Novel Immunotherapies

CAR-T Cells in AML

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Disclosures

- I have the following relevant financial relationships to disclose:

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  - GEN-DX B.V., Utrecht, The Netherlands: Research support, Royalties

- I will discuss the following off label use and/or investigational use in my presentation:

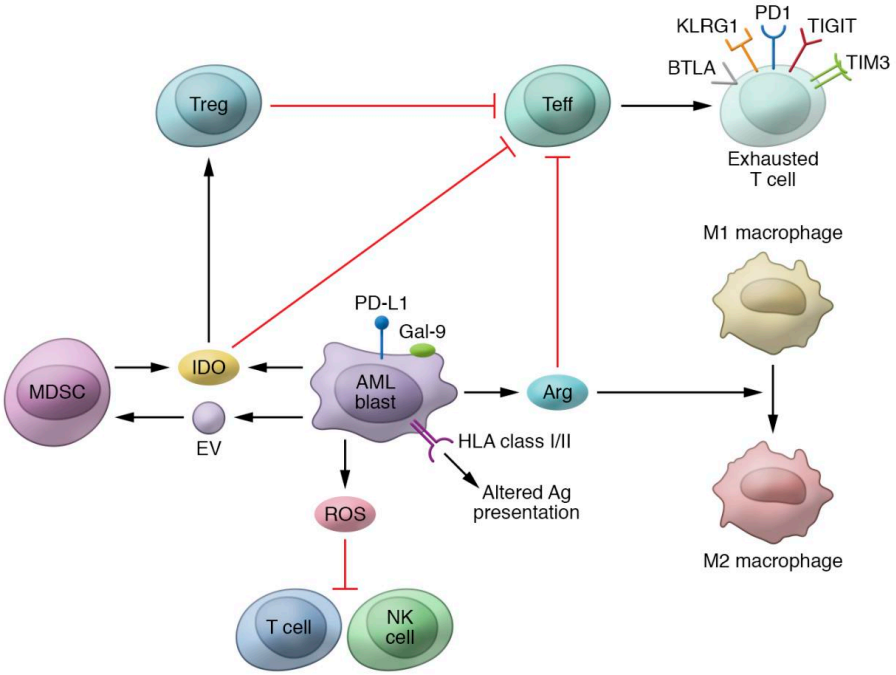
  HLA-KMR, GEN-DX

AML as a Target for Immunotherapy

Allogeneic Hematopoietic Cell Transplantation (allo-HCT) provides the irrefutable proof that AML blasts can be controlled or eradicated by immune cells

BUT

- the Graft-versus-Leukemia effect leverages mostly on donor-recipient genetic **incompatibilities** (minor histocompatibility antigens, HLA mismatches)
- The **rapid growth kinetics** of the disease limit the effectiveness of immune-based approaches if disease burden is significant
- Leukemic cells can directly of indirectly **impair their recognition** by immune effectors

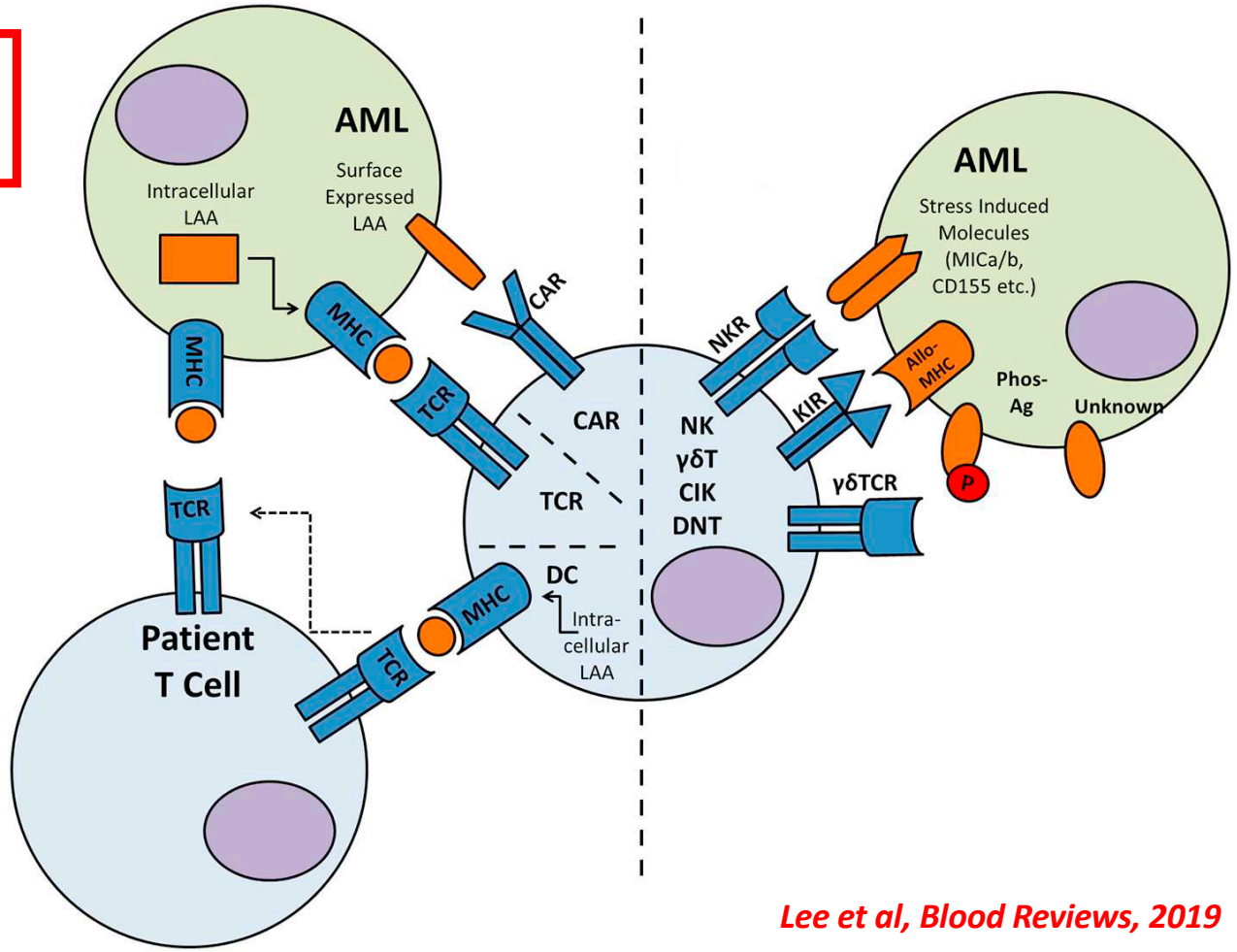


Vago and Gojo, J Clin Invest, 2020

Immune Strategies to Treat AML

Disease Specific

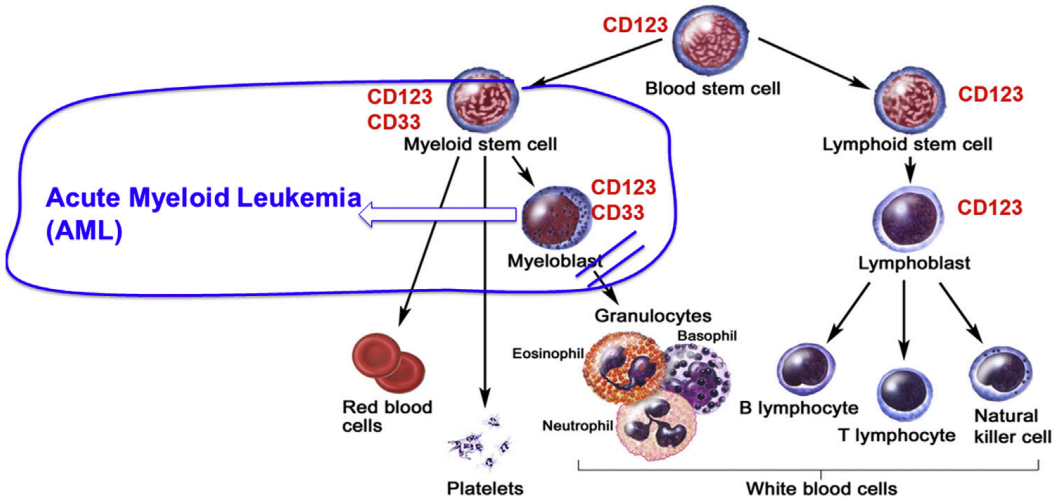
Non Disease Specific



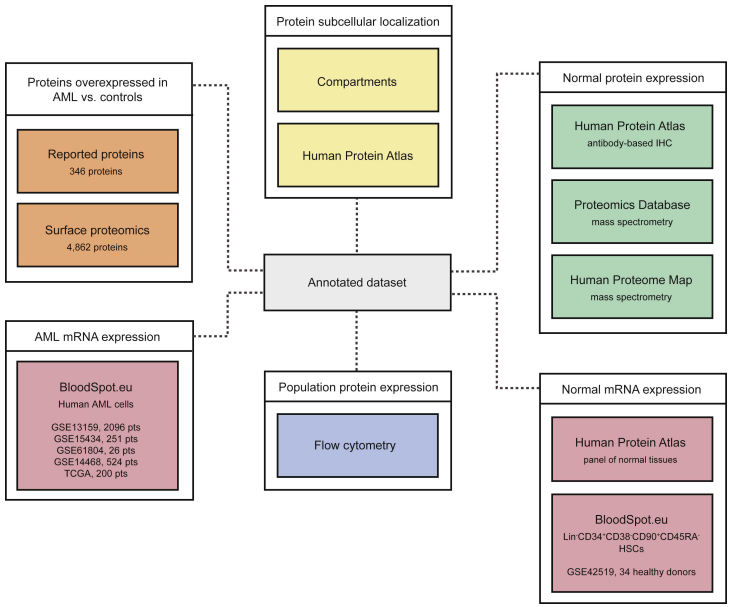
Lee et al, Blood Reviews, 2019

Issues in Identifying a Disease-Specific Antigen in AML

- AML is a phenotypically heterogeneous disease (both inter-patient and intra-patient)
- Most (all?) of its surface antigens are shared with HSCs and/or monocytes, that can not be ablated for long periods of time



Gill, Best Pract & Res Clin Haematol, 2019



Perna et al, Cancer Cell, 2017

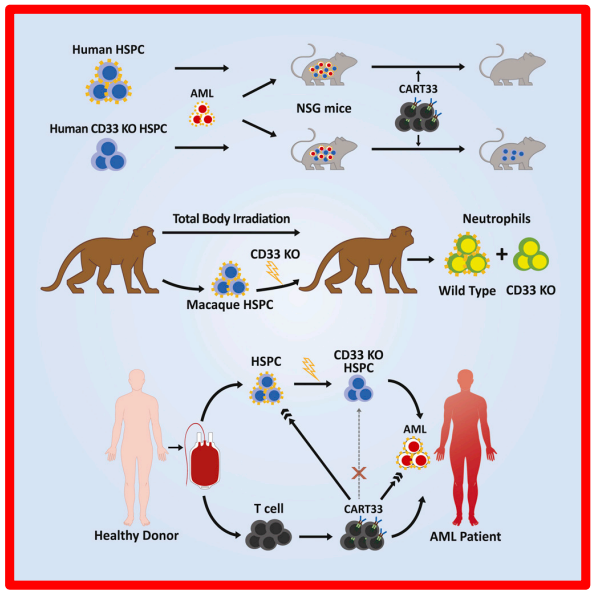
Possible Solutions to the Issue of Shared Antigens

Today:

- CAR-T cell therapy as conditioning for subsequent allo-HCT
- Use of short-term persisting effectors or CAR constructs (non-integrating)
- Inclusion of a suicide gene in the vector

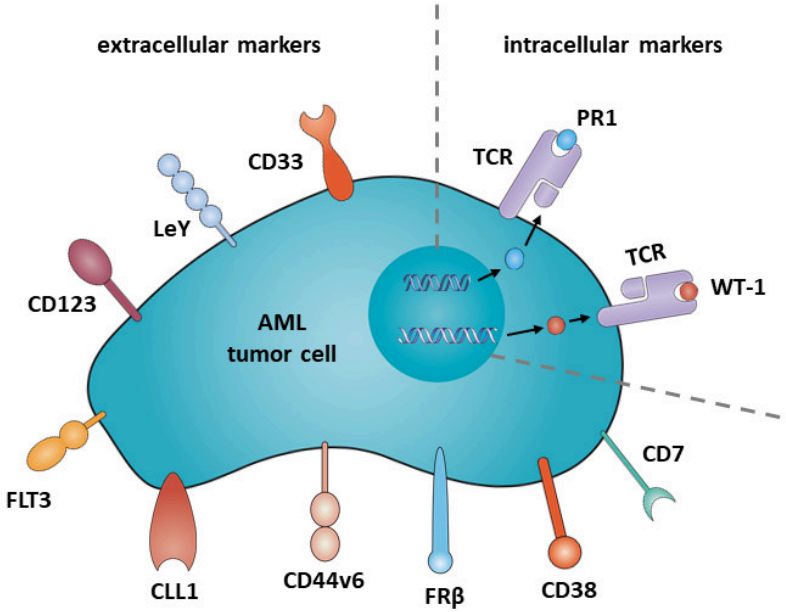
Tomorrow:

- Combinatorial CARs (“AND” or “NOT” gate)
- uCAR-T cells
- Co-infusion of CAR-T cells and gene edited HSCs



Kim et al, Cell, 2018

AML Surface Antigens: An Overview



Hofmann et al, J Clin Med, 2019

Table 1. Select lineage-restricted surface AML antigen targets

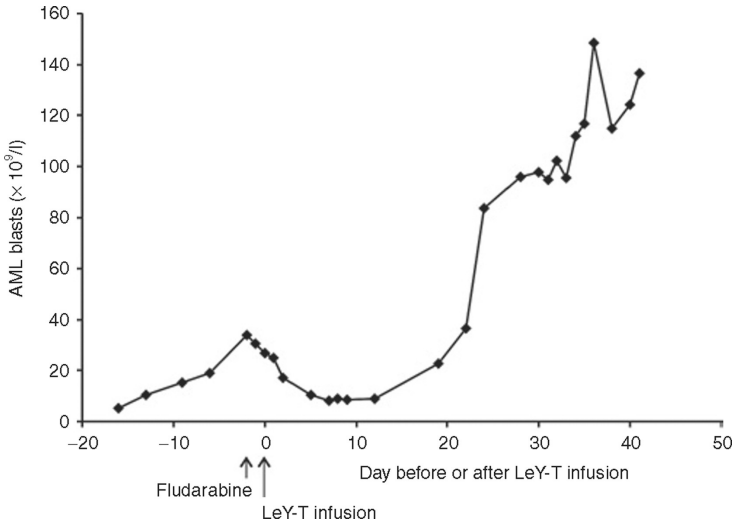
Antigen	Description	AML blast expression	LSC expression	Normal tissue expression
CD33 (Siglec-3)	Transmembrane receptor	90%	Yes	HSCs; myeloid progenitors, monocytes, mast cells, Kupffer cells, microglial cells in the brain
CD123 (IL-3Rα)	IL-3 receptor-α	50%–100%	Yes	HSCs (little or no); myeloid progenitors, monocytes, basophils, dendritic cells, epithelial cells (respiratory, gastrointestinal)
CLL1 (CLEC2A)	Transmembrane receptor	77%–100%	Yes	Monocytes, granulocytes, tissue-resident lung macrophages
CD44v6	Transmembrane receptor/splice variant	64%	Yes	Monocytes, keratinocytes; different epithelial tissues (respiratory, gastrointestinal, genitourinary)
CD56 (neural cell adhesion molecule [NCAM])	Member of the immunoglobulin superfamily CAM	28%	Possibly	Dendritic, NK, and T cells, monocytes, neural and neuroendocrine tissues
Lewis Y (CD174)	Blood group carbohydrate antigen	50%	Likely	HSCs; intestinal epithelial cells
FLT3 (CD135)	Type III receptor tyrosine kinase	70%–100%	Yes	HSCs; myeloid progenitors, neurons
CD7	Transmembrane protein; member of the immunoglobulin superfamily	30%	Possibly	T cells
FOLR2 (folate receptor-β)	Folate-binding protein receptor	70%	Possibly	Myeloid cells, macrophages
CD25	IL-2 receptor-α	20%	Yes	Activated T cells

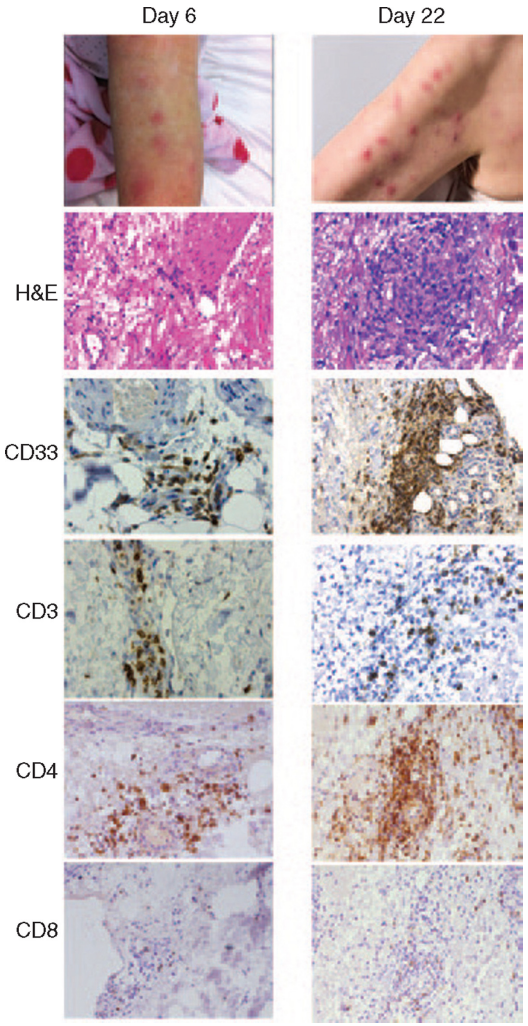
Others include IL1RAP (IL-1 receptor accessory protein), CD64 (Fc receptor that binds IgG), CD13 (type II membrane glycoprotein–aminopeptidase N), CD38 (cyclic ADP ribose hydroxylase), CD45 (tyrosine phosphatase), CD15 (adhesion molecule, carbohydrate antigen), and NKG2D/NKG2DL (C-type lectin–like transmembrane receptor protein). LSC, leukemia stem cell.

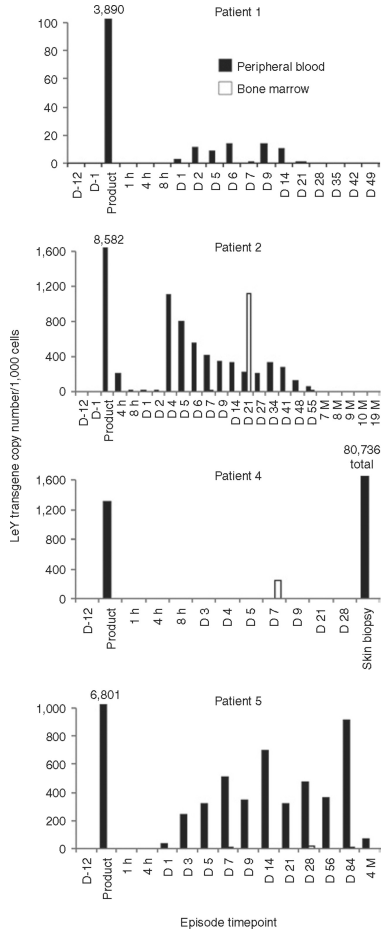
Vago and Gojo, J Clin Invest, 2020

First Experience in AML Patients: anti-LeY Ag CAR-T cells

- Four patients treated
- Second generation CAR-T
- 5-10x10^8 CAR-T cells/kg infused after FLA-Ida pre-conditioning
- Modest clinical response
- No CRS, hematological or extrahematological toxicity



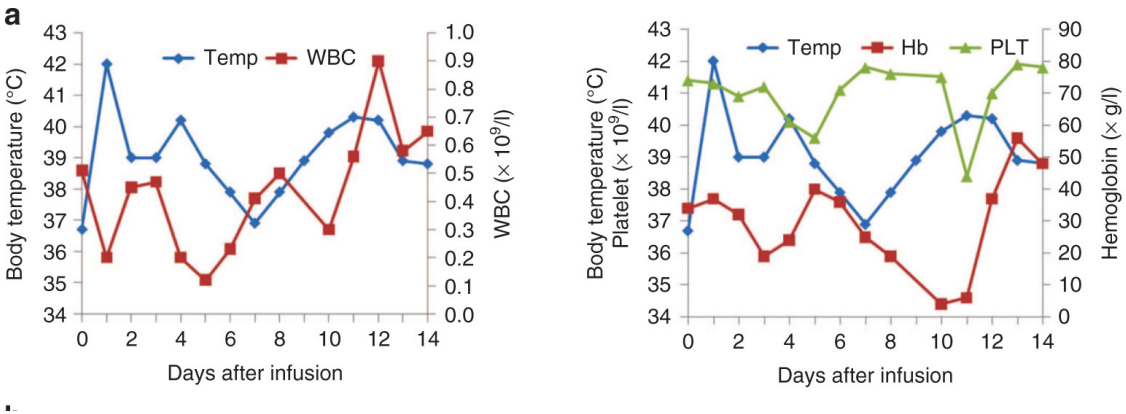


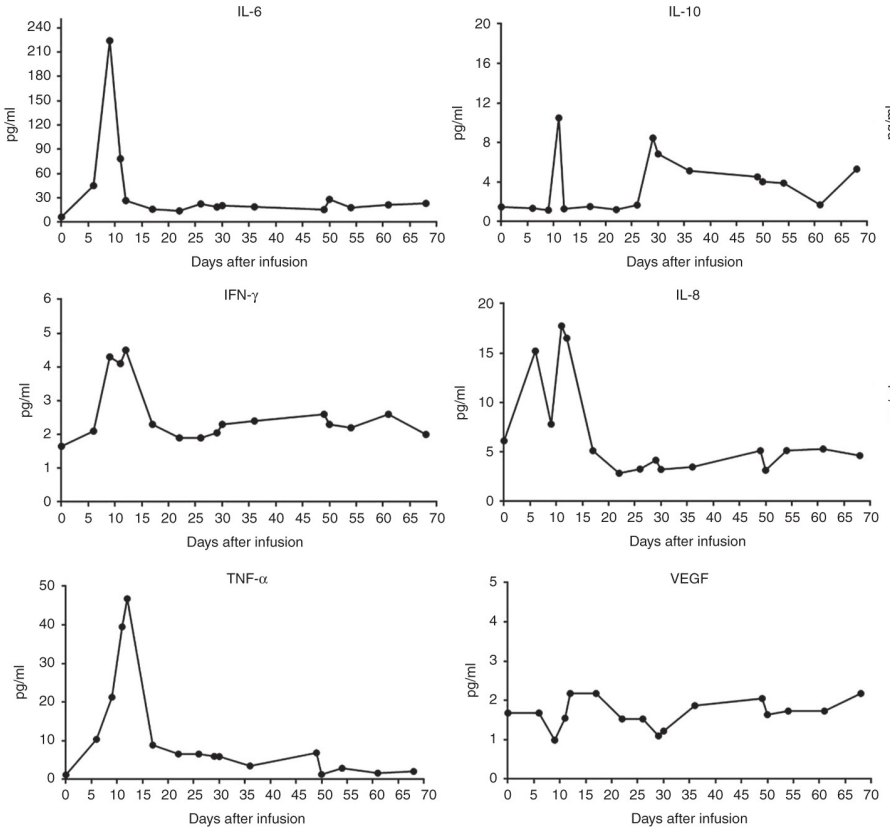


Ritchie et al, Mol Ther, 2013

anti-CD33 CAR-CIK cells

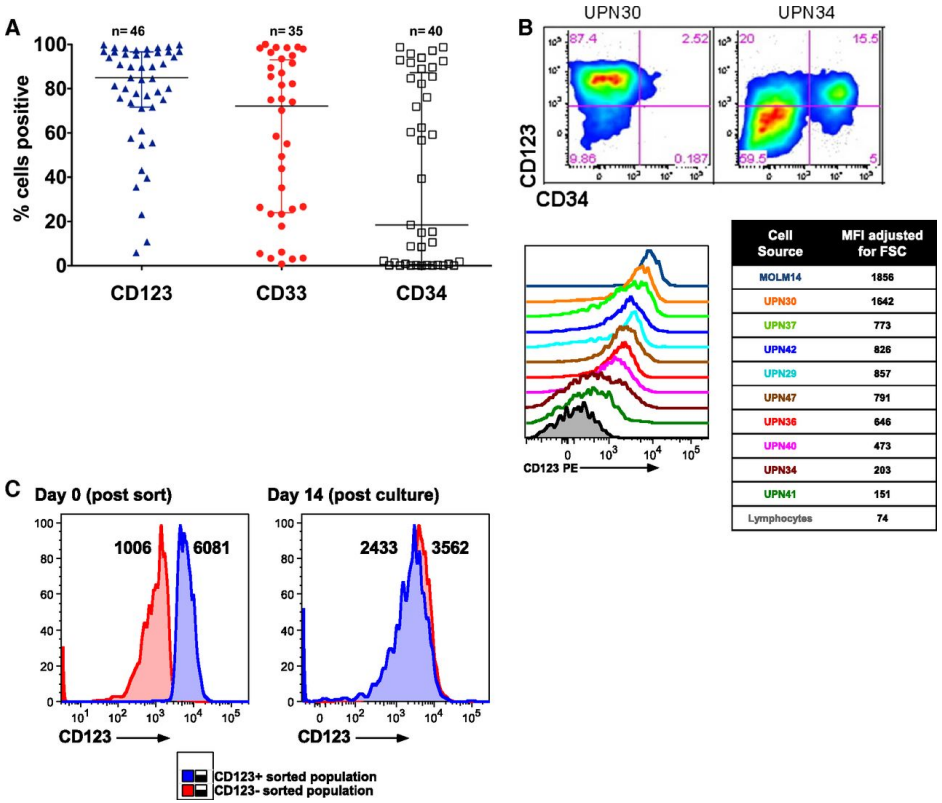
- One single published report
- $10x10^8$ CAR-CIK cells/kg infused without pre-conditioning
- Modest clinical response
- Evidence of CRS, pancytopenia (?), no extrahematological toxicity





Wang et al, Mol Ther, 2015

anti-CD123 CAR-T cells



Cell Source	MFI adjusted for FSC
MOLM14	1856
UPN30	1642
UPN37	773
UPN42	826
UPN29	857
UPN47	791
UPN36	646
UPN40	473
UPN34	203
UPN41	151
Lymphocytes	74

Gill et al, Blood, 2014

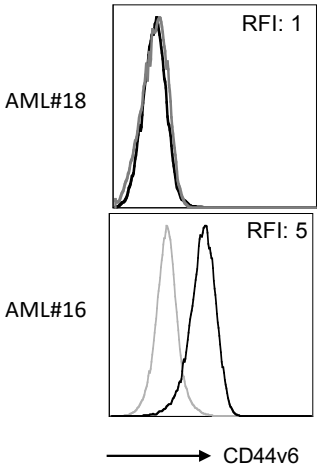
NCT02623582 (U Penn): mRNA electroporation for transient CAR expression. No disease responses, no overt vascular, hematological or neurological toxicities, laboratory and clinical CRS in all treated patients. Prompted the ongoing NCT03766126 study, using a LV vector.

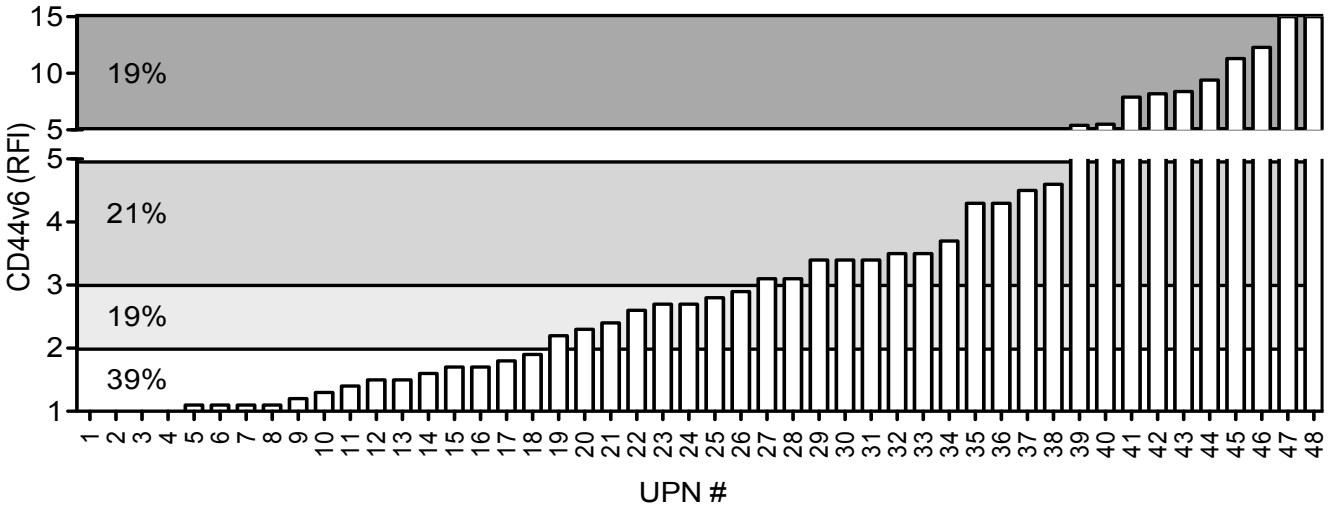
Cummins et al, ASH 2017

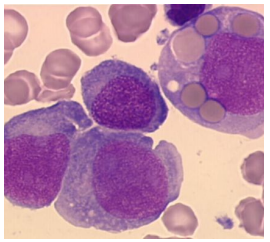
NCT02159495 (City of Hope): phase I study of a second generation LV anti-CD123 CAR (MB-102) in AML and BPDCN. Some complete remissions without dose-limiting toxicities: FDA's orphan drug designation for MB-102, expansion phase ongoing

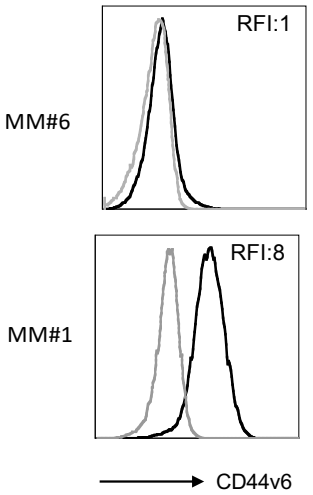
Budde et al, AACR, 2018

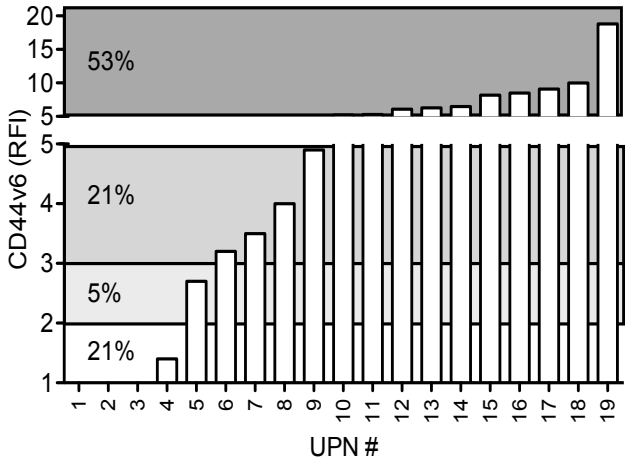
CD44v6: The Antigen

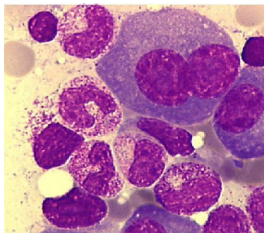










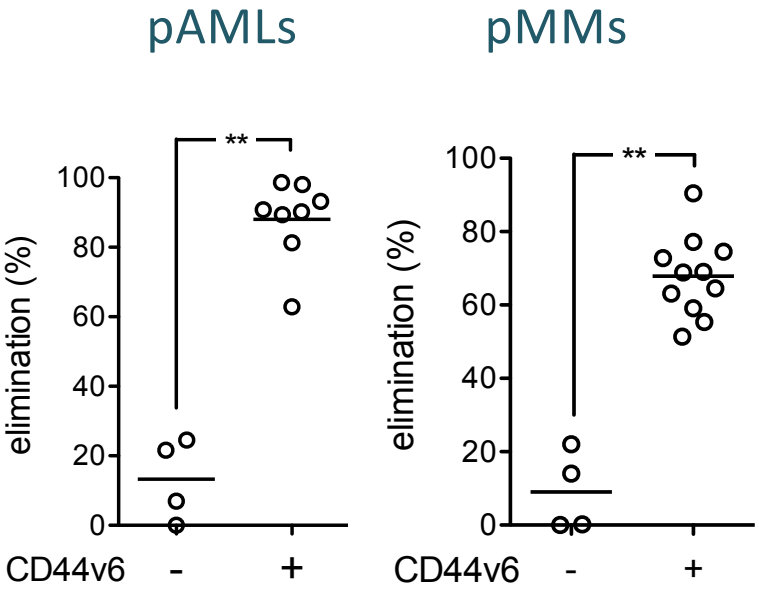


- Splicing variant of CD44, involved in interactions with the microenvironment
- Expressed on AML, MM and epithelial cancers
- Expressed on circulating monocytes and keratinocytes

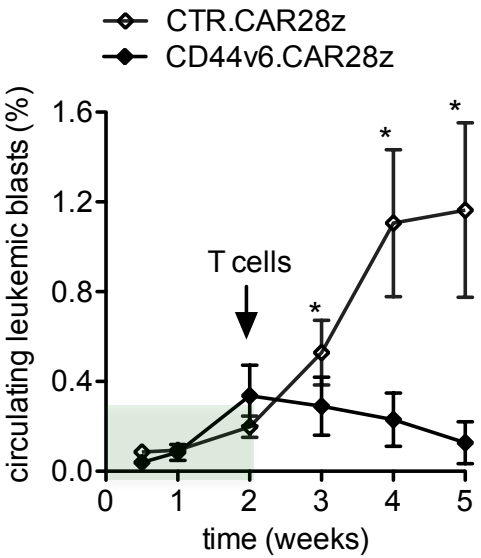
Casucci et al, Blood, 2013

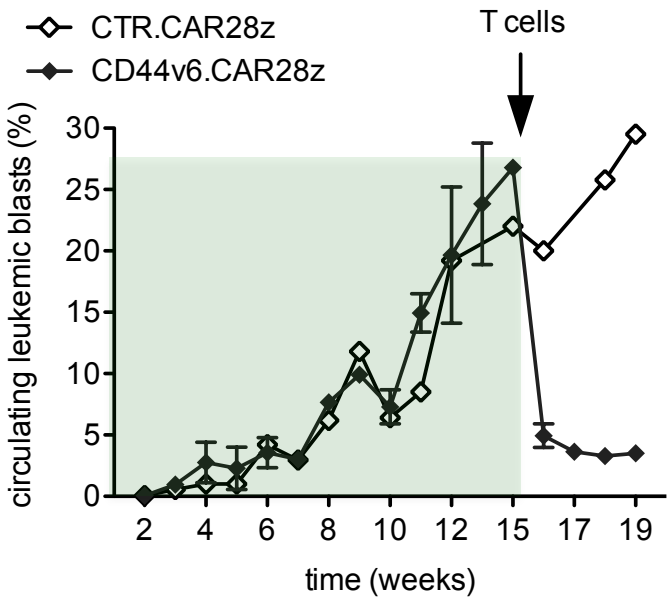
anti-CD44v6 CAR-T cells: on target activity

In vitro



In vivo

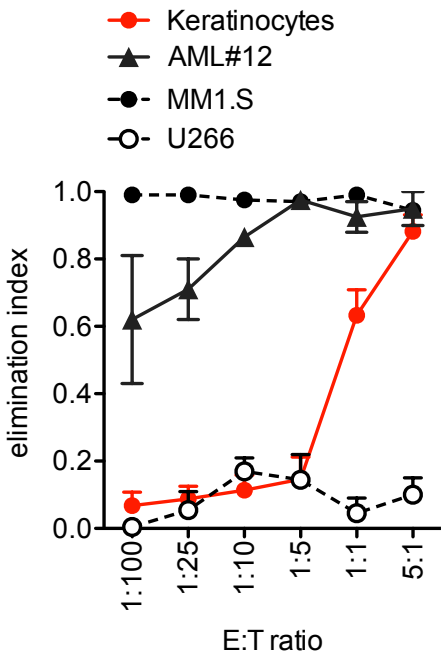




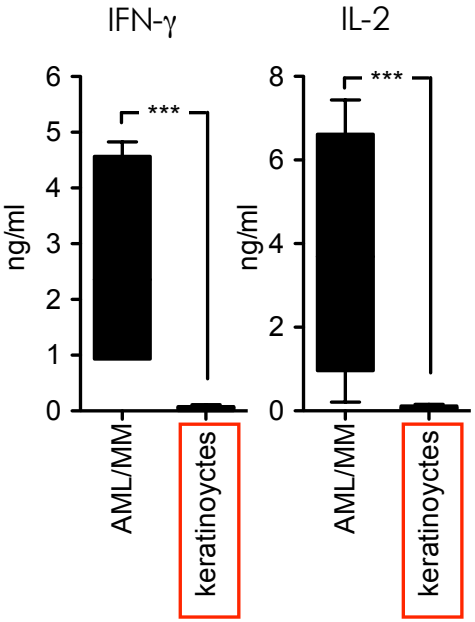
Casucci et al, Blood, 2013

anti-CD44v6 CAR-T cells: Sparing of Keratinocytes

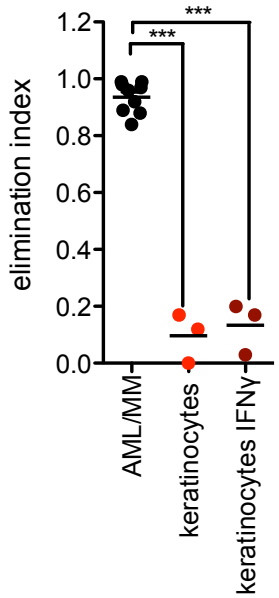
Elimination



Cytokines



Elimination (inflammation)



Casucci et al, Blood, 2013

EURECART Clinical Trial

Multi-centre, first-in-man Phase I/IIa clinical trial to demonstrate the safety and the efficacy of CAR-CD44v6 T-cell immunotherapy in:

- Acute Myeloid Leukemia (**AML**)
- Multiple Myeloma (**MM**)

Phase I - Dose escalation

Objectives: *Maximum Tolerated Dose and Clinical Activity*

exact number of pts will be determined according to the BOIN Adaptive design

Phase IIa - Dose expansion

Objectives: *Confirmation of Clinical Activity and Safety Profile*

14 Pts. (1 Dose level selected in Phase I) per indication (Simon design)

EURECART Clinical Trial: Partecipating Centres

- Ospedale San Raffaele - Milano (Italy)
- Universitätsklinikum Würzburg (Germany)
- Ospedale Pediatrico Bambino Gesù – Roma (Italy)
- Hospital de la Santa Creu i Sant Pau – Barcelona (Spain)
- Poliklinikou Ostrava Foundation (Czech Republic)

- Istituto Superiore di Sanità (Italy)

- Acromion GMBH (Germany)
- ARTTIC SAS (France)

Final Considerations and Key Future Challenges

- CAR-T cell development in AML is mainly hampered by issues related to the identification of appropriate targets
- Few small and early phase clinical reports, difficult to interpret both in terms of efficacy and of hematological toxicities
- CRS might be even more prevalent than in lymphoid diseases
- On target toxicities might be poorly predicted by expression profiling and in vitro studies, prompting development of new and improved models

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Innovative Immunotherapies Unit