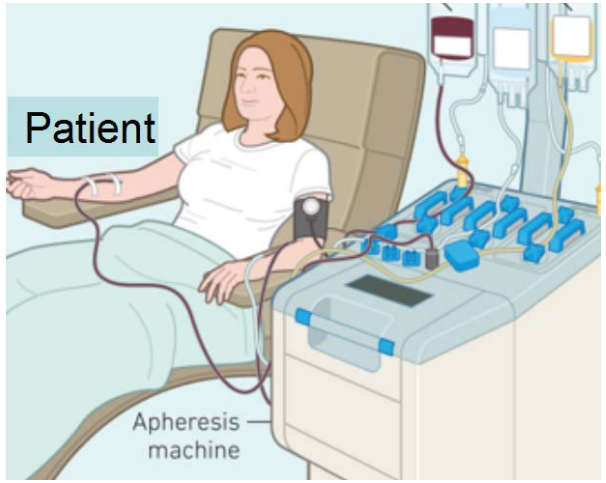
CARs combine an antibody binding domain (scFv) that recognizes a desired tumor associated antigen with one or more T-cell receptor signaling endodomains. Forced expression of CAR on the T-cell surface leads to activation of T cells through CD3ζ CD28 and/or CD137.

2nd generation CD19 CAR T cells in clinic



Gene transfer	Retrovirus	Retrovirus	Lentivirus	Sleeping beauty
	Juno Therapeutics	Kite Pharma	Novartis	Ziopharm
	JCAR	KTE-C19	CTL-019	

Overall Patient Flow and CAR T Cell Therapy Schedule



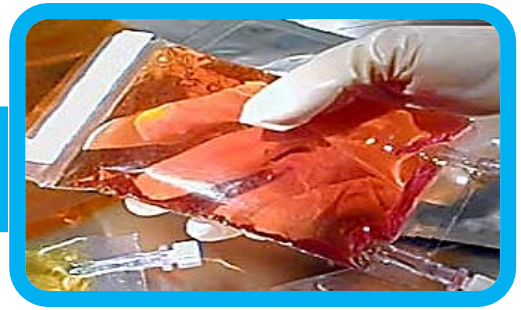
Apheresis Product

To Lab

T cell separation

Cell transfer to bag for growth

Viral transduction



To Clinical Facility

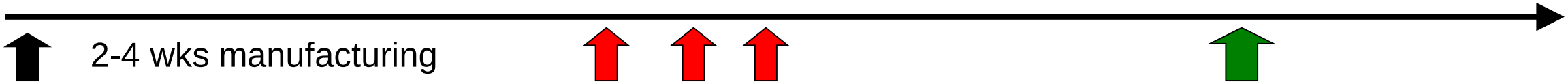
Wash, Concentrate & Freeze

Expand cells

Ready for bedside use

- The CAR is introduced into T cells using viruses and other means

Treatment Schema



Apheresis /
Blood draw

Days -5 to -3

Conditioning chemotherapy
Cyclophosphamide
Fludarabine

Day 0

CAR T cells
(100-500 million cells)

- Patient-specific product; infused in <30 mins
- CAR T cells frozen at ≤ -150^{o} C in GMP facility until the subject is ready for infusion
- Subject will be hospitalized for infusion of CAR T cells and remain hospitalized for at least 7 days following treatment

CD19 CAR Therapy for ALL

Publication/meeting date	Number/age of subjects	Complete remission rate
Brentjens, *Sci Transl Med*, March 21, 2013	5 adults	100%
Grupp, *New* April 18, 20		
Davila, *Sci* February 1		
Lee, *Lance* October 13		rden) urden)
Maude, *N E* 2014, ASH		
Park and Curran, *ASH 2015*,	58 adults	76%
Frey, *ASH 2014*,	12 adults	89%
Gardner/Turtle ASH 2015	>50 adults and children	90%

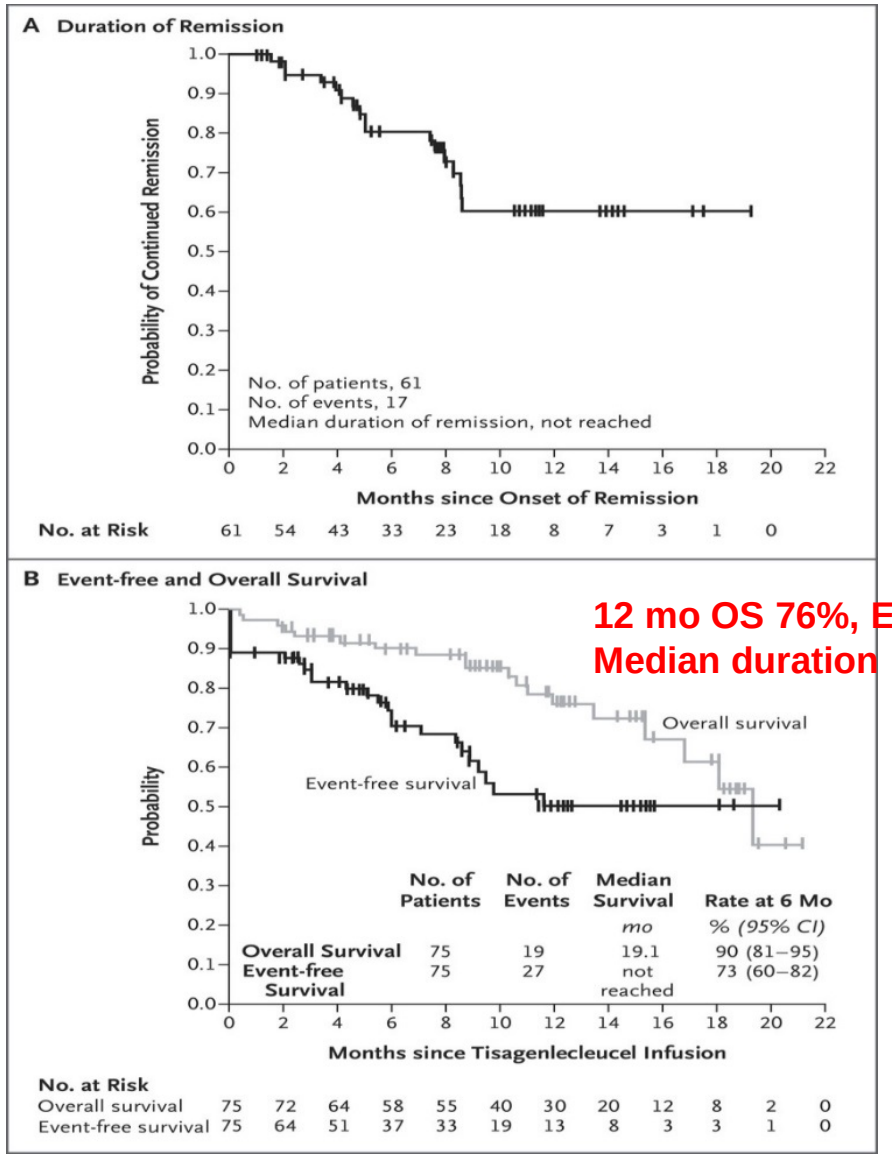
CR rates - Approximately 90%

Eliana Study: Patient characteristics and outcomes

Numbers: 107 screened, 92 enrolled, 75 infused

Patient characteristics, response	Treated patients N=75
Median age in years (range)	11 (3-23)
Female:Male	45/55
Median prior lines of therapy (range)	3 (1-8)
Prior transplant	61%
Med days from enrollment to infusion	45 (30-105)
Manufacture failure	8%
ORR (CR+CRi) w/i 3 months, all MRD^{neg}	81%
ORR, Intent to treat	66%
22 relapses: 15 $CD19^{neg}$, 1 $CD19^{pos}$, 6 unknown	
Rate of SCT; all alive	13%

Maude, et al. NEJM 2018; 378:439



12 mo OS 76%, EFS 50%;
Median duration not reached

FDA Approved August 30, 2017

Relapsed/Refractory Pediatric and Young Adult ALL up to age 25 years

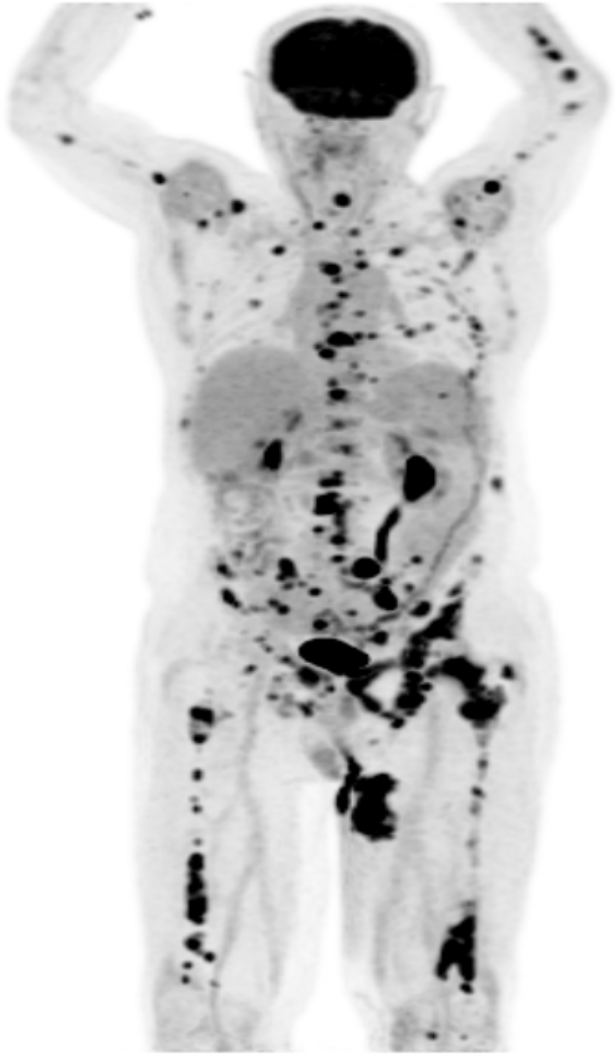
$475,000 per product

Risk Evaluation and Mitigation Strategy (REMS) mandated by FDA for CRS and neurotox

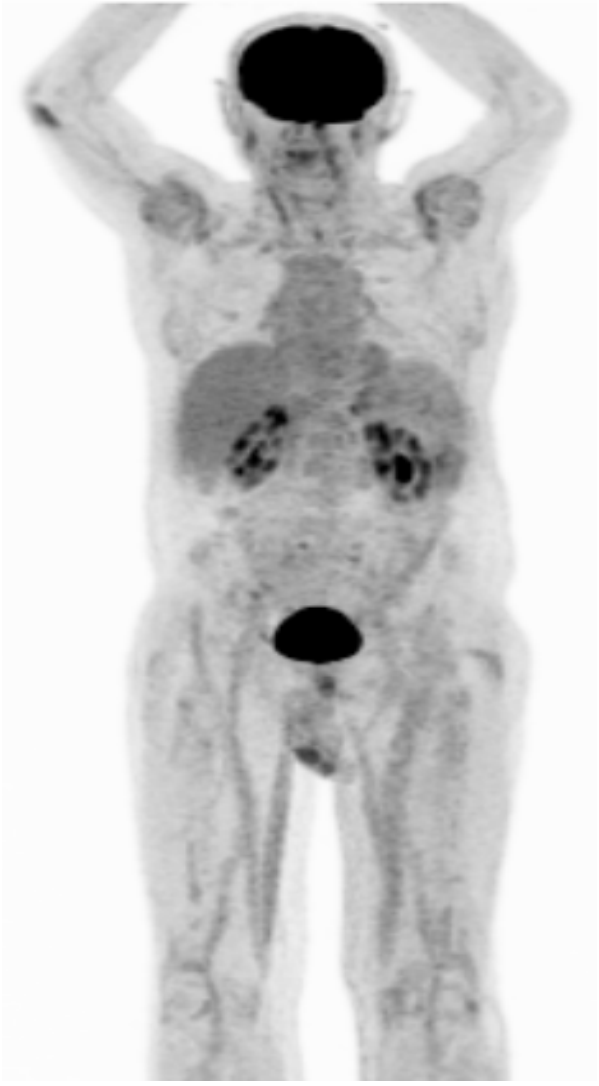
- **Dedicated prescribers who are trained in the toxicities**
- **Ensure that hospitals and clinics have immediate access to tocilizumab**

DLBCL – Resolution of multiple bony lesions

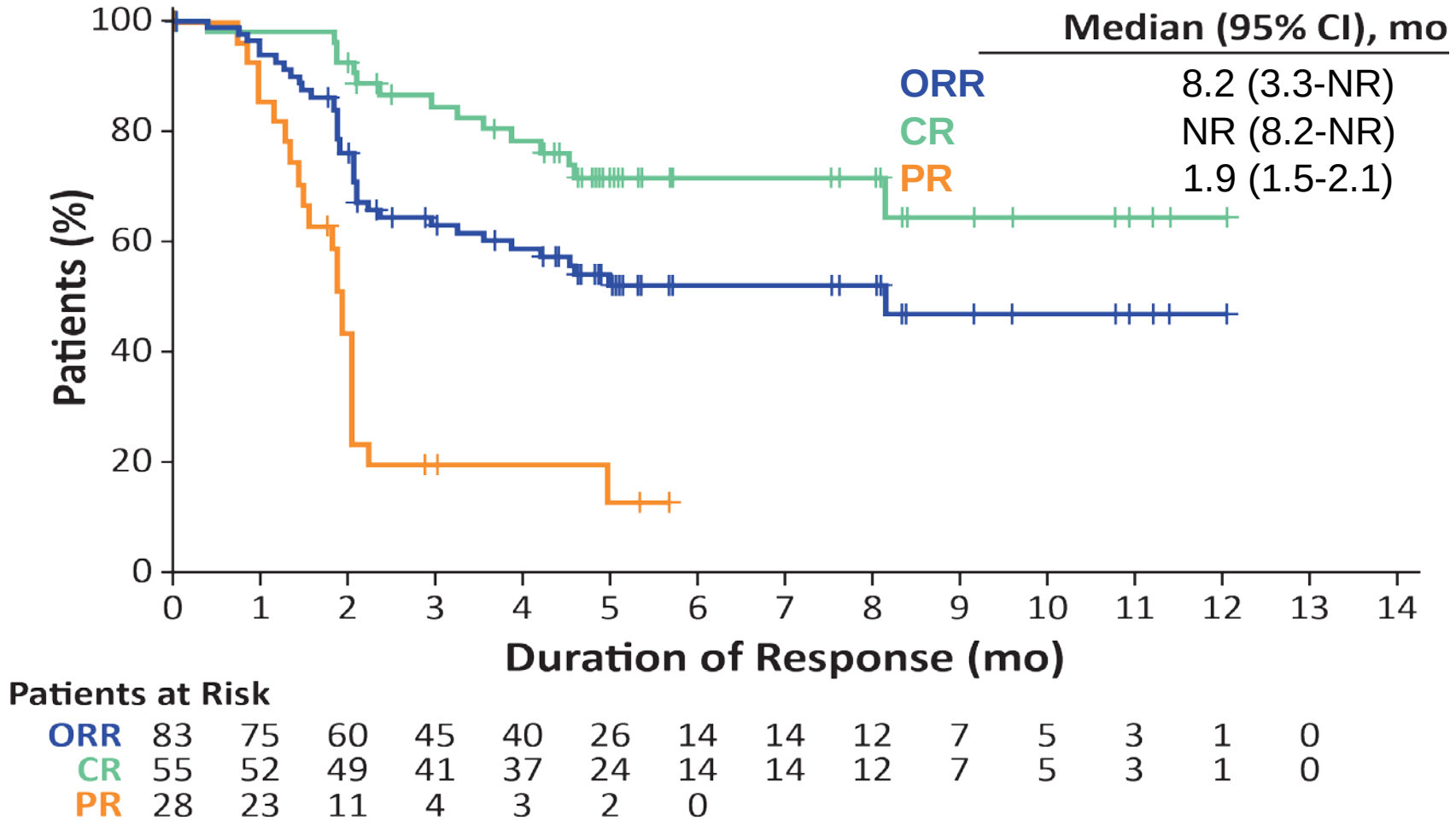
Before treatment



1 month after treatment



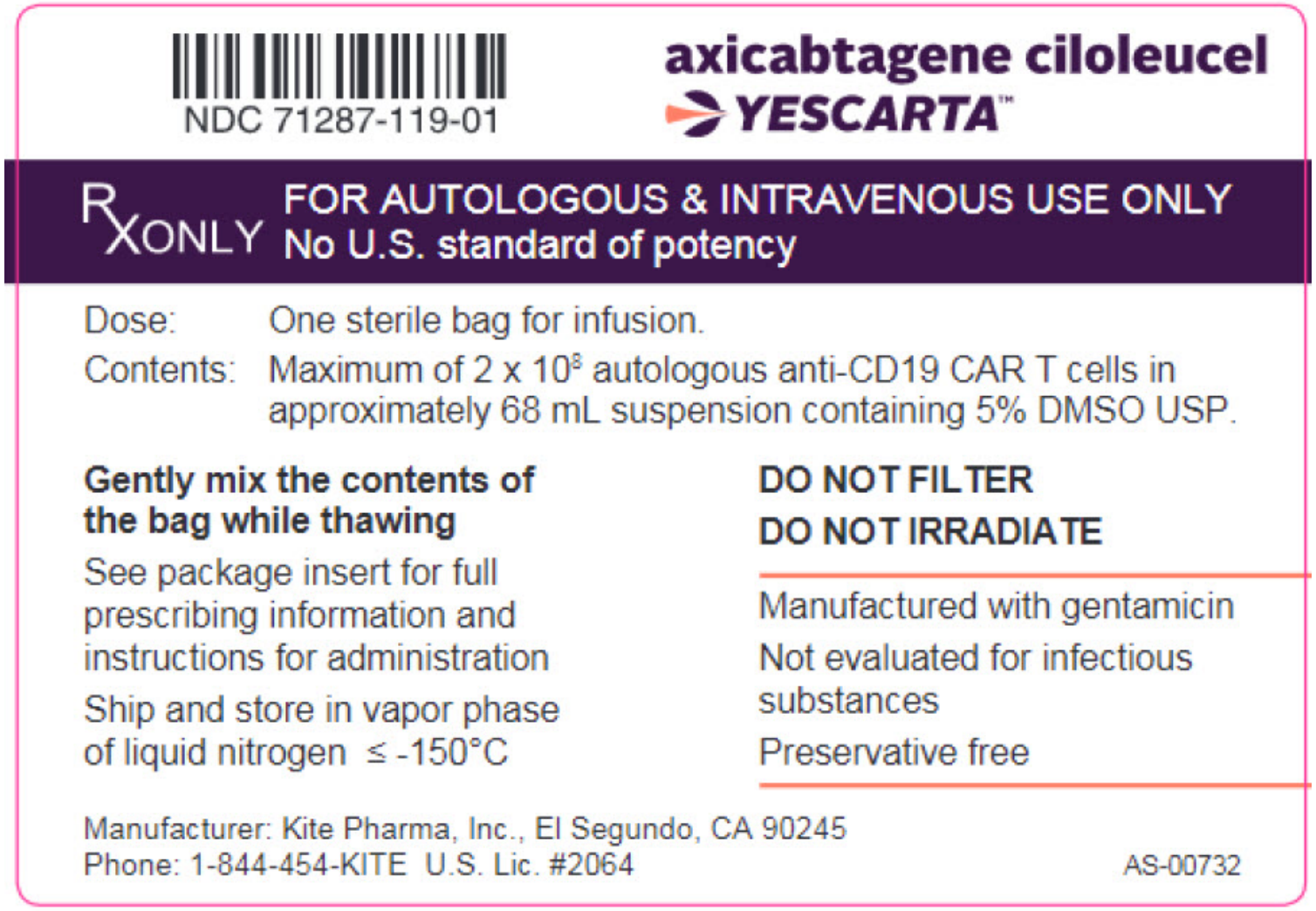
Zuma-1 Duration of Remission
Patients ≥18 years with Refractory NHL



CR, complete response; NR, not reached; ORR, objective response rate.

Neelapu et al. NEJM 2018

FDA Approved 10-17-2017 $373,000 per product



NDC 71287-119-01

axicabtagene ciloleucel

YESCARTA™

℞ ONLY FOR AUTOLOGOUS & INTRAVENOUS USE ONLY

No U.S. standard of potency

Dose: One sterile bag for infusion.

Contents: Maximum of 2 x 10^8 autologous anti-CD19 CAR T cells in approximately 68 mL suspension containing 5% DMSO USP.

Gently mix the contents of the bag while thawing

See package insert for full prescribing information and instructions for administration

Ship and store in vapor phase of liquid nitrogen ≤ -150°C

DO NOT FILTER

DO NOT IRRADIATE

Manufactured with gentamicin

Not evaluated for infectious substances

Preservative free

Manufacturer: Kite Pharma, Inc., El Segundo, CA 90245

Phone: 1-844-454-KITE U.S. Lic. #2064

AS-00732

Adults ≥18 yrs. with Relapsed/Refractory:

- **DLBCL**
- **1^0 Mediastinal LCL**
- **DLBCL arising from Follicular NHL**

REMS program mandated by FDA

Safety of CAR T Cell Therapy

Cytokine release syndrome (CRS) (up to 90%)

- Mediated by high levels of inflammatory cytokines, such as IL-6
- Symptoms include fever, tachycardia, hypotension, and hypoxia

Neurologic Events (up to 65%)

- Events are associated with high CAR T cell levels and/or high cytokine levels
- Symptoms include confusion, tremor, aphasia, encephalopathy, and seizures

CRS (1-14 days)

May occur within minutes or hours but generally appear within days or weeks

Neurologic events

Generally reversible in most patients; rare cases of long-term symptoms[1]

CAR-T Cell Therapy: Current State of the Art

- 40-80% of patients with refractory B cell malignancies showing durable complete responses to CD19-CAR T cell therapy
  - CD19 negative antigen escape (up to 1/3 or higher)
  - Contaminating leukemic cell in product may contribute to relapse (Ruella et Nat Med 2018)
- Potent therapy, but associated with unique toxicities:
  - Cytokine Release Syndrome (high fevers, hypotension, hypoxia, multi-organ failure) CAR-Related neurotoxicity (aphasia, encephalopathy, seizures, cerebral edema)
  - ~50% of patients require ICU management and fatalities have oocured
- Patient-specific and therefore expensive

An off-the-shelf product that can use one donor to treat multiple patients may overcome some of these limitations

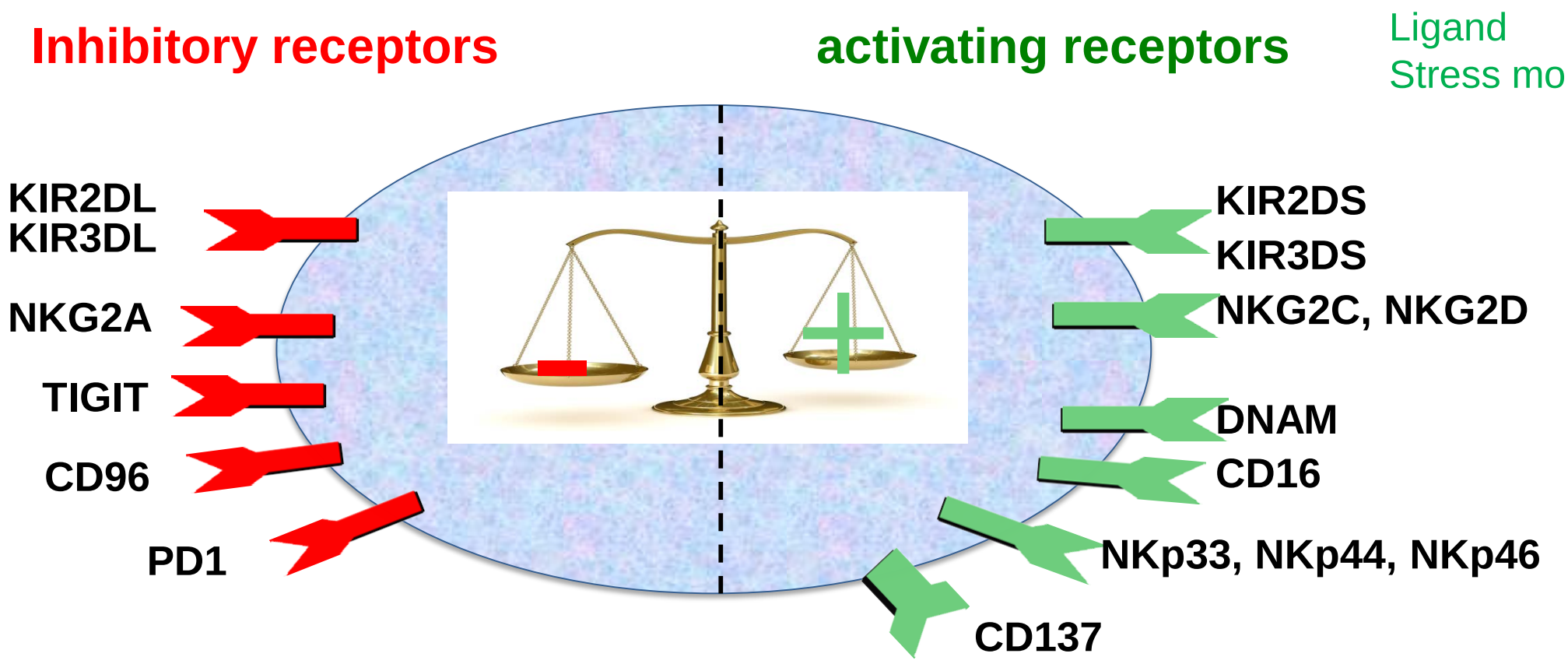
NK Cells

- Innate immune system
- CD56+CD3-
- Differentiate in the BM
- No antigen priming
- Primarily in blood
- Recognition takes place through complex array of receptors

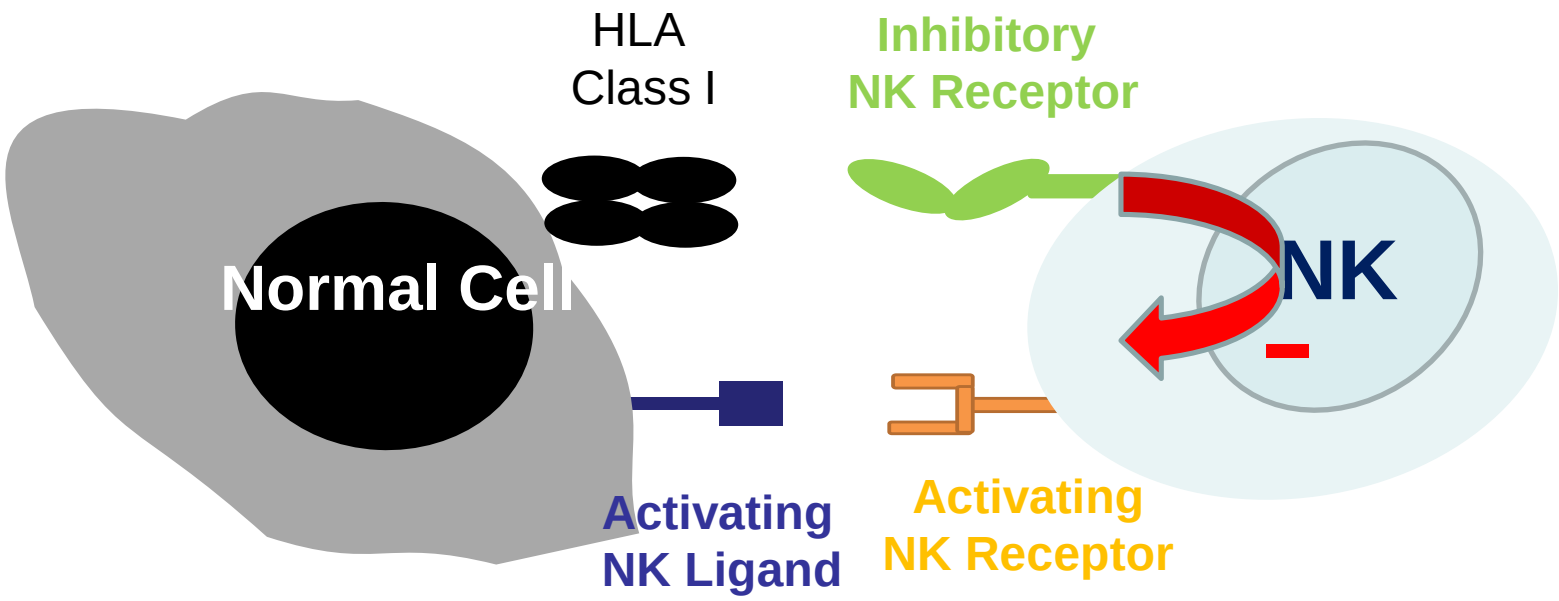
T Cells

- Adaptive immune system
- CD3+CD4+ or CD3+CD8+
- Differentiate in the thymus
- Antigen priming required
- Antigen specific
- Recognize targets through TCR rearrangement

NK cell activation depends on the intricate balance between activating and inhibitory receptors

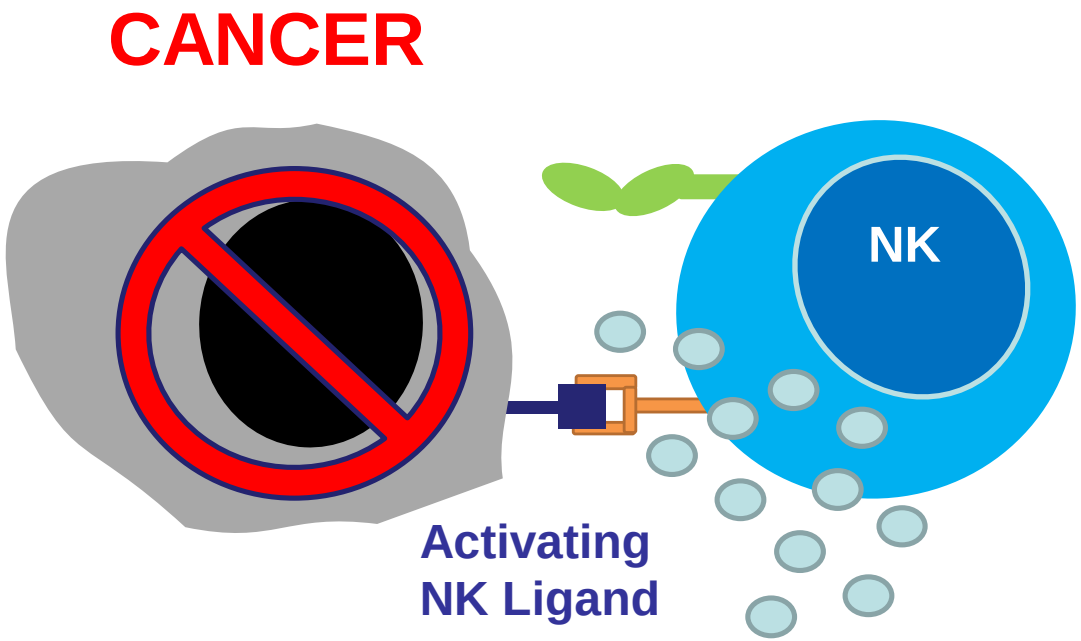


Integration of inhibitory and activating NK cell receptor signals regulates NK decision to kill



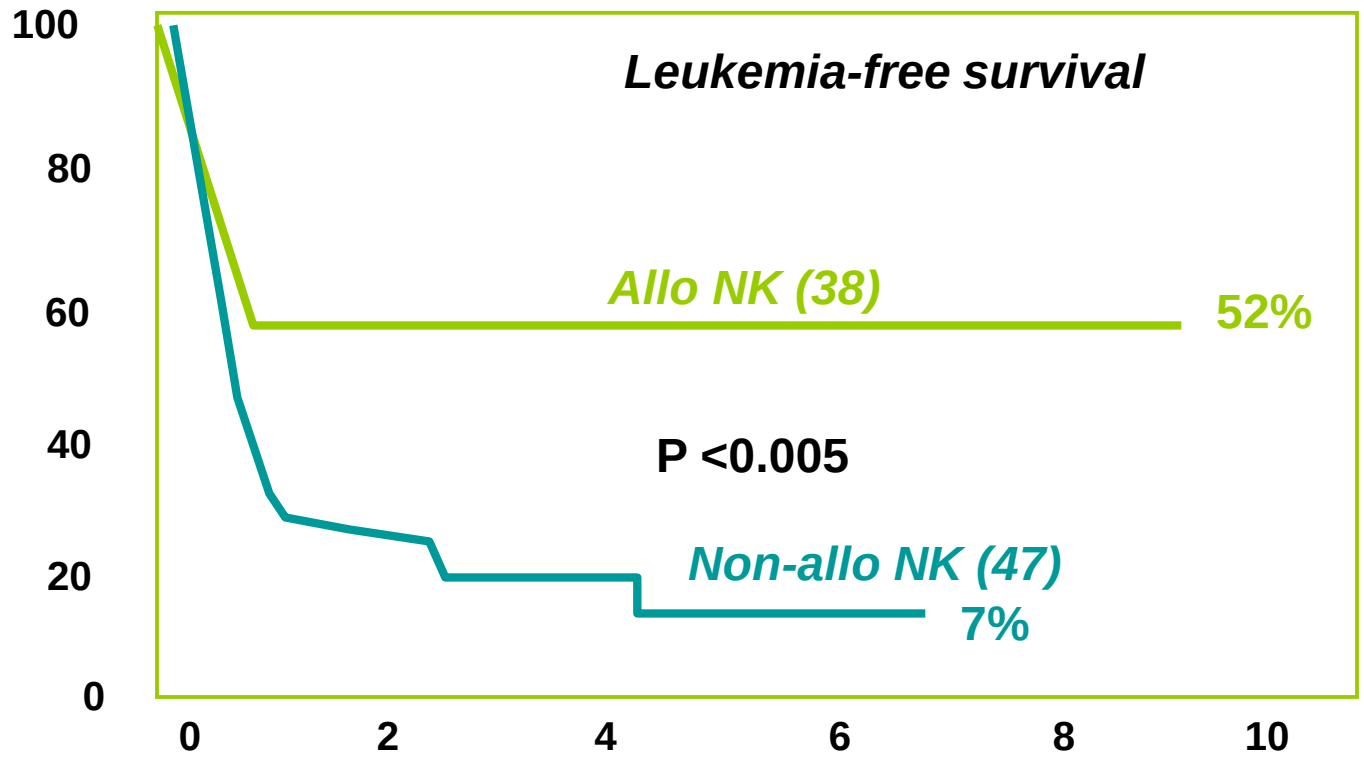
In normal cells, the inhibitory signals triggered by KIR-HLA-I molecules engagement overrides activating signals.

Integration of inhibitory and activating NK cell receptor signals regulates NK decision to kill



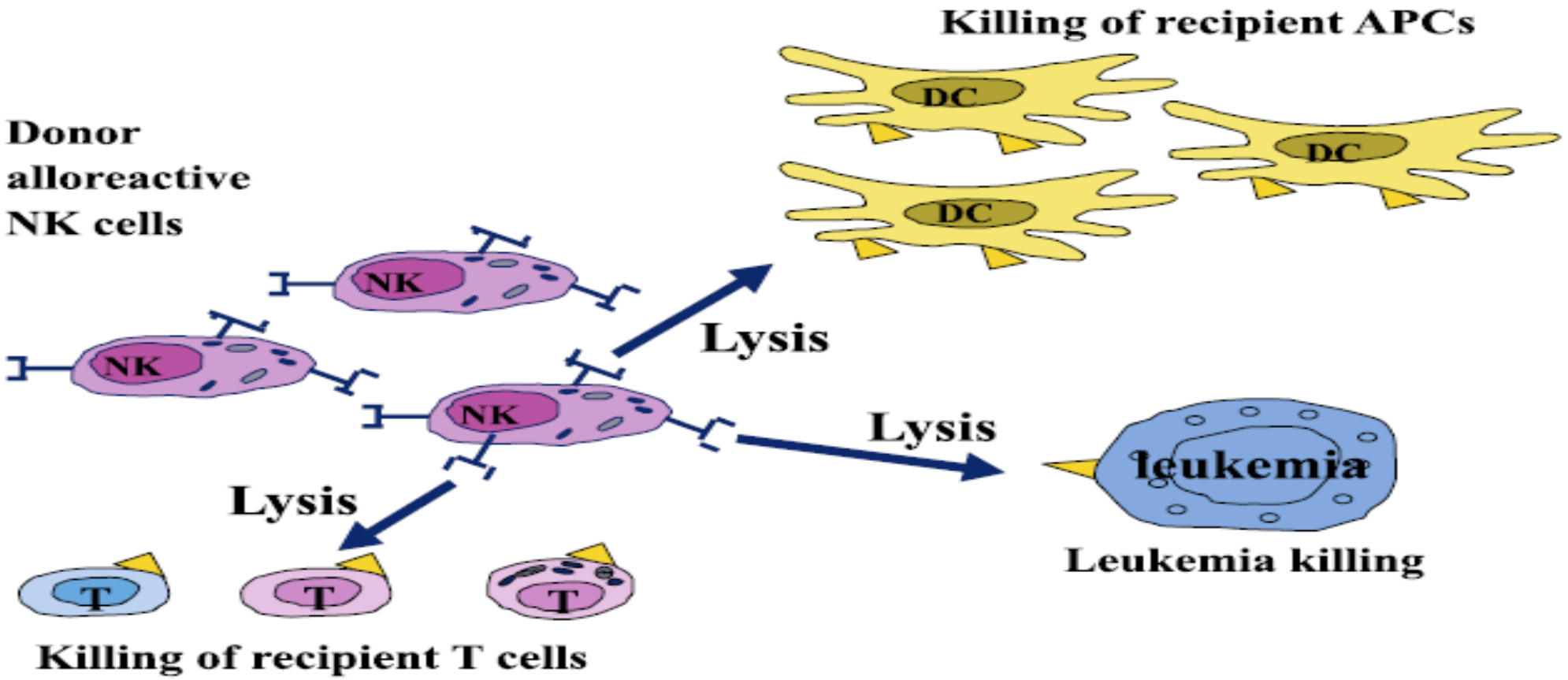
In the context of cancer, expression of stress ligands for activating receptors, in conjunction with low expression of HLA-I molecules attenuates the triggering of inhibitory receptors and results in an activating signal

An NK mediated GVL effect in Haploidentical T cell depleted SCT for AML



Ruggeri et al, Blood 1999; 94, 339

Allogeneic NK-cells mediate anti-tumor response without inducing GVHD



Ruggeri et al, *Science 1999*

Advantages of NK cells over T cells for CAR therapy

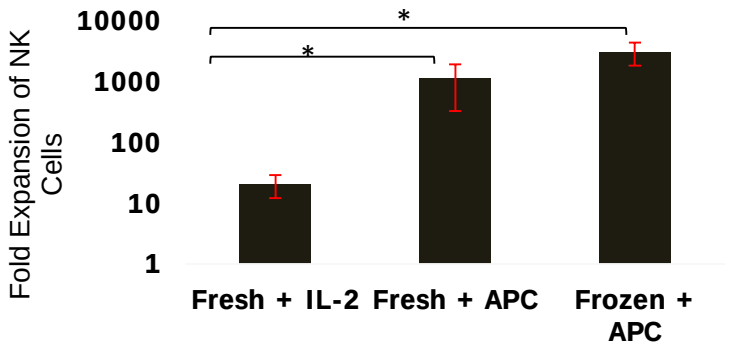
- NK cells:
  - Low/absent risk of GVHD
  - activity through their native receptors-
    - synergize with CAR
    - may prevent tumor escape by downregulation of CAR target antigen
  - Potential for ’off-the-shelf’ allogeneic product, thus increasing accessibility and reducing cost

Limitations of NK cell immunotherapy in the treatment of cancer

- Logistics: NK cells need to be collected on an individual case basis:
  - From a healthy donor (allogeneic source) – haploidentical donor or **cord blood** (MDACC CB Bank)
  - Others use NK92 cell line, HSC or iPSCs
  - From the patient (autologous- *less effective*)
- Limited persistence of adoptively infused NK cells
- Not antigen-specific

Impressive CB-NK expansion from fresh or cryopreserved CB units

A



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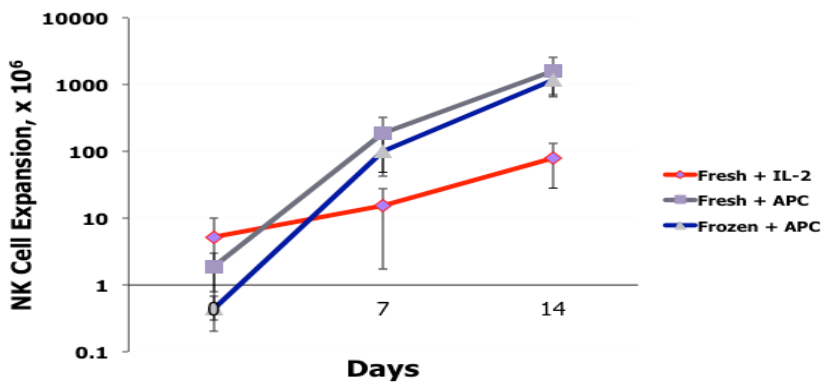

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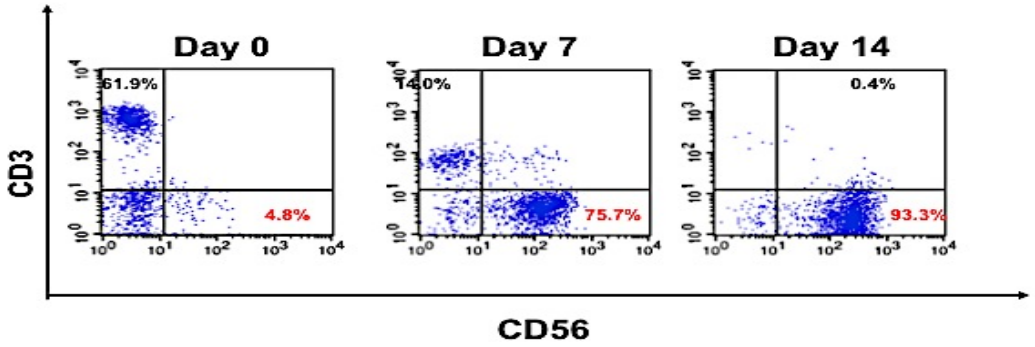
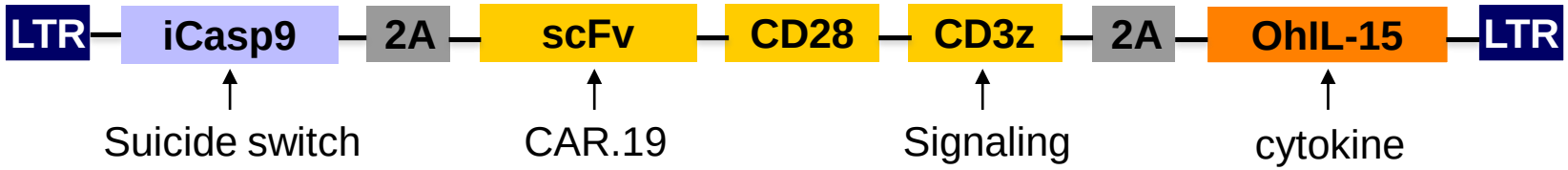


fold NK increase	absolute NK produced (x 10e6)	absolute CD3 (x 10e6)
4093	1471	4.50

CAR NK cells persist & control Raji tumor

Enli Liu

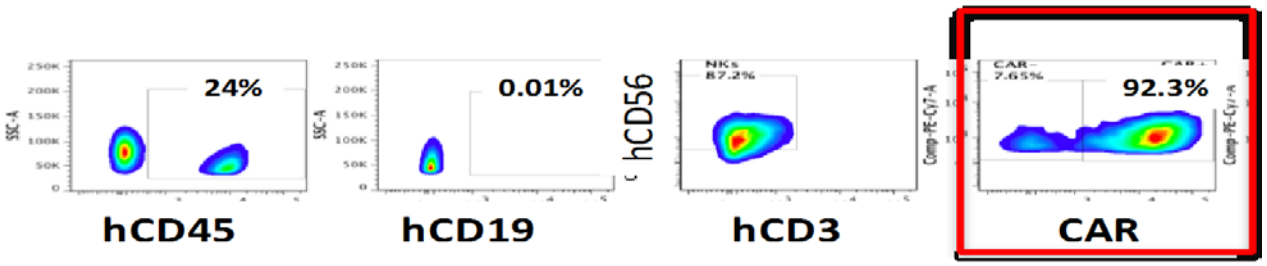
Armored CAR

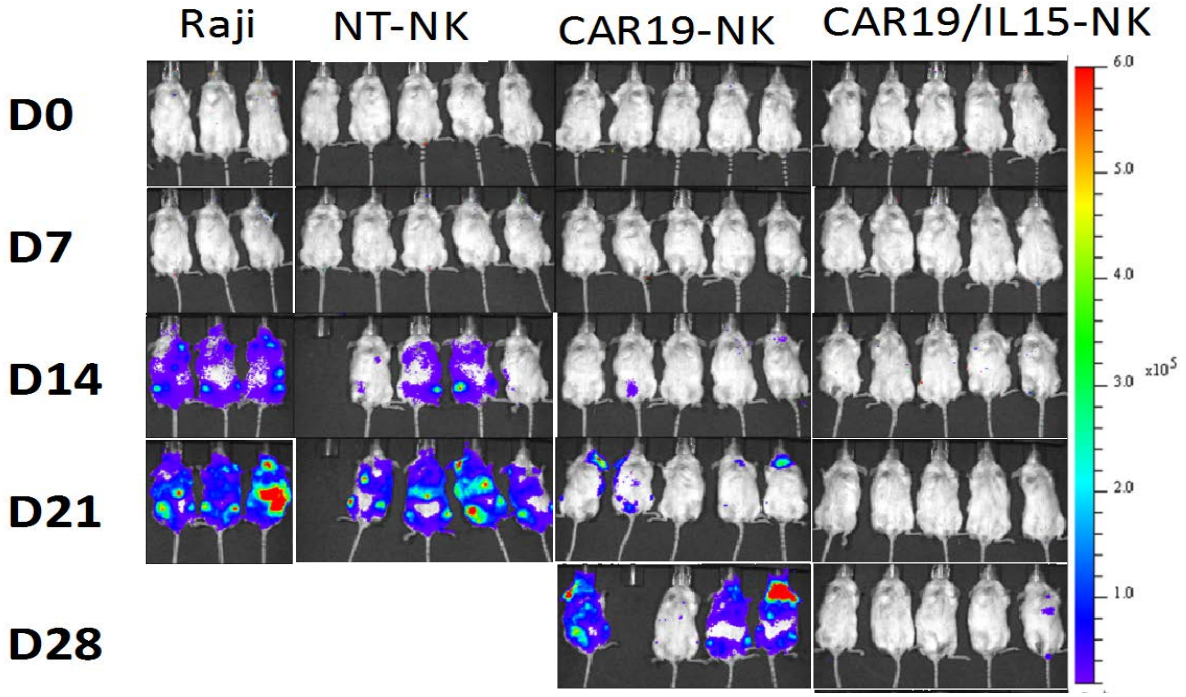


Pietro Dotti- BCM

Day 70 Post Infusion

Blood



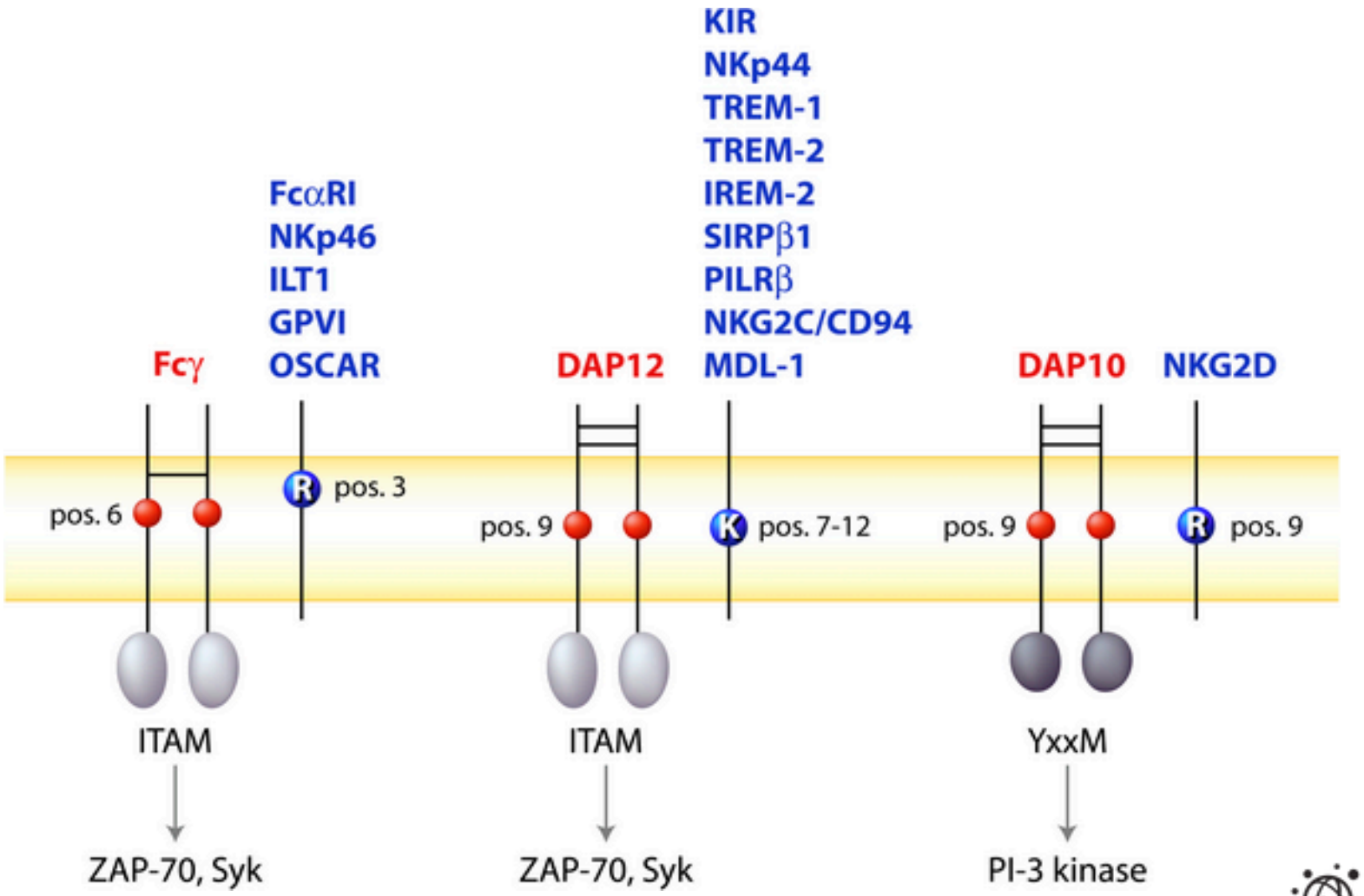


Liu et al. Leukemia 2017

Next generation CAR NK cell therapies

- Is CD3zeta-CD28 the best signaling domain for CAR NK cells?
- Can we move beyond CD19 CAR?

Alternative NK signaling domains



Feng J, Call ME, Wucherpfennig KW (2006)

Can we apply CAR NK cell therapy beyond lymphoid malignancies?

- Can we target antigens beyond CD19?
  - T-ALL
  - AML

Features of an Ideal CAR Target

Goal	Activity	Expression
Efficient recognition and targeting by CAR T or NK cells	high on-tumor	- in all tumor cells - at high level - In many patients
Safe discrimination of target cells by CAR T or NK cells	Low off-tumor	NOT in: - any normal tissue, especially vital tissues - normal counterparts (e.g., HSPCs for AML) - resting/activated T cells

Adapted from Perna 2017, Cancer Cell

Targeting T-cell leukemia

- CAR-mediated therapy against T-cell antigens-
  - Directing CAR-modified T-cells against shared T-cell antigens present on malignant cells could result in self-targeting and fratricide of CAR T-cells, thus compromising therapeutic ability of CAR T cells.
  - higher immunodeficiency impact of T-cell depletion vs B-cell aplasia

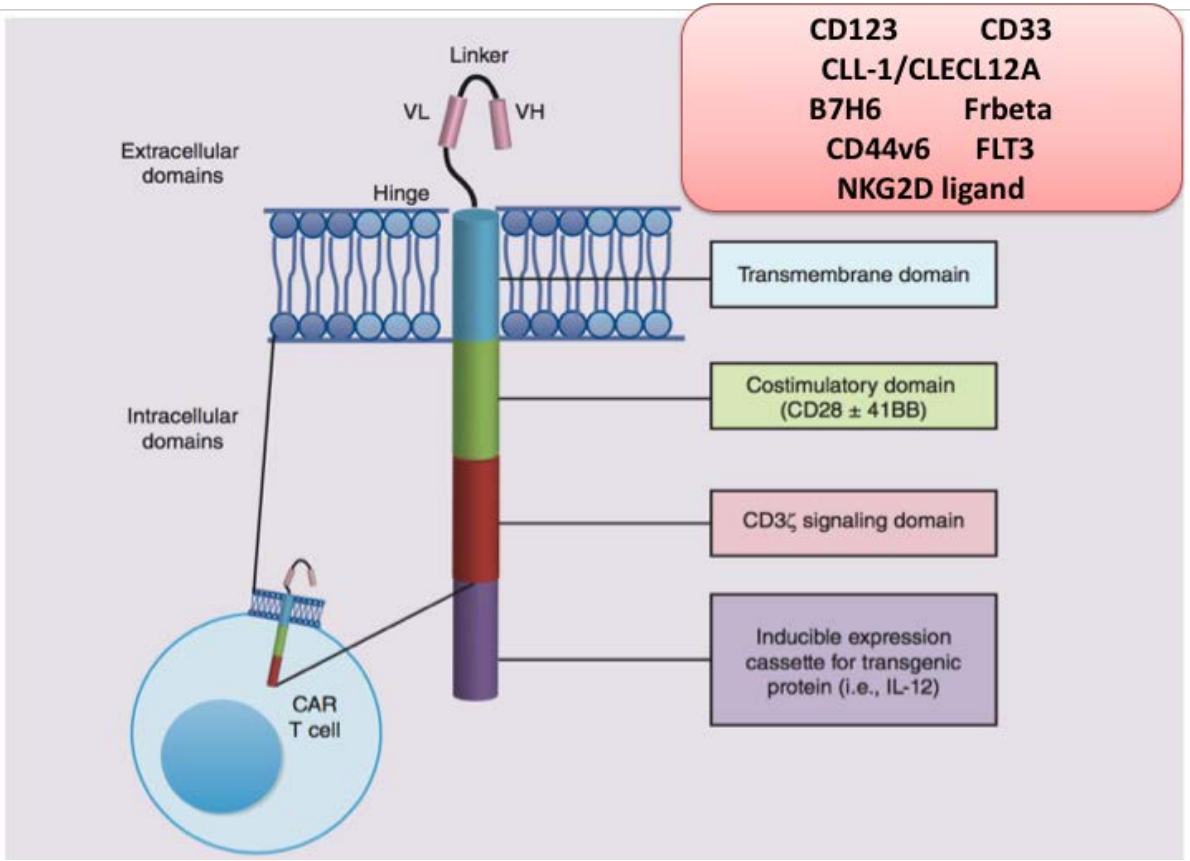
Self-targeting and fratricide could potentially be mitigated by using NK cells.

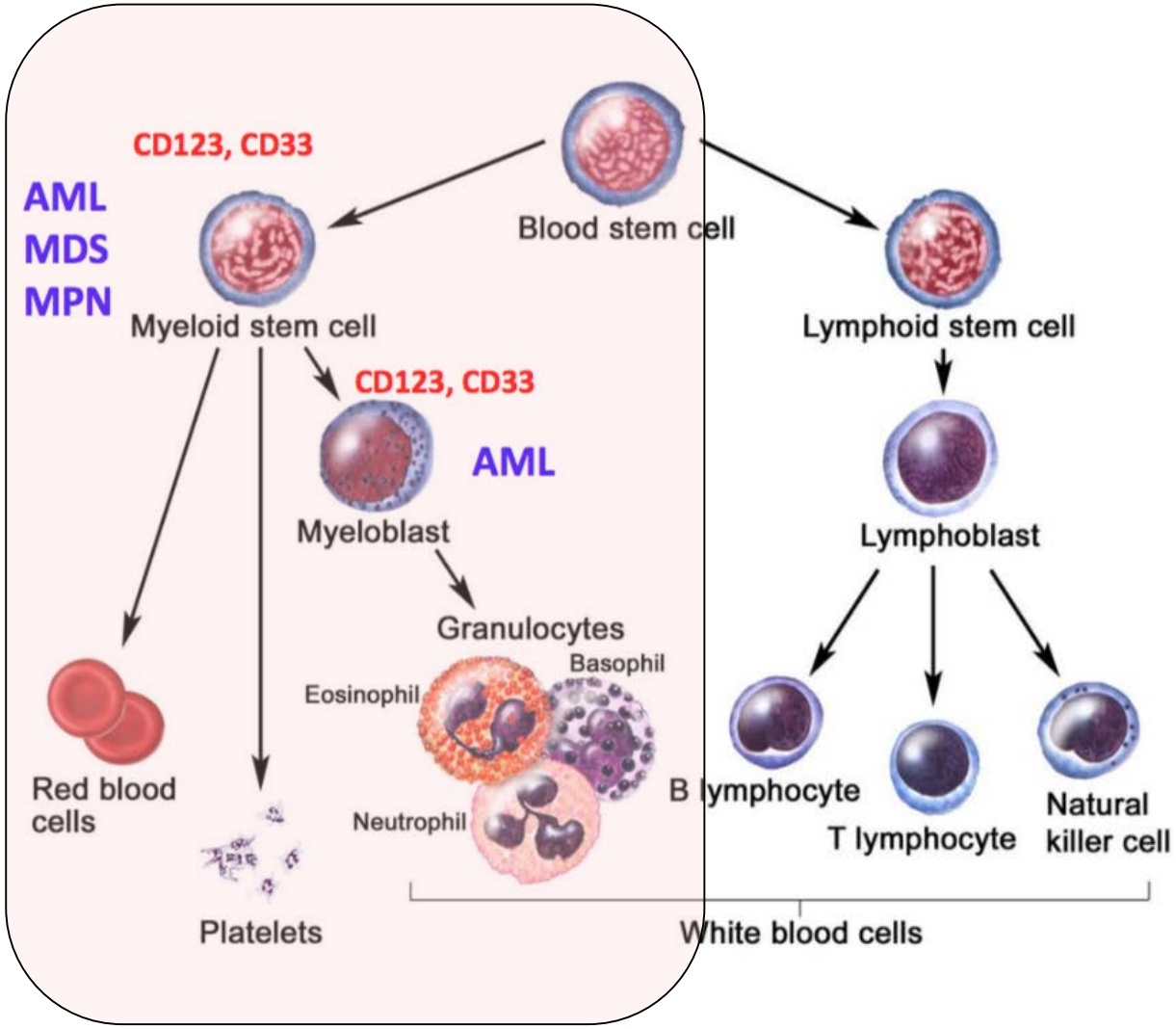
Can we apply CAR NK cell therapy beyond lymphoid malignancies?

- What is the role of checkpoint molecules in NK biology?
  - Other genes: TIGIT, LAG3, NKG2A, PD1, Adenosine receptor 2

- Can we target antigens beyond CD19?
  - T-ALL
  - AML

Potential AML CAR Targets





Adapted fromPrzespolewski A, Szeles A, Wang Es. Future Oncology. 2018

Potential limitations of current AML CAR Targets

Antigen	Expression	references
CD33	high on bulk AML cells but lower on LSC → difficulty to specifically target LSC	Kenderian Leukemia. 2015 O'Hear Haematologica. 2015
CD123	High on bulk AML and LSC, as well as normal HSC and multiple normal tissues	Gill, Blood. 2014 Mardiros, Blood. 2013 . 2013
CLL1		2016
TIM3	AML bulk cells and LSC, also T cells	Kikushige Y, Cell Stem Cell. 2010
FLT3	On normal hematopoietic progenitors as well as on most AML blasts	Chien, ASH 2016
Lewis Y (LeY) antigen	overexpressed on AML cells, with limited expression on normal tissues. → toxicity	Peinert, Gene Ther 2010

No CD19 equivalent

Clinical trials of CAR T cells in AML

CAR	Author/Center	Results
Anti-Ley-CD28-CD3-ζ chain CAR T	Ritchie/Peter Mc Callum Center 2013	N=4 • 1 cytogenetic relapse -> transient cytogenetic remission • 2 stable disease • No significant toxicity
Anti-CD33-41BBζ CAR T	Wang/Chinese PLA General Hospital	N=1 • Transient reduction in blasts • Cytokine release syndrome
CM-CS1 T cells which recognize NKG2D-ligands	Nikiforow/Dana-Farber Cancer Institute National Heart, Lung, and Blood Institute (NHLBI)	N=6 (AML/MDS) • No response
CAR123	• Off-the-shelf allogeneic UCAR123 in r/r AML (NCT03190278). Cornell and MDACC • Donor-derived CART-123 cells in relapsed AML following allogeneic transplant (NCT03114670) - China • CD123/CD28 CAR T cells (NCT02159495). COH	• Not published yet • Presented at ASH 2017

CD123 CAR T results in AML and Blastic Plasmacytoid Dendritic Cell Neoplasm: 3 of 7 responses

(Budde et al. City of Hope ASH 2017)

UPN	Age/Sex; Dx	Prior lines Allo (donor)	BM Blasts; CD123	Cytogenetics/ Molecular	Lympho-depletion	CAR T dose	Response
136	44/F AML/MDS	6; Y (MRD)	20%; -ve to dim	-7 inv(3)	Flu/Cy	50M donor	PD 40% blasts
138	54/F AML/MPD	4; Y (MRD)	18%; dim to mod	IDH1	Flu/Cy	50M donor	Morphologic leukemic free state
167	43/F AML	4; Y (MRD)	20%; dim	nl	Flu/Cy	200M donor	CRi (MRD-ve by flow on 14)
178	54/F AML	7; Y (MUD)	37%; mod	t(3;7) +21	Flu/Cy	200M Donor (DLI)	SD 20% blasts
195	42/F AML/MDS	6, Y (MRD)	41%; mod	-7.+8	Flu/Cy decitabine	187M Donor(DLI)	SD 46% blasts
200	28/M AML	7, Y (MRD)	3% dim	Complex FLT3-TKD N-RAS	Flu/Cy	200M Donor	CR (MRD+, 0.10% day 28)
203	74/m / BPDCN	1 (sl401 x6 -> PR); No	NED	nl	Flu/Cy	100M	CR

Summary of Adverse Events

Adverse Events	
CRS	Grade 1, N=3 Grade 2, n=1
Neurotoxicity	Dizziness grade 1, N=1; grade 2, n=2 Headeache grade 1, n=5; grade 2 Somnolence grade 1, n=1; grade 1, n=2
Infection	Lung infection grade 3, n=1; grade 4, n=1 Others grade 3, n=1
Most common ≥ grade 3 AEs	Lymphopenia, n=7 Thrombocytopenia, n=6 Febrile neutropenia, n=6

No grade 5 events or DLTs

No myeloablation

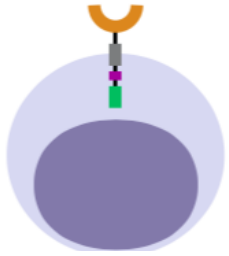
Challenges to the application of CAR therapy in AML

- Heterogeneous antigen expression on diverse AML cell populations
- Potential for off-target toxicities to normal myeloid progenitor and hematopoietic stem cells in patients
- Potential for life-threatening complications – CRS/neurotoxicity

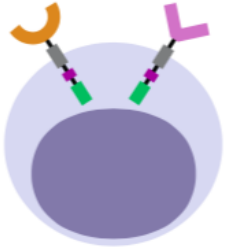
Overcoming clonal heterogeneity and antigen escape

Single target strategy

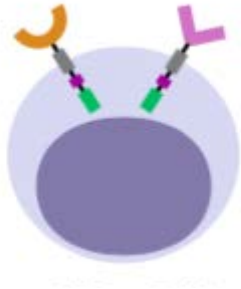
Combinatorial strategy



CD19



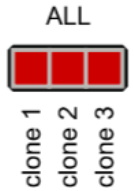
CAR + CAR



CAR + CAR

Overcome clonal heterogeneity

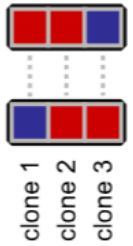
Antigen A



ALL

Antigen A

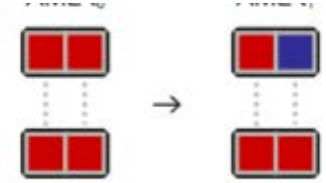
Antigen B



AML

Antigen A

Antigen B



AML

Perna et al Cancer Cell 2017

Broadening the targeting milieu of CAR T-cell therapies

Generate a comprehensive set of AML surface proteins
• Surface biotinylation & MS identification in panel of AML cell lines (4,862) +
• Previously reported AML surface targets (346)

Select molecules overexpressed in AML vs normal counterparts
• Analysis of antigen expression in AML vs normal HSPCs

Quality control
• Selection of membrane-associated molecules +
• Selection of molecules with ≥ 2 normal tissue proteomics annotation sources

Select molecules with minimal expression in normal tissues
• Exclusion of proteins with high expression across all normal tissues

Flow cytometric analysis
• in primary AML patient samples

Flow cytometric analysis
• primary healthy BM HSPCs • primary healthy T cells

Annotated dataset

4,942

682

361

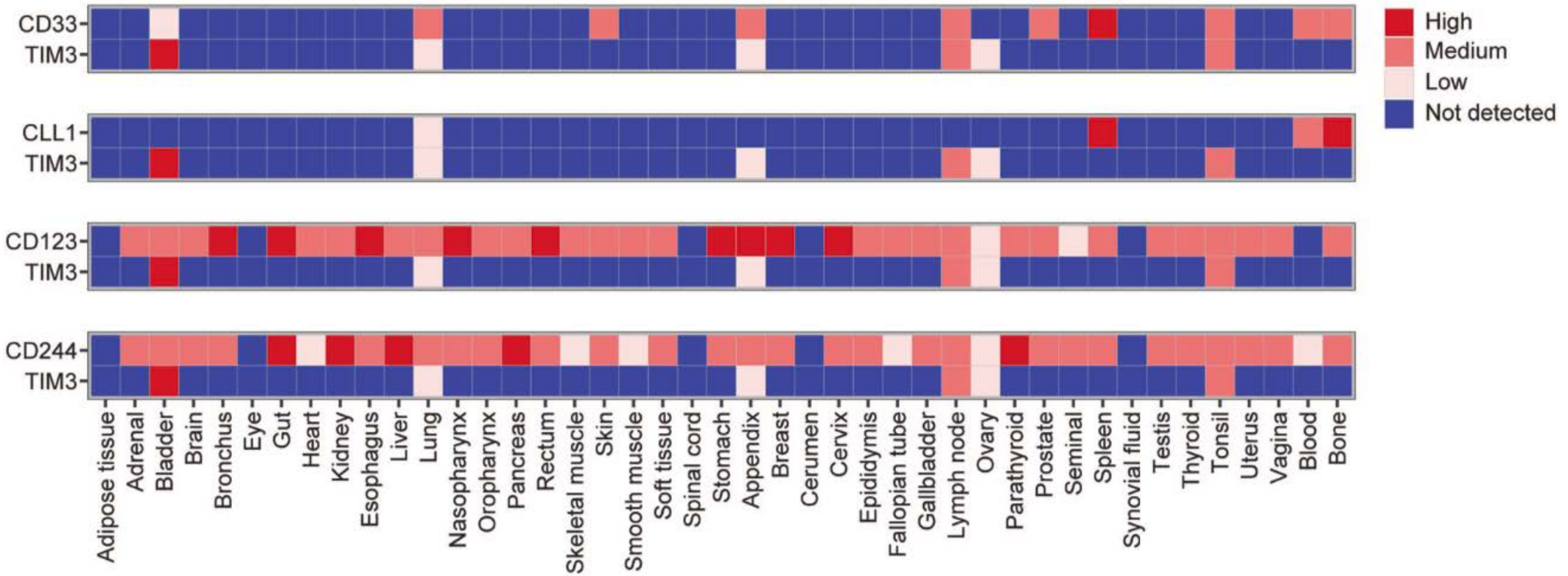
24

9

4 **ADGRE2, CCR1, CD70, and LILRB2**

Adapted from Perna et al Cancer Cell 2017

Broadening the targets of CAR T-cell therapy in AML – antigen combinations expressed on AML cells



S. Haubner et al. Leukemia 2018

NK cells as an alternative to T cells for CAR therapy in AML

- Exploit the innate features of NK cells along with the CAR for potential synergism.
  - AML cells are highly susceptible to NK mediated killing
    - express ligands for NK activating receptors- MICA, MICB, ULBP
  - Harness ‘missing-self’ effect - select NK cells that are KIR-ligand mismatched with the recipient

→ activity through native NK receptors may prevent tumor escape by downregulation of CAR target antigen

Conclusions

- Clinical trials of CAR therapy in AML are underway at a number of center
- Challenges include choice of antigen and on-target/off-tumor toxicity
- Next generation:
  - Use of a combinatorial CAR T strategy targeting two or more antigens or CAR NK cells
  - Suicide approaches to improve safety profile
  - Bridge to transplant

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