Approaches to overcome CAR-T cell toxicities: anti-cytokine antibodies and suicide genes

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A. Bondanza receives research funding from Molmed Spa and is the inventor of CAR-T cell technologies acquired by and/or licensed to Molmed Spa

The risk of severe cytokine release syndrome (CRS) after CAR-T cell therapies depends on:

1) Type of costimulatory endodomain (CD28 > 4-1BB)
2) Nature of the targeted antigen, *e.g.* CD19
3) Tumor burden (high > low)
4) CD4/CD8 composition

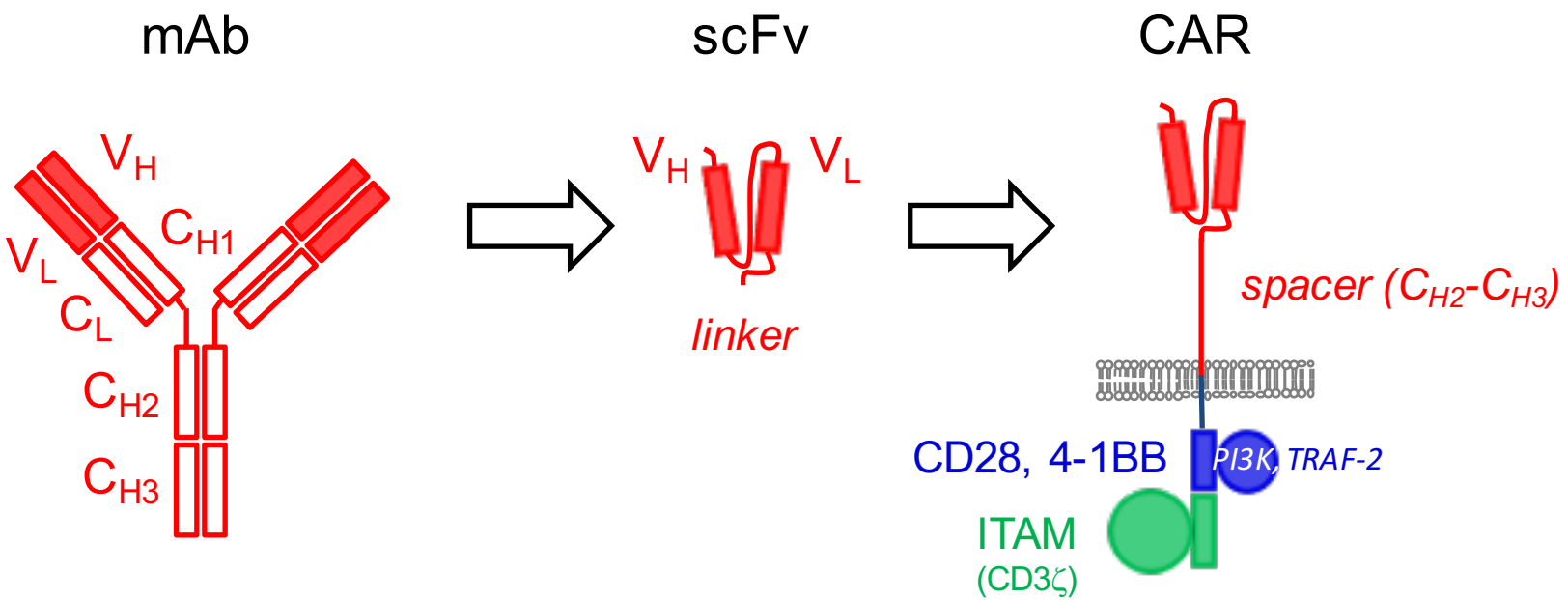
Severe neurotoxicties (*e.g.* brain edema) after CAR-T cell therapies are:

1) More likely in certain tumors than in others (ALL > NHL)
2) Due to fludarabine conditioning
3) Independent from CRS
4) Effectively controlled by tocilizumab

The “perfect” suicide gene to be implemented in CAR-T cell therapies needs to be:

1) Non-immunogenic
2) Fast-acting
3) Actionable with prodrugs that cross the blood-brain barrier
4) Effective and highly penetrant

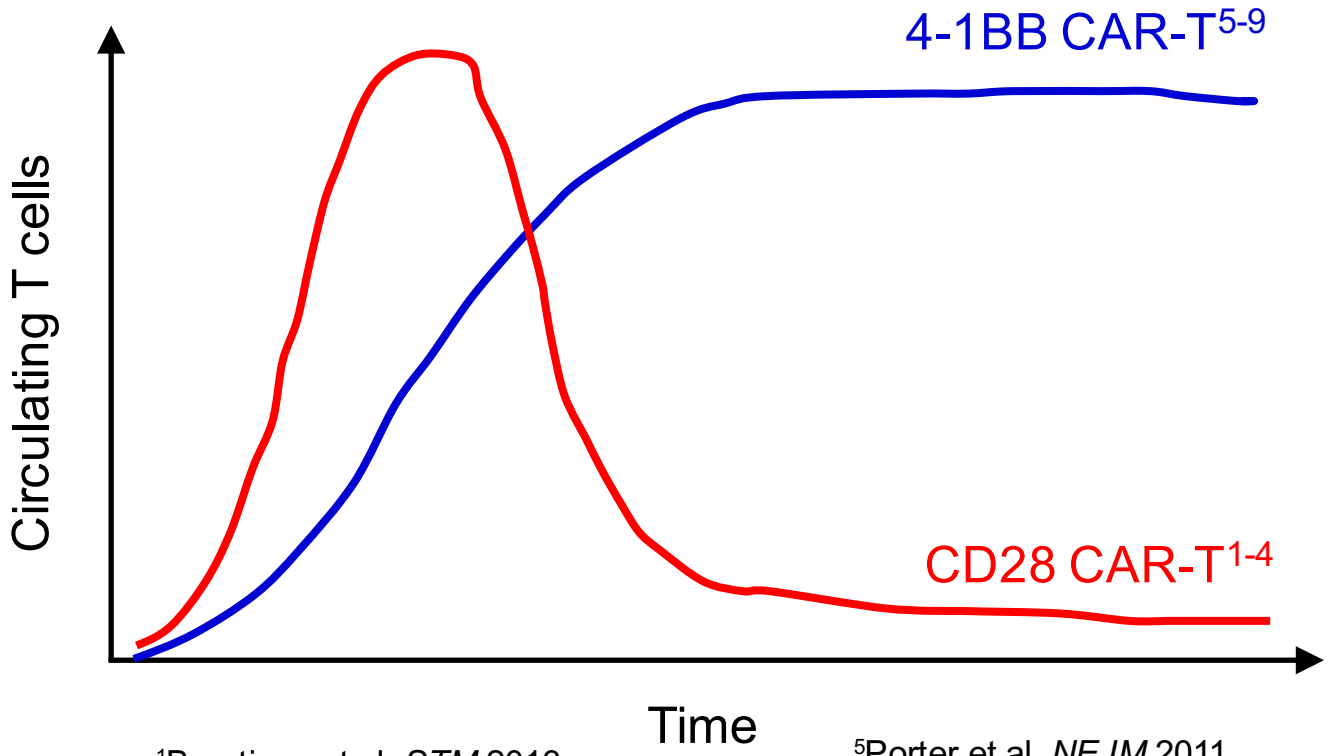
CARs are synthetic biology receptors made up of mAb-derived targeting motifs and TCR/costimulatory endodomains



TCRs
HLA-dependent
Intracellular Ags
Protein Ags
Low affinity (10^{-3}-10^{-4})
Killing and proliferation

CARs
HLA-independent
Surface Ags
Protein, sugar and lipid Ags
High affinity (10^{-8}-10^{-10})
Killing

CD28 and 4-1BB differently affect the pharmacokinetics of CAR-T cells



[1]Brentjens et al, *STM* 2013
[2]Kochenderfer et al, *JCO* 2014
[3]Lee et al, *Lancet Oncol* 2015
[4]Wang et al, *Blood* 2016

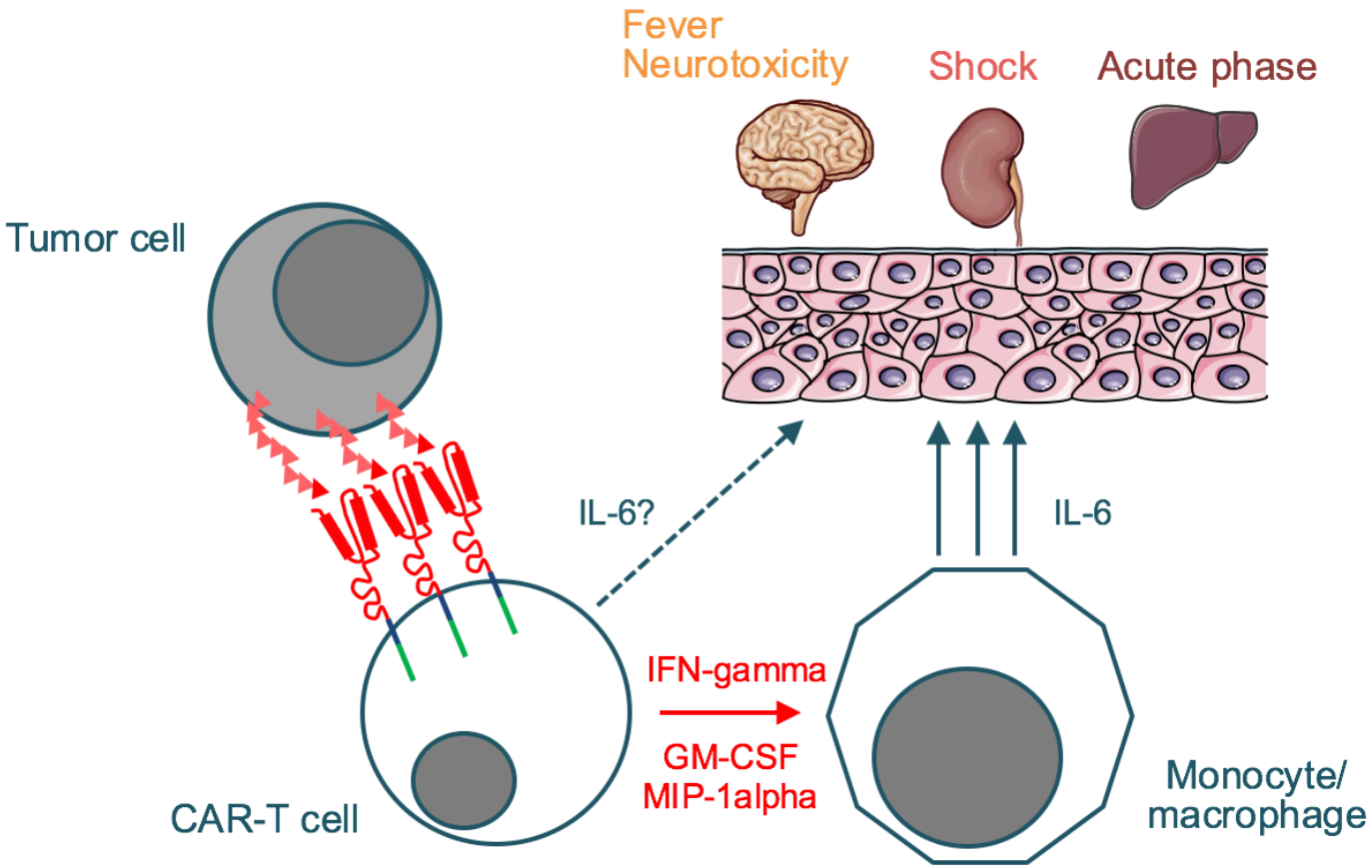
[5]Porter et al, *NEJM* 2011
[6]Porter et al, *STM* 2011
[7]Grupp et al, *NEJM* 2013
[8]Turtle et al, *JCI* 2015
[9]Maude et al, *NEJM* 2014

Antitumor responses by CD19 CAR-T differ between B-cell tumors but not between costimulatory endodomains

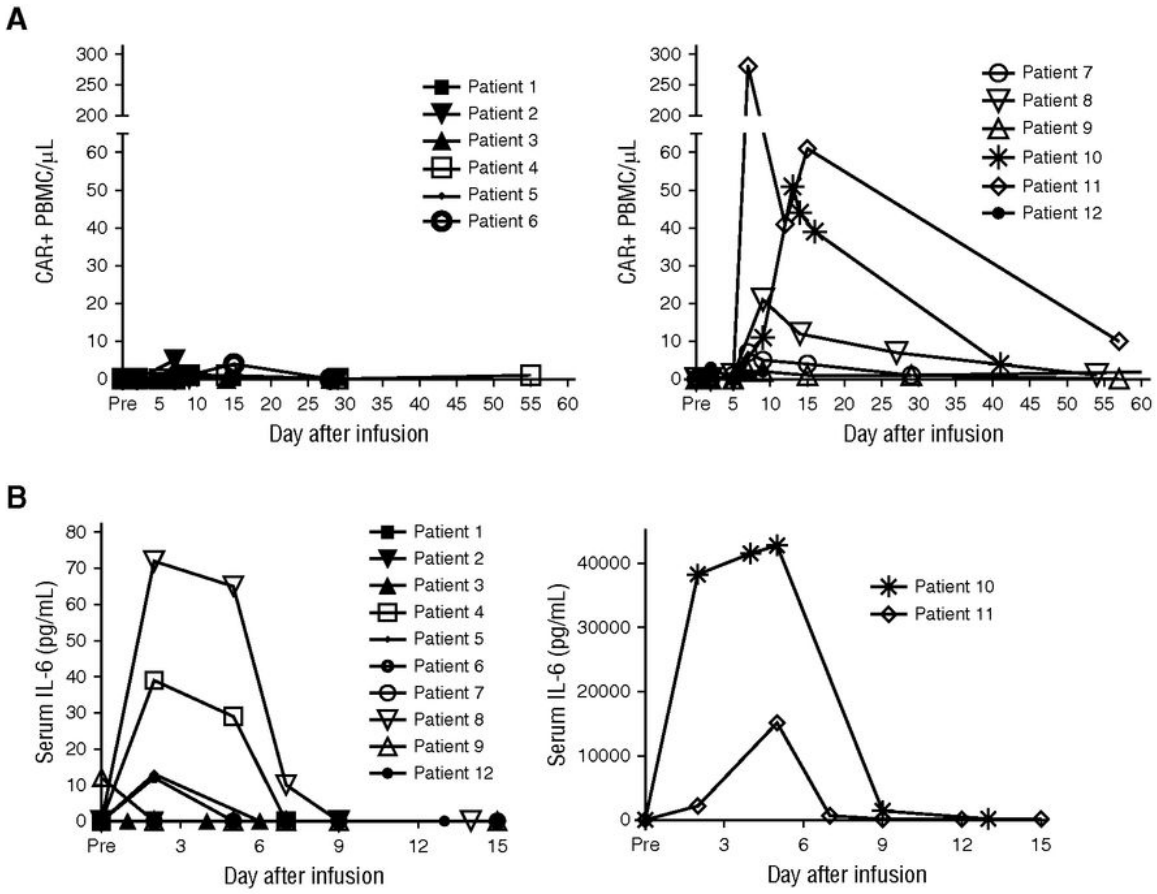
Disease	Complete response (CR) rate
Chronic lymphocytic leukemia (CLL)[1,2]	30-40%
Non-Hodgkin lymphoma (NHL)[3,4]	50-70%
Acute lymphoblastic leukemia (ALL)[5-9]	80-90%

[1]Porter et al, *NEJM* 2011
[2]Porter et al, *STM* 2011
[3]Kochenderfer et al, *JCO* 2014
[4]Wang et al, *Blood* 2016
[5]Grupp et al, *NEJM* 2013
[6]Brentjens et al, *STM* 2013
[7]Maude et al, *NEJM* 2014
[8]Lee et al, *Lancet Oncol* 2015
[9]Turtle et al, *JCI* 2015

Cytokine release syndrome (CRS) is caused by by-stander activation of innate immunity



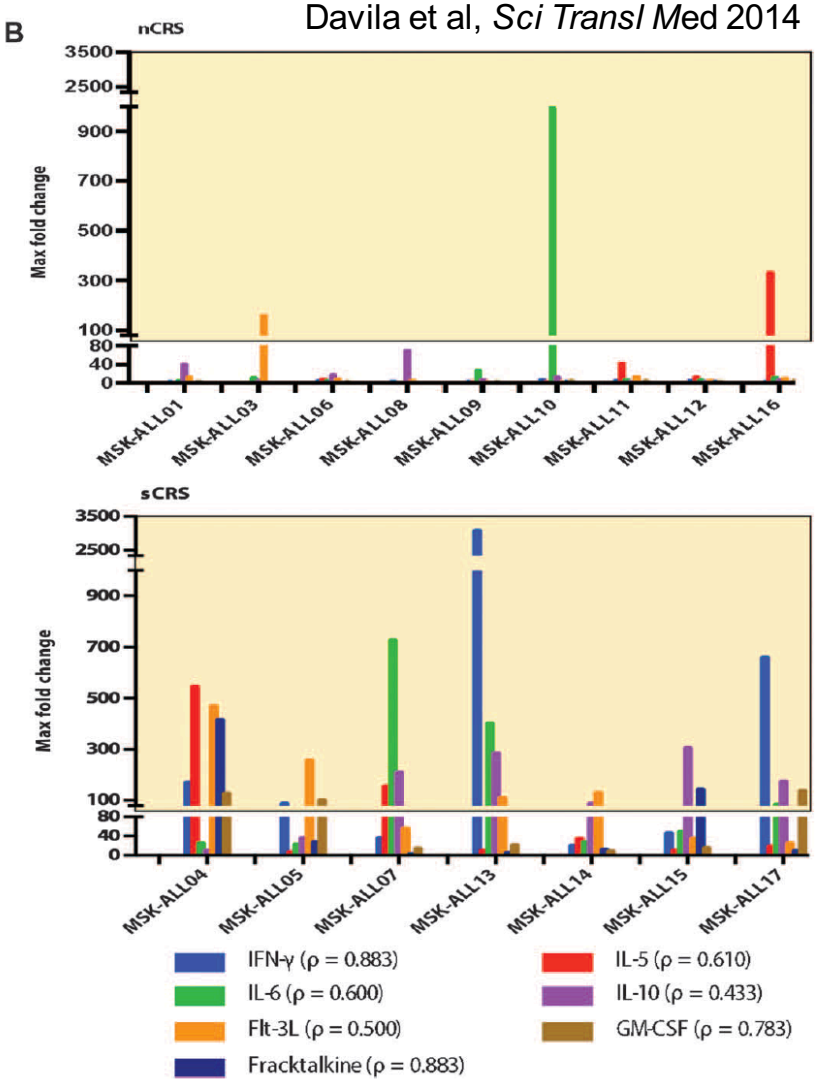
Severe CRS has also been observed with BCMA CAR-T cells



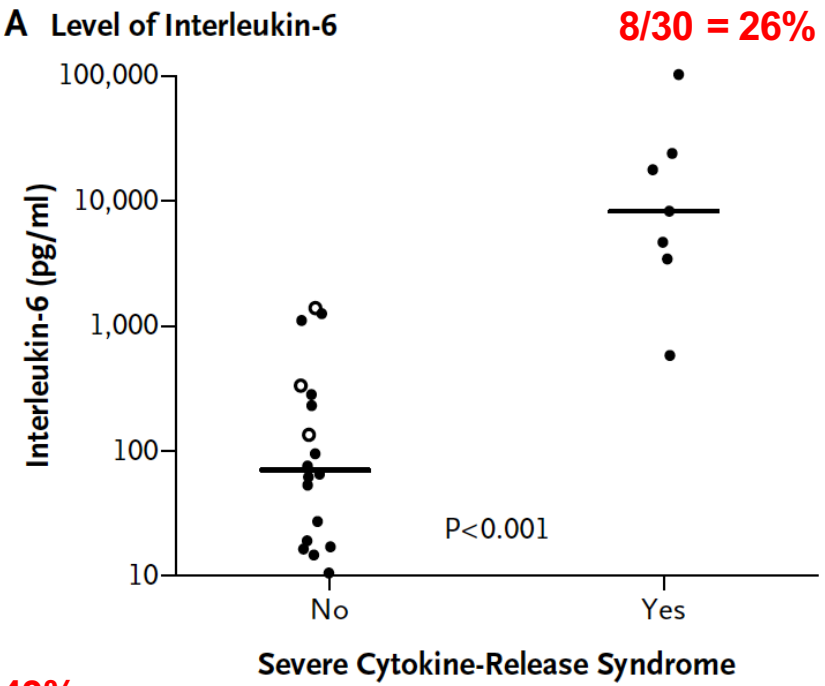
Abbas-Ali et al, *Blood* 2016

Severe CRS is equally frequent with CAR-T cells having CD28 or 4-1BB costimulatory endodomains

Davila et al, *Sci Transl Med* 2014

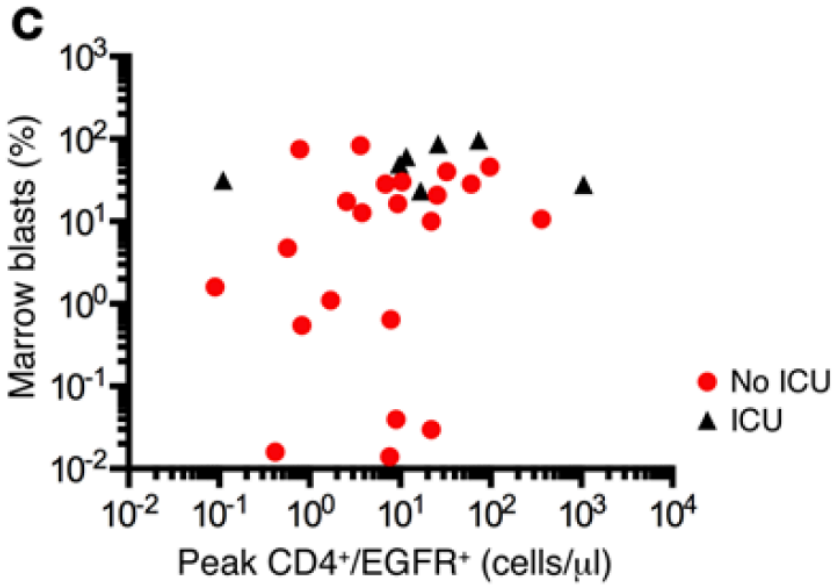


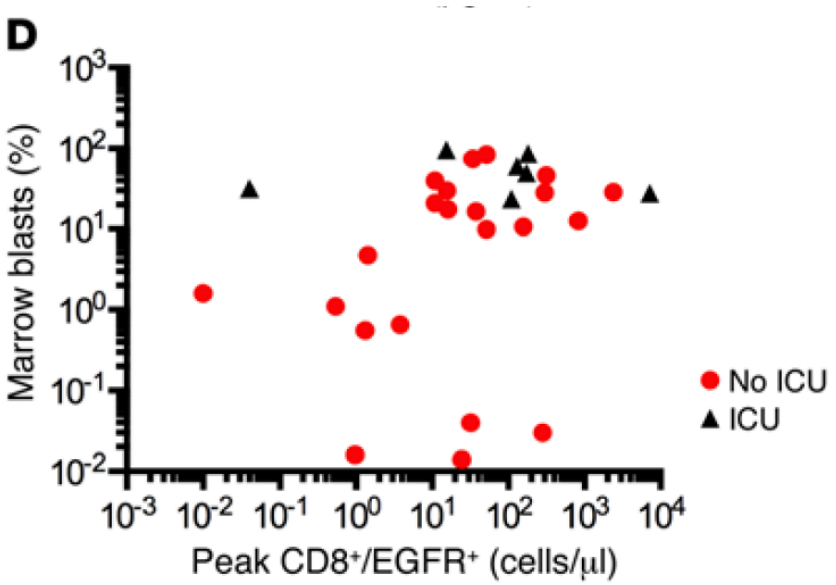
7/16 = 43%



Maude et al, *N Eng J Med* 2014

Severe CRS is more likely in the case of high tumor burdens regardless of CD4/CD8 composition





Turtlle et al, *J Clin Invest* 2016

The risk of severe CRS after CAR-T cell therapies depends on:

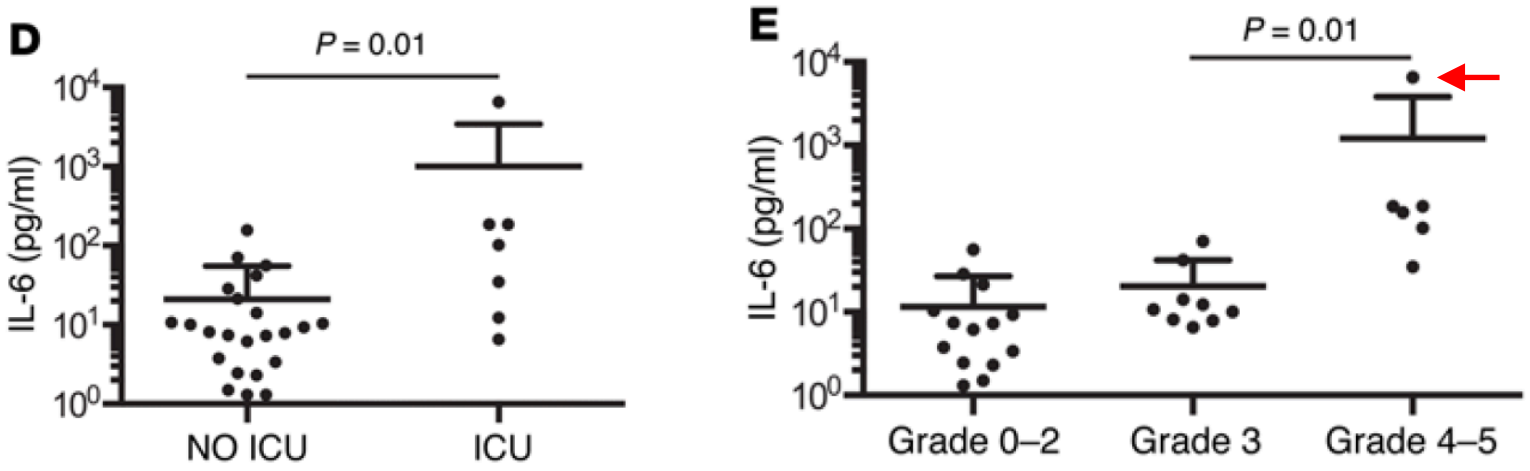
1) Type of costimulatory endodomain (CD28 > 4-1BB)
2) Nature of the targeted antigen, *e.g.* CD19
3) **Tumor burden (high > low) – better, E:T ratio!**
4) CD4/CD8 composition

Severe neurotoxicties (*e.g.* brain edema) after CAR-T cell therapies are:

1) More likely in certain tumors than in others (ALL > NHL)
2) Due to fludarabine conditioning
3) Independent from the cytokine release syndrome
4) Effectively controlled by tocilizumab

Severe neurotoxicities are preceded by cytokine release syndrome and ineffectively controlled by tocilizumab

15/29 = 51%



Turtlle et al, *J Clin Invest* 2016

Severe neurotoxicities are independent from fludarabine conditioning and apparently less frequent in NHL

Table 1. Patient Clinical Characteristics

Patient No.	Age (years)	Sex	Malignancy	No. of Prior Therapies[a]	sAAIPI Risk Group	Total Cyclophosphamide Dose (mg/kg)[b]	No. of CAR-Positive T Cells Infused ($\times 10^6$/kg)	Response[c]		Grade ≥ 3 Toxicities[d]	
								Type	Duration (months)		
1[e]	56	Male	SMZL	4	NA	120	5	PR	23+[f]	Hypotension, confusion, acute renal failure, fever	
2	43	Female	PMBCL[g]	4	Low	60	5	CR	22+[f]	Fever, confusion, aphasia, facial nerve palsy, headache, urinary tract infection	
3	61	Male	CLL (FR)	2	NA	60	4	CR	23+[f]	Headache, fever, confusion, hypotension	
4	30	Female	PMBCL[g]	3	High	120	2.5	NE		Nausea, hypoxia, dyspnea, tachycardia, fever, bacteremia, malaise, vascular leak syndrome, death	
5[e]	63	Male	CLL	4	NA	120	2.5	CR	15+[f]	None	
6	48	Male	CLL (FR)	1	NA	60	2.5	CR	14+[f]	None	
7	42	Male	DLBCL NOS[g]	5	High	60	2.5	CR	9+[f]	Influenza, fever, headache, bacteremia	
8	44	Female	PMBCL[g]	10	High	60	2.5	CR	12+[f]	Fever, pneumonitis, hypotension, hypoxia, bacteremia, obtundation, elevated creatinine	←
9	38	Male	PMBCL[g]	3	High	120	2.5	SD	1	Fever, aphasia, myoclonus	←
10	57	Female	Low-grade NHL[h]	4	NA	60	1	CR	11+[f]	Bacteremia, fever, fatigue	
11	58	Female	DLBCL[g] from CLL	12	High	60	1	PR	1	Bacteremia, urinary tract infection, fever	
12	60	Female	DLBCL NOS[g]	3	High	60	1	NE[i]		Fever, urinary tract infection, bacteremia, upper extremity thrombosis	
13	68	Male	CLL	4	NA	60	1	PR	4	Dyspnea, upper extremity thrombosis, urinary tract infection, creatinine increase, hypotension	
14	43	Male	DLBCL NOS[g]	2	High	60	1	CR	6	Fever	
15	64	Female	DLBCL NOS[h]	3	Intermediate	60	1	PR	6+[f]	Fever, aphasia, encephalopathy, neuropathy, gait disturbance	←

3/15= 20%

Kochenderfer et al, *J Clin Oncol* 2015

Severe neurotoxicties (brain edema) after CAR-T cell therapies are:

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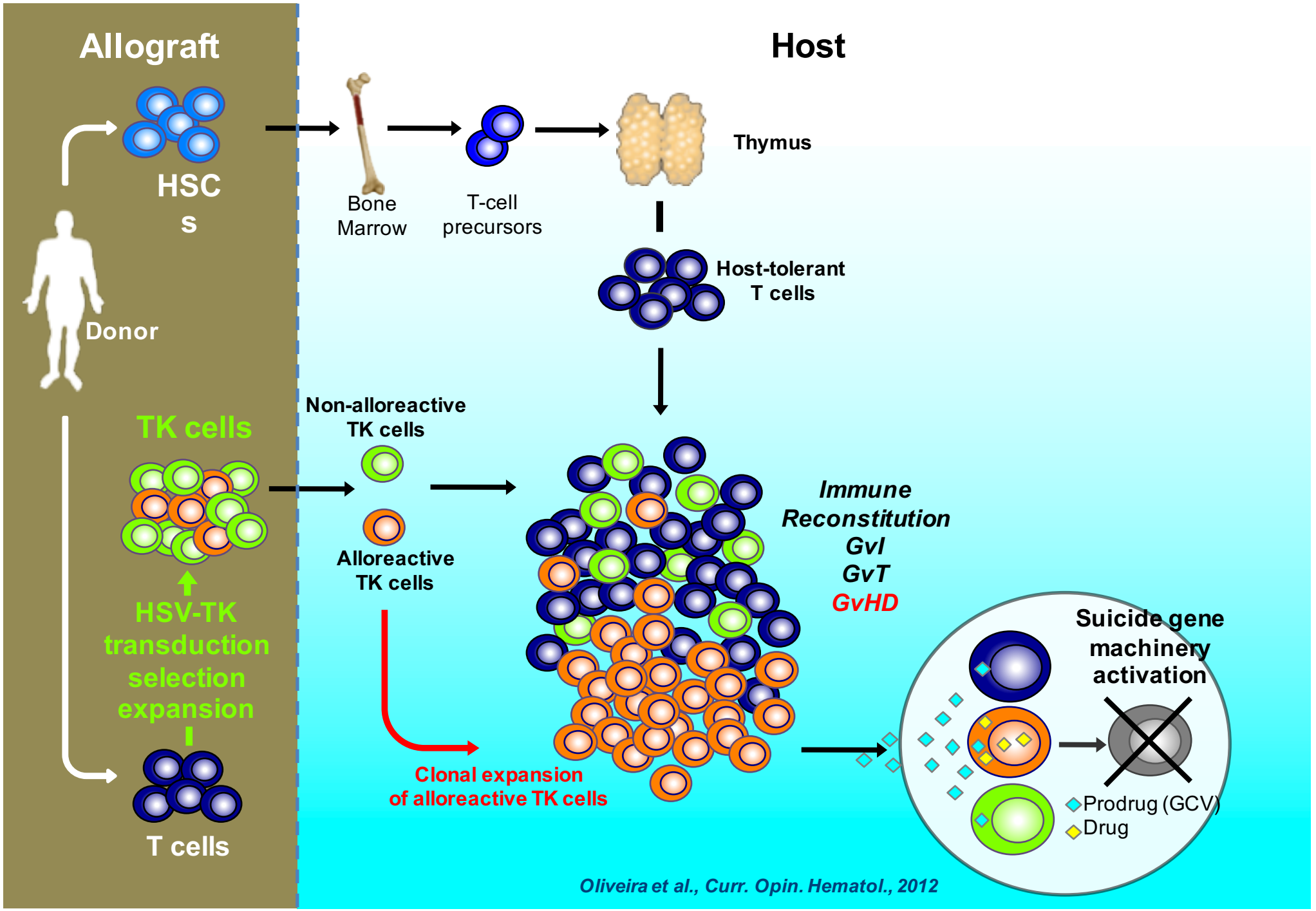
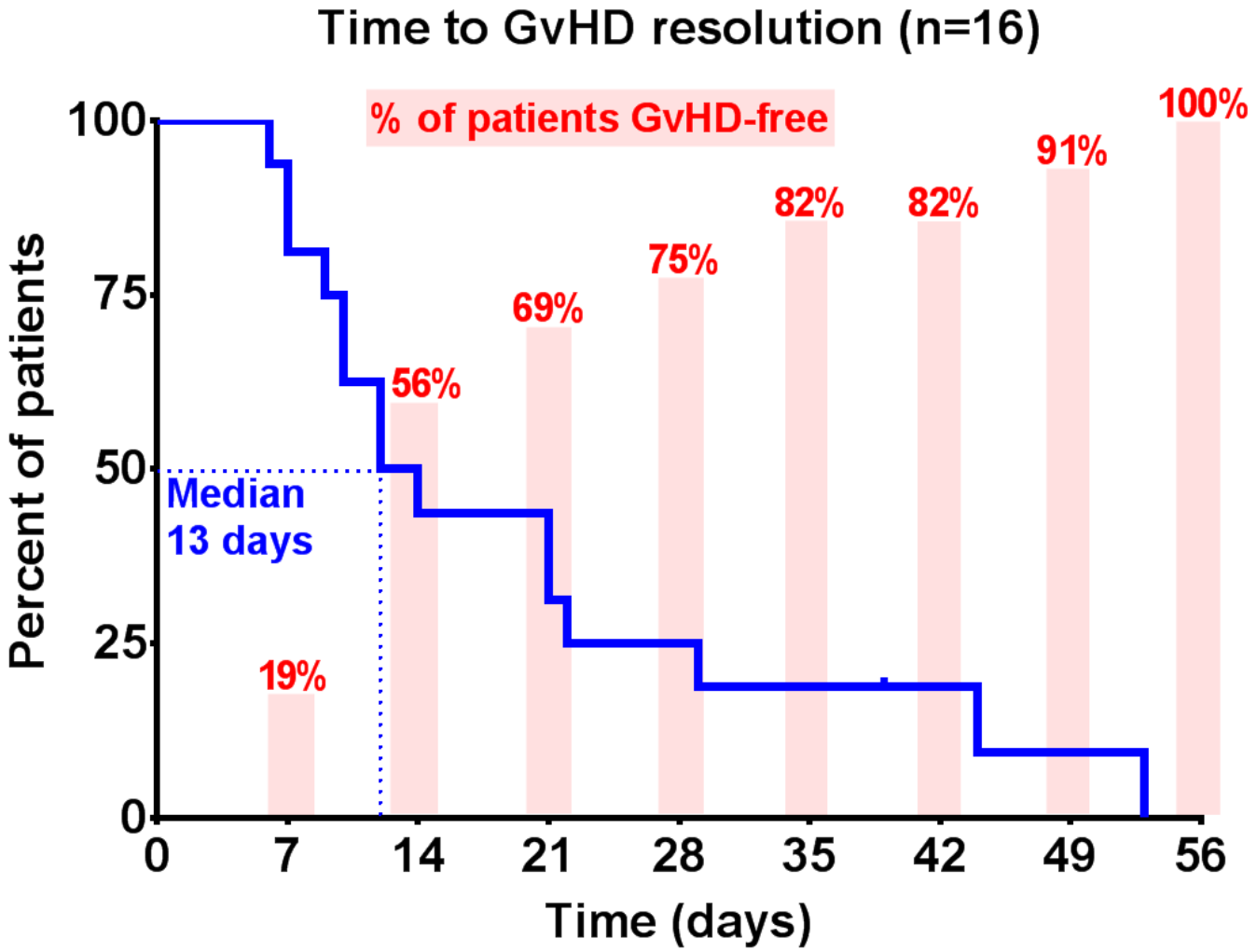
2) Due to fludarabine conditioning
3) Independent from cytokine release syndrome
4) Effectively controlled by tocilizumab

The “perfect” suicide gene to be implemented in CAR-T cell therapies needs to be:

1) Non-immunogenic
2) Fast-acting
3) Used for managing cytokine release syndrome/neurotoxicities
4) Effective and highly penetrant


Allograft
Host
HSCs
Donor
Bone Marrow
T-cell precursors
Thymus
Host-tolerant T cells
TK cells
HSV-TK transduction selection expansion
T cells
Non-alloreactive TK cells
Alloreactive TK cells
Clonal expansion of alloreactive TK cells
Immune Reconstitution
GvI
GvT
GvHD
Suicide gene machinery activation
Prodrug (GCV)
Drug
Oliveira et al., Curr. Opin. Hematol., 2012

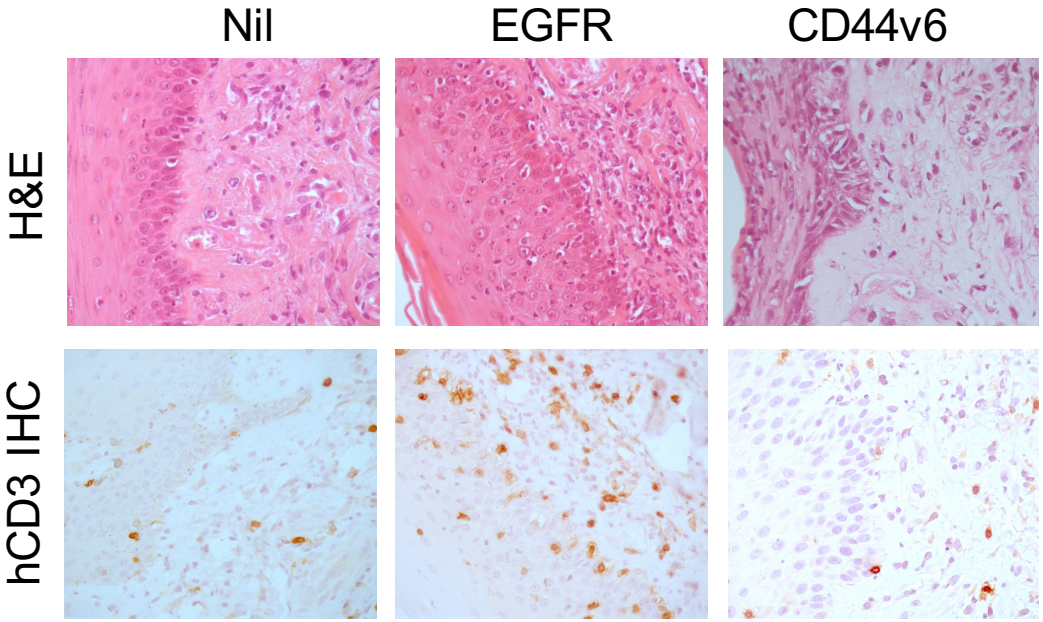
TK is a slow, yet highly penetrant suicide gene capable of reverting GVHD

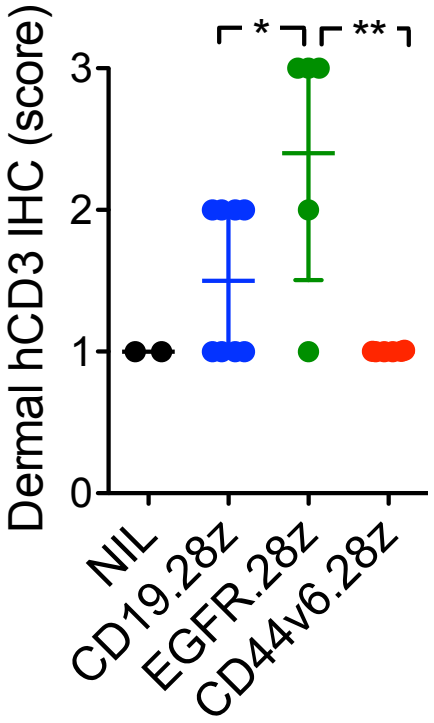


Ciceri et al, *Lancet Oncology* 2009
Lupo-Stanghellini et al, *ASH 2014*

CD44v6 CAR-T cells are not toxic to human skin engrafted onto NSG mice

NSG mice +
Full-thickness human skin +
$T_{SCM/CM}$ CAR-T cells (5x10E6)

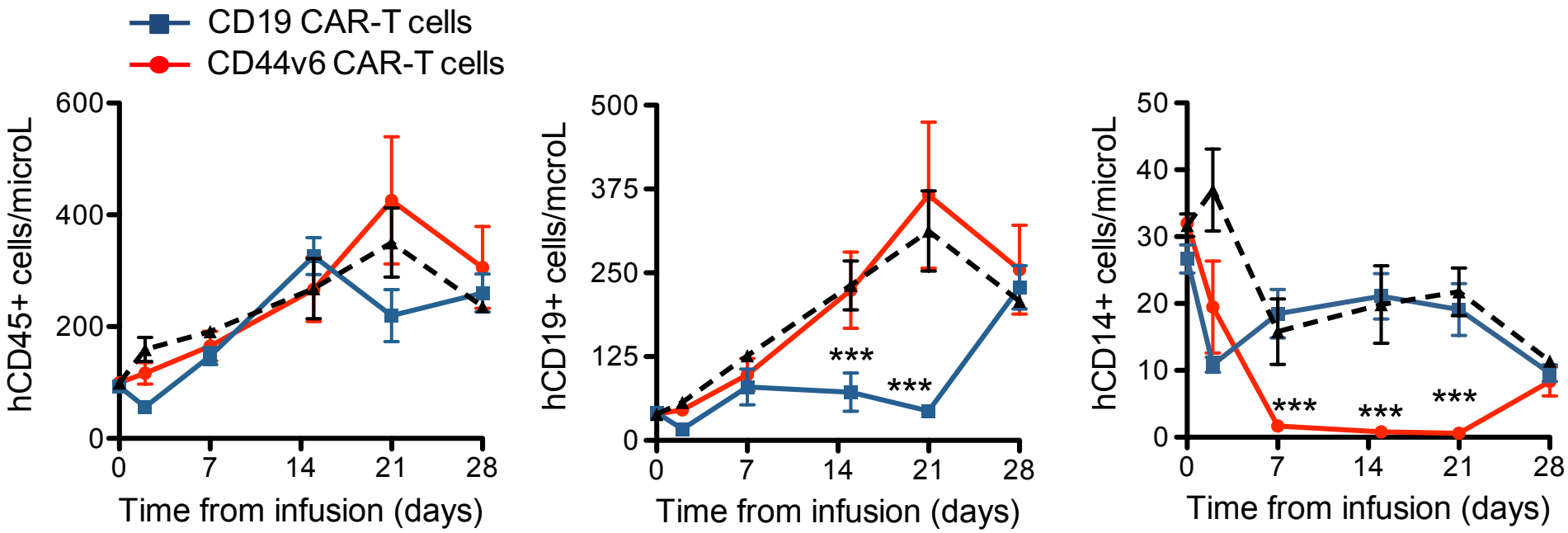




CD44v6 CAR-T cells cause selective monocytopenia in NSG mice reconstituted with human HSCs

NSG-SGM3 mice (human SCF, GM-CSF, IL-3)
HSCs (cord blood, 50,000/mouse)
CD44v6 CAR-T cells (cord blood, 2x10E6/mouse)

Casucci et al, *Blood* 2013



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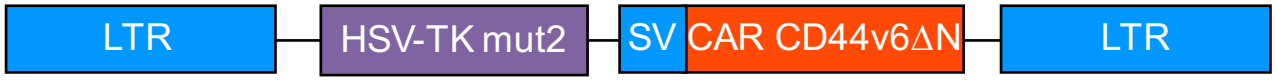

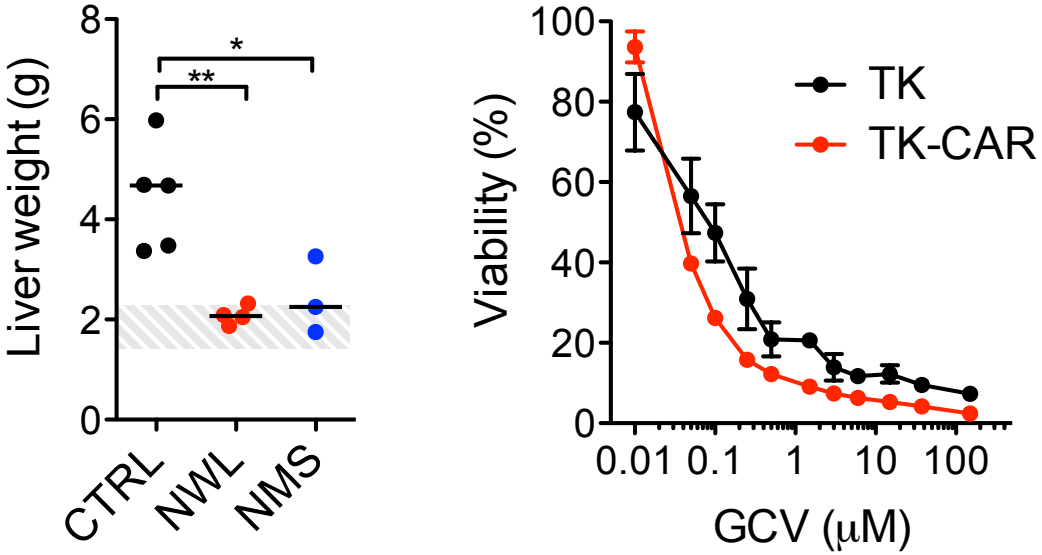

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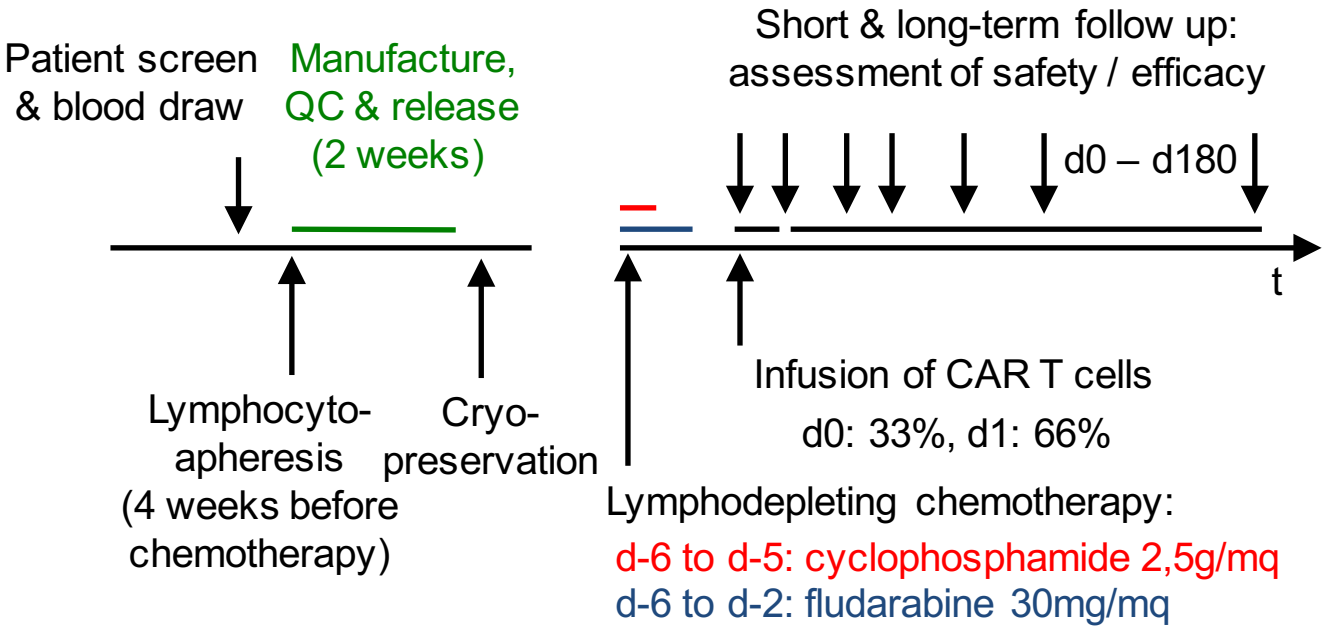
A phase I/IIa clinical trial of anti-CD44v6 CAR-T cells in relapsed/refractory AML and MM will begin in 2018

Centers:

San Raffaele, Milano (A Bondanza, F Ciceri)
Wurzburg University (H Einsele)
Ospedale Pediatrico Bambino Gesù, Roma (F Locatelli)
Sant Pau Hospital, Barcelona (J Sierra)
University Hospital Ostrava (R Hajek)



Acknowledgements

Monica Casucci
Laura Falcone
Barbara Camisa
Maddalena Noviello
Benedetta Nicolis di Robilant (now at Uni Stanford US)
Fabiana Gullotta (now at Uni Basel CH)
Margherita Norelli
Edoardo Galli (now at ETH Zurich CH)
Beatrice Greco
Matteo Doglio
Ayurzana Purevdorj (now at Uni Wien AT)
Silvia Arcangeli
Marco Montagna
Marta Biondi
Marta Moresco
Antonella Antonelli
Ilaria Palamà (Joint Lab at CNR-Nanotec, Lecce IT)
Luca Cossa

Collaborations

Fabio Ciceri
Chiara Bonini
Luigi Naldini
Bernhard Gentner
Pietro Genovese

Gianpietro Dotti (Uni North Carolina US)
Aurore Saudemont (Anthony Nolan, London UK)
Giuseppe Gigli (CNR-Nanotec, Lecce IT)
Sara Deola (Sidra Medical Center, Doha QATAR)

Claudio Bordignon (Molmed Spa, Milano IT)
Francois Meyer (TxCell SA, Valbonne FR)
Christian Klein (Roche Glycart, Schlieren CH)
Daniel Olive (ImmCheck Therapeutics, Marseille FR)