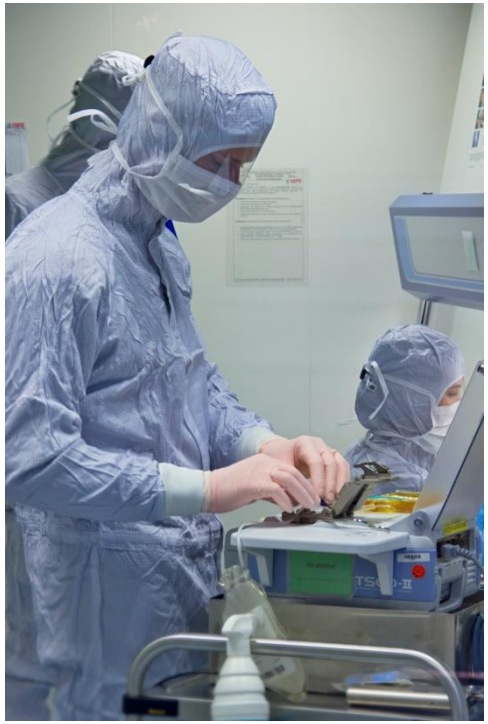
CAR NK cell therapy

Ulrike Koehl



Fraunhofer Institute for Cellular Therapeutics and Immunology (IZI),
Institute of Clinical Immunology, University of Leipzig (UL/UKL),
Institute of Cellular Therapeutics, Hannover Medical School (MHH), Germany

Disclosure

In relation to this presentation, I declare that there are no conflicts of interest.*

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GEFÖRDERT VOM

Bundesministerium für Bildung und Forschung

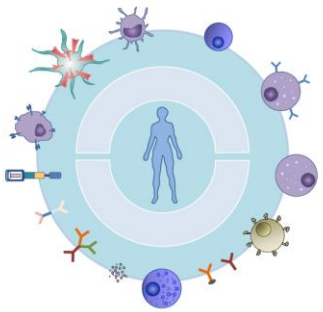
DLR Projektträger

-

- CTL019 European study trial NOVARTIS Kymriah®
- Consulting: AstraZeneca, Affimed, Glycostem

* A conflict of interest is any situation in which a speaker or immediate family members have interests, and those may cause a conflict with the current presentation. Conflicts of interest do not preclude the delivery of the talk, but should be explicitly declared. These may include financial interests (e.g. owning stocks of a related company, having received honoraria, consultancy fees), research interests (research support by grants or otherwise), organisational interests and gifts.

The era of Advanced Therapy Medicinal Products



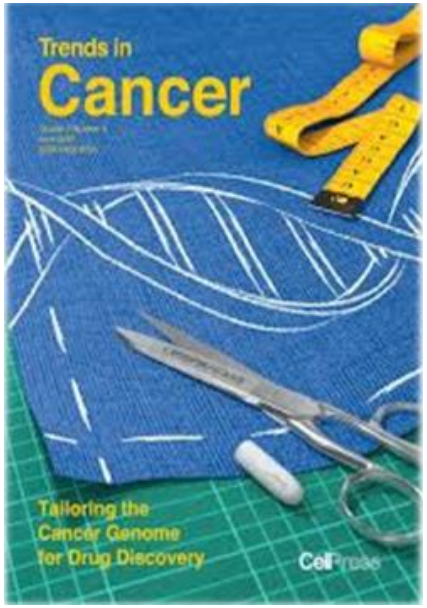
Imlygic®
Holoclar®

Zalmoxis®
Strimvelis®

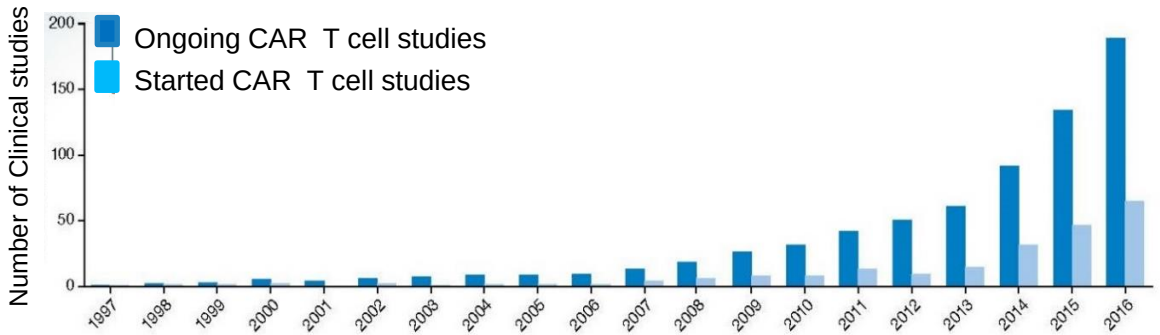
Yescarta® (FDA)
Kymriah® (FDA)
Spherox®

Kymriah® (EMA)
Yescarta® (EMA)
Alofisel®
Luxturna®

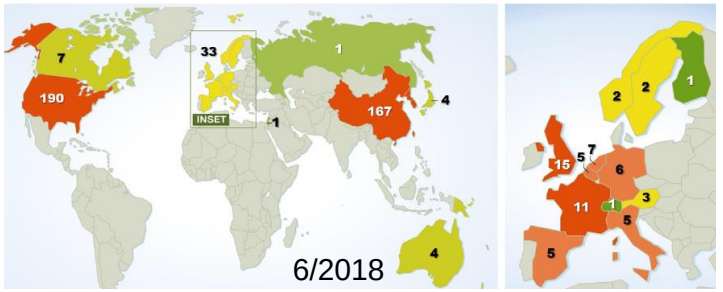
2009 2010 2011 2012 2013 2014 2015 2016 2017 2018 2019



Clinical CAR T cell studies

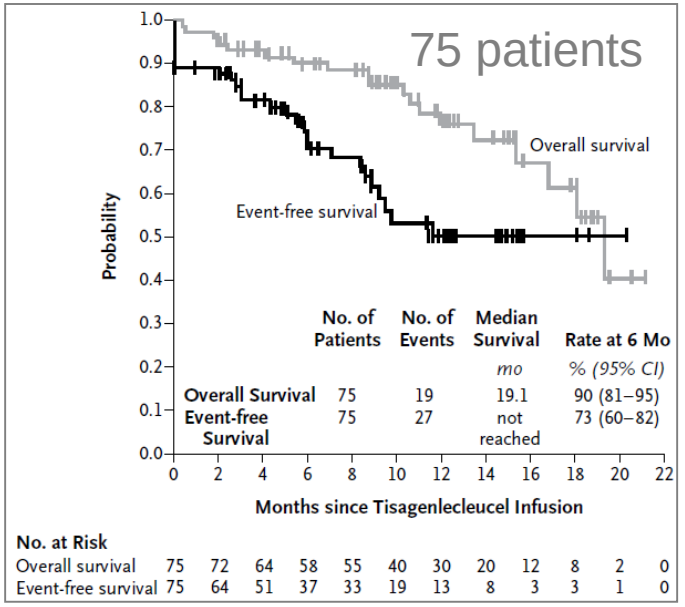


Hartman J, et al. EMBO Mol Med, 8 2017



Clinicaltrials.gov

Paediatric r/r ALL – ELIANA

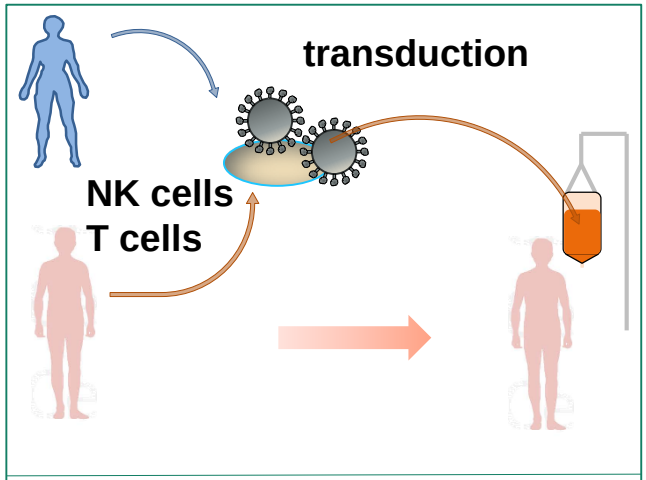


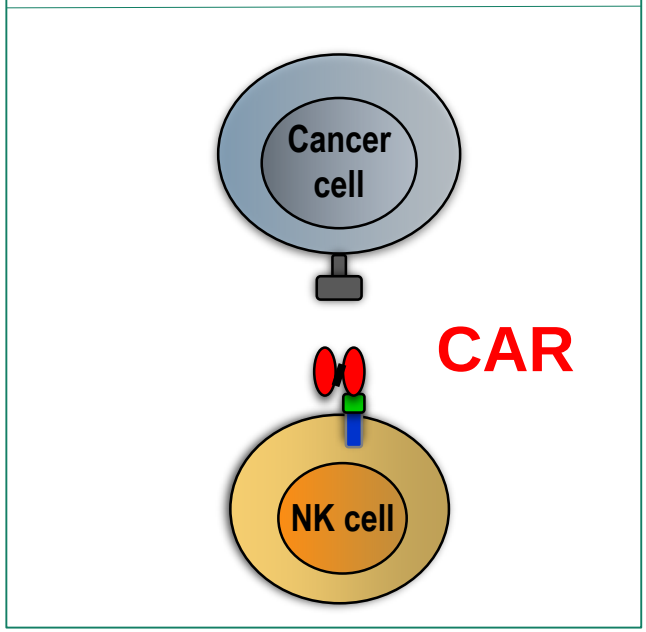
Maude SL *et al.* New Engl J Med 2018

- > 450 clinical trials (2/2019)
- 10% of the studies in Europe, only
- To date, 2 products on the market
- To date, **successful results in hematological disorders** (most experience in CD19+ diseases)
- But very limited efficacy in solid tumors

CAR = chimeric antigen receptor

Overview – CAR NK cells





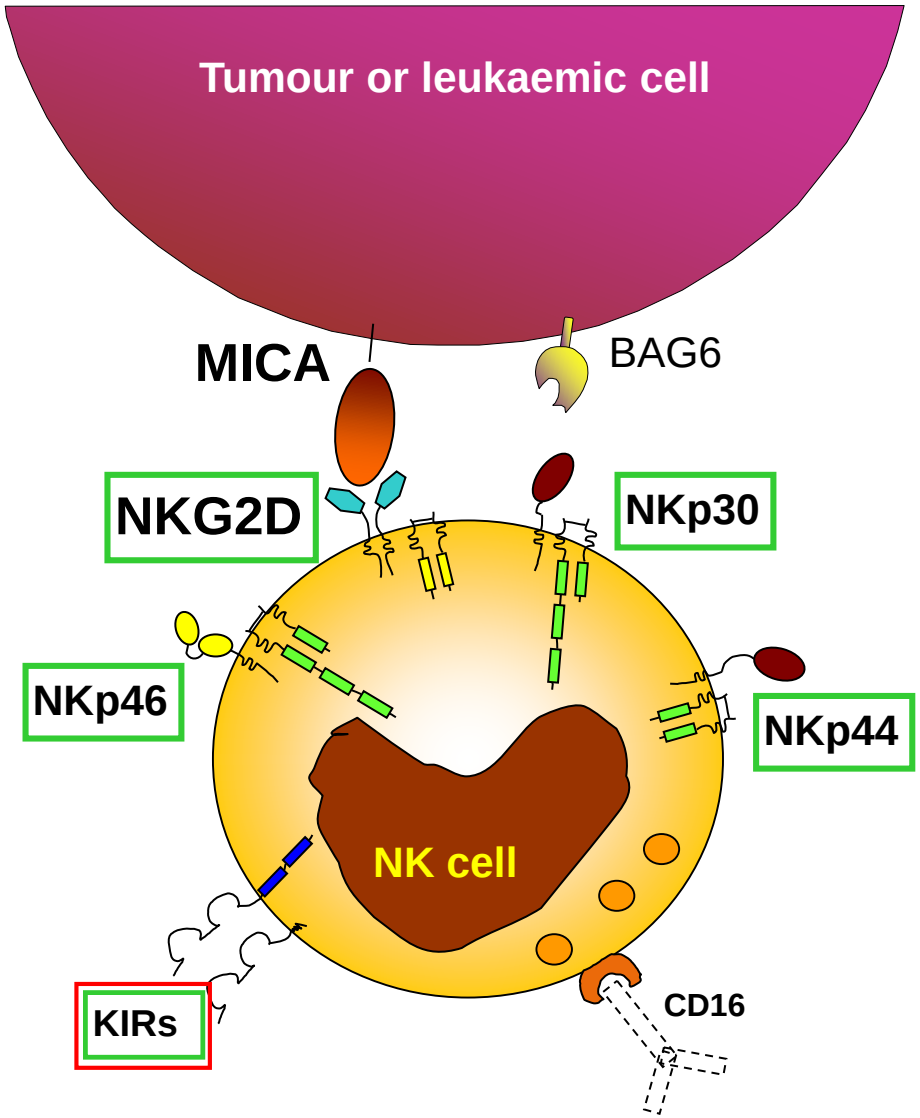
- Autologous CAR T cells – other limitations:
  - manufacturing time consuming, expensive
  - in some cases failure in manufacturing
  - relapse due to contaminating leukemic cells in the product *(Ruella M, Nature Medicine 2018)*

- **CAR NK cells for advanced strategies**
  - Allogeneic donor NK cells as an „off the shelf“ therapy
  - CAR NK cells for improved killing functionality
  - Possibility to overcome tumour immune escape?

CAR = chimeric antigen receptor
NK = natural killer cells

Fraunhofer IZI

Natural Killer (NK) cells

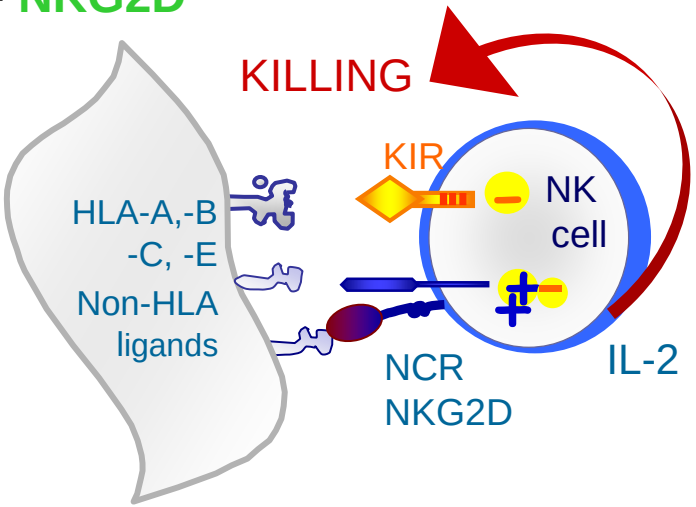


IgV IgC ITAM immunoreceptor tyrosine-based activation motifs)

- $CD56^{+}CD3^{-}$ NK cells comprise 2-18% of lymphocytes in the peripheral blood
  $CD56^{bright}CD16^{negative}$ (immunregulatory)
  $CD56^{dim}$ $CD16^{positive}$ (cytotoxic)

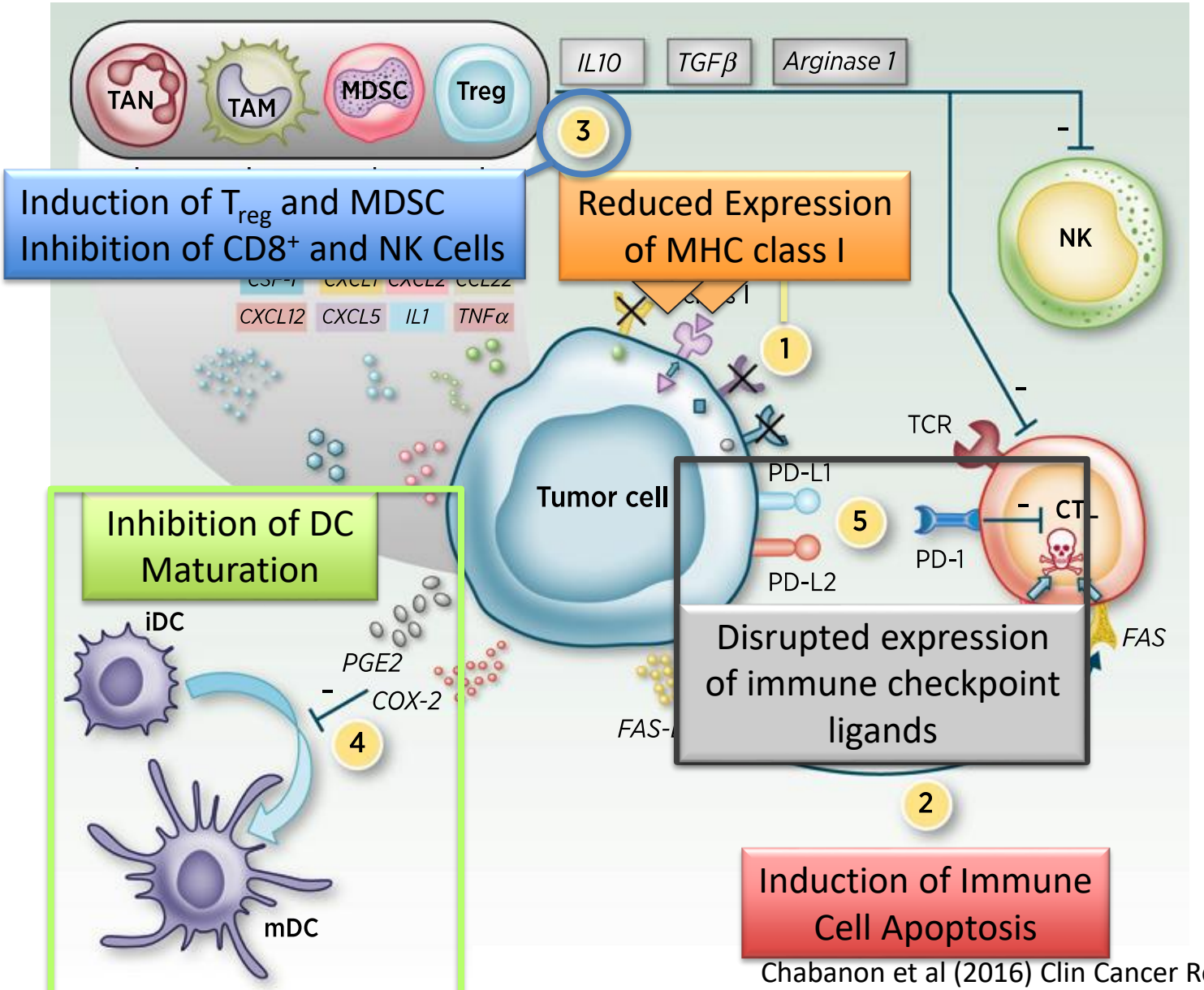
- Major role in killing of tumour cells best in case of MHC negative targets

- Inhibitory and activating receptors:
  - KIRs
  - **NCRs** (NKp30, NKp46, NKp44)
  - **NKG2D**

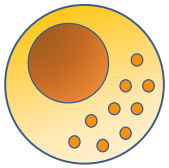


KIR (killer immunglobulin like receptors)
NCR (natural cytotoxicty receptors)

Mechanisms of immune escape in the tumor microenvironment

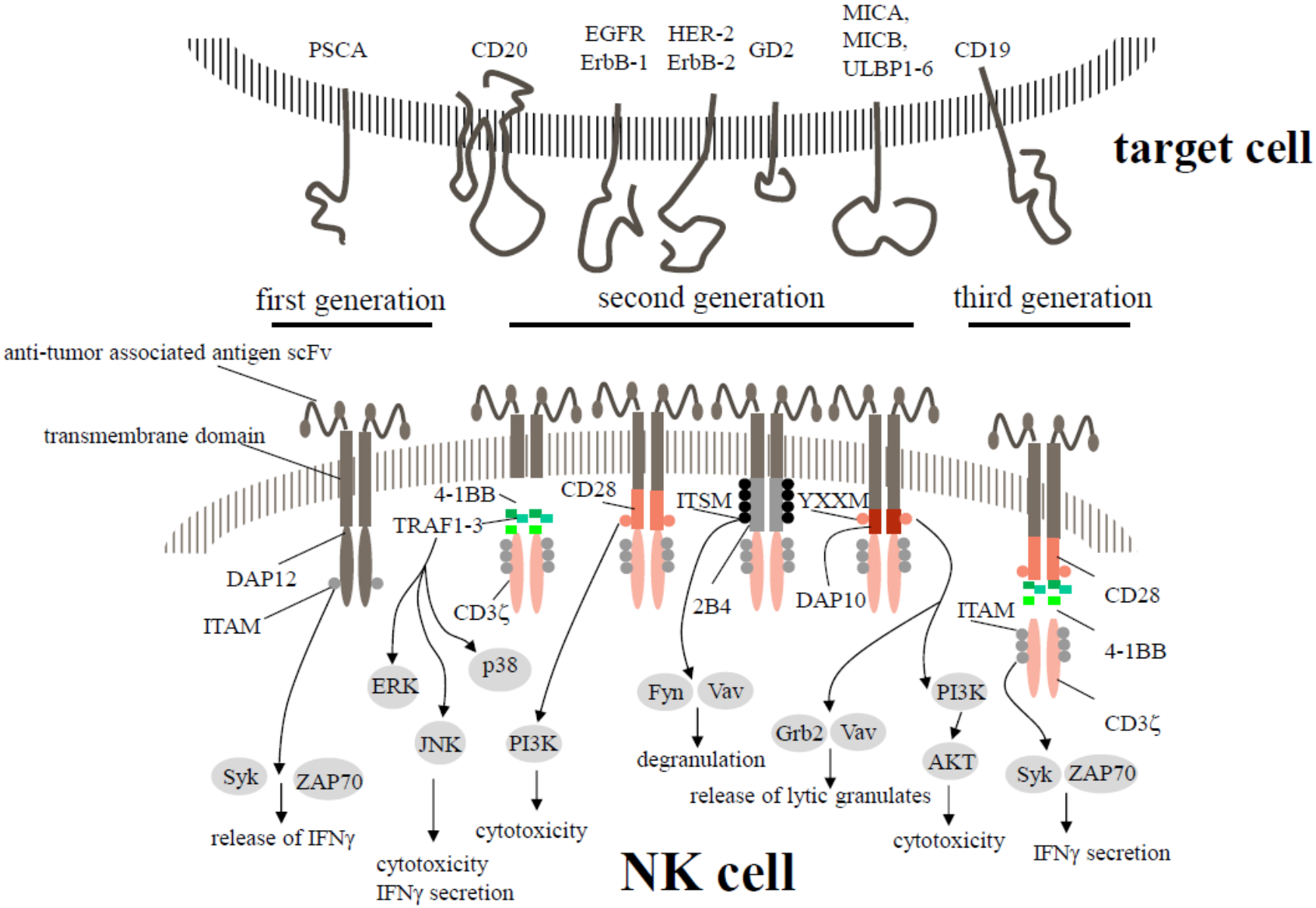


However: Improved NK cell attack



Chabanon et al (2016) Clin Cancer Res; 22, 4310

Signaling and CARs in primary human NK cells



Oberschmidt O, Kloess S, Koehl *U.* Front Immunol 2017

NK cell immunotherapy

results

NK-DLI = NK donor lymphocyte infusion

REVIEW

OPEN ACCESS

Advances in clinical NK cell studies: Donor selection, manufacturing and quality control

U. Koehl[a], C. Kalberer[b], J. Spanholtz[c], D. A. Lee[d], J. S. Miller[e], S. Cooley[e], M. Lowdell[f], L. Uharek[g], H. Klingemann[h], A. Curti[i], W. Leung[j,*], and E. Alici[k,l,m,*]

ONCOIMMUNOLOGY
2016, VOL. 5, NO. 4, e1115178 (11 pages)
http://dx.doi.org/10.1080/2162402X.2015.1115178

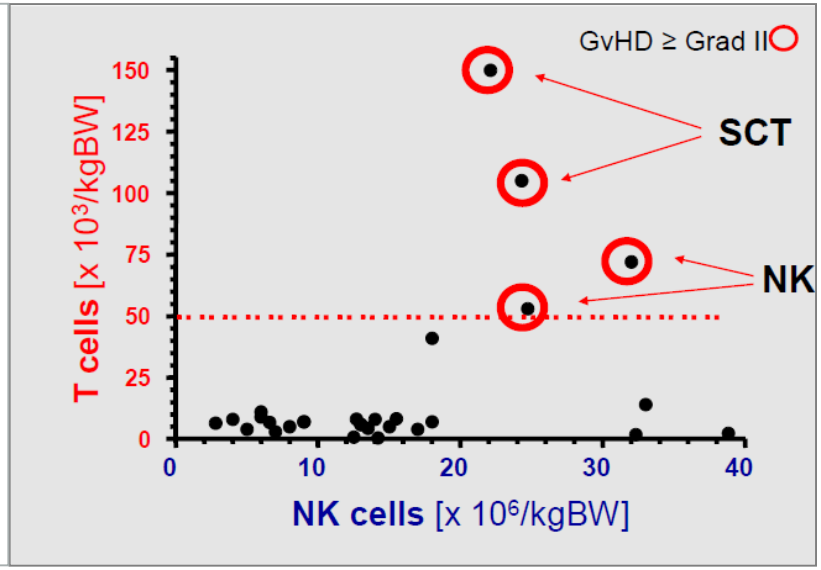
Advantage

- No severe adverse events in patients
- Primary aim >10x10^6 CD56$^+$CD3$^-$/kgBW: 41/49
- No graft versus host disease if T cells < 25x10^3/kg
- IL-2 stimulation → improved NK cell cytotoxicity

Disadvantage

- Tumor immune escape mechanism (TIEMs)

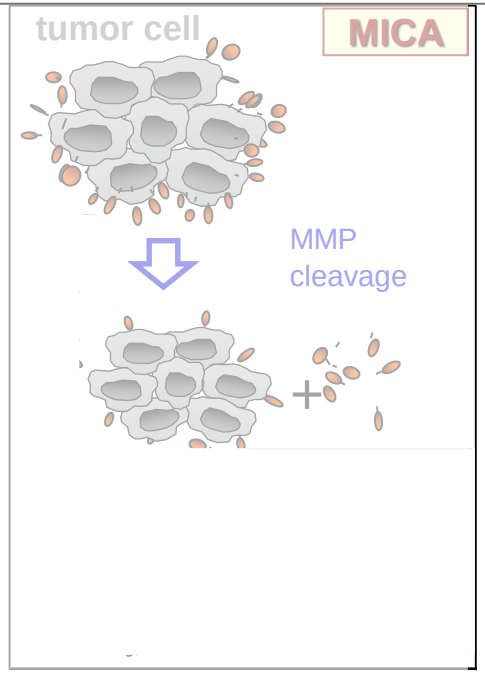
Kloess *et al.* Eur J Immunol 2010; Kloess *et al.* Oncoimmunol 2015



Koehl U *et al.* Blood Cell Mol Dis 2004; Koehl U *et al.* Klin Päd 2005; Koehl U *et al.* Front Oncol 2013, Stern M *et al.* BMT 2013

$_{sol}$MICA dependent tumor immune *escape* inhibits NK cells in patients with Neuroblastoma

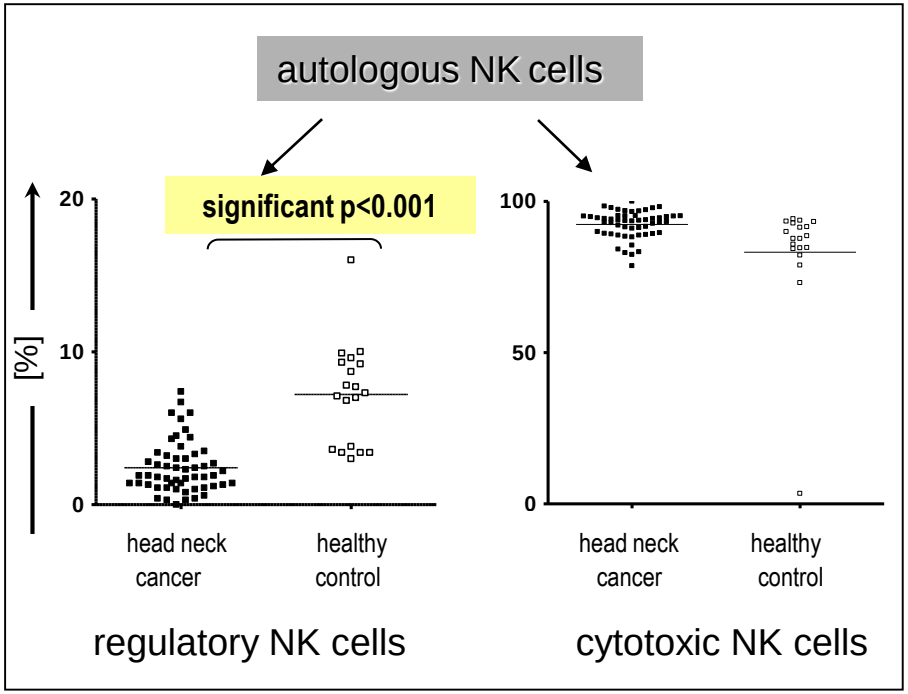
results

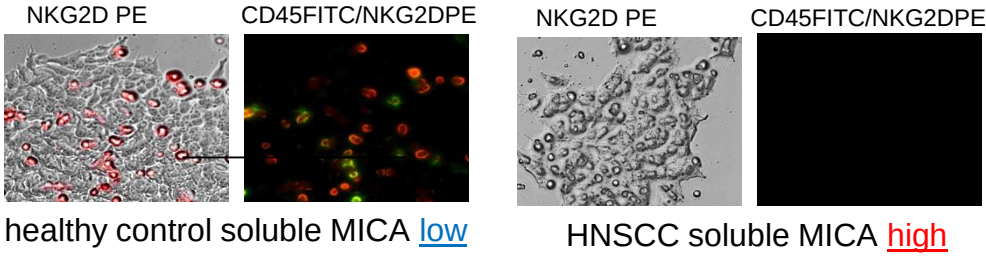


➔ IL-2 activated NK cells improve NKG2D mediated cytotoxicity via scavanging of $_{sol}$MICA in plasma

Impaired NK cell cytotoxicity in patients with head and neck cancer (HNSCC)

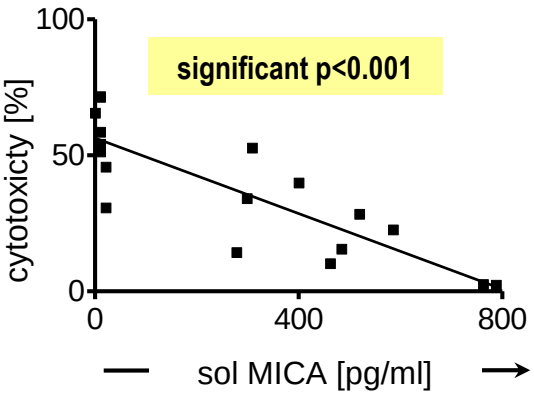
n=67 patients with HNSCC
peripheral blood screening

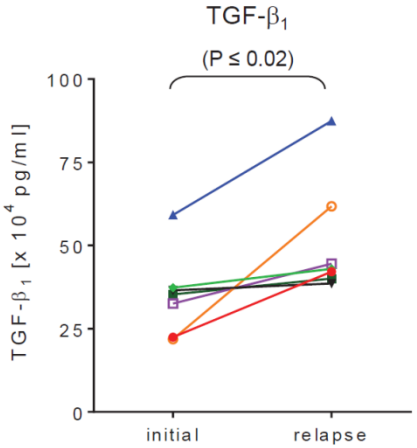




healthy control soluble MICA low

HNSCC soluble MICA high





To overcome those hurdles: CAR NK cells ?

Kloess S *et al.* Oncoimmunology 2016

Clinical trials with CAR expressing NK cells

Clinical trial identifier	Target	Condition/disease	Origin of NK cells	Phase	Status	Location
NCT03056339	CD19	Lymphoma and leukaemia (relapsed/ refractory B-cell malignancy)	Cord blood	I/II	recruiting	Houston, Texas, United States
NCT01974479	CD19	ALL	Haploidentical donor NK cells	I	suspended	Singapore, Singapore
NCT00995137	CD19	ALL	Expanded donor NK cells	I	completed	Memphis, Tennessee, United States
NCT02892695	CD19	Lymphoma and leukaemia	NK92	I/II	recruiting	Suzhou, Jiangsu, China
NCT02742727	CD7	Lymphoma and leukaemia	NK92	I/II	recruiting	Suzhou, Jiangsu, China
NCT02944162	CD33	Acute myeloid leukaemia	NK92	I/II	recruiting	Suzhou, Jiangsu, China
NCT02839954	MUC1	Solid tumours	Not specified	I/II	recruiting	Suzhou, Jiangsu, China
NCT03415100	NKG2D ligands	Solid tumours	autologous or haploidentical NK cells	I	recruiting	Guangzhou, Guangdong, China
NCT03383978	HER2	Glioblastoma	NK92	I		Frankfurt, Germany
NCT03579927	**CD19**	**Lymphoma and leukaemia**	**Cord blood NK cells**	**I/II**	**not yet recruiting**	**MD Anderson C. Houston, USA**
NCT03656705	**CCCR**	**Non-small Cell Lung**	**NK92**	**I**	**recruiting**	**Hospital of Xinxiang Henan, China**

CAR: CD19-CD28-zeta-2A-iCasp9-IL15 (K. Rezvani)

CCCR: Chimeric Costimulatory Converting Receptor

Kloess S … Koehl U. Transfusion Medicine and Hemotherapy 2019

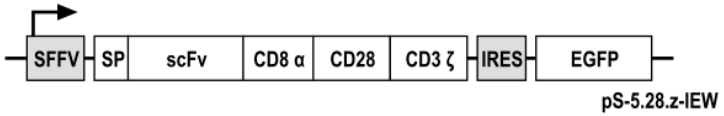
Redirected “CAR” NK-92 cell line

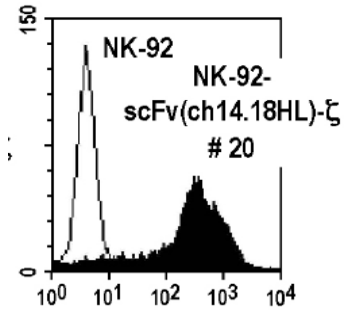
results

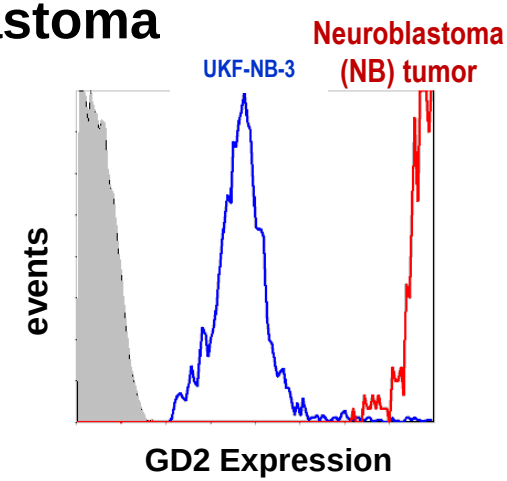
R. Esser

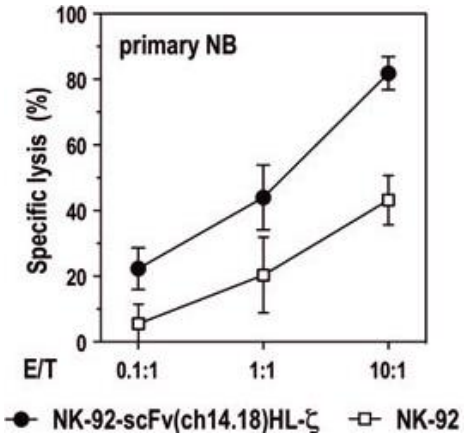
anti-GD2

Neuroblastoma









Esser R *et al.* J of Cellular and Molecular Medicine 2011

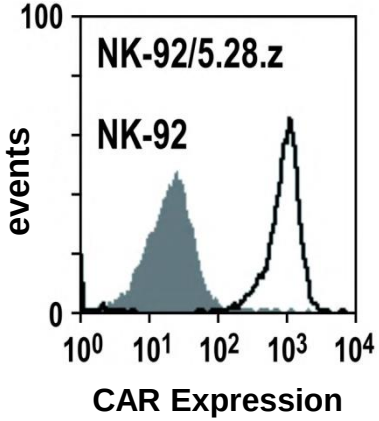
coop. U. Köhl (MHH), W. Wels (FFM), T. Tonn *(Dresden)*

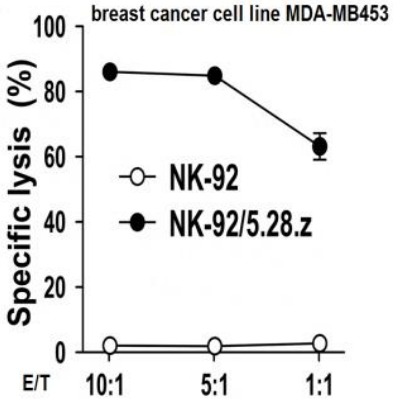
anti-ErbB2/HER2

NK-92

NK-92/5.28.z

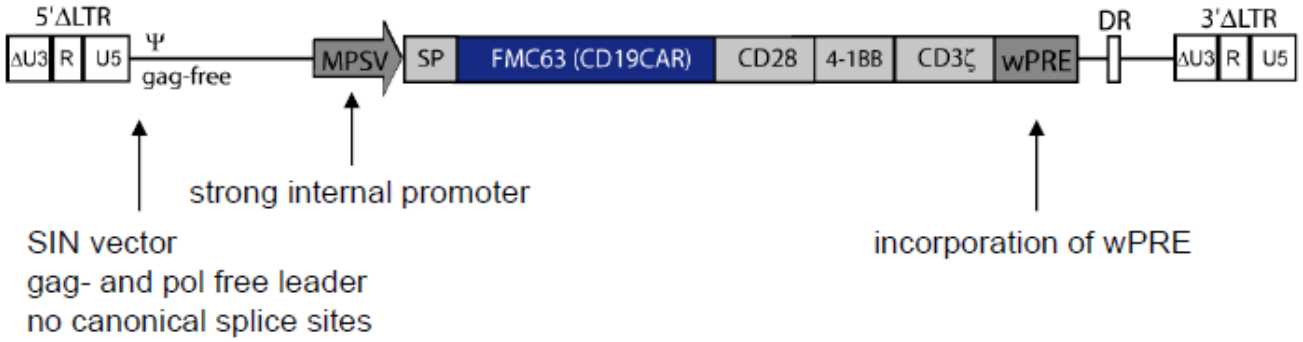
Schönfeld *et al.* Molecular Therapy 2014

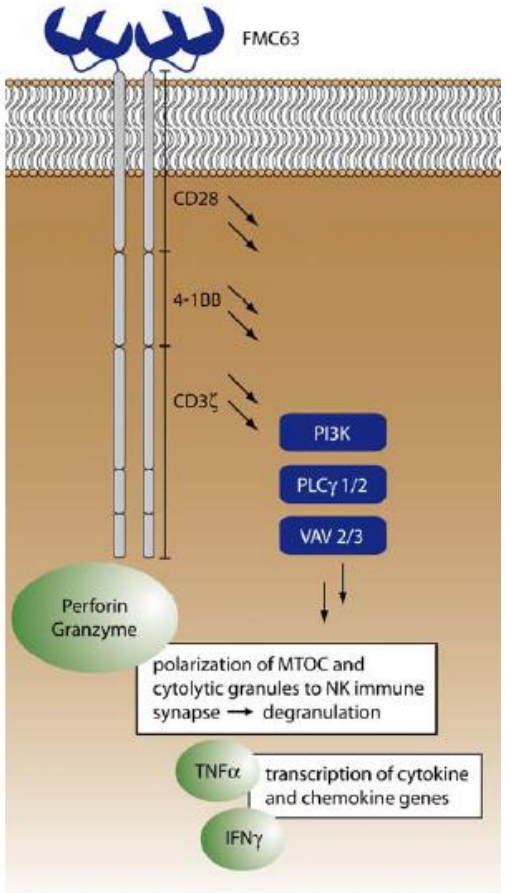




Chimeric Antigen Receptor Vector Design for primary human NK cells

A. Schambach





- Endodomain: FMC63 → CD19
- Endodomain: CD28 + 4-1BB(CD137) +CD3 ζ
- Codon-optimization: removal of cryptic splice sites, polyadenlyation signals and other inhibitory sequences

→ CD19 binding leads to signal transduction

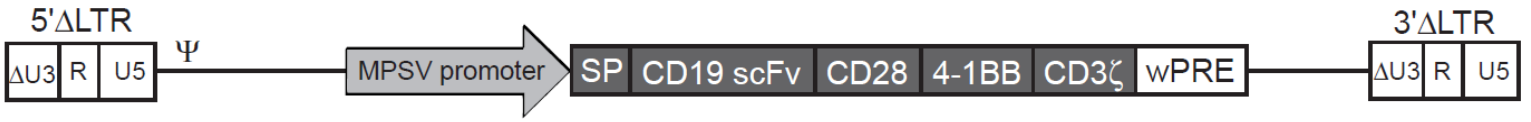
→ Enhanced cytotoxicity

coop.: A. Schambach, MHH

Fraunhofer IZI

CAR expressing NK cells redirected against CD19

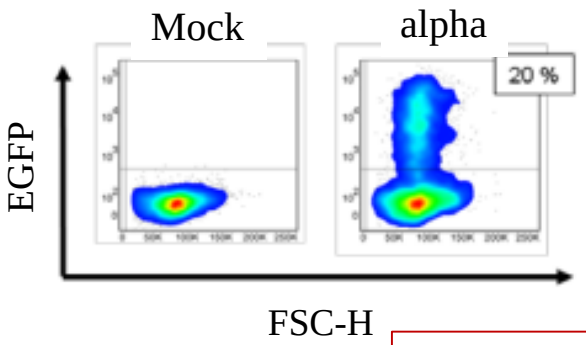
results

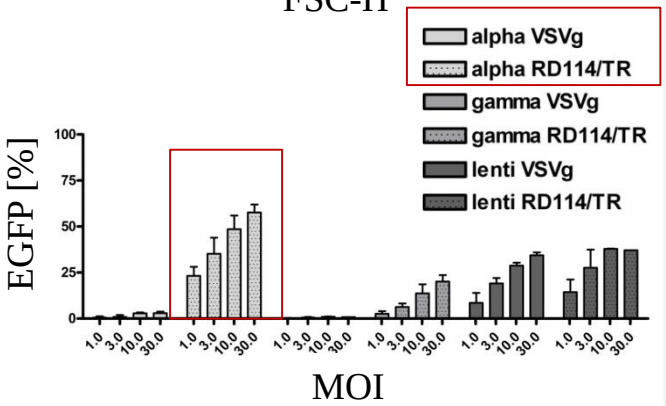


MOI1

Alpha SIN vector

Transduction of mature primary human dNK cells feasible

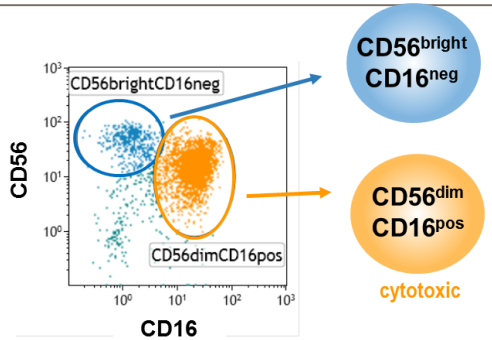




Suerth J et al. J Mol Med August 2015

Secretions of cytokines and pro-apoptotic molecules by CAR NK cells

results

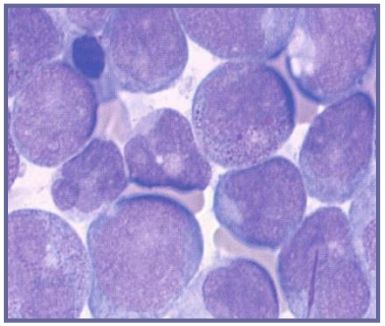


CD56^{bright}CD16$^{dim\&neg}$ (immune regulatory) E/T 1:1	5:1		CD56^{dim}CD16^{pos} (cytotoxic) E/T 1:1	5:1
++	0	**anti-inflammatory:**		
+	+	IL-4	+	+
		IL-10	+	+
		pro-inflammatory:		
++	+	IL-6	+	+
++	+	IL-17A	+	-
+	+	IFNγ	-	-
++	++	TNFα	+	+
		pro-apoptotic:		
+	0	GrA	++	+
0	0	GrB	++	++
-	-	Perforin	+	+
0	+	Granulysin	0	++

Primary CAR expressing NK cells

redirected against AML cell lines and

patients own leukemic cells

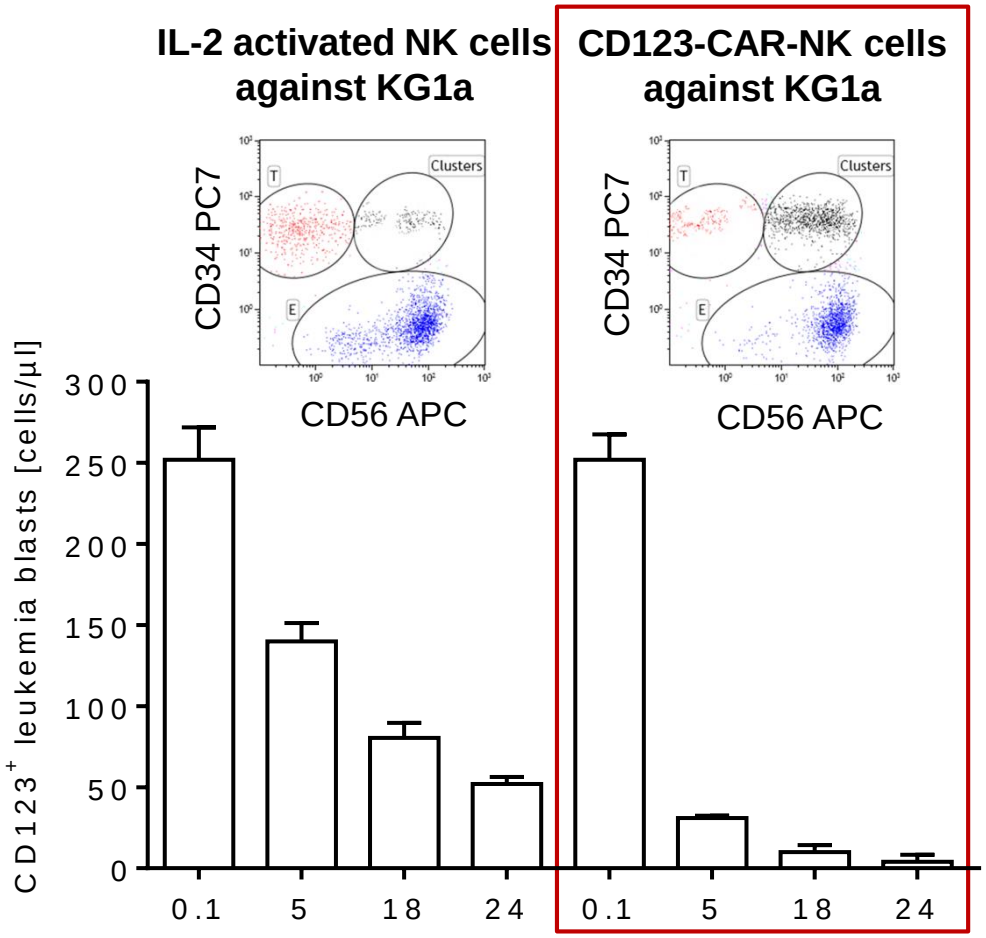


Coop.: M. Morgan, A. Schambach, M. Heuser, M. Sauer

Fraunhofer IZI

CAR expressing NK cells redirected against CD123+

results



NK cells : CD123+ KG1a incubation time [h]; E:T ratio: 10:1

Kloess et al. Human Gene Therapy 2017

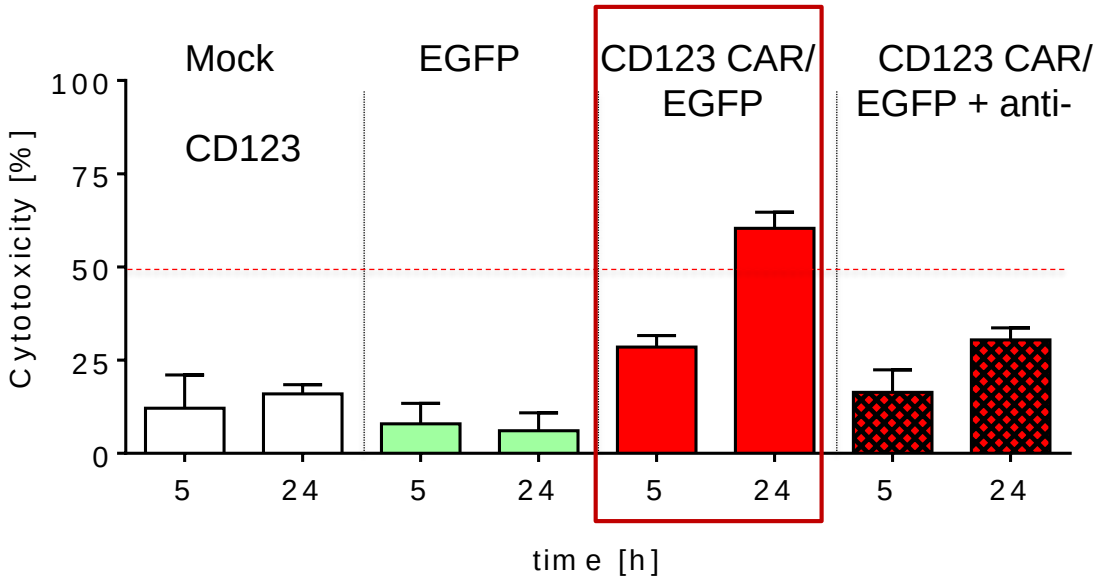
Coop.: A. Schambach, MHH

CAR NK cells against patient´s CD123^{+}AML

results

S. Kloess

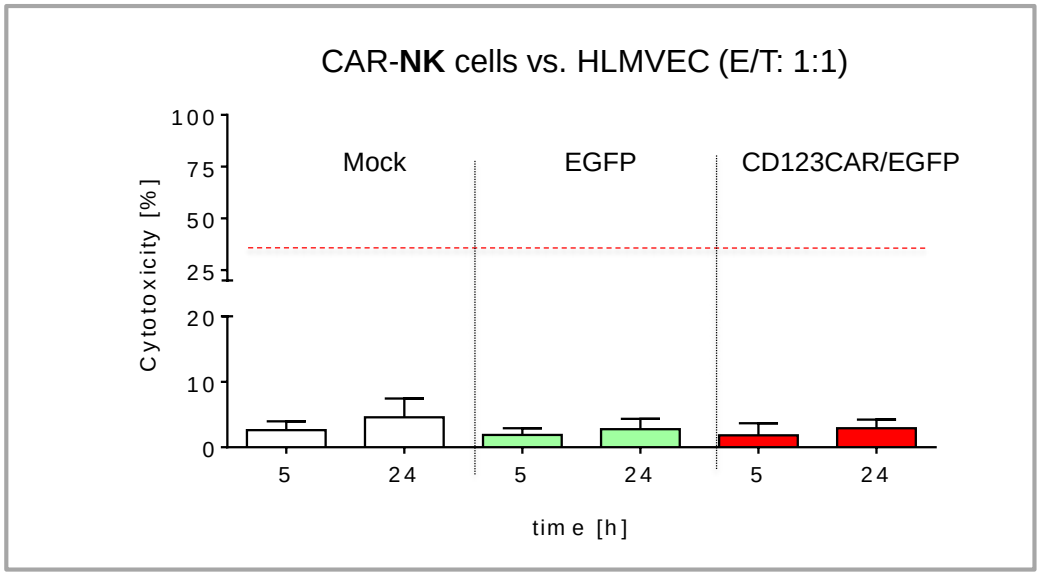
Cytotoxicity



MOI1

CAR-**NK** cells
vs.
patient´s AML
(E/T: 1:1)

Side effects



Coop.:
M. Heuser,
A. Schambach MHH

Kloess et al. Human Gene Therapy 2017

Kloess et al. Human Gene Therapy 2019

CD123CAR expressing NK cells and EGFP$^+$ mock NK cells aginst CD123 positive KG1α targets

anti-CD123-CAR NK cells EGFP$^+$

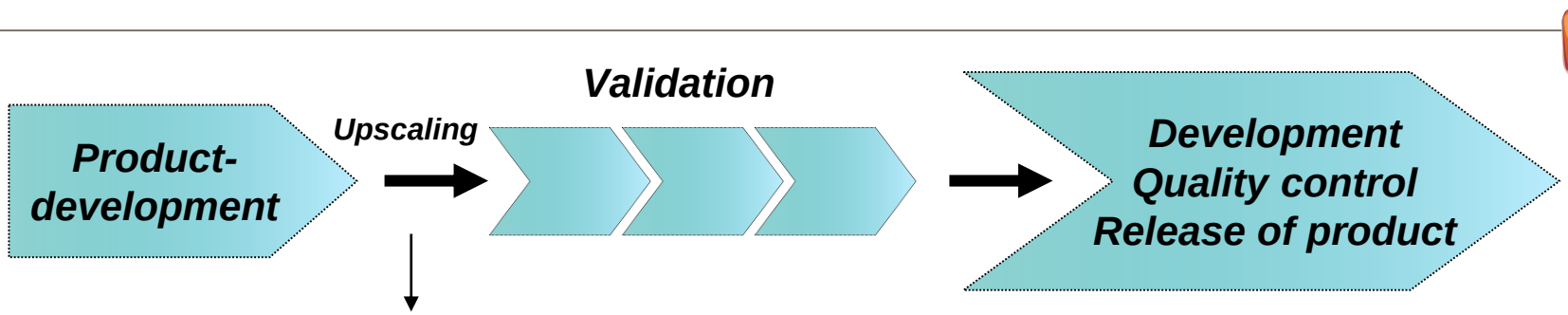
anti-CD16-APC, EGFP$^+$ NK cells

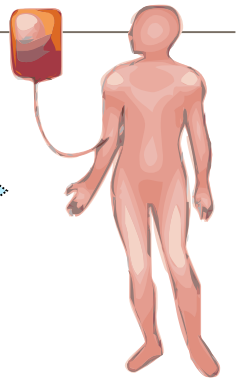
CD123$^+$KG1α cell proliferation dye: eFluor®450, anti-CD34-PE

NK:KG1 E:T ratio: 5:1; MOI1

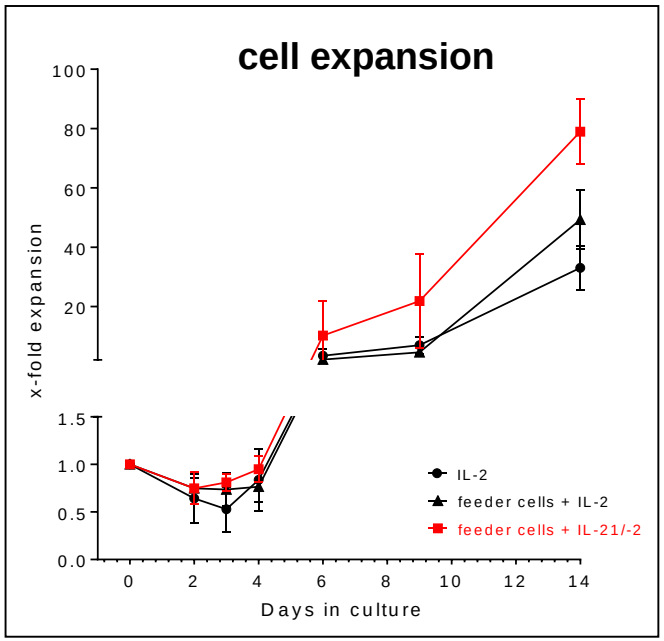
Clinical scale – CAR expressing NK cells

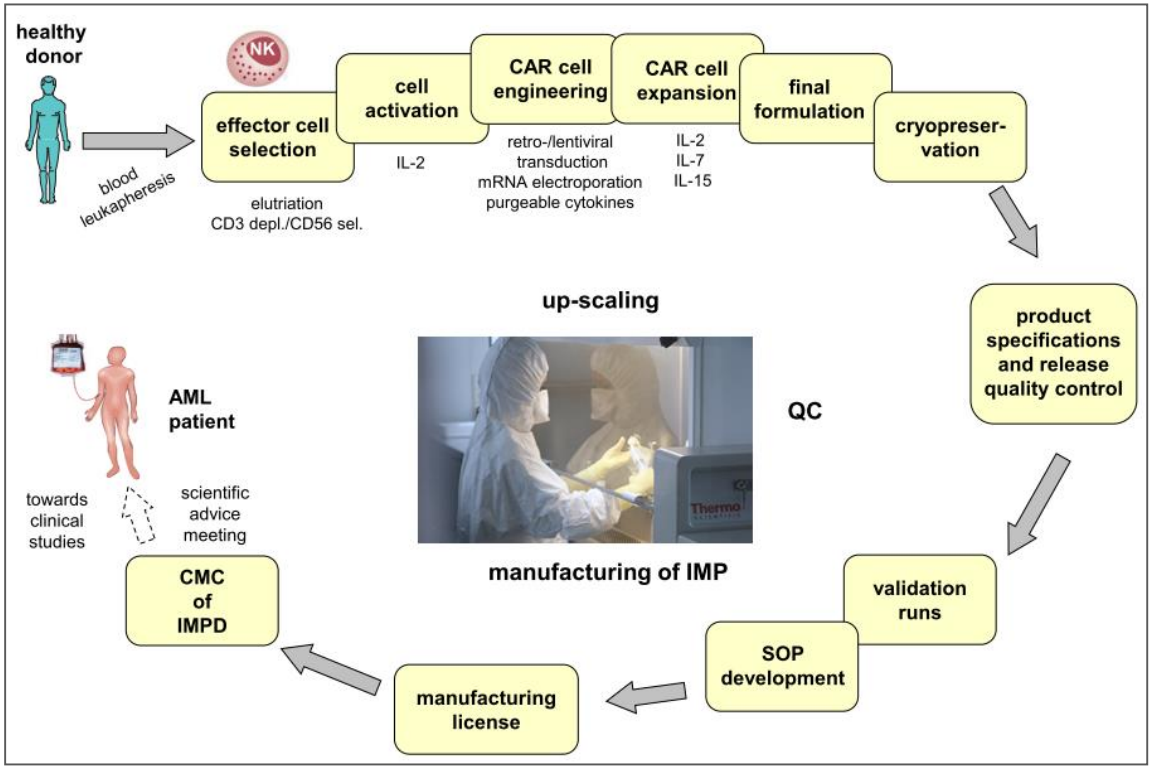
results





GMP-compliant protocol





„Off the shelf product“ Advanced Therapy Medicinal Product

SOP=standard operation protocol CMC=chemical manufacturing and control IMPD=investigational medicinal product dossier

CAR expressing effector cells

CAR T cells:

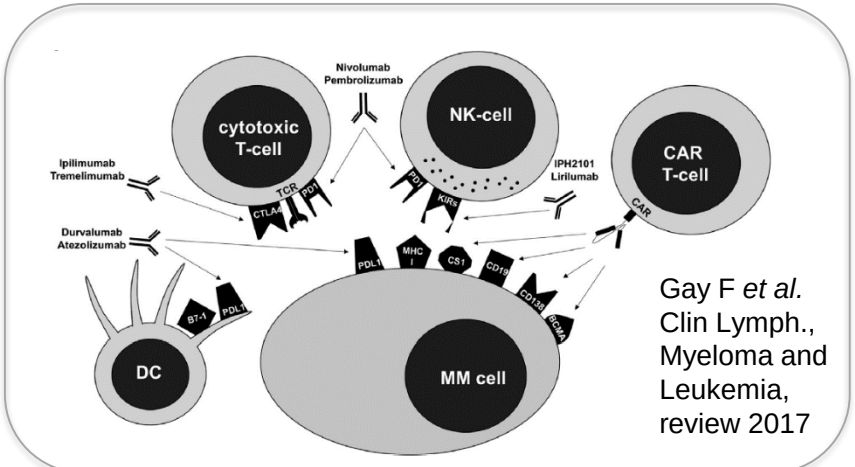
- Successful clinical CAR T cells studies (~ 450 documented world wide)
- Manufacturing failure of autologous CAR T cells needs complementary concepts

Primary human CAR NK cells:

- Patients can receive allogeneic haploidentical or „third party NK cells“ without severe side effects → good candidates for „off the shelf CAR products“
- CAR NK cells (alpha retroviral SIN vectors) reached a nearly complete elimination of CD19+ and CD123+ leukemic cells after 48 h

Improvement in future studies:

- CAR expressing cells and checkpoint inhibitors → combination
- CAR effector cells with transient cytokine secretion



Gay F *et al.* Clin Lymph., Myeloma and Leukemia, review 2017

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