

Automatisierung von CAR Effektorzellen

Ulrike Köhl



Disclosure

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

“CD20CAR-TIME“ is a joint research project partly funded by the German ministry of education and research (ref. 01EK1507A-C) within the research programme “Innovations in Personalised Medicine“.



UNIKLINIK
KÖLN

MHH

GEFÖRDERT VOM



Bundesministerium
für Bildung
und Forschung



DLR Projektträger

* 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.

Network cell and gene therapy - manufacturing

Director/Head ICT, ICI, IZI: Prof. Dr. U. Köhl

I. Hannover Medical School, Hannover (MHH)

Institute of Cellular Therapeutics (ICT)

*Transplantation
Regeneration*



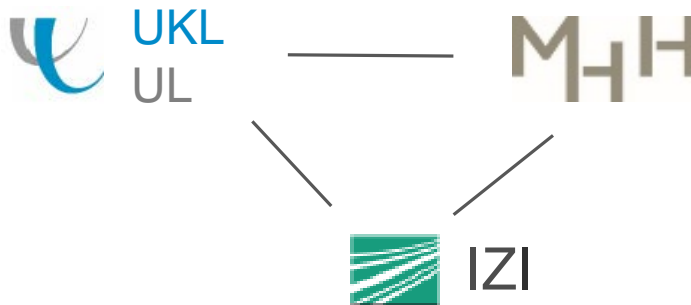
II. University and University Hospital Leipzig (UL/UKL)

Institute of Clinical Immunology (ICI)

*Cancer
Adipositas*



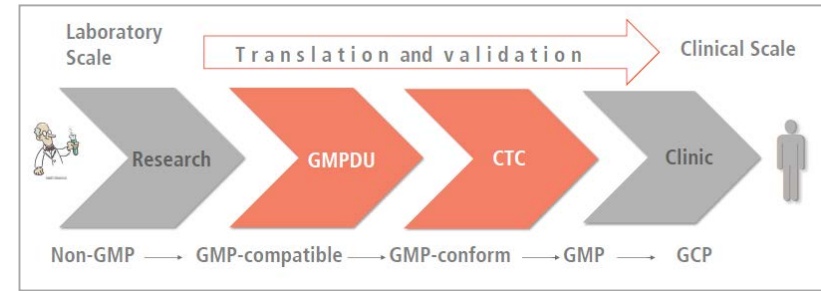
III. Fraunhofer Institute for Cellular Therapeutics and Immunology (IZI), Leipzig



GMP Cell and Gene Therapy Manufacturing Units

MHH GMP Core facility, ICT

- 3 clean rooms “A in B”
- Head: Dr. L. Arseniev
- 21 qualified staff members
- GMP Development Unit (GMPDU): Up-Scaling
- Cellular Therapy Centre (CTC): Manufacturing



MHH



IZI GMP Core facility

- 3 facilities, 21 clean rooms („A in B“, level S2)
- Head: Dr. G. Schmiedeknecht
- > 100 qualified staff members
- Cross-link to the IZI development units



Development up to translation into the clinic

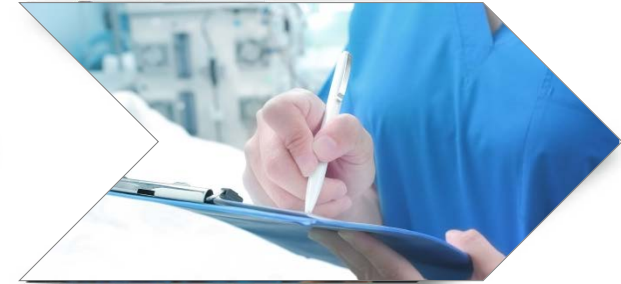
M-H-H



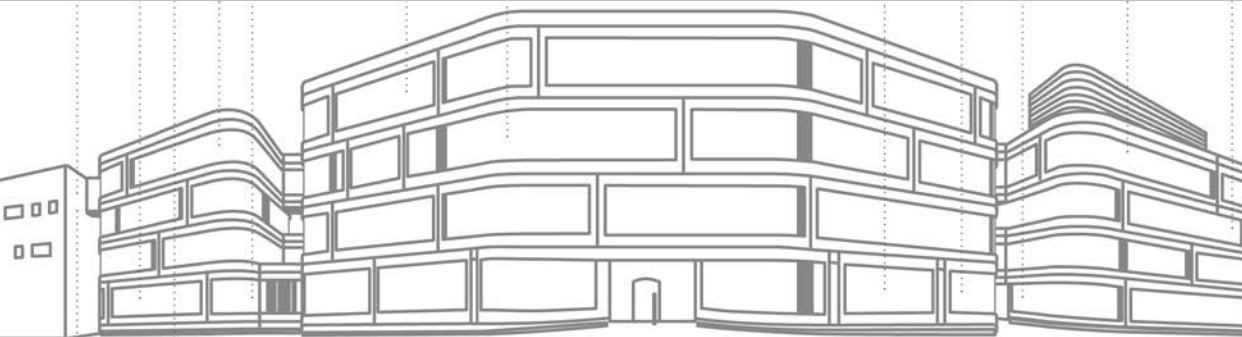
Pre-clinical development



GMP Manufacturing



Clinical Studies
Phase I/ II/ III/ IV



IZI: > 600 staff members/ 3.508 m² lab/ additional 5 sub institutes

Manufacturing of cell-based therapies

Pre-clinical

Manufacturing and proof „AMG“

„First in man“ study

Late clinical studies

Routine

Blood and bone marrow stem cells (incl. CD34 sel., CD3/CD19 depl., TCR α/β depl.)

Antigen-specific T cells (CMV-, ADV-, EBV-, multivirus-spec. CTL)

CD19 CAR T cells (CTL019)

CAR T cells Melanoma

CAR NK cells

Regulatory T cells

Mesenchymal stroma cells

Dendritic cells

iPS-cardiomyocytes

iPS-macrophages

Europe wide

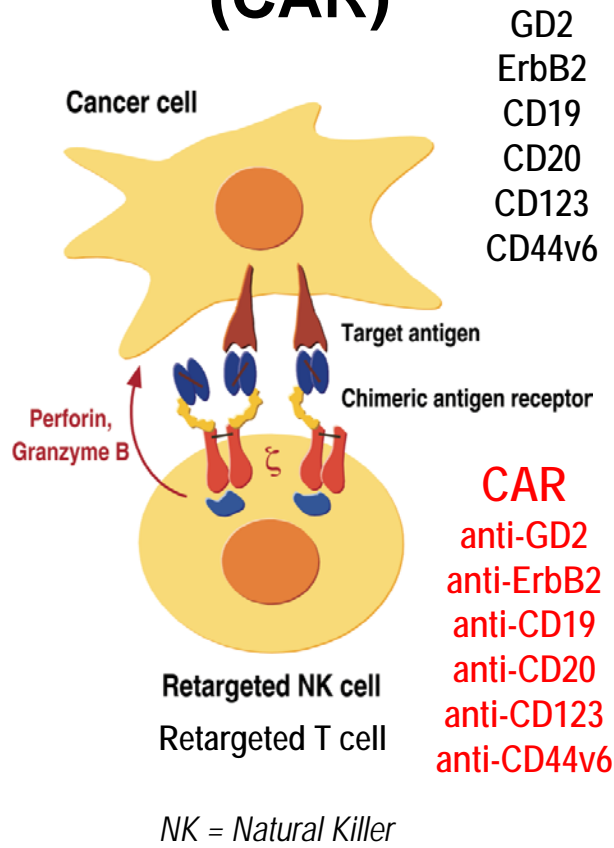
Clinical and Industrial partners



> 350 cellular products/ year

“CAR” expressing effector cells

Chimeric antigen receptor (CAR)



➤ CAR engineered T cells

- clinical studies
- manufacturing challenge

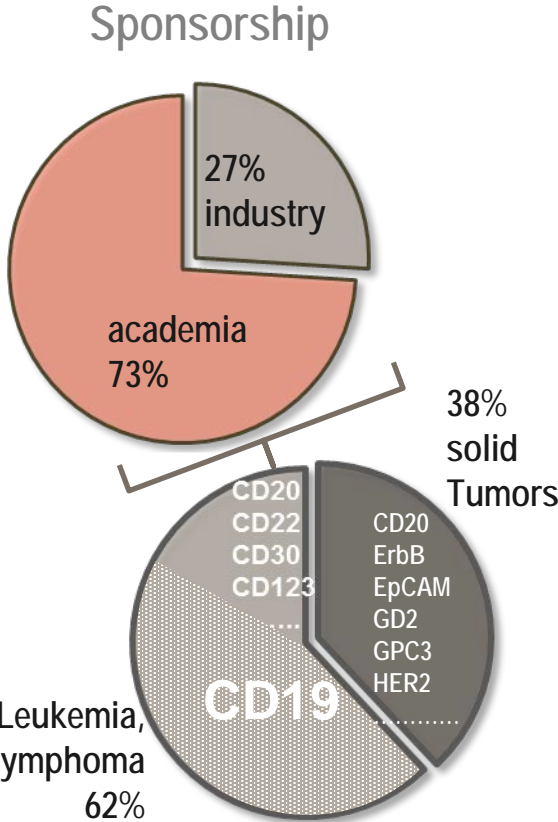
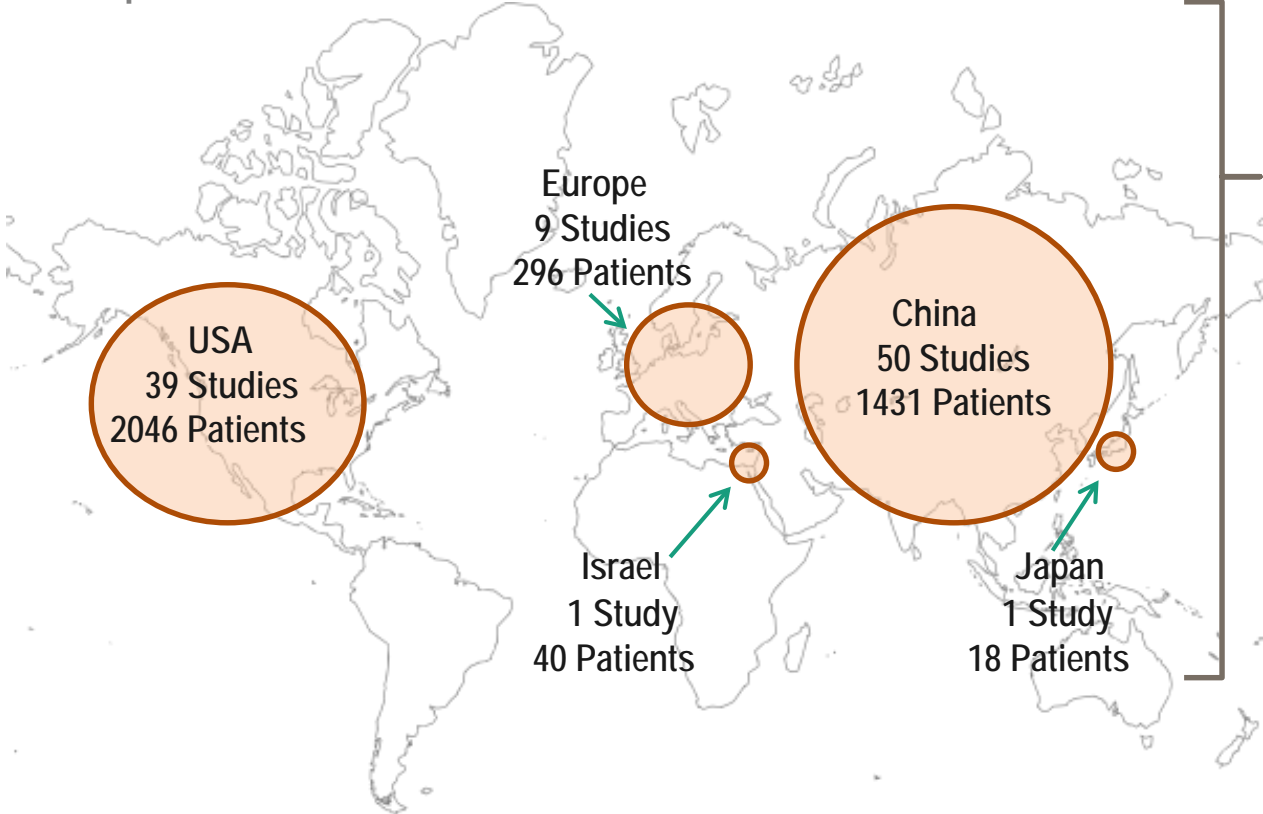
➤ „off the shelf“ CAR NK cells

- clinical NK cell studies
- primary human „CAR“ NK cells
- lytic activity
- benefit and side effects

Clinical trials with CAR expressing T cells

(clingov. Q4/2016)

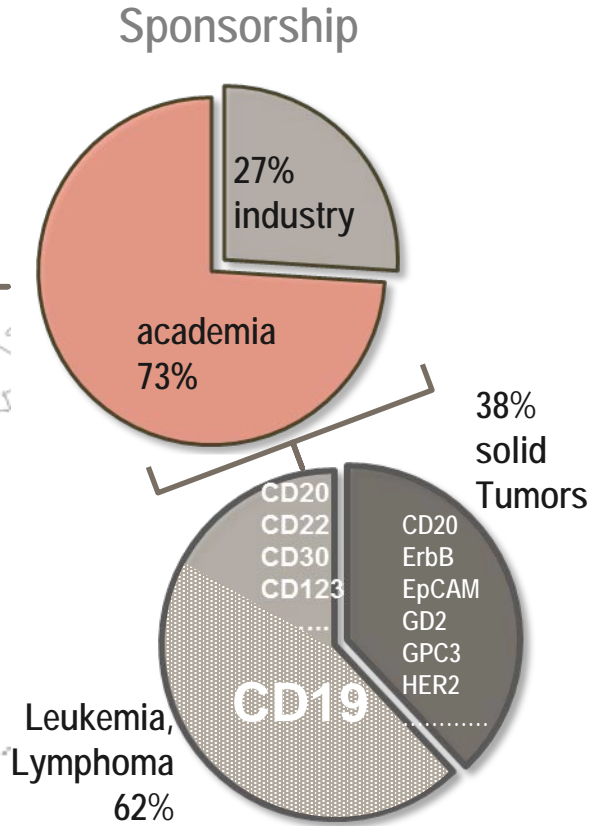
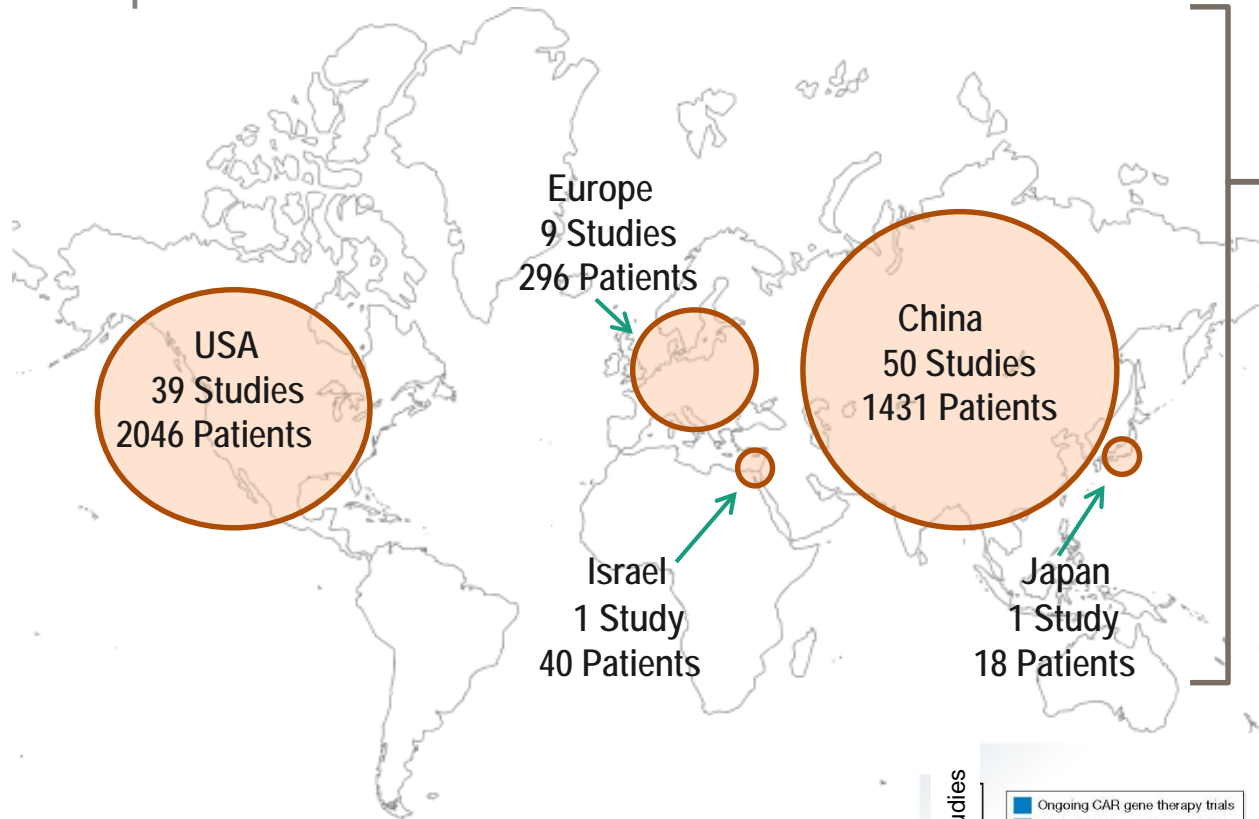
100 open clinical trials with CAR T cells registered worldwide
 3831 patients should be included:



Clinical trials with CAR expressing T cells

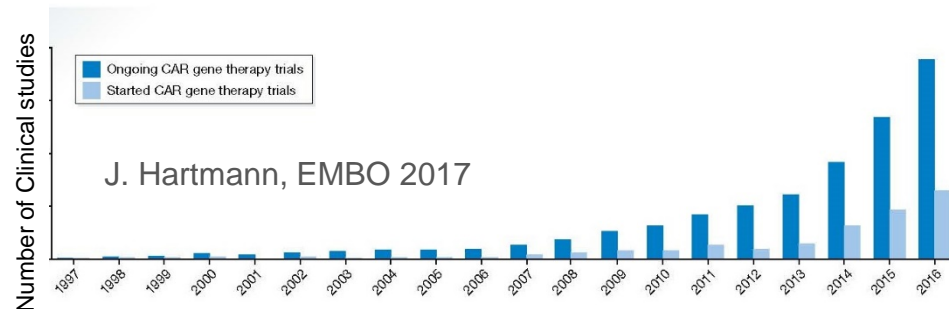
(clingov. Q4/2016)

100 open clinical trials with CAR T cells registered worldwide
 3831 patients should be included:



Update 9/2018: n > 390 studies

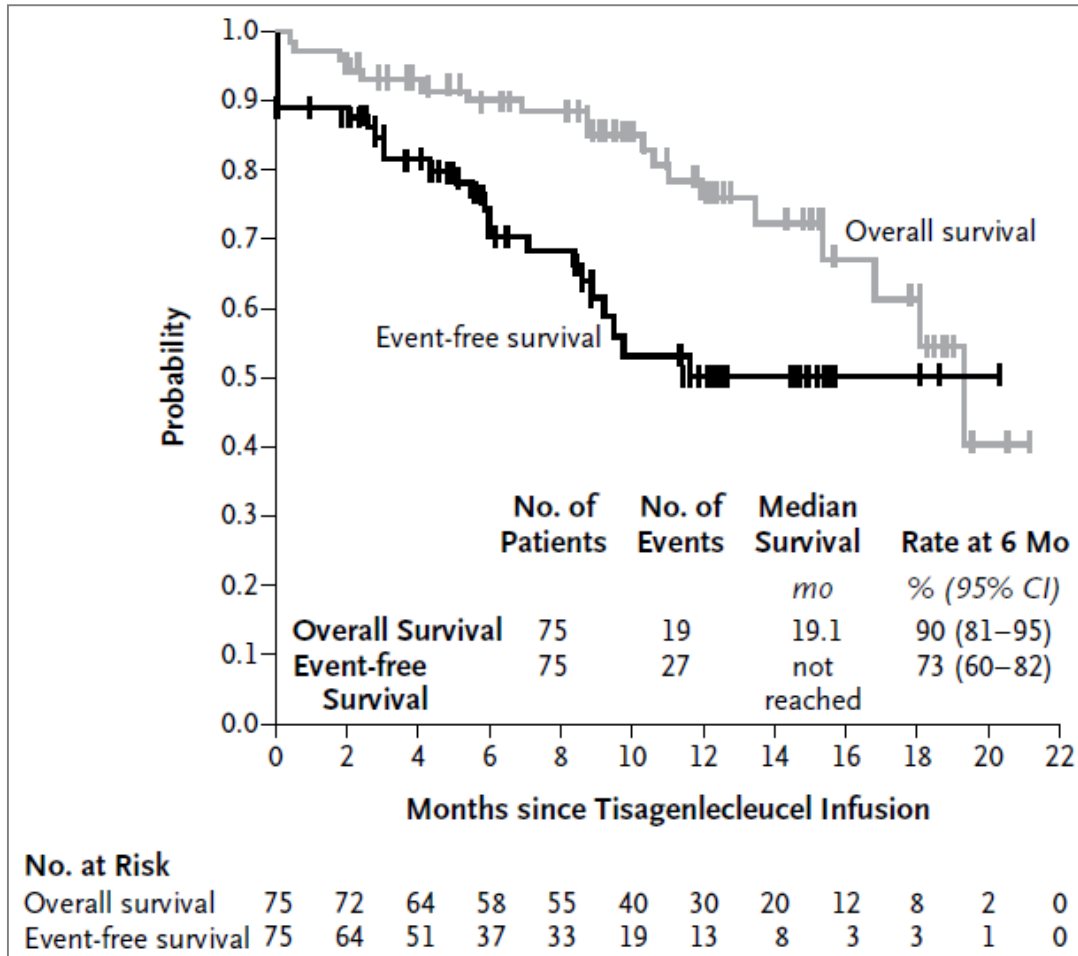
Zuther K ... Koehl U. J Onkologie 2017



CTL019 pediatric r/r ALL – ELIANA

”Event-free” and “overall survival”

75 patients



Now > 5 years cured

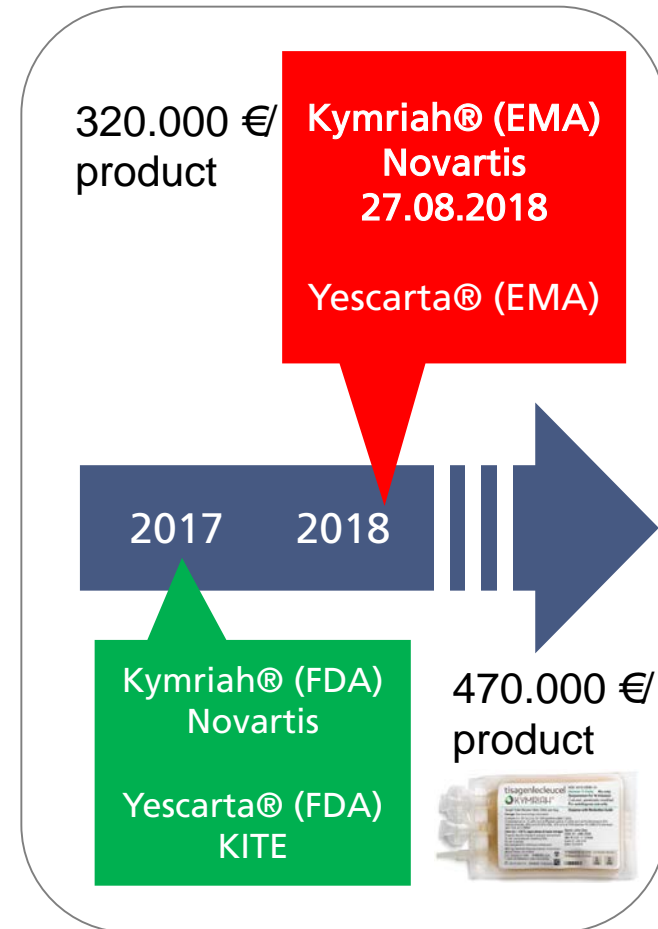
- **Event-free survival**
 - 73% post 6 months
 - 50% post 12 months

- **Overall survival**
 - 90% post 6 months
 - 76% post 12 months

Marketing authorization in Europe



- Marketing authorization CAR T cells (Kymriah®), EU
- Press release NOVARTIS - Fraunhofer IZI, 30.8.2018



CAR T Cells in Trials: Recent Achievements and Challenges that Remain in the Production of Modified T Cells for Clinical Applications



H Abken

Ulrike Köhl,¹⁻³ Stanislava Arsenieva,¹⁻³ Astrid Holzinger,^{4,5} and Hinrich Abken^{4,5,*}

¹Institute of Cellular Therapeutics, Hannover Medical School, Hannover, Germany; ²Institute of Clinical Immunology, University Hospital Leipzig, Leipzig, Germany; ³Fraunhofer Institute for Cell Therapy and Immunology, Leipzig, Germany; ⁴Center for Molecular Medicine Cologne, University of Cologne, Cologne, Germany; and ⁵Department I for Internal Medicine, University Hospital Cologne, Cologne, Germany.

The adoptive transfer of chimeric antigen receptor (CAR)-modified T cells is attracting growing interest for the treatment of malignant diseases. Early trials with anti-CD19 CAR T cells have achieved spectacular remissions in B-cell leukemia and lymphoma, so far refractory, very recently resulting in the Food and Drug Administration approval of CD19 CAR T cells for therapy. With further applications and increasing numbers of patients, the reproducible manufacture of high-quality clinical-grade CAR T cells is becoming an ever greater challenge. New processing techniques, quality-control mechanisms, and logistic developments are required to meet both medical needs and regulatory restrictions. This paper summarizes the state-of-the-art in manufacturing CAR T cells and the current challenges that need to be overcome to implement this type of cell therapy in the treatment of a variety of malignant diseases and in a greater number of patients.

Human Gene Therapy online 1. May 2018

State of the art: hands on manufacturing



excubate



transfer & tare



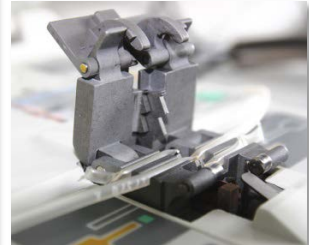
centrifugate



extract



resuspend



dis-/connect

(45 min + 3 dis-/connections) x 15/process = **11¼ hrs + 45 dis-/connections**



COBE 2991
(Terumo BCT)



SEPAX 2
(Biosafe SA)



SEFIA
(Biosafe SA)



WAVE
(GE Healthcare)

(non-exhaustive)

e.g. PERFUSE / WASH / FORMULATE

Automated manufacturing?

ENGINEERING EFFECTIVITY

LAB BENCH

BEDSIDE

WORKBENCH

TOOLMAKER

✓ efficacy

- limitations
- toxicities
- timeframes

- rapid prototyping
- robust manufacture

- smallest batches
- personalized
- regulated environment

- vectors (GMP compliant)
- raw materials (GMP compatible)
- platforms (GMP compatible)

- improved CARs
- safer vectors
- new / alternative targets

MANUFACTURING EFFICIENTLY

Automated manufacturing of CAR T cells

Automated manufacturing of chimeric antigen receptor T cells for adoptive immunotherapy using CliniMACS Prodigy

ULRIKE MOCK^{1,*}, LAUREN NICKOLAY^{1,*}, BRIAN PHILIP²,
GORDON WENG-KIT CHEUNG², HONG ZHAN¹, IAN C.D. JOHNSTON³,
ANDREW D. KAISER³, KARL PEGGS², MARTIN PULE², ADRIAN J. THRASHER¹ &
WASEEM QASIM¹

Cytotherapy, 2016; 18: 1002–1011

[Start in
the Prodigy:
Apheresis
product](#)

Automated Enrichment, Transduction, and Expansion of Clinical-Scale CD62L⁺ T Cells for Manufacturing of Gene Therapy Medicinal Products

Christoph Priesner,^{1,†} Krasimira Aleksandrova,^{1,†} Ruth Esser,² Nadine Mockel-Tenbrinck,³
Jana Leise,¹ Katharina Drechsel,³ Michael Marburger,² Andrea Quaiser,² Lilia Goudeva,⁴
Lubomir Arseniev,¹ Andrew D. Kaiser,^{3,‡} Wolfgang Glienke,^{2,‡} and Ulrike Koehl^{1,2,‡,*}

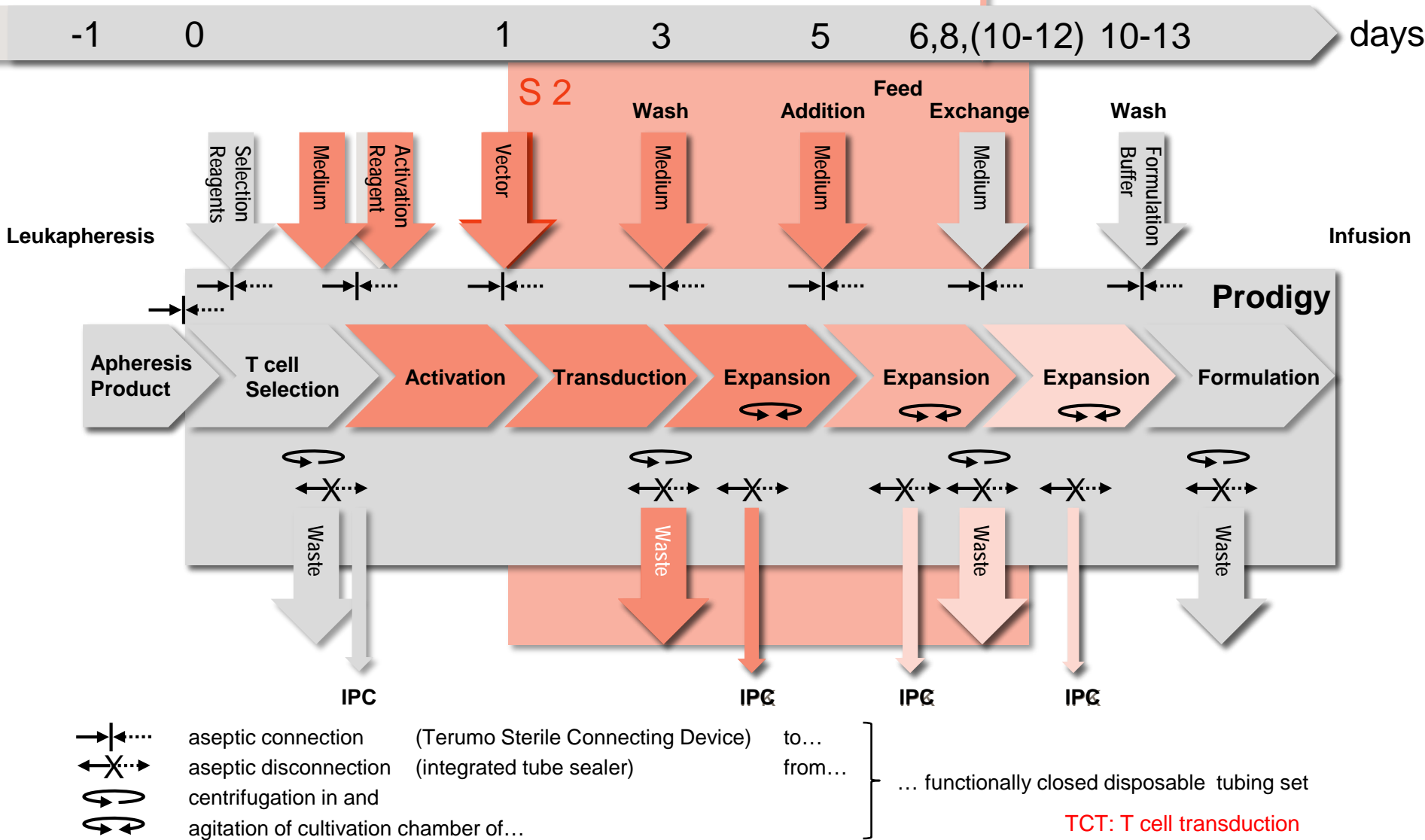
[Start in
the Prodigy:
Immunomagnetic
selected T cells](#)

HUMAN GENE THERAPY, VOLUME 27 NUMBER 10

Published online: August 25, 2016.

Automated manufacturing of CAR T cells

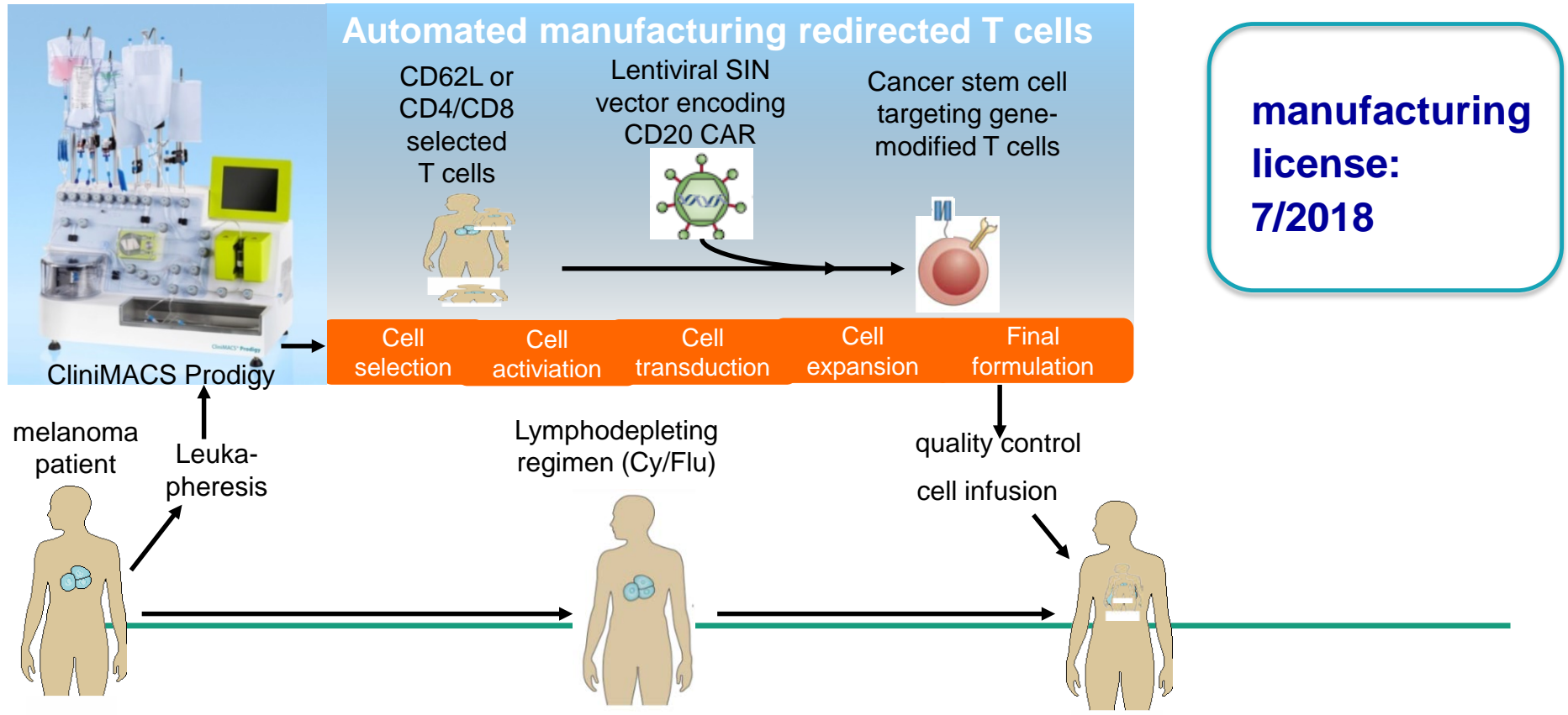
results



Automated manufacturing of CAR T cells

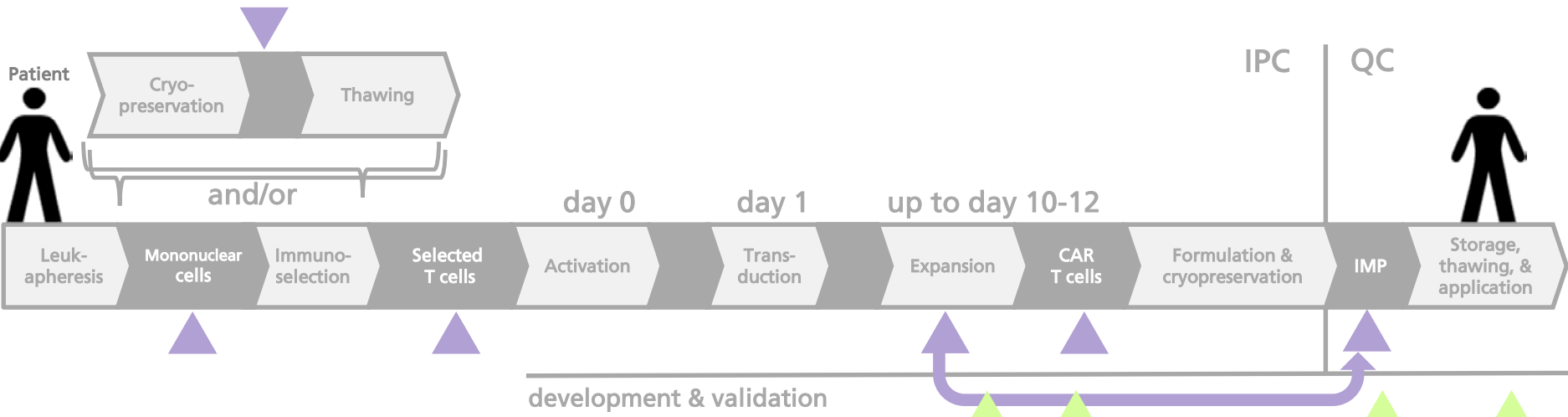
BMBF funded multicenter trial: **Refractory metastatic Melanoma**

cooperation: Miltenyi Biotec, Bergisch Gladbach
H. Abken, Cologne (Research development) and
U. Köhl, MHH, ICT, Hannover (Manufacturing)



Quality Control of CAR-transduced T cells

Cytological in-process (IPC), quality (QC), and complementary controls



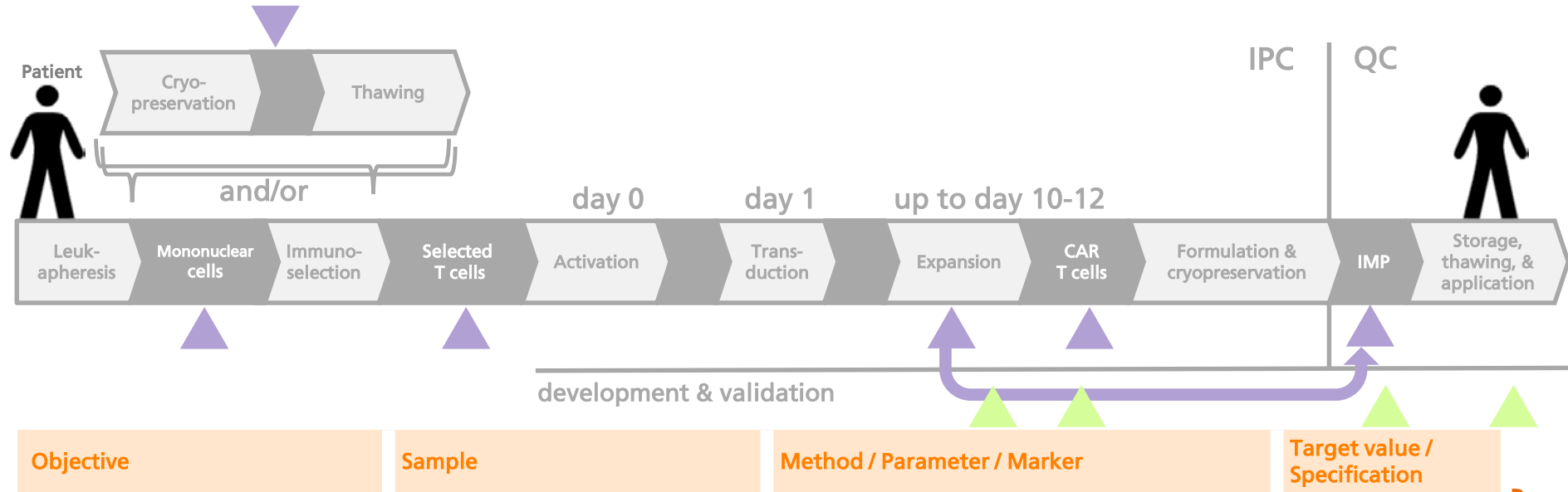
Objective	Sample	Method / Parameter / Marker	Target value / Specification
IPC QC: purity / content / identity	starting material, intermediates thawed IMP	CD3, 4, 8, 14, 45, 62L	to be defined
IPC QC: purity	intermediates after enrichment thawed IMP	%T cells of viable mononucleated cells: %(CD3+CD45+) of (CD45+ 7AAD-)	≥80% to be defined
IPC QC: content / identity	starting material, intermediates thawed IMP reference sample	viability of T cells: %7AAD- of (CD3+CD45+)	≥80% ≥50%
IPC QC: content / identity	sample of expansion culture (day 6), cultured in parallel (serum free)	transduction frequency: %CAR+ of (CD3+ CD45+ 7AAD-)	≥10%
advanced identity / purity	starting material, intermediates thawed IMP	CD45RA, 45RO, 27, 28 / 19, 56	to be defined
potency evaluation	intermediates thawed IMP	ELISPOT IFN-γ secretion with malignant cells	to be defined
potency evaluation	intermediates thawed IMP	Cytotoxicity Assay lysis of malignant target cells	to be defined

Flow Cytometry (EP 2.7.23)



Quality Control of CAR-transduced T cells

Cytological in-process (IPC), quality (QC), and complementary controls

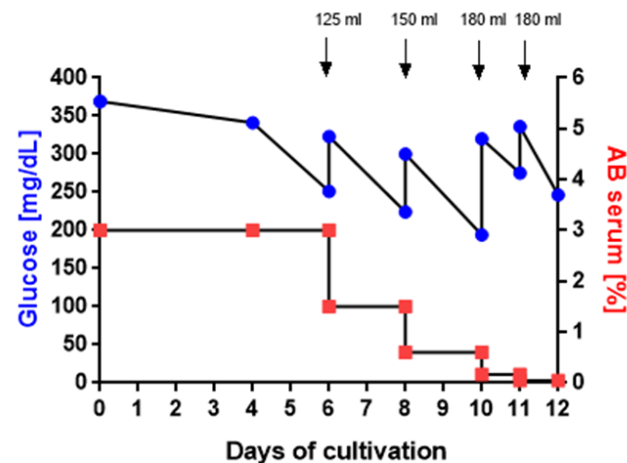
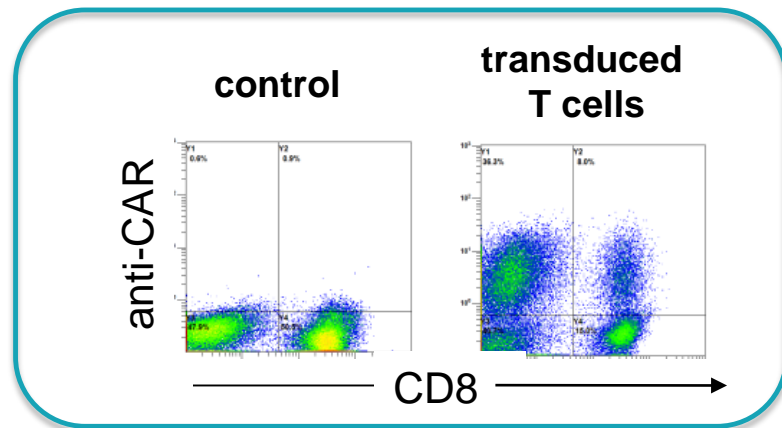
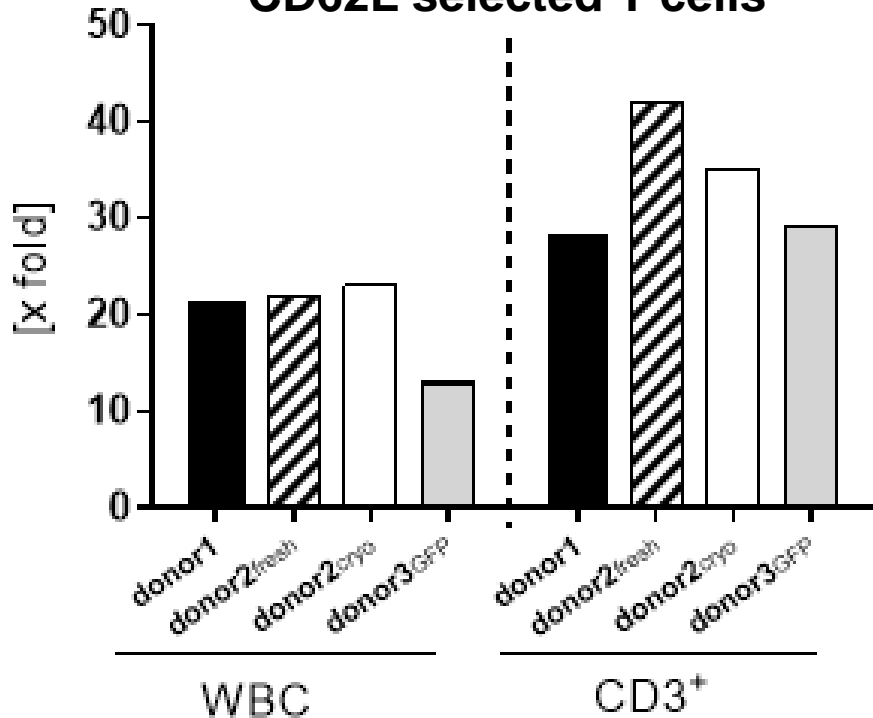


- EP 2.7.23 Flowcytometric analysis
- EP 2.6.21 Realtime qPCR for Vector copy number (VCN)
- EP 2.6.14 Bacterial Endotoxine test (BET)
- EP 2.6.27 Microbiological control
- EP 2.6.21 Mycoplasmas

Final end product at harvesting

results

Cell expansion
CD62L selected T cells

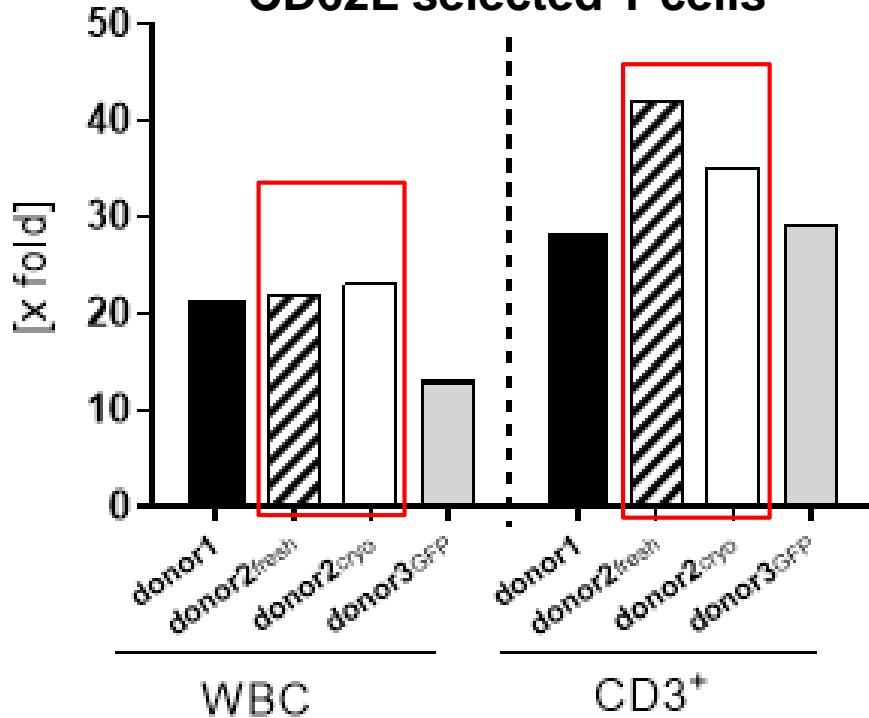


● Glucose ■ AB serum ↓ Medium exchange (without AB serum)

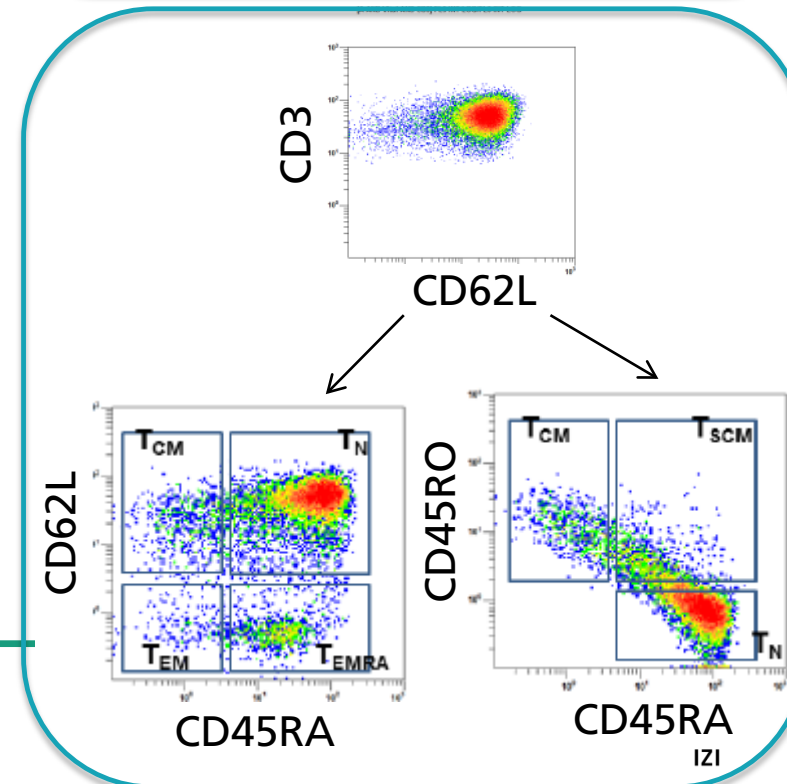
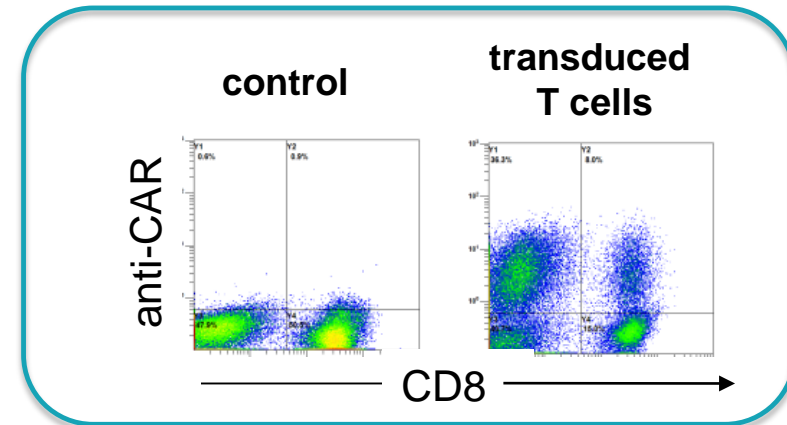
Final end product at harvesting

results

Cell expansion
CD62L selected T cells



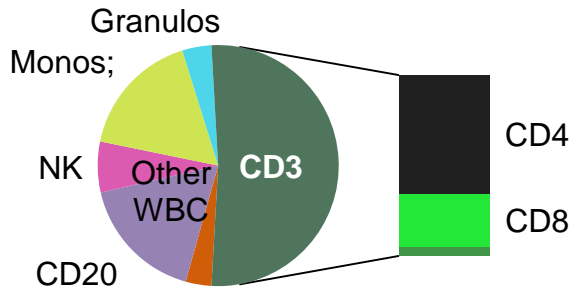
- uniform T cell expansion rate; > 30-fold
- No difference cryopreserved versus fresh
- Naive (T_N) and central memory (T_{CM}) T cells as well as T stem cell like memory cells (T_{SCM})



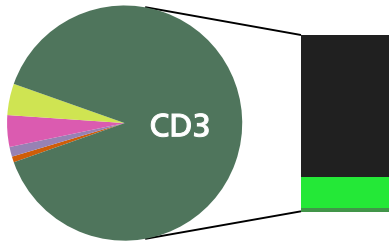
Cellular composition (CD4/CD8 sel.)

results

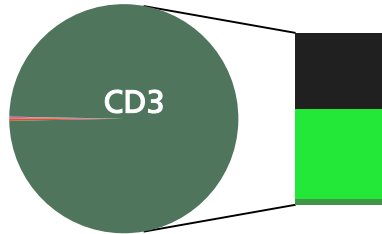
Apheresis



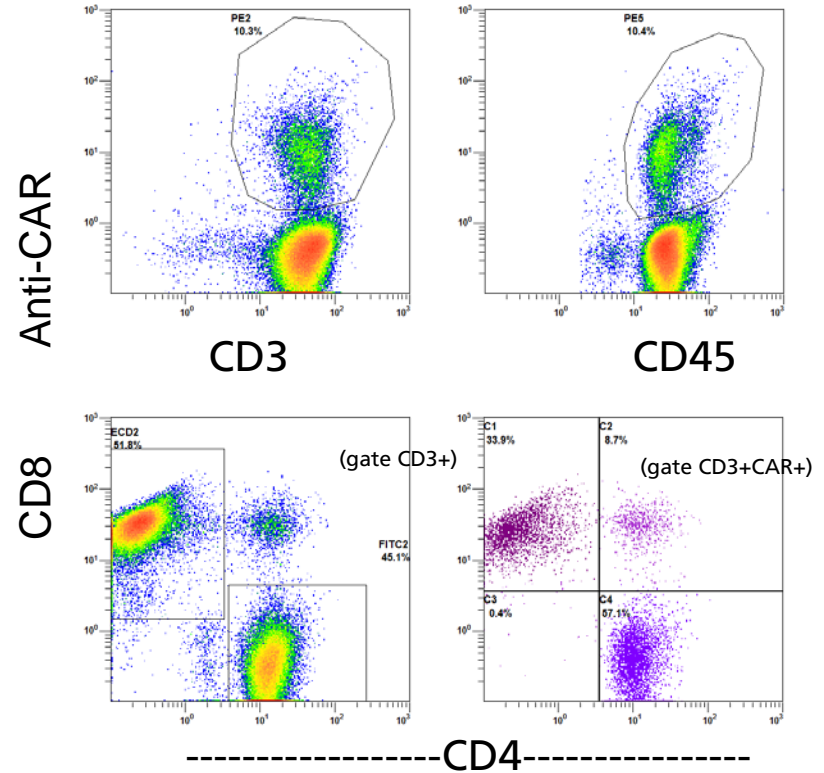
CD4/CD8 selected cells



Day 12 harvesting



Flow Cytometry day12



© F →

Pure T cells at harvesting

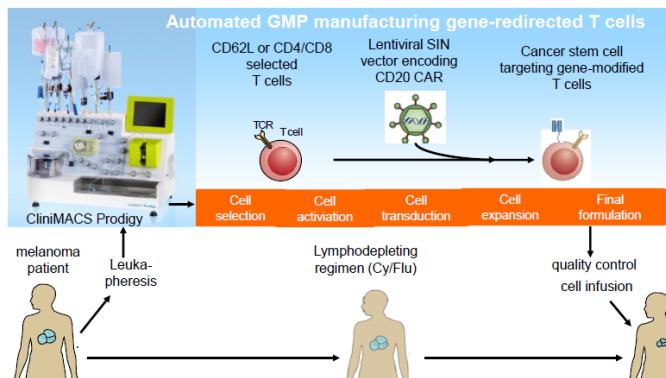


Successful transduction efficiency

Bench to bedside and back to the lab

➤ Automation – CAR T cells

M_HH



➤ Clinical CTL019 study

CAR T cells
development/
manufacturing EU

Fraunhofer
IZI



8/2017 (FDA) Kymriah™

NOVARTIS

➤ New „CAR“ technologies

Proof of concept 2018-2022
Sleeping beauty
target antigen: ROR1
Helmholtz Berlin
Fraunhofer IZI/
University of Würzburg

➤ TRUCKs

T cells Redirected
for Universal
Cytokine Killing

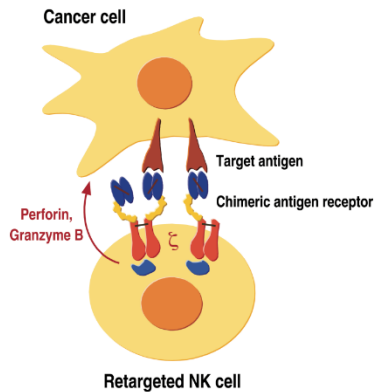
7 Universities

➤ „CAR“ NK cells

EU consortium:
2018-2022;
mono and bi-specific
CAR NK cells
NK cell signalling
Speaker: U. Köhl

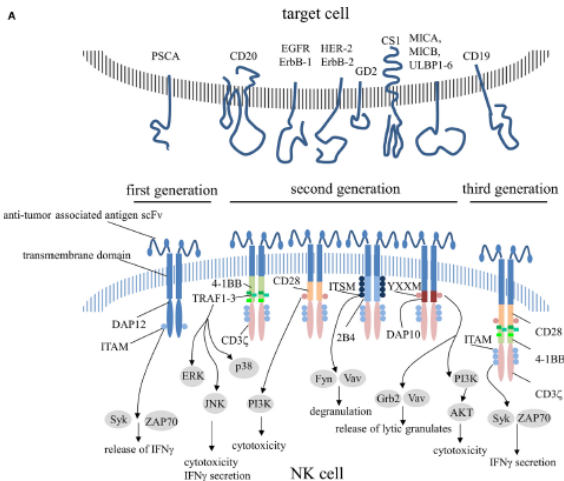


“CAR” expressing effector cells



➤ CAR engineered T cells

- Clinical studies
- Manufacturing challenge
- **in some patients: failure in manufacturing (heavily pre-treated)**



Oberschmidt O, Kloess S and Koehl U.
Frontiers Immunology 2017

➤ „off the shelf“ CAR NK cells

- Clinical NK cell studies
- Primary human „CAR“ NK cells
- lytic activity
- Benefit and side effects

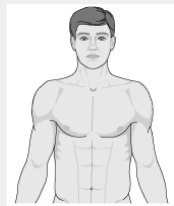
NK cell immunotherapy

results

NK-DLI = NK donor lymphocyte infusion

Allogeneic NK-DLI post haplo-SCT (Clin Gov No NCT 01386619)

Patients with high risk leukaemia and malignant tumours



Donor /
Parents

Leukapheresis



NK cell purification
(CD3 depl./ CD56 sel.)

GMP



IL-2 expansion
(1000 U/ml, 10 days)

GMP



NK cell application
patient



haplo-SCT

CD34 sel./
CD3/CD19 depl.

0

1st NK cell
application

(+3)

2nd NK cell
application

+40

3rd NK cell
application

+100

[days post SCT]

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. Curtiⁱ, 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>

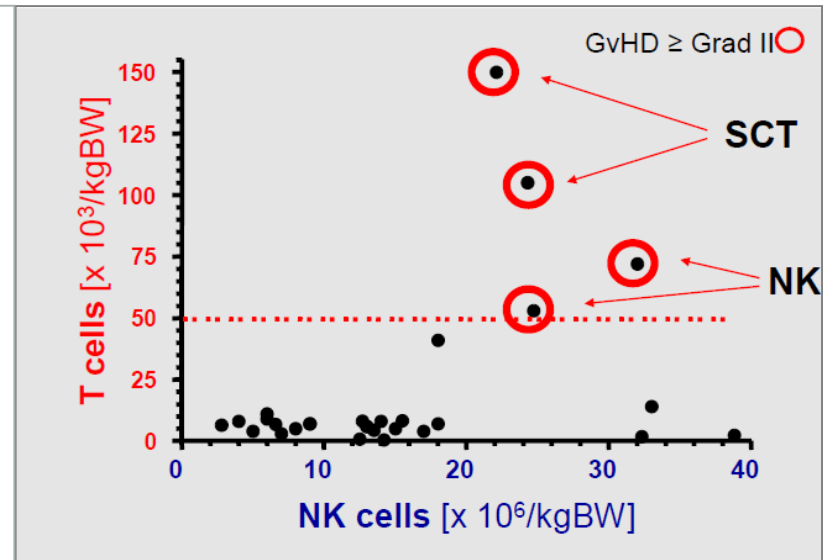
Advantage

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

Disadvantage

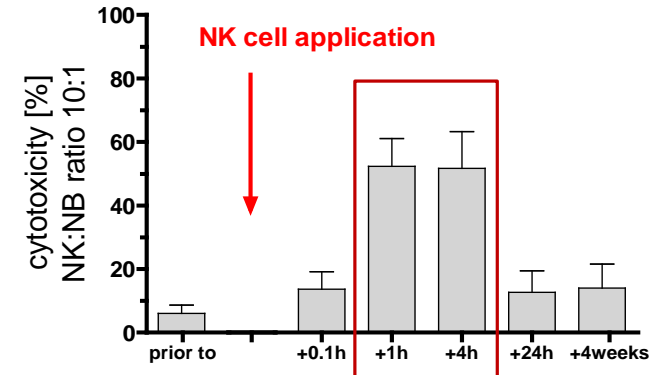
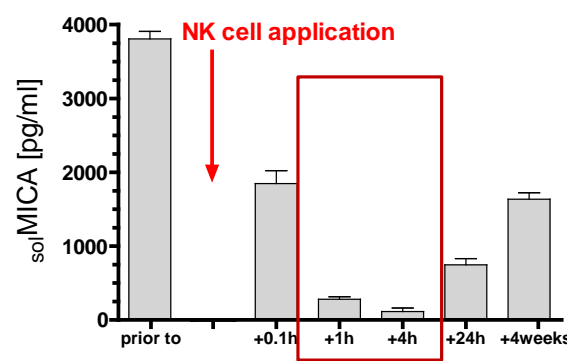
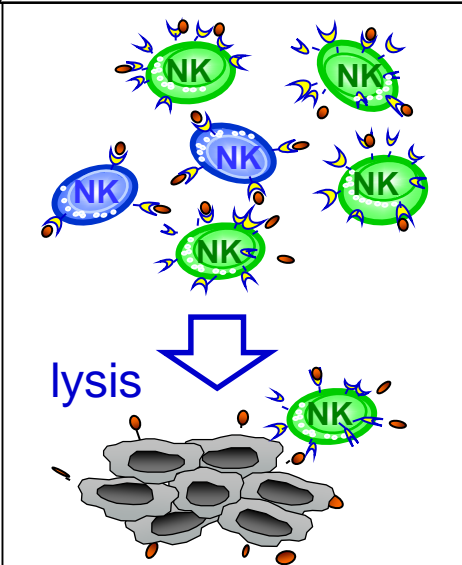
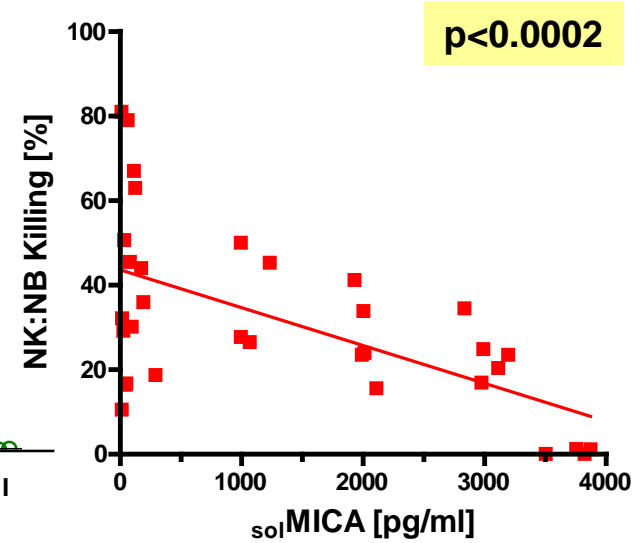
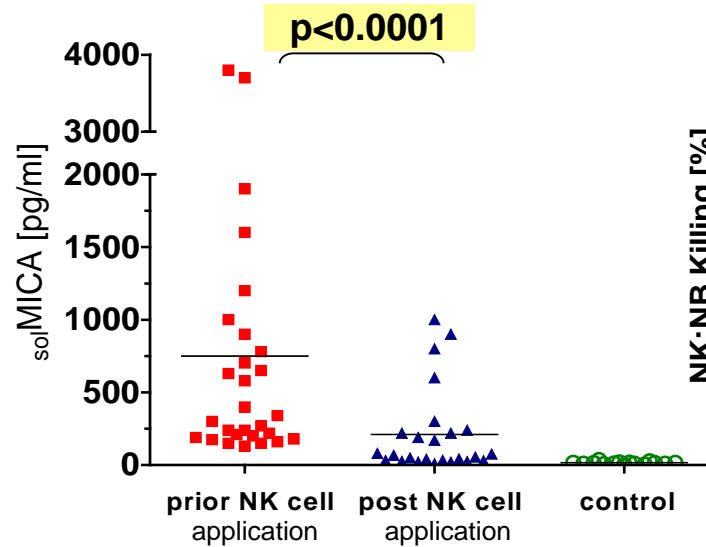
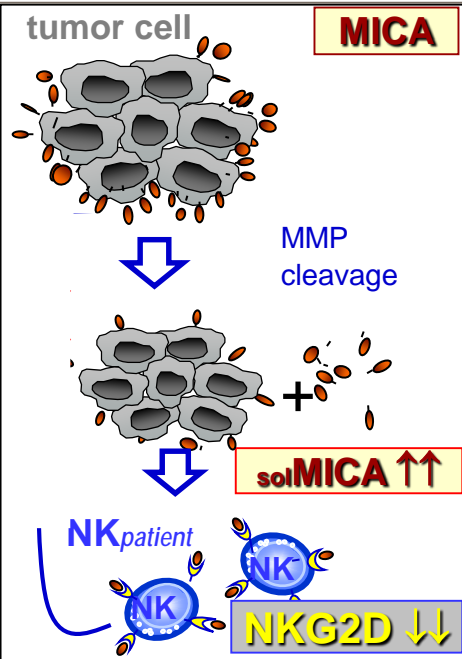
- Tumor immune escape mechanism (TIEMs)

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



solMICA dependent tumor immune escape inhibits NK cells in patients with Neuroblastoma

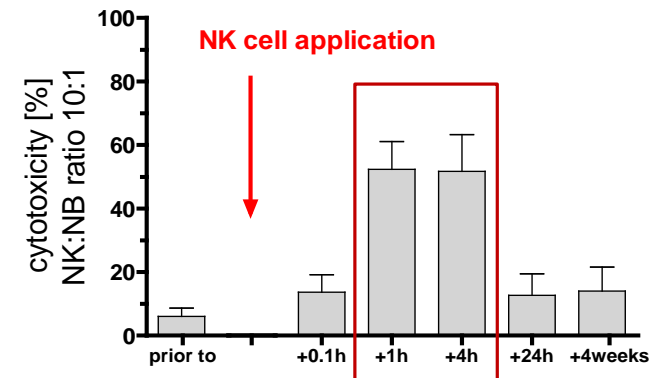
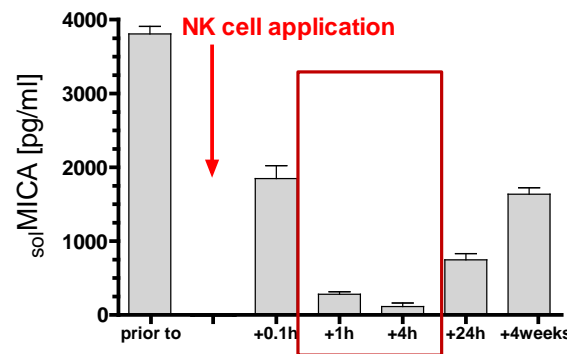
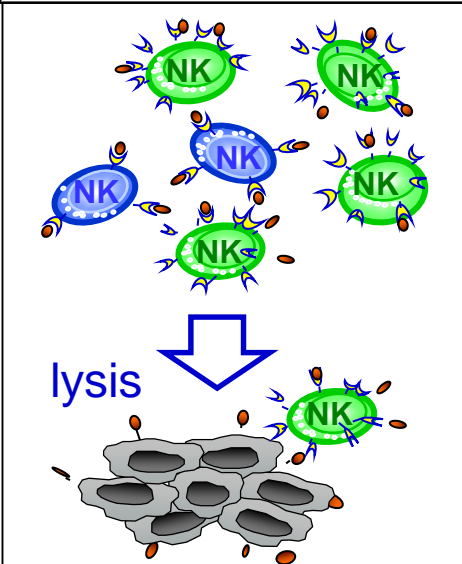
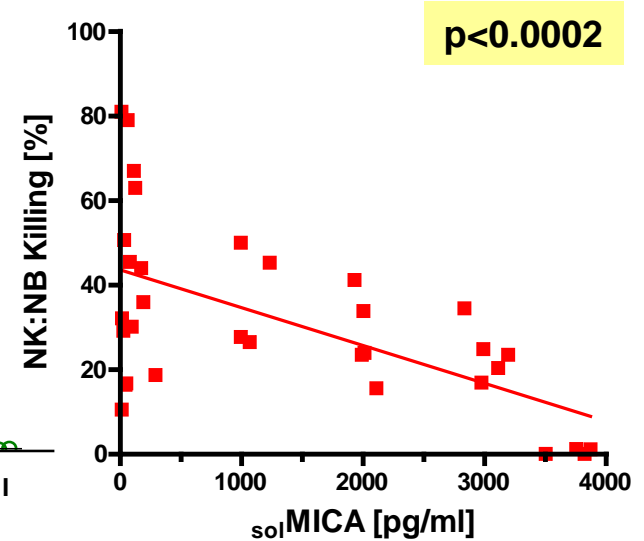
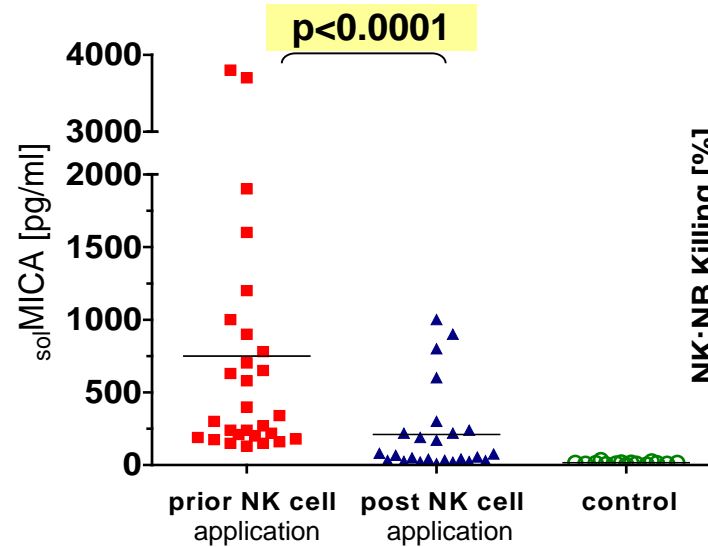
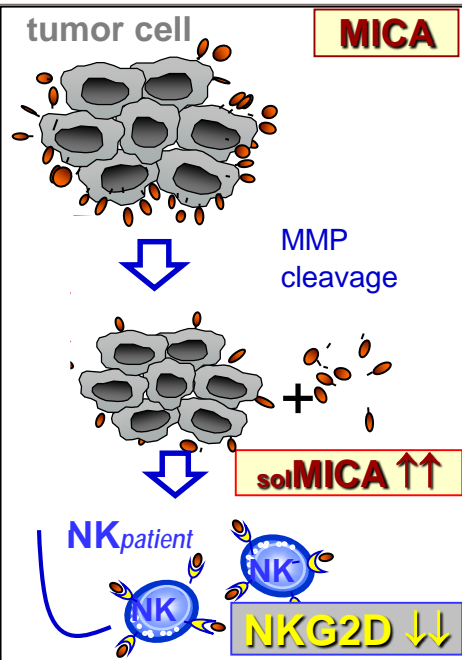
results



➔ IL-2 activated NK cells improve NKG2D mediated cytotoxicity via scavenging of solMICA in plasma

solMICA dependent tumor immune escape inhibits NK cells in patients with Neuroblastoma

results



To overcome those hurdles: CAR NK cells ?

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

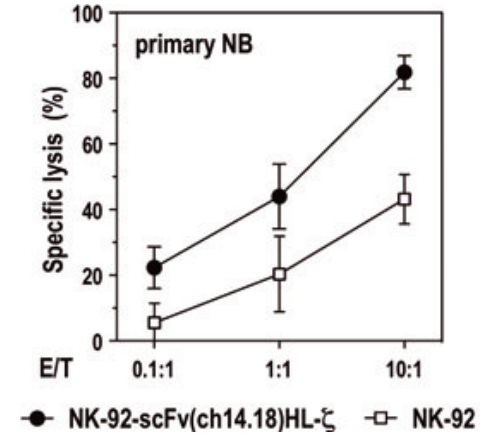
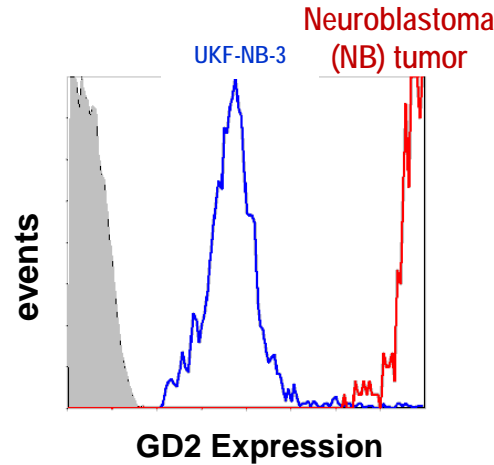
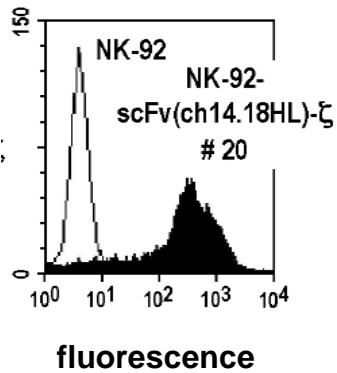
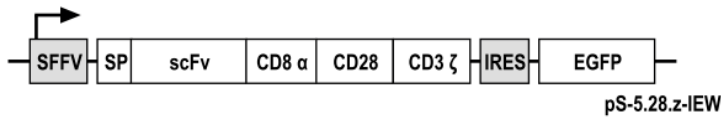
CAR:
CD19-CD28-zeta-
2A-iCasp9-IL15

CCCR: Chimeric
Costimulatory
Converting Receptor

Redirected "CAR" NK-92 cell line

results

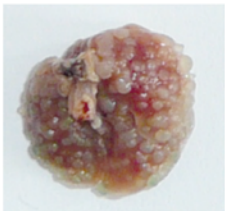
anti-GD2



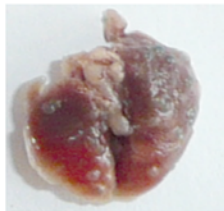
Esser R *et al.* J of Cellular and Molecular Medicine 2011

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

anti-ErbB2/HER2

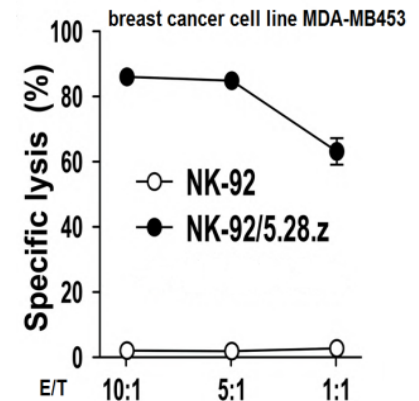
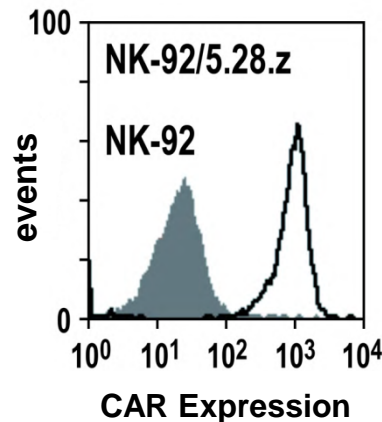


NK-92



NK-92/5.28.z

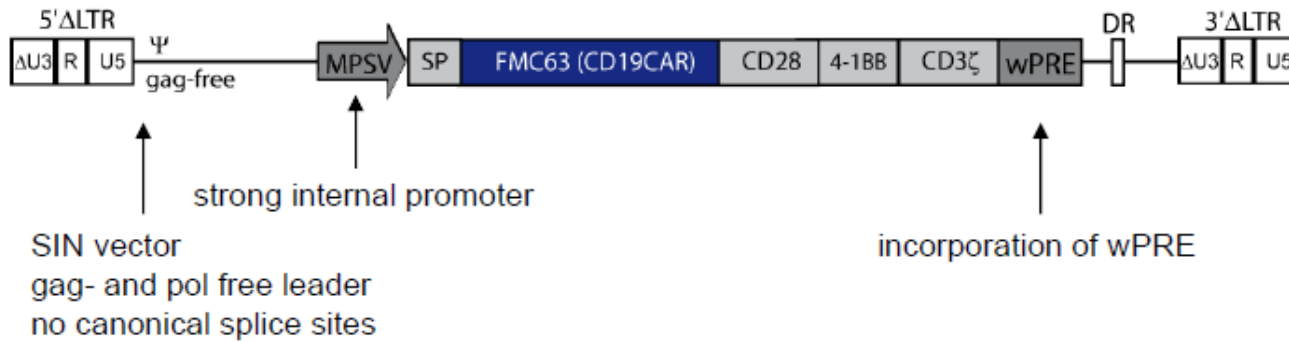
Schönfeld *et al.* Molecular Therapy 2014



Chimeric Antigen Receptor Vector Design for primary human NK cells



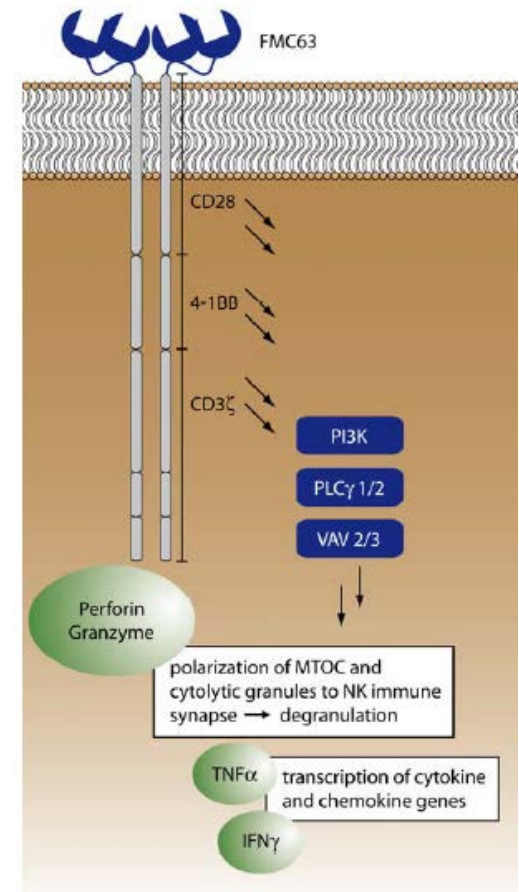
A. Schambach



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

→ CD19 binding leads to signal transduction

→ Enhanced cytotoxicity

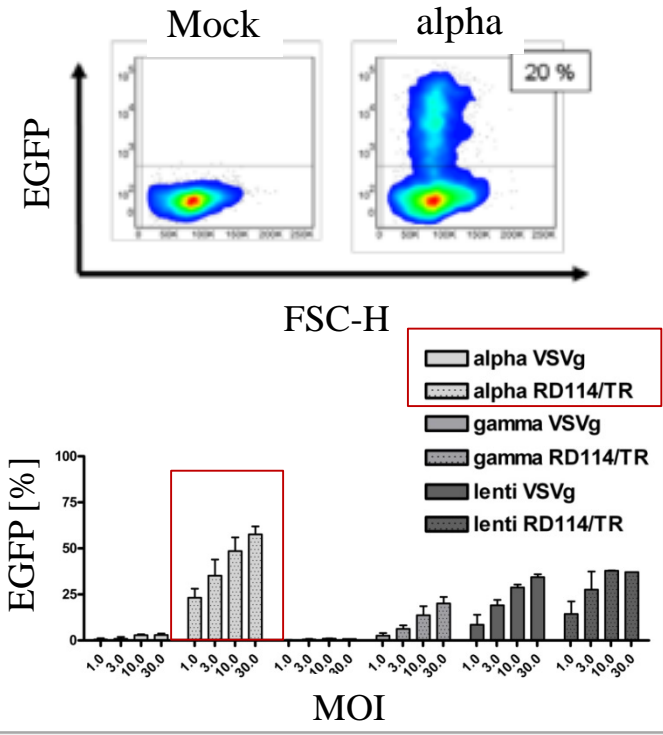


CAR expressing NK cells redirected against CD19

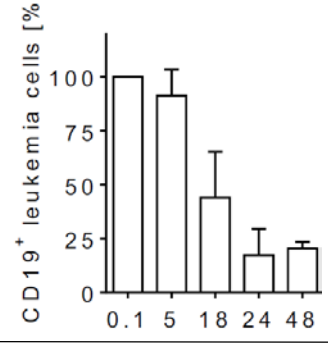
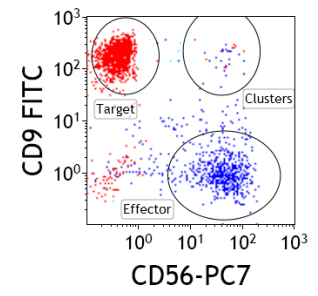
results



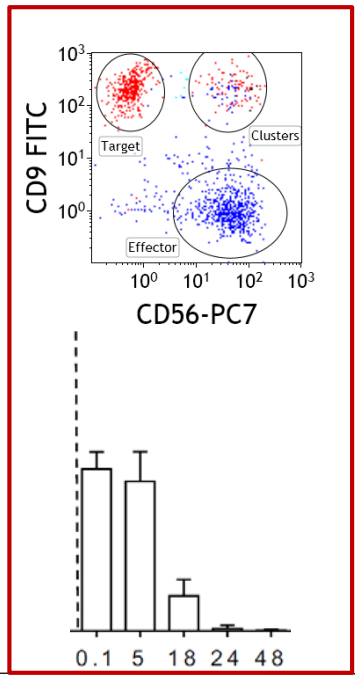
Alpha SIN vector
Transduction of mature primary human dNK cells feasible



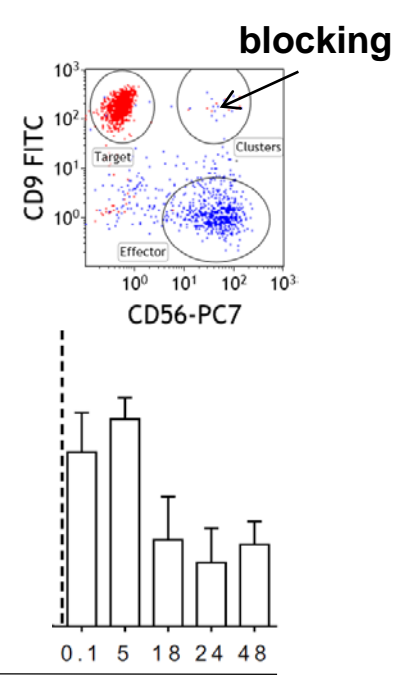
dNK cells vs. BV173



CD19-CAR-dNK cells vs. BV173



CD19-CAR-dNK cells vs. BV173+CD19mAB

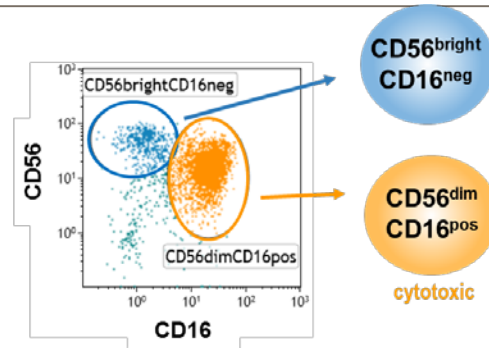


dNK cells : CD19+ BV173 incubation time [h]; E:T ratio: 5:1

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

results

CD56^{bright}CD16^{dim&neg}
(immune regulatory)



CD56^{dim}CD16^{pos}
(cytotoxic)

E/T
1:1 5:1

++ 0
+ +

++ +
++ +
+ +
++ ++

+ 0
0 0
- -
0 +

anti-inflammatory:

IL-4
IL-10

pro-inflammatory:

IL-6
IL-17A
IFN γ
TNF α

pro-apoptotic:

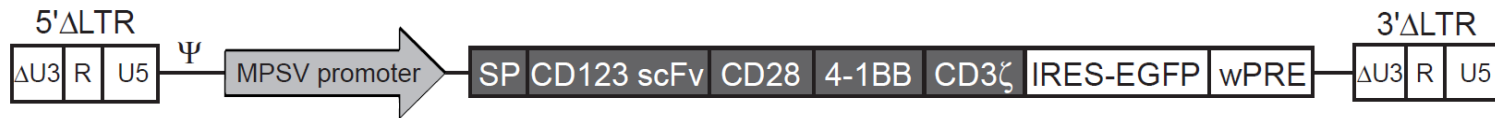
GrA
GrB
Perforin
Granulysin

E/T
1:1 5:1

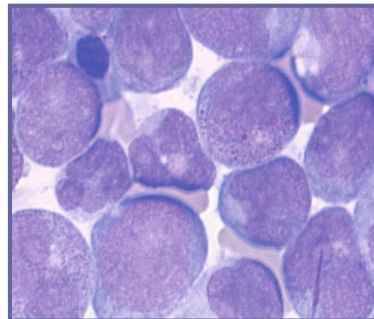
+ +
+ +

+ +
+ -
- -
+ +

++ +
++ ++
+ +
0 ++

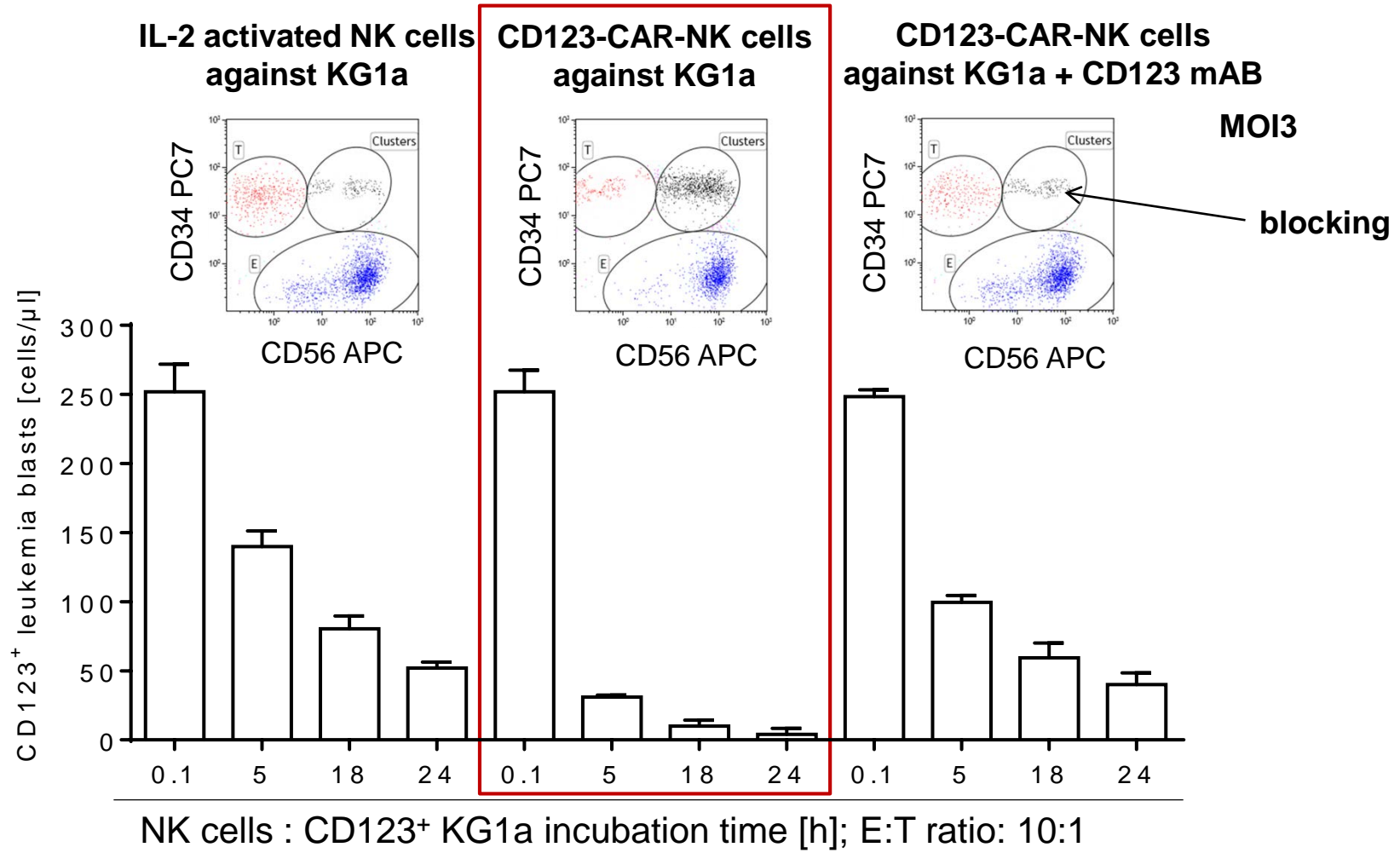


Primary CAR expressing NK cells
 redirected against AML cell lines and
 patients own leukemic cells



CAR expressing NK cells redirected against CD123⁺

results



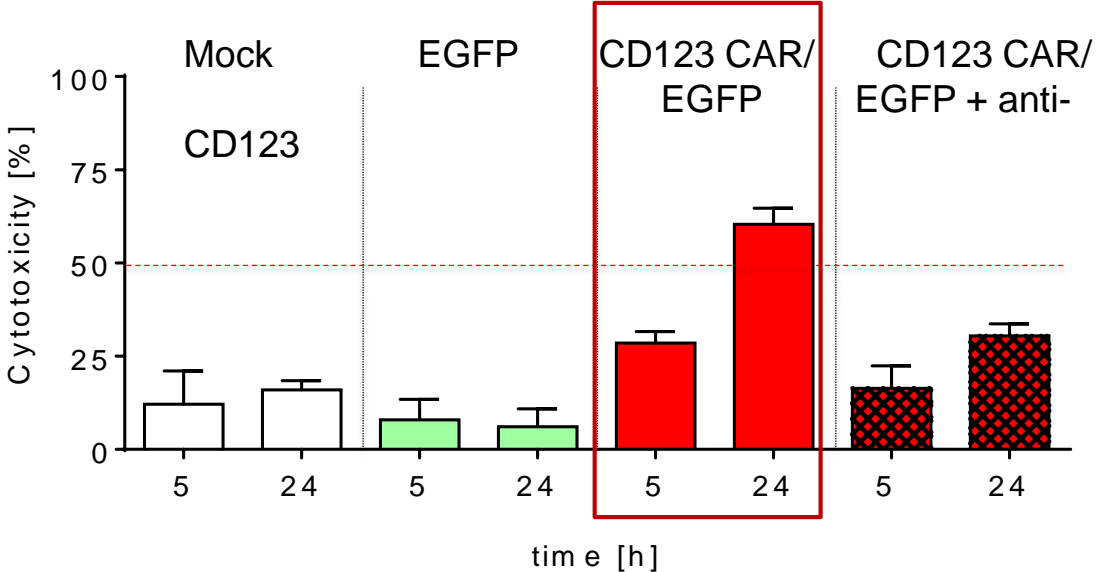
CAR NK cells against patient's CD123+AML

results



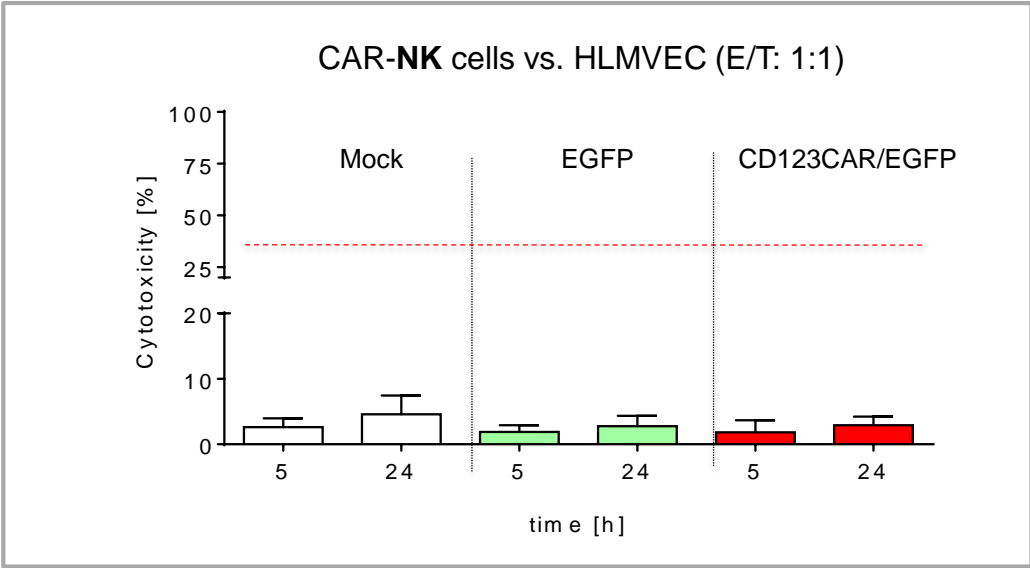
S. Kloess

Cytotoxicity



MOI1
 CAR-NK cells
 vs.
 patient's AML
 (E/T: 1:1)

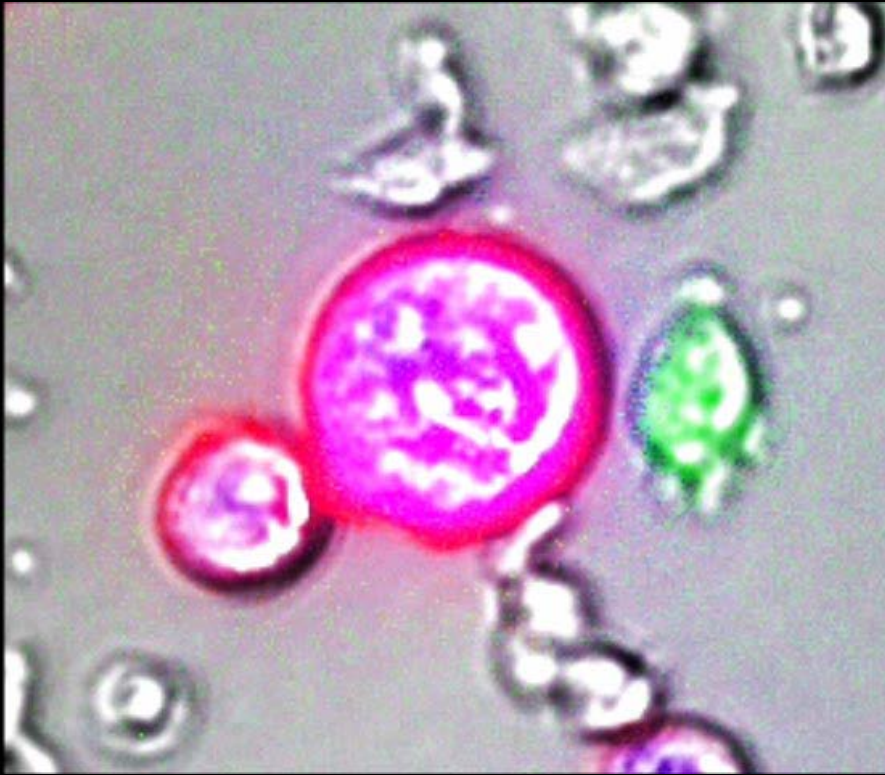
Side effects



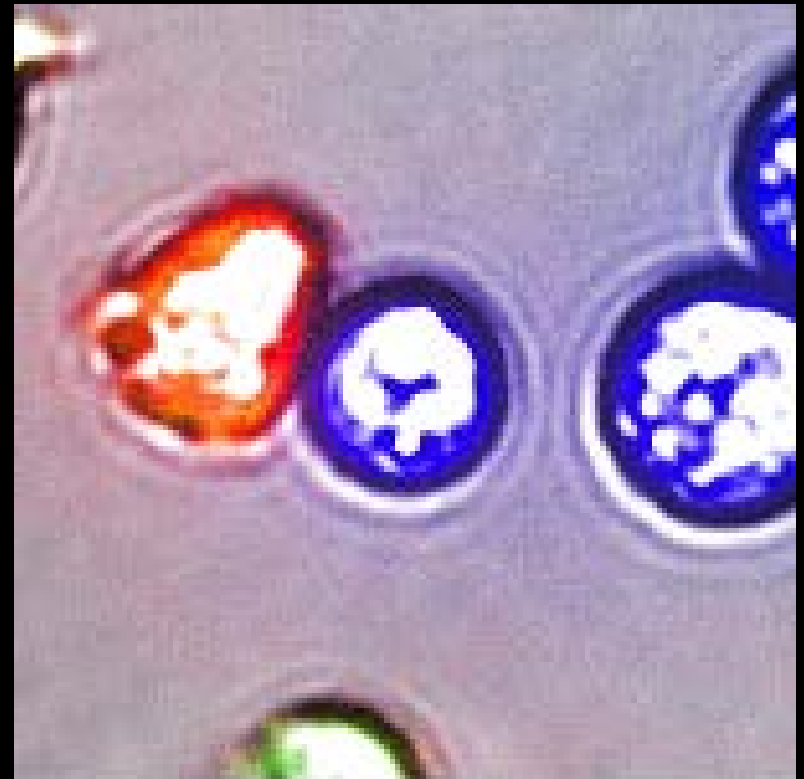
Coop.:
 M. Heuser,
 A. Schambach MHH

CD123CAR expressing NK cells and EGFP⁺ mock NK cells against CD123 positive KG1 α targets

anti-CD123-CAR NK cells EGFP⁺



anti-CD16-APC, EGFP⁺ NK cells

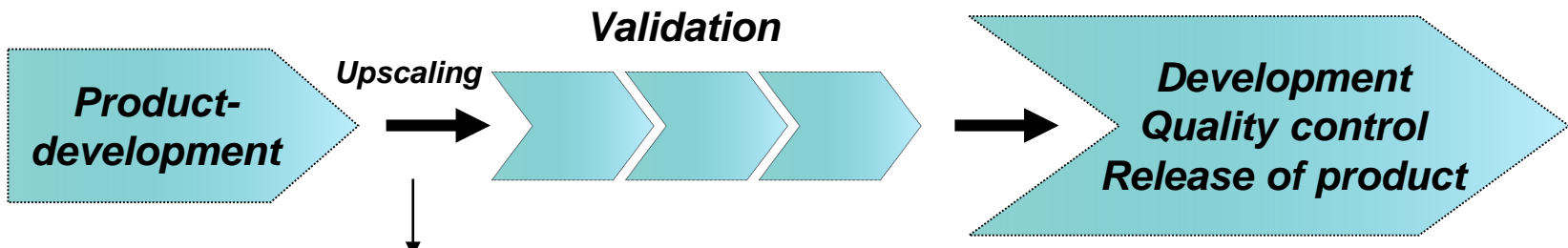


CD123⁺KG1 α cell proliferation dye: eFluor[®]450, anti-CD34-PE

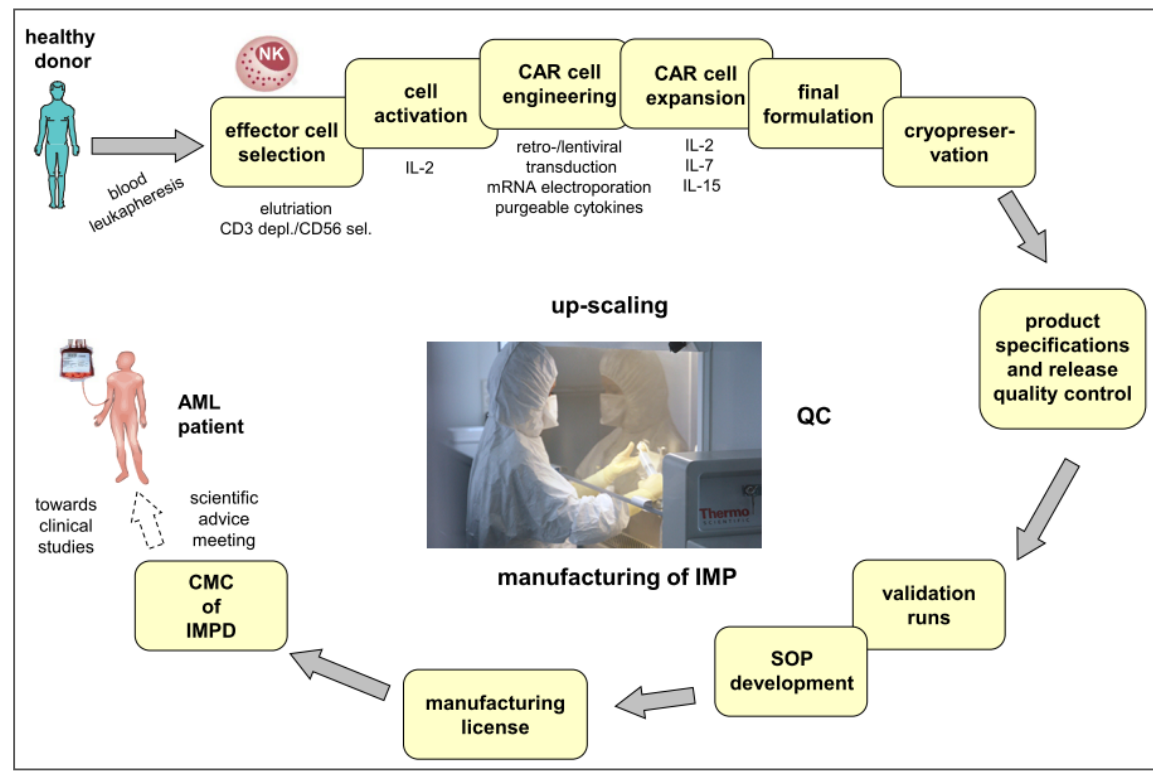
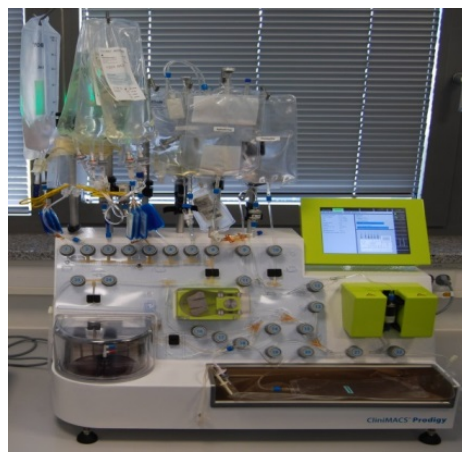
NK:KG1 E:T ratio: 5:1; MOI1

Clinical scale – CAR expressing NK cells

results



GMP-compliant protocol

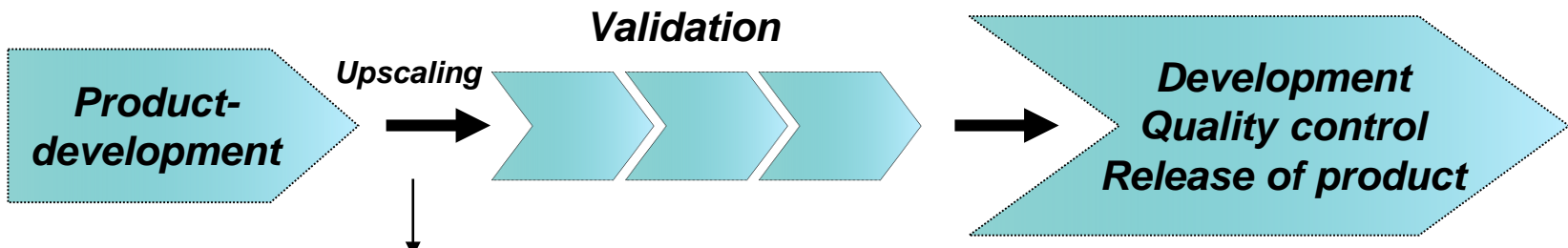


„Off the shelf product“ Advanced Therapy Medicinal Product

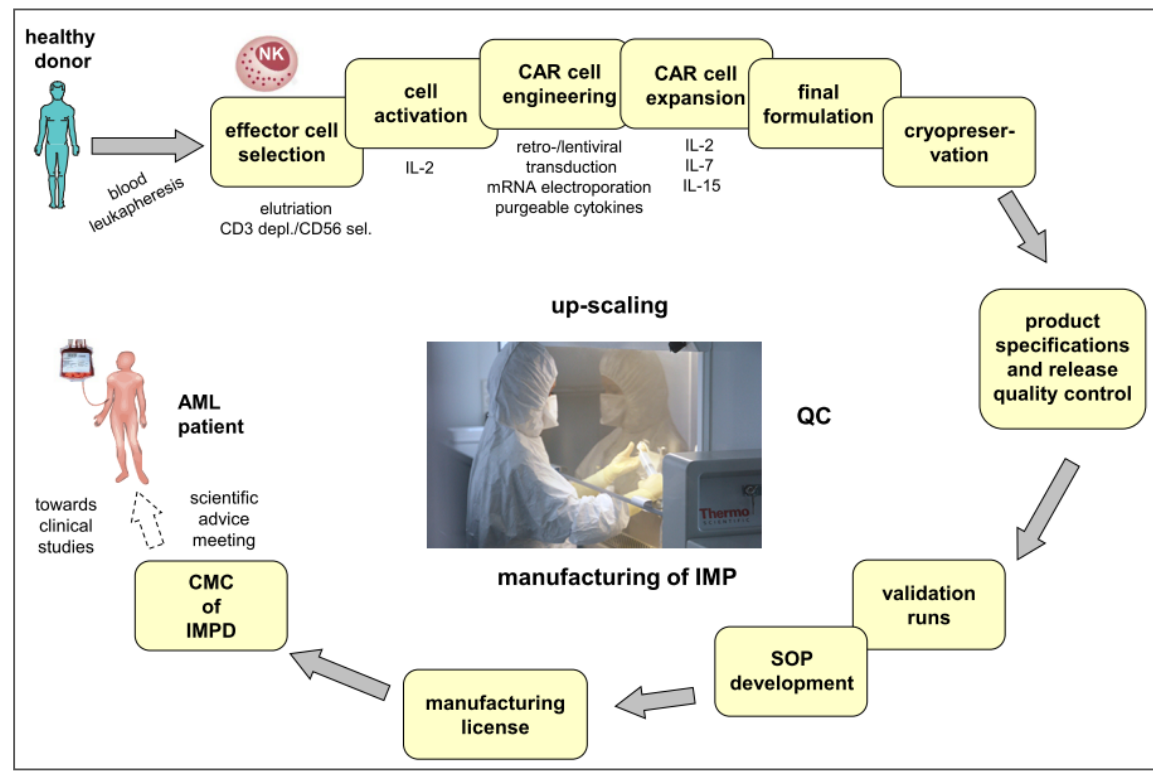
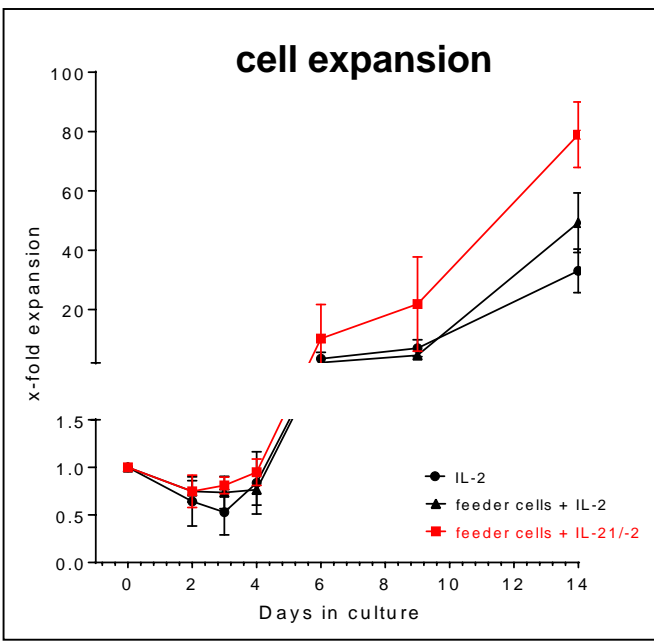
SOP=standard operation protocol CMC=chemical manufacturing and control IMPD=investigational medicinal product dossier

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 T cells:

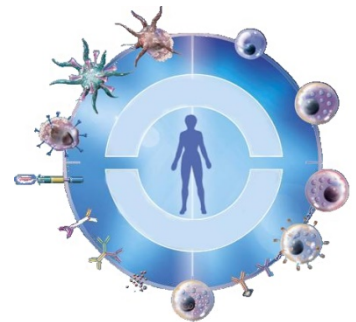
- Successful clinical CAR T cells studies (385 documented world wide)
- Our data about automated manufacturing of CAR expressing T cells gives rise for harmonized protocols in future clinical studies
- Manufacturing failure of autologous CAR T cells needs complementary concepts

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



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S. Mitzner	S. Fricke

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Georg Speyer House Frankfurt, D

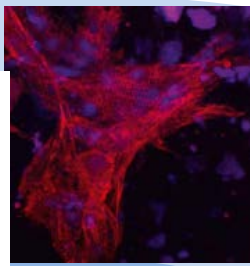
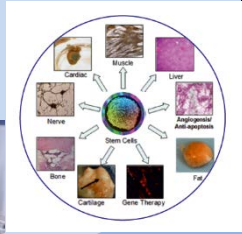
W. Wels

Haematol. /Oncol./ SCT, MHH, D

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Internal Medicine, Würzburg, D

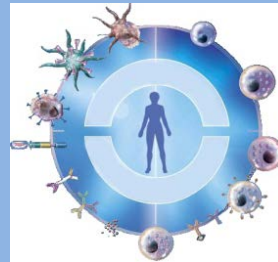
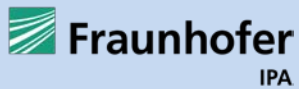
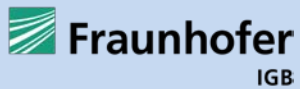
H. Einsele M Hudecek



Diagnostic - devices cell-based therapies

Havard Medical
School * Boston
Gene Therapy

International
partners



Technologies
Automation

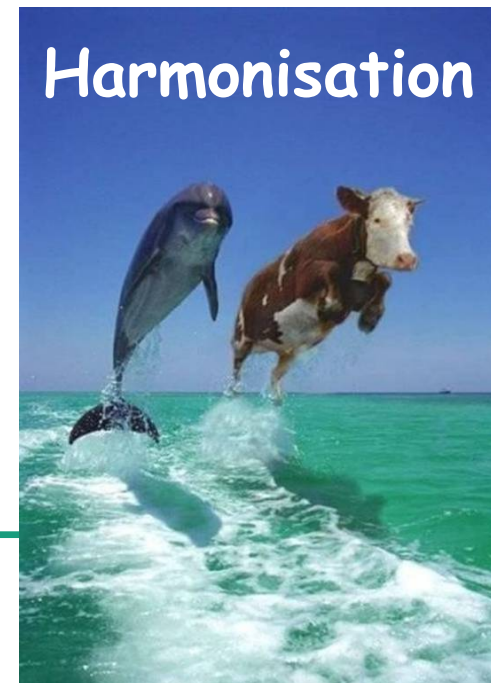
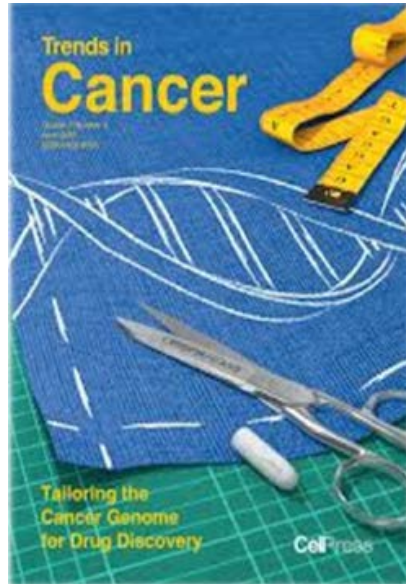
72 Institutes

> 25.000 staff members





Network for individualised stratified medicine using cell-based therapies



.... and thanks for listening