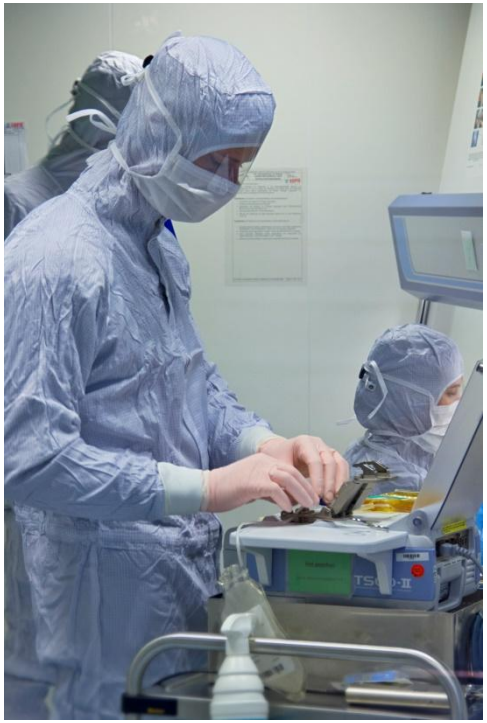
Automatisierung von CAR Effektorzellen

Ulrike Köhl

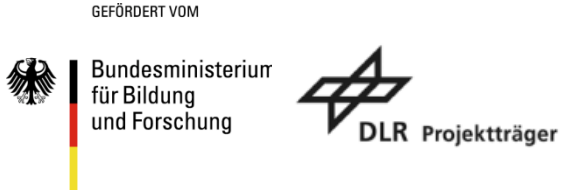


Institute of Cellular Therapeutics, Hannover Medical School (MHH)
Fraunhofer Institute for Cellular Therapeutics and Immunology (IZI),
Institute of Clinical Immunology, University of Leipzig (UL/UKL), Germany

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“.



* 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

UKL
UL
MHH
IZI

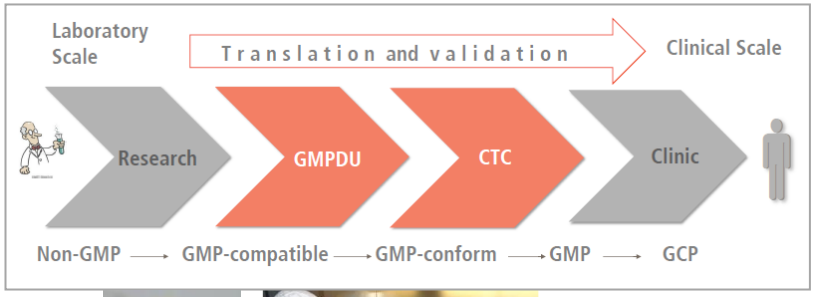
Germany

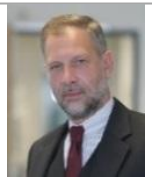
Fraunhofer IZI

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





L. Arseniev

IZI GMP Core facility

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

G. Schmiedeknecht Dipl. Ing. K. Kebbel

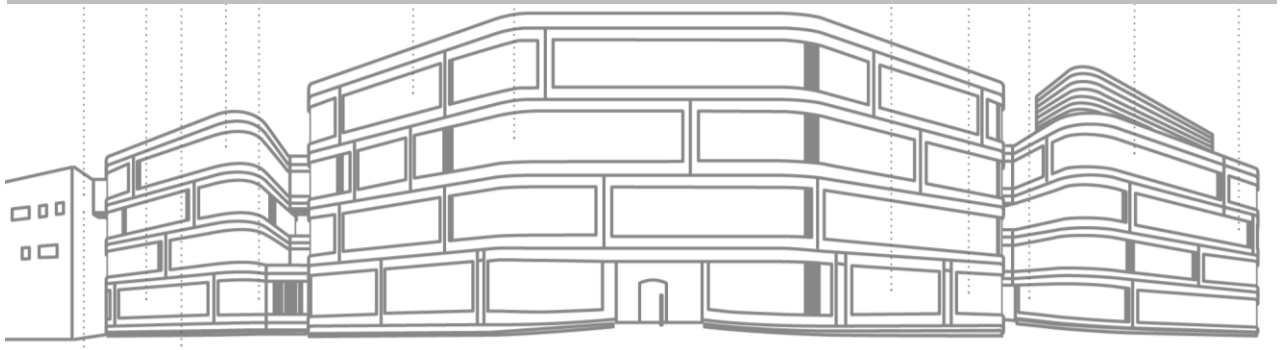
Development up to translation into the clinic

MHH

Pre-clinical development

GMP Manufacturing

Clinical Studies
Phase I/ II/ III/ IV



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

Fraunhofer IZI

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

Europe wide

- CD19 CAR T cells (CTL019)
- CAR T cells Melanoma
- CAR NK cells
- Regulatory T cells
- Mesenchymal stroma cells
- Dendritic cells
- iPS-cardiomyocytes
- iPS-macrophages

Clinical and Industrial partners

NOVARTIS

Cognate BioServices, Inc.

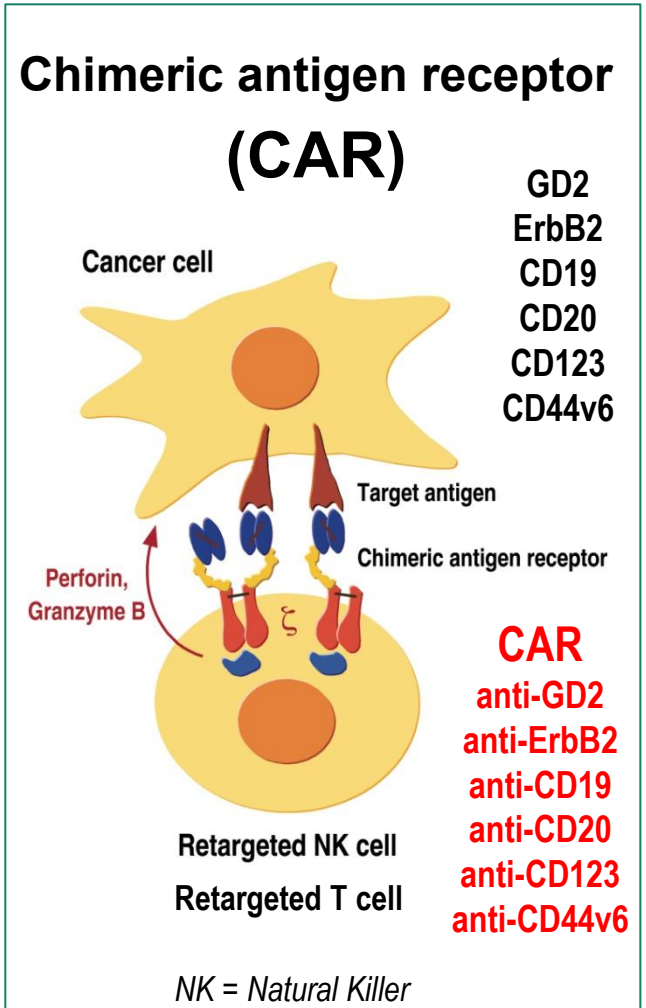
Northwest Biotherapeutics

PRIMABioMed LIMITED

> 350 cellular products/ year

CAR = Chimeric Antigen Receptor iPS = induced pluripotent stem cells

“CAR” expressing effector cells

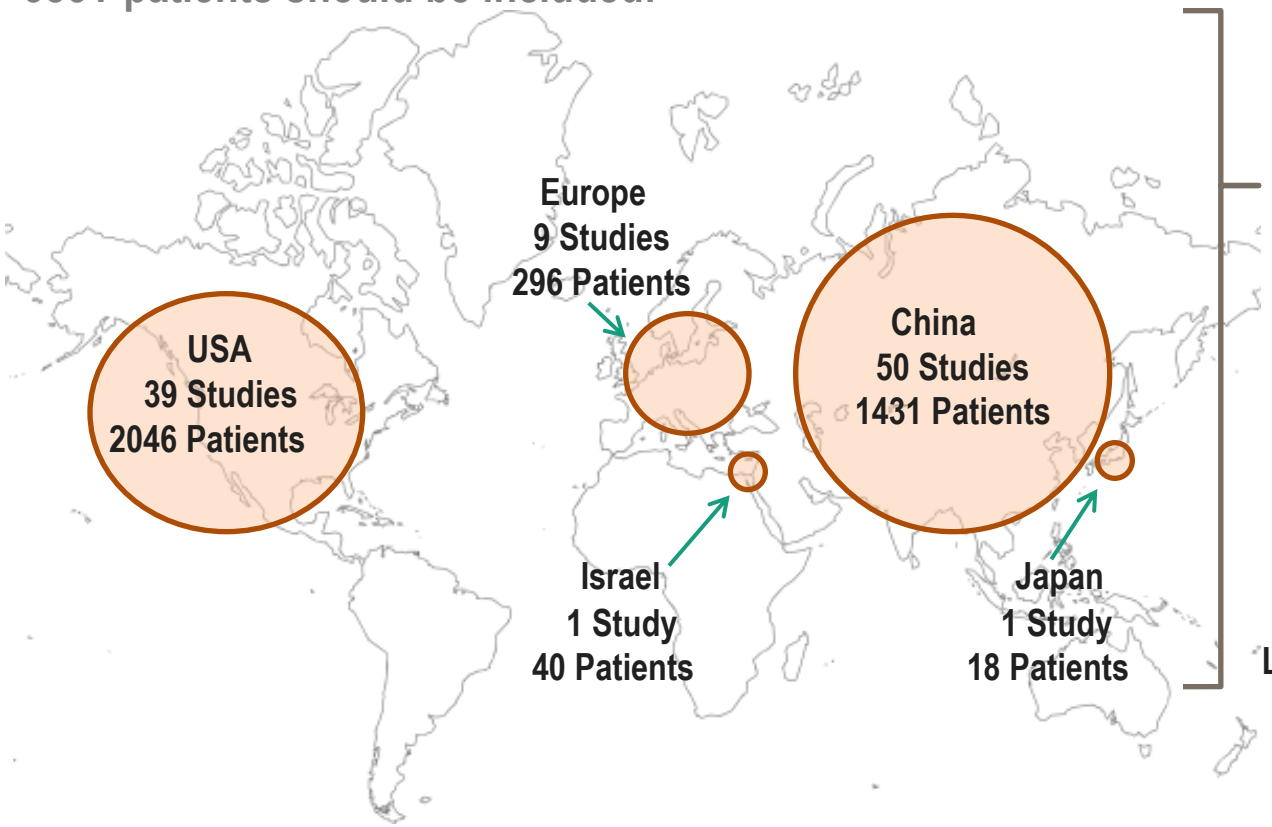


- **CAR engineered T cells**
  - clininical studies
  - manufacturing challenge

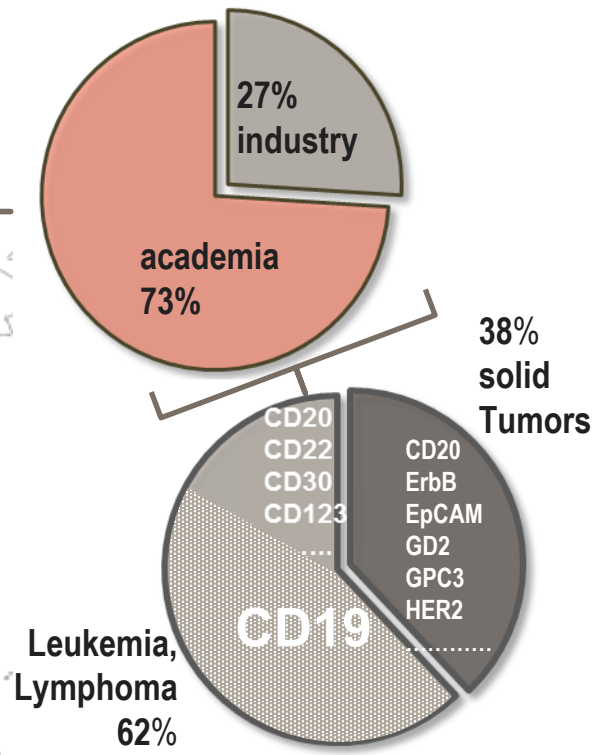
- **„off the shelf“ CAR NK cells**
  - clinical NK cell studies
  - primary human „CAR“ NK cells
  - lytic activity
  - benefit and side effects

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:



Sponsorship



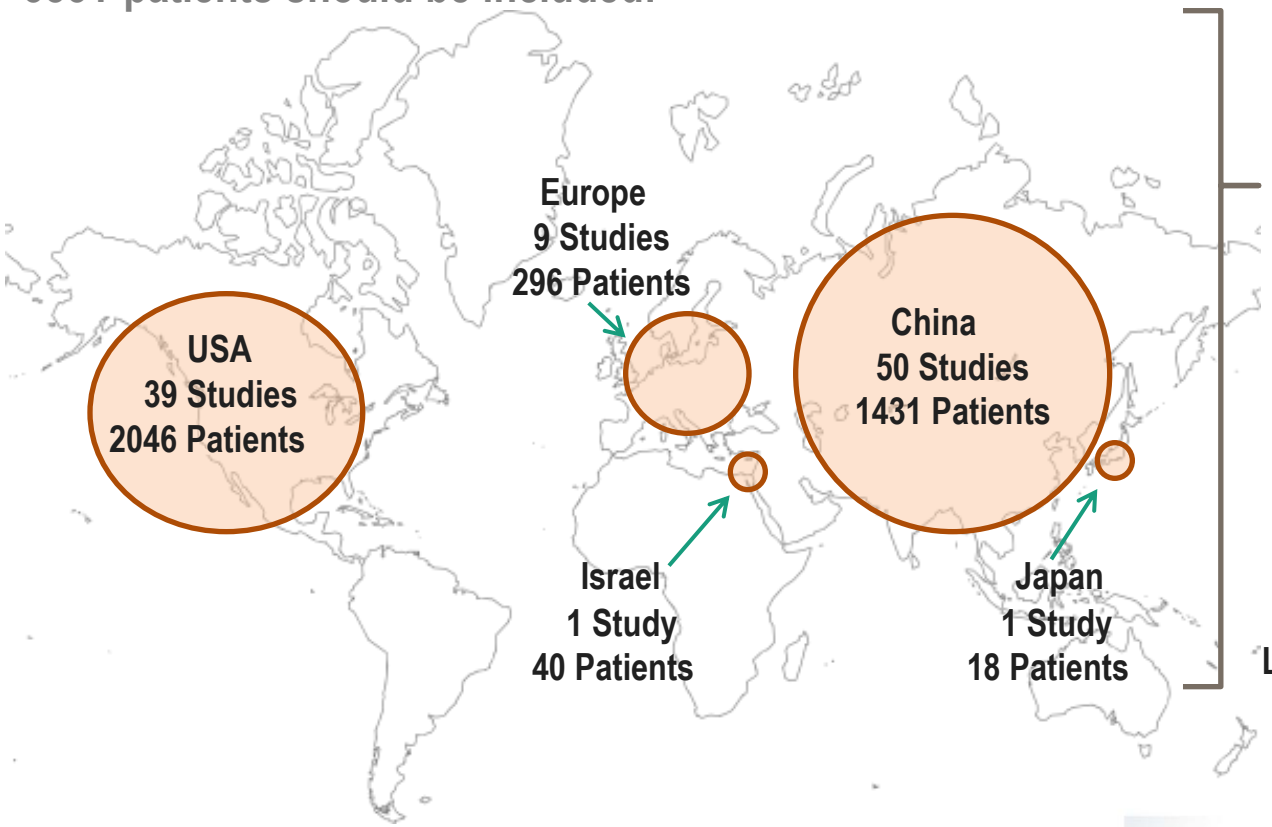
Zuther K … Koehl U. J Onkologie 2017

Indications and Target-Antigens

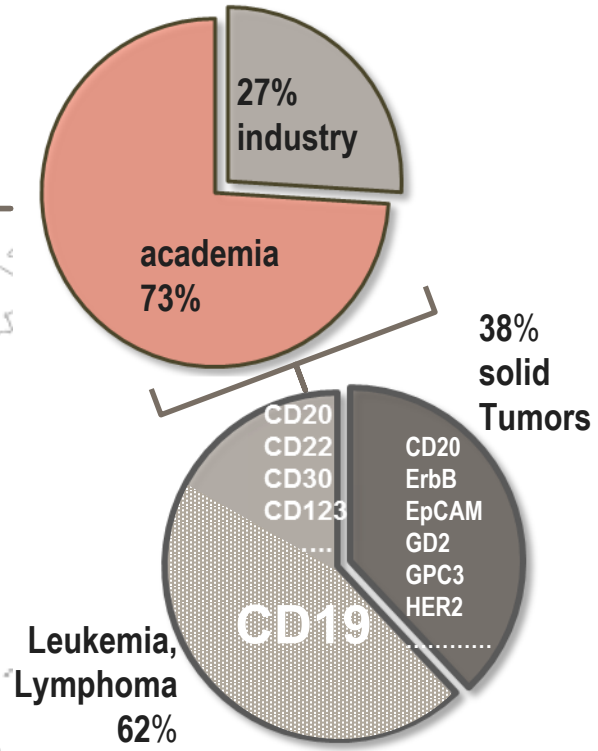
Fraunhofer IZI

Clinical trials with CAR expressing T cells
(clingov. Q4/2016)

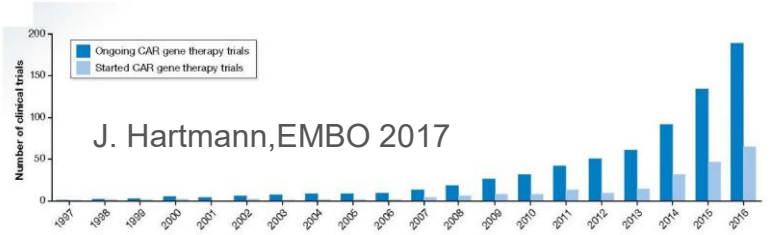
100 open clinical trials with CAR T cells registered worldwide
3831 patients should be included:



Sponsorship



Update 9/2018: n > 390 studies

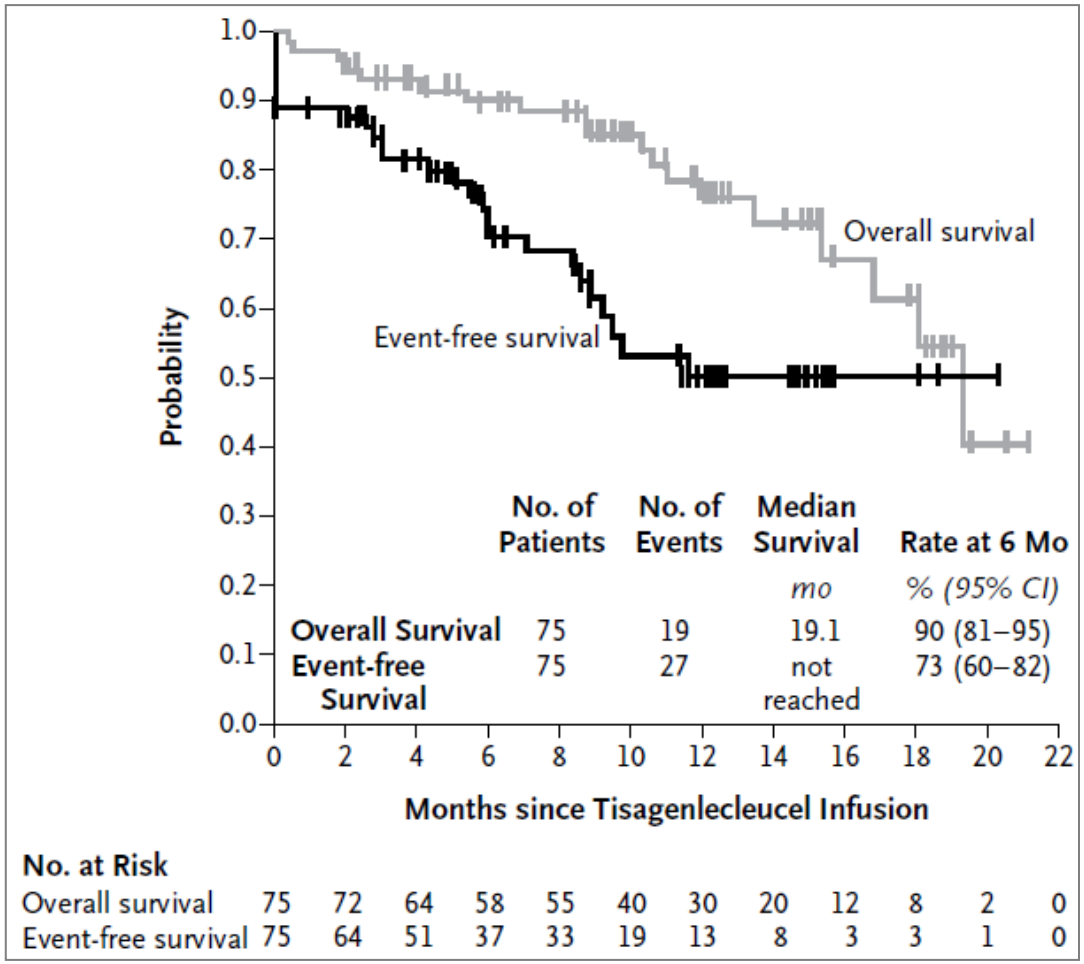


CTL019 pediatric r/r ALL – ELIANA

"Event-free" and "overall survival"

Now> 5 years cured

75 patients



- **Event-free survival**
  - 73% post 6 months
  - 50% post 12 months
- **Overall survival**
  - 90% post 6 months
  - 76% post 12 months

Maude SL *et al.* Februar 2018 The NEW ENGLAND JOURNAL of MEDICINE

Marketing authorization in Europe

320.000 €/ product

Kymriah® (EMA)
Novartis
27.08.2018

Yescarta® (EMA)

2017 2018

Kymriah® (FDA)
Novartis

Yescarta® (FDA)
KITE

470.000 €/ product

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

Fraunhofer IZI

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,*]

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

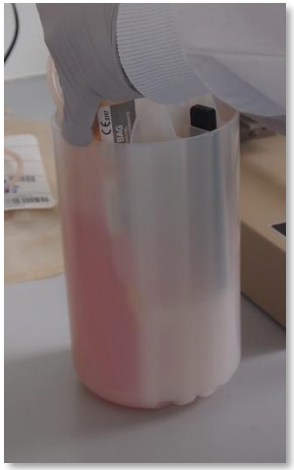
The adoptive transfer of chimeric antigen receptor (CAR)-modified T cells is attracting growing interest for the treatment of malignant diseases. Early trials with anti-CD19 CAR T cells have achieved spectacular remissions in B-cell leukemia and lymphoma, so far refractory, very recently resulting in 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

Fraunhofer IZI

State of the art: hands on manufacturing

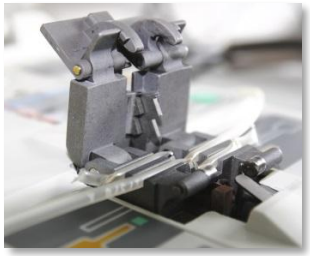

excubate


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)

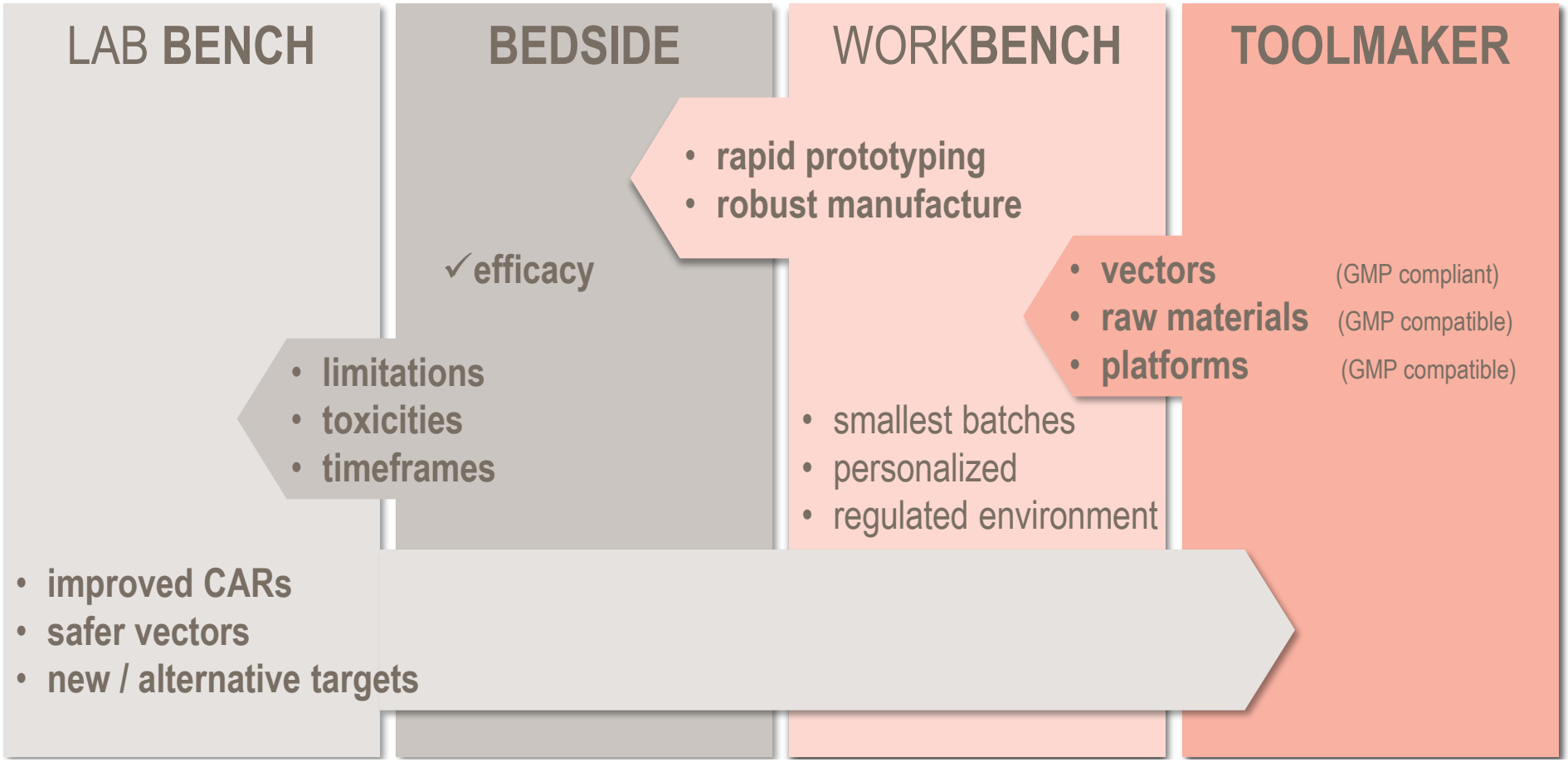

SEFIA
(Biosafe SA)


WAVE
(GE Healthcare)

(non-exhaustive)

e.g. PERFUSE / WASH / FORMULATE

Automated manufacturing?



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 PHILIP[2], GORDON WENG-KIT CHEUNG[2], HONG ZHAN[1], IAN C.D. JOHNSTON[3], ANDREW D. KAISER[3], KARL PEGGS[2], MARTIN PULE[2], ADRIAN J. THRASHER[1] & WASEEM QASIM[1]

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

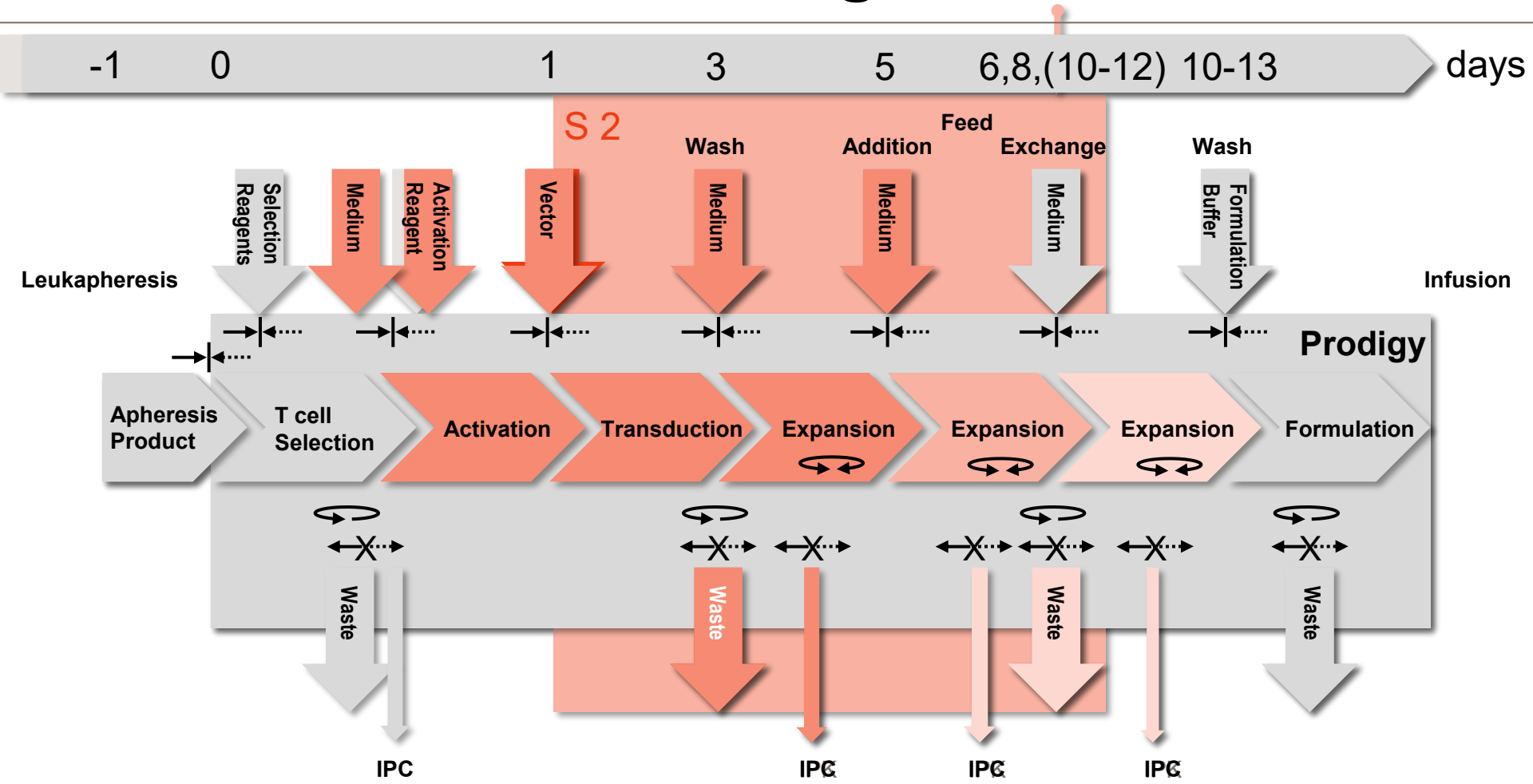
Christoph Priesner,[1,†] Krasimira Aleksandrova,[1,†] Ruth Esser,[2] Nadine Mockel-Tenbrinck,[3] Jana Leise,[1] Katharina Drechsel,[3] Michael Marburger,[2] Andrea Quaiser,[2] Lilia Goudeva,[4] Lubomir Arseniev,[1] Andrew D. Kaiser,[3,‡] Wolfgang Glienke,[2,‡] and Ulrike Koehl[1,2,‡,*]

HUMAN GENE THERAPY, VOLUME 27 NUMBER 10 Published online: August 25, 2016.

Start in the Prodigy: Immunomagnetic selected T cells

Fraunhofer IZI

Automated manufacturing of CAR T cells



Universitätsklinikum Leipzig
Medizin ist unsere Berufung.
UNIVERSITÄT LEIPZIG
Medizinische Fakultät

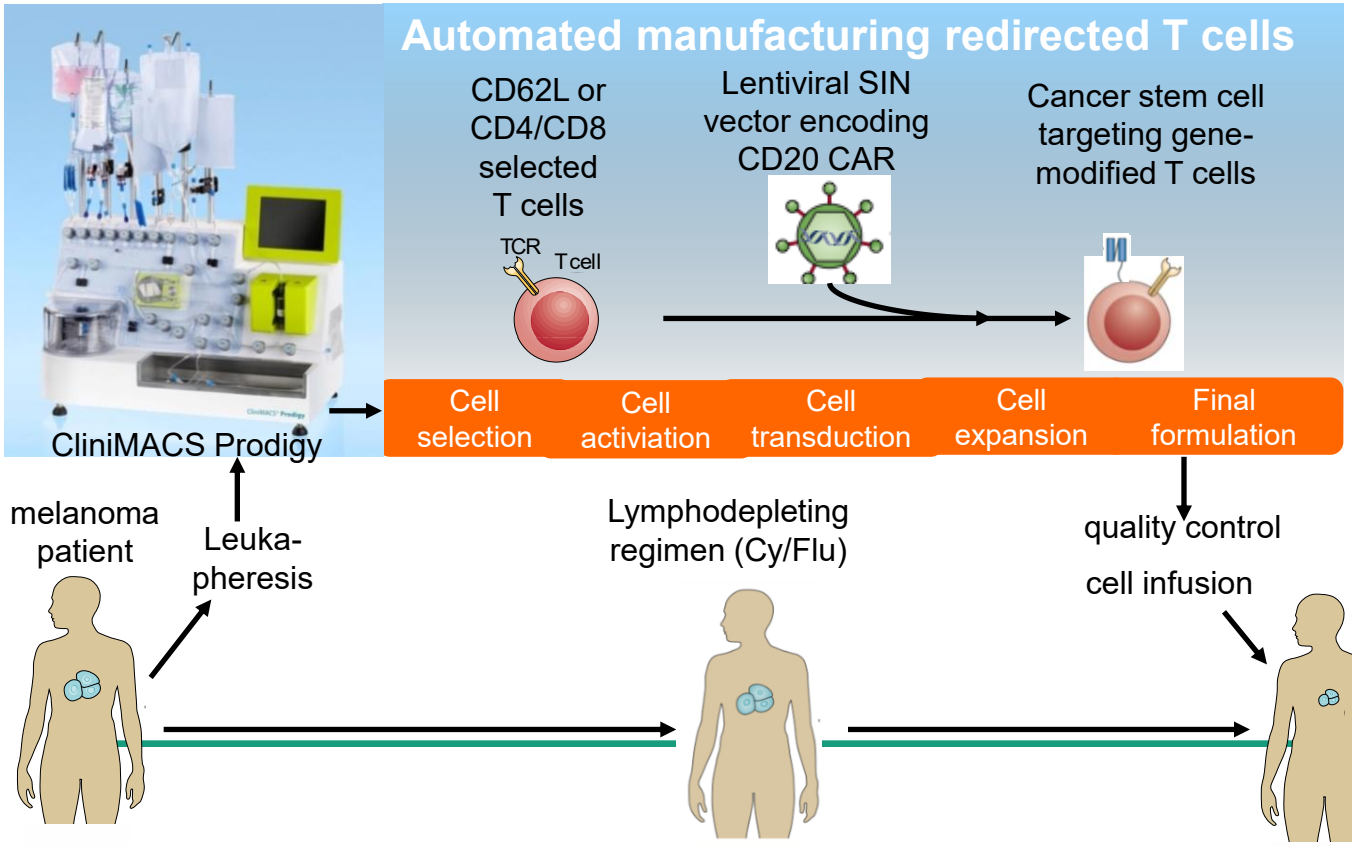
MHH

Fraunhofer IZI

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)



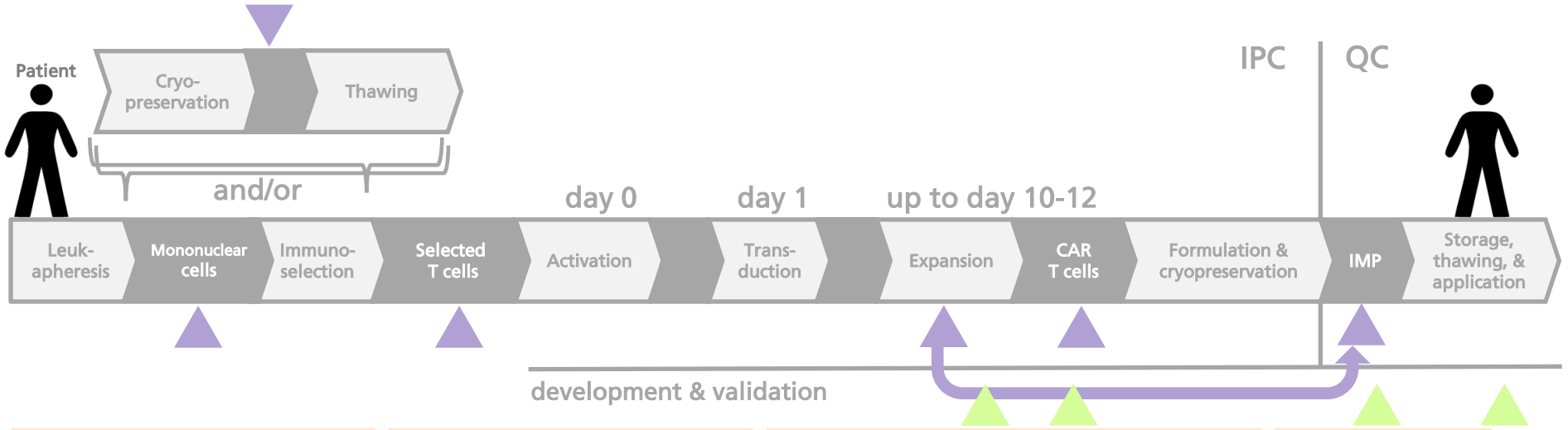
manufacturing license: 7/2018

Priesner C … Koehl U. HGT 2016

Quality Control of CAR-transduced T cells

results

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

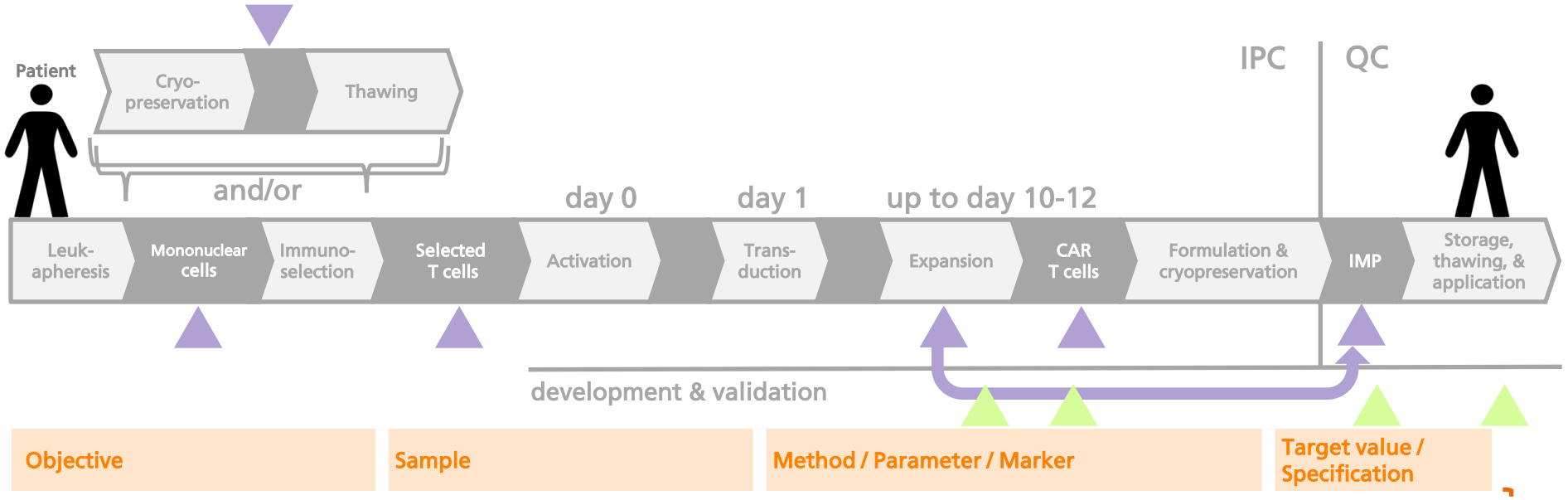


Objective		Sample	Method / Parameter / Marker	Target value / Specification
IPC QC: identity	purity / content /	starting material, intermediates thawed IMP	CD3, 4, 8, 14, 45, 62L	to be defined
IPC QC:	purity	intermediates after enrichment thawed IMP	%T cells of viable mononucleated cells: %(CD3+CD45+) 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)

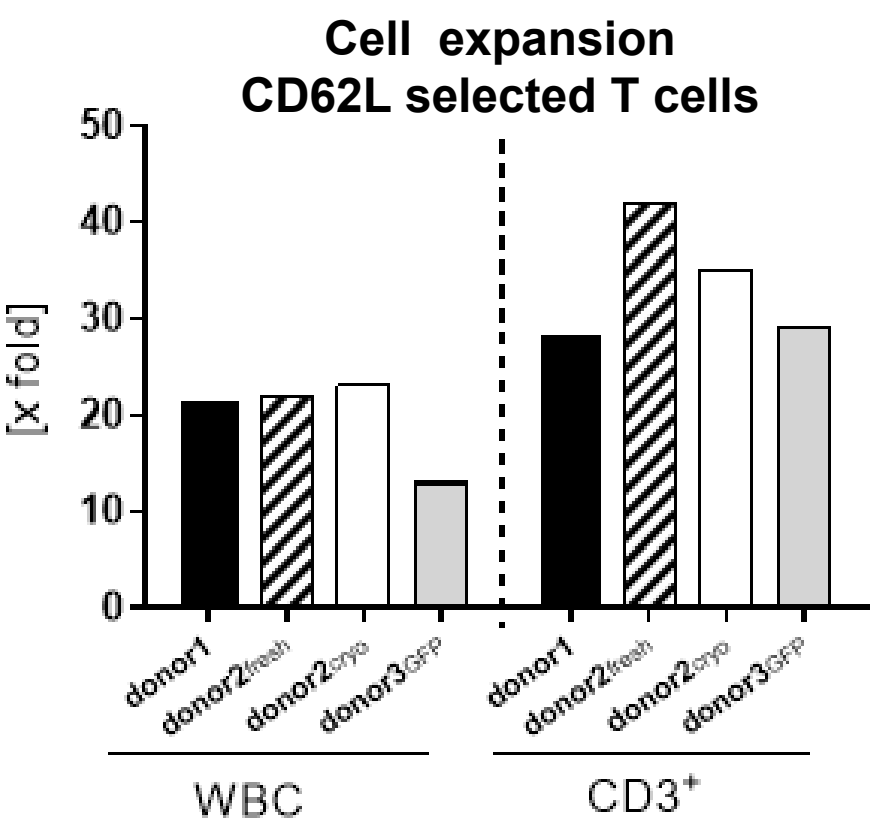
Quality Control of CAR-transduced T cells

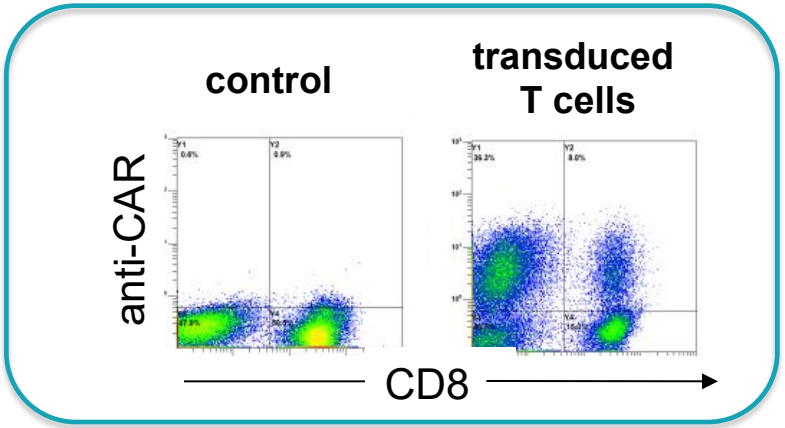
Cytological in-process (IPC), quality (QC), and complementary controls

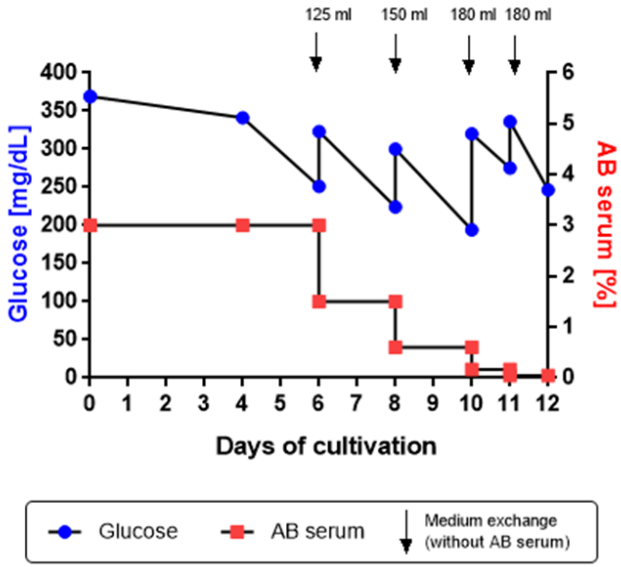


- EP 2.7.23 Flowcytometric analysis
- EP 2.6.21 Realtime qPCR for Vector copy number (VCN)
- EP 2.6.14 Bacterial Endotoxine test (BET)
- EP 2.6.27 Microbiological control
- EP 2.6.21 Mycoplasmas

Final end product at harvesting

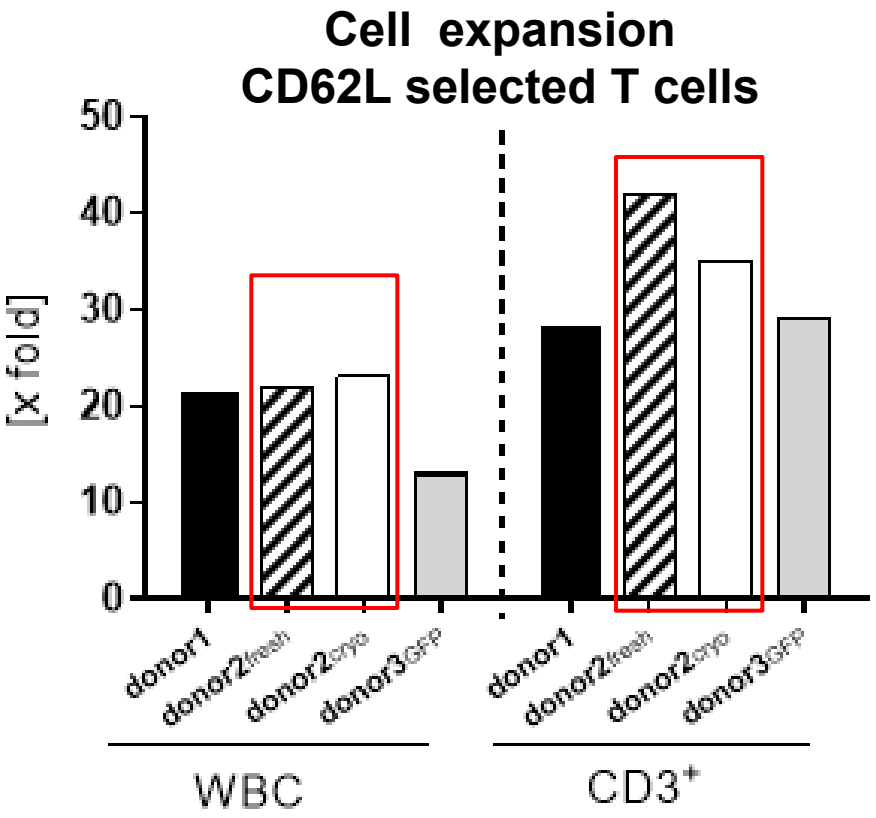


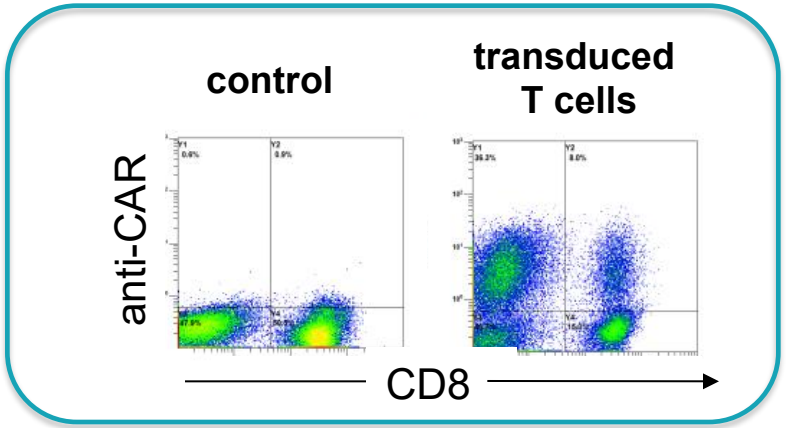


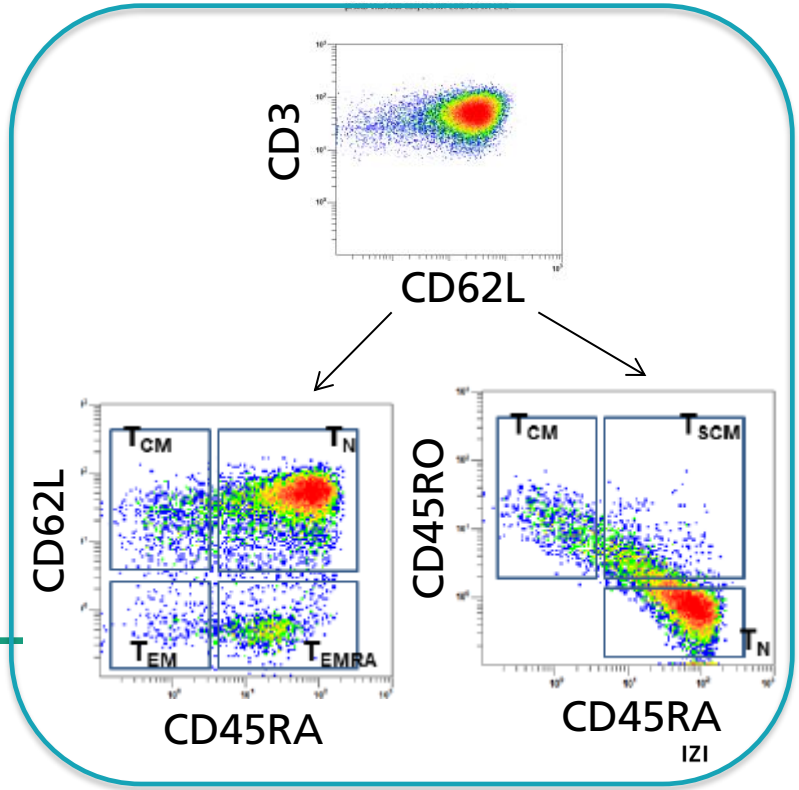


Priesner C … Koehl U. HGT 2016

Final end product at harvesting





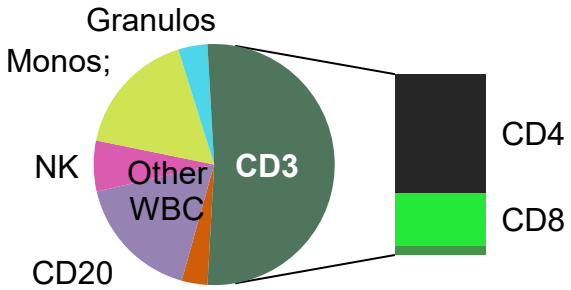


- uniform T cell expansion rate; > 30-fold
  No difference cryopreserved versus fresh
- Naive (T_N) and central memory (T_{CM}) T cells as well as T stem cell like memory cells (T_{SCM})

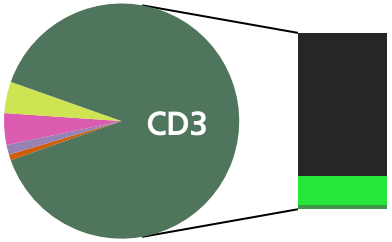
Priesner C … Koehl U. HGT 2016

Cellular composition (CD4/CD8 sel.)

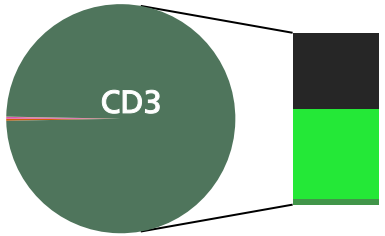
Apheresis



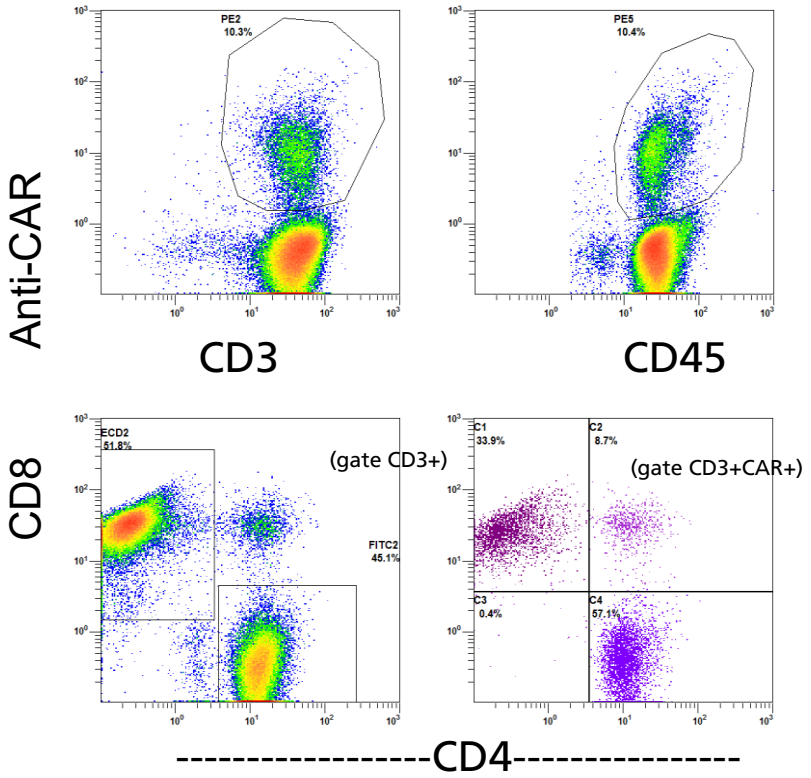
CD4/CD8 selected cells



Day 12 harvesting



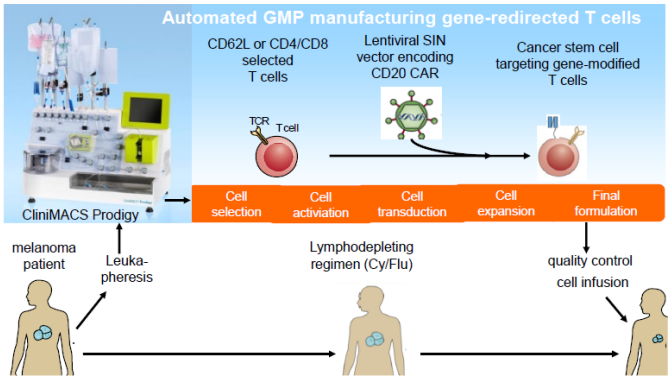
Flow Cytometry day12



Pure T cells at harvesting → Successful transduction efficiency

Bench to bedside and back to the lab

Automation – CAR T cells



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
Helmholtz Berlin
Fraunhofer IZI/
University of Würzburg

TRUCKs

T cells **R**edirected for **U**niversal **C**ytokine **K**illing

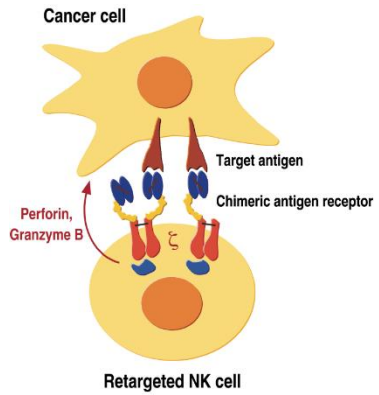
7 Universities

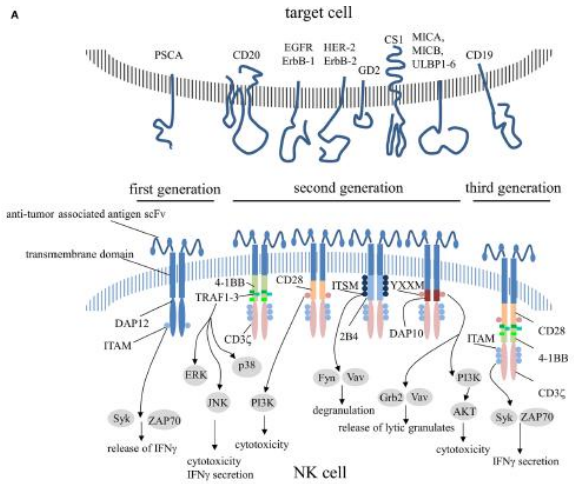
„CAR“ NK cells

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

Fraunhofer IZI

“CAR” expressing effector cells





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

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

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

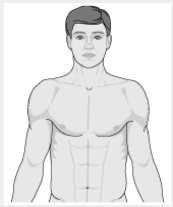
Fraunhofer IZI

NK cell immunotherapy

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

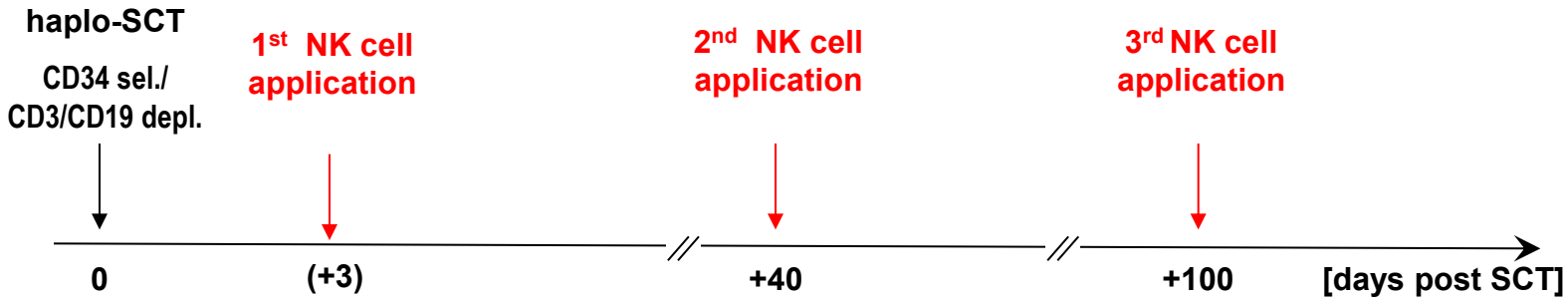
NK cell purification (CD3 depl./ CD56 sel.)

GMP

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

GMP

NK cell application patient



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

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

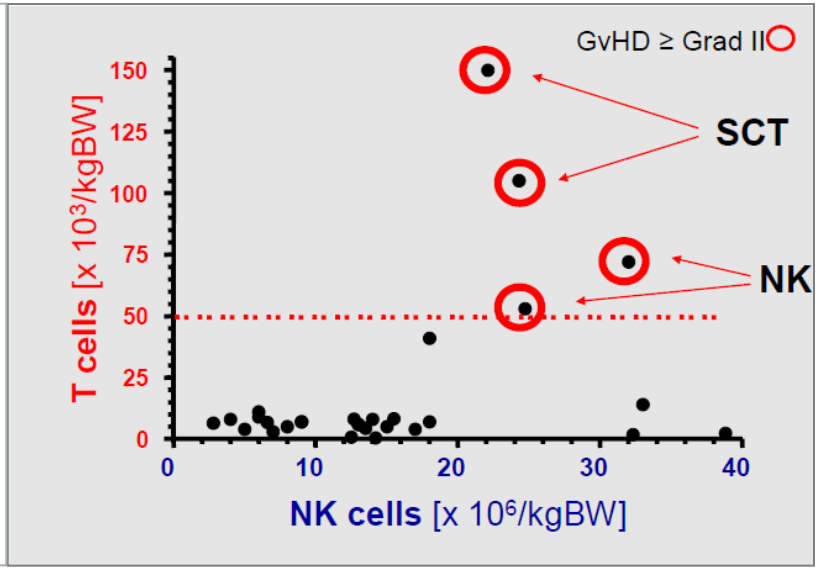
Advantage

- 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

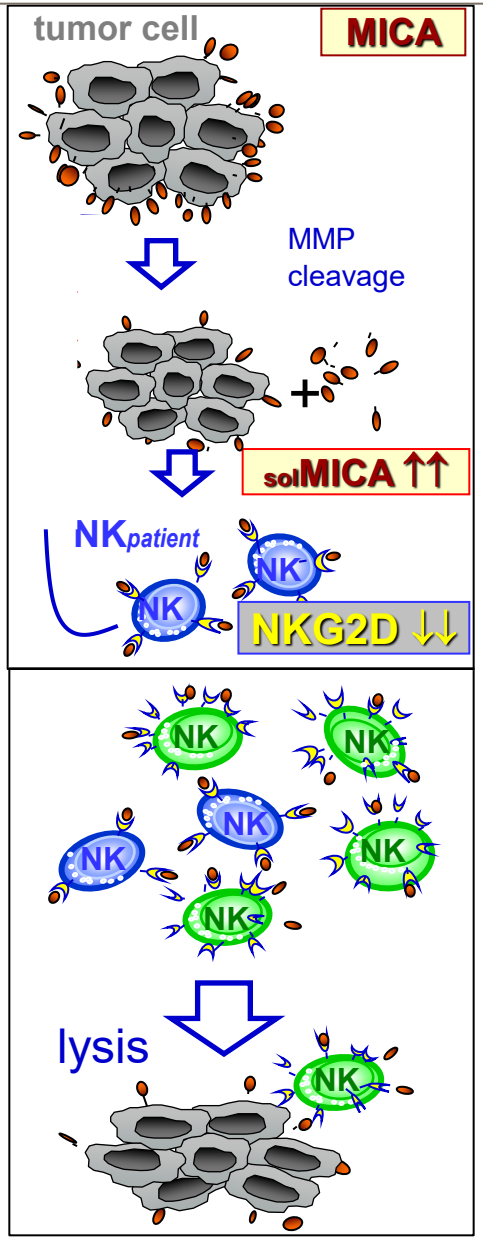
- Tumor immune escape mechanism (TIEMs)

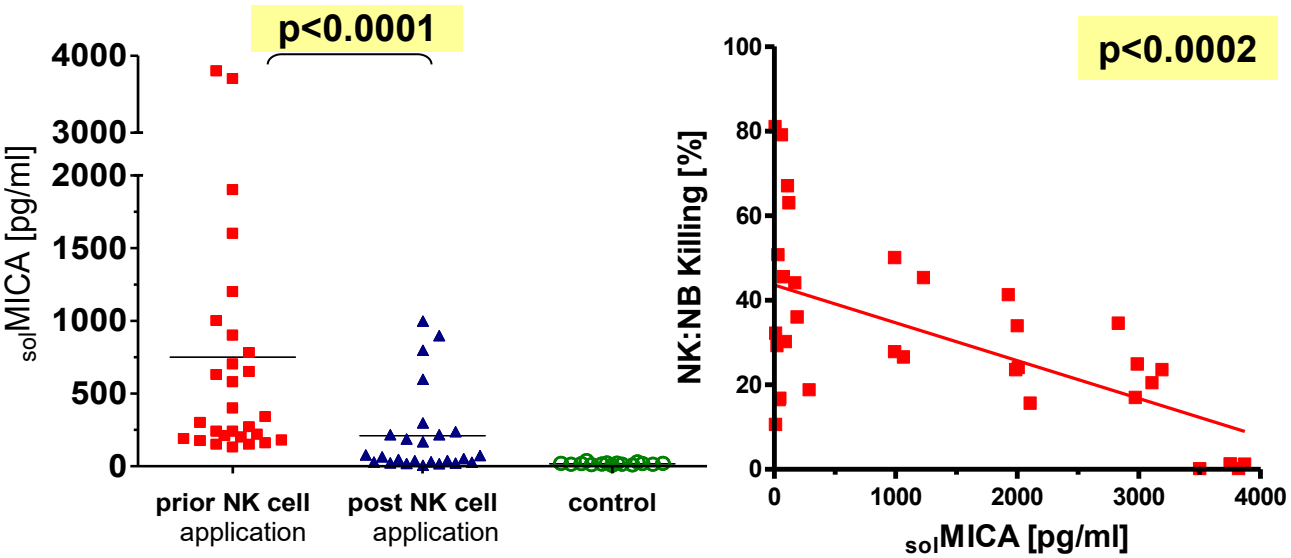
Kloess *et al.* Eur J Immunol 2010; Kloess *et al.* Oncoimmunol 2015

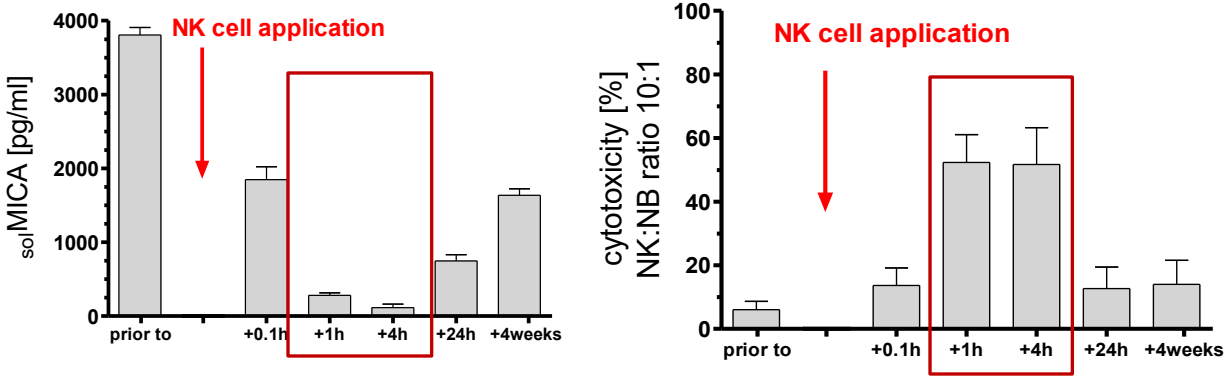


Koehl U *et al.* Blood Cell Mol Dis 2004; Koehl U *et al.* Klin Päd 2005; Koehl U *et al.* Front Oncol 2013, Stern M *et al.* BMT 2013

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

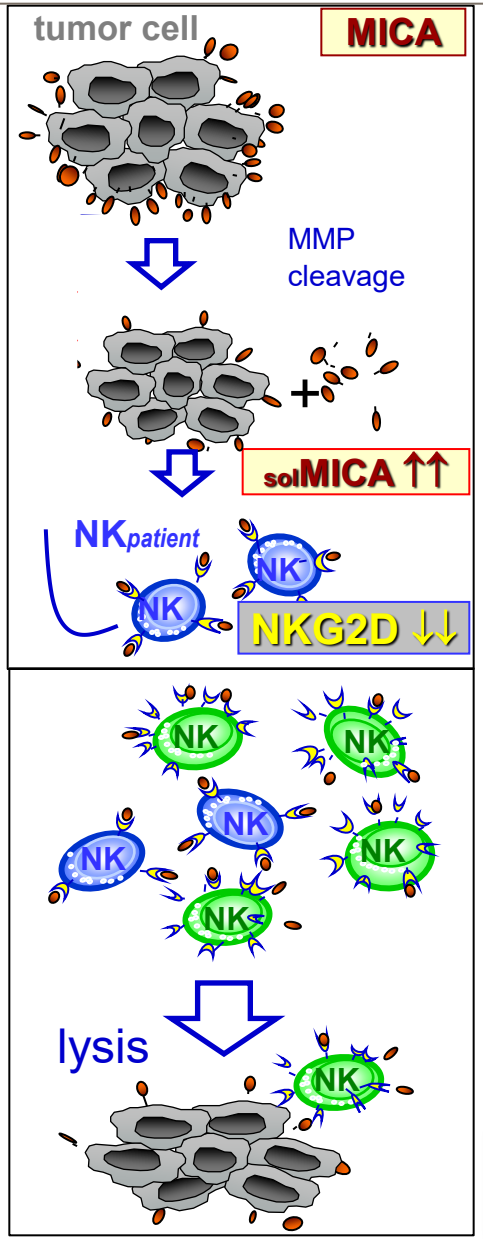


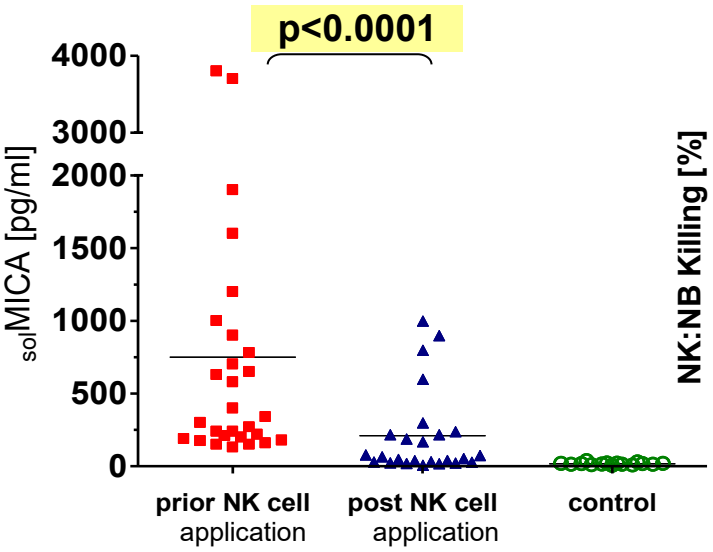


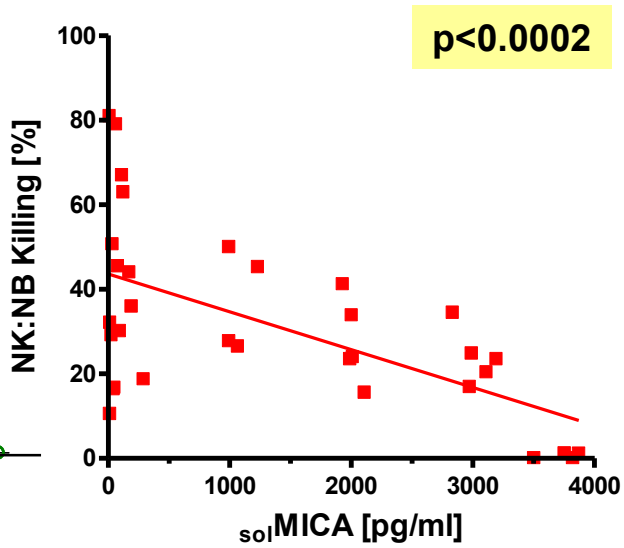


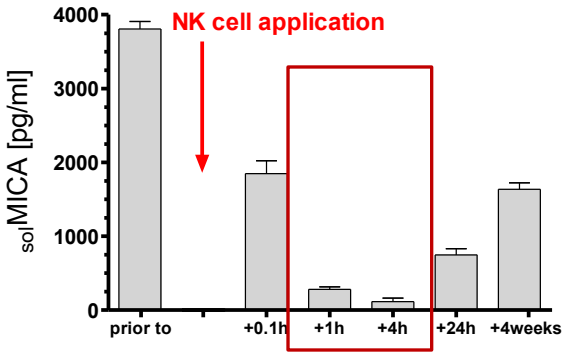
IL-2 activated NK cells improve NKG2D mediated cytotoxicity via scavanging of $_{sol}$MICA in plasma

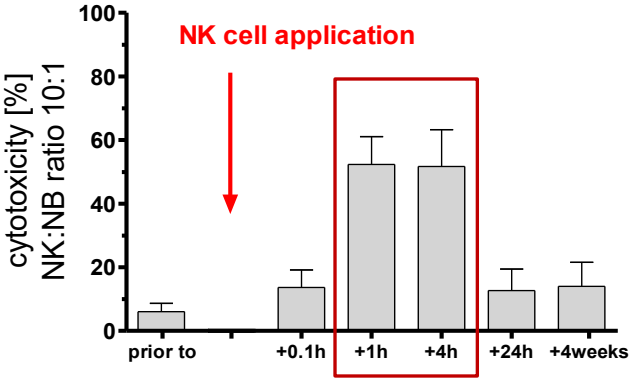
$_{sol}$MICA dependent tumor immune *escape* inhibits NK cells in patients with Neuroblastoma











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**

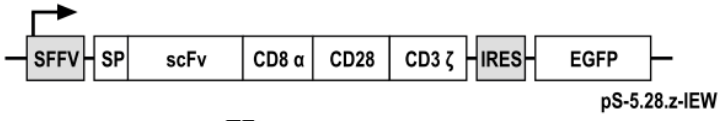
CAR: CD19-CD28-zeta-2A-iCasp9-IL15

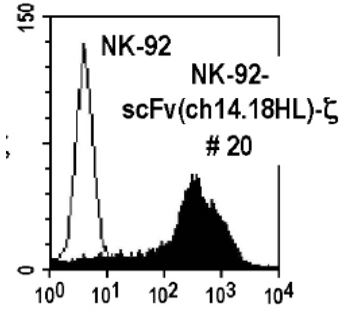
CCCR: Chimeric Costimulatory Converting Receptor

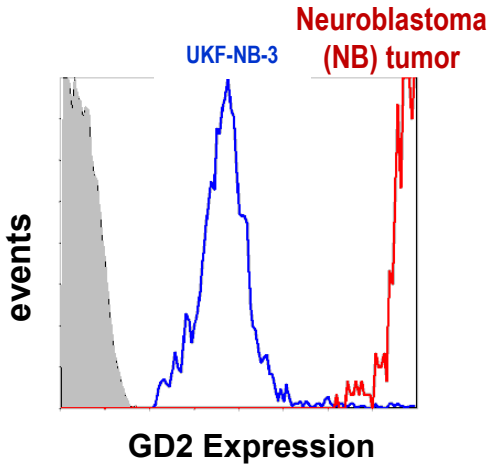
Redirected “CAR” NK-92 cell line

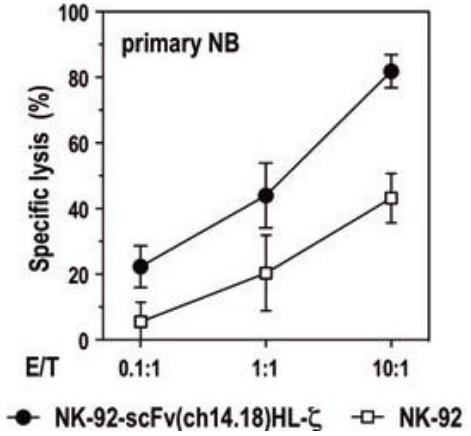
results

anti-GD2





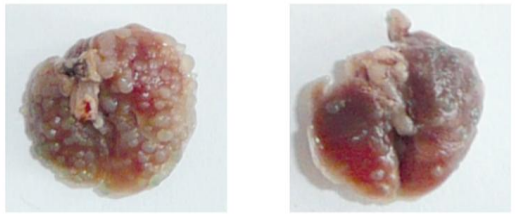




Esser R *et al.* J of Cellular and Molecular Medicine 2011

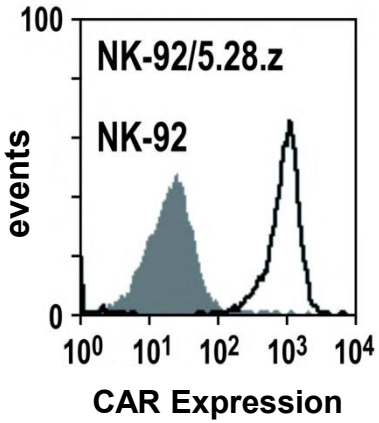
coop. U. Köhl (MHH), W. Wels (FFM), T. Tonn *(Dresden)*

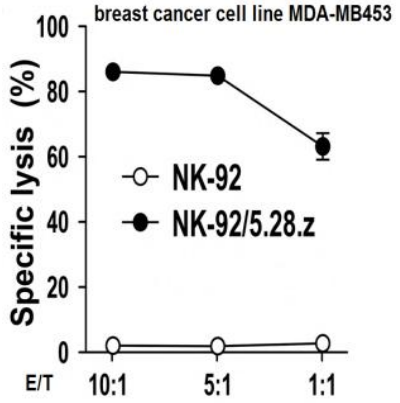
anti-ErbB2/HER2



NK-92 NK-92/5.28.z

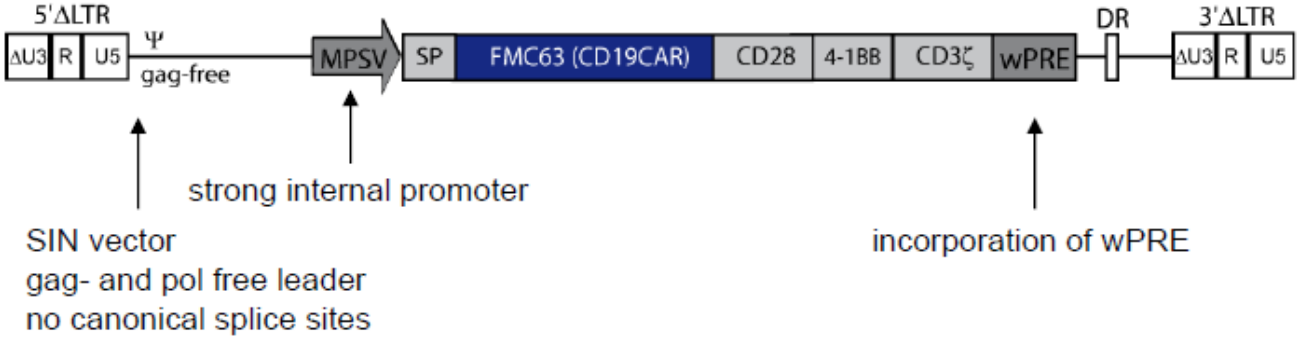
Schönfeld *et al.* Molecular Therapy 2014

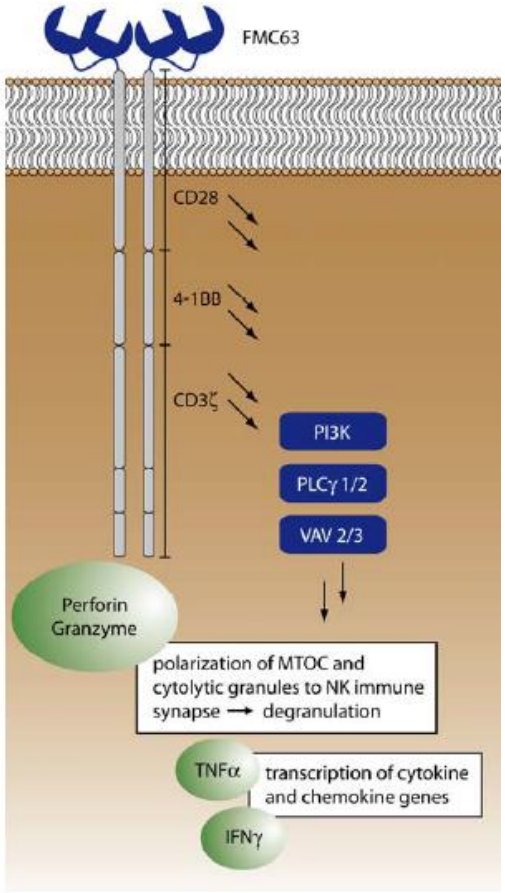




Chimeric Antigen Receptor Vector Design for primary human NK cells

A. Schambach





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

→ CD19 binding leads to signal transduction

→ Enhanced cytotoxicity

coop.: A. Schambach, MHH

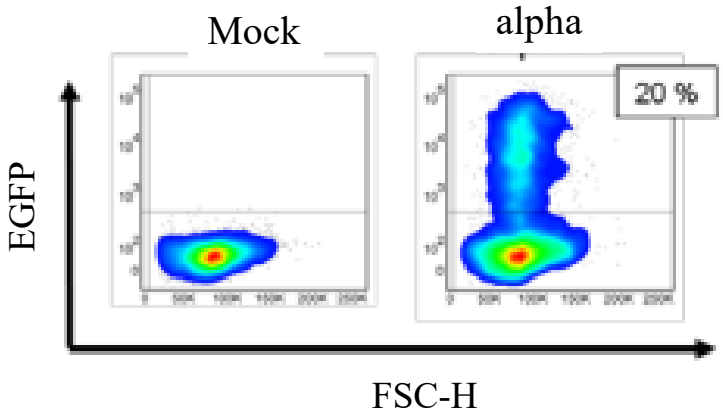
Fraunhofer IZI

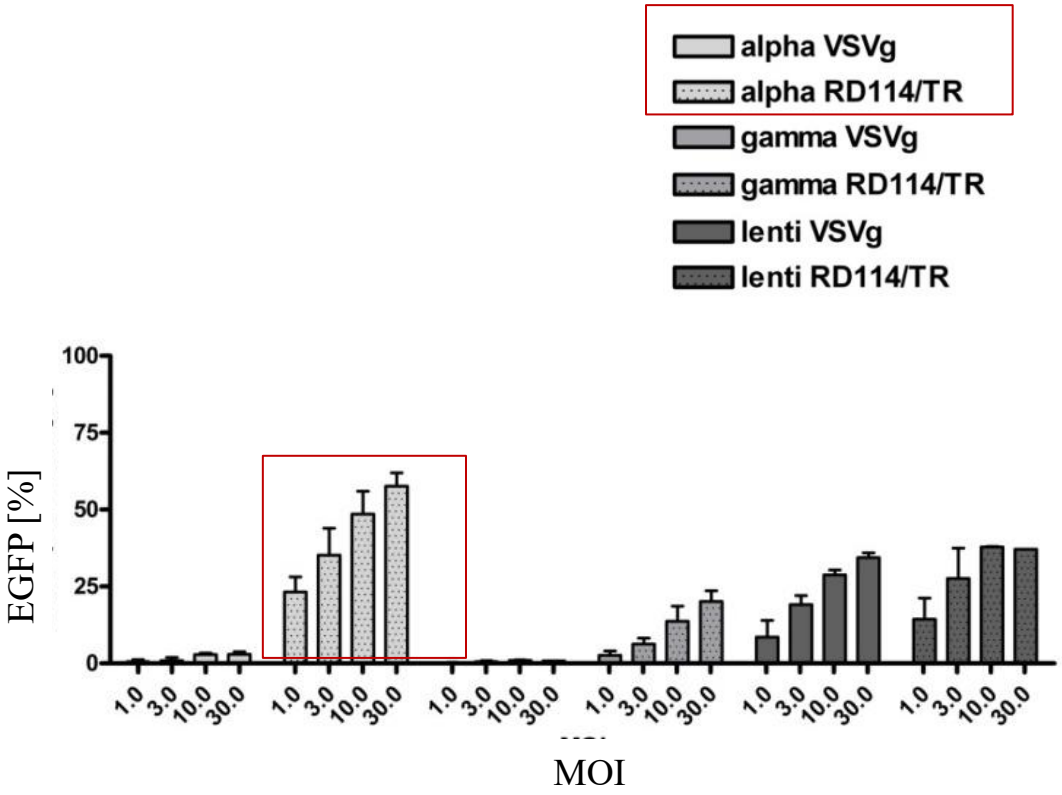
CAR expressing NK cells redirected against CD19

MOI1

Alpha SIN vector

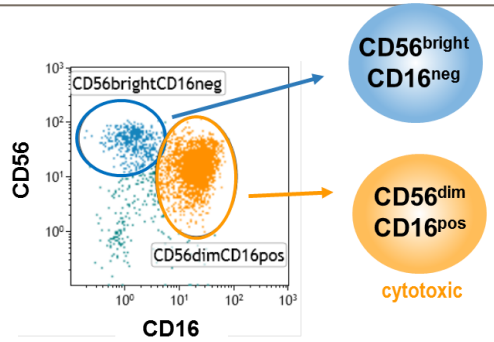
Transduction of mature
primary human dNK cells feasible





Suerth J et al. J Mol Med 2016

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

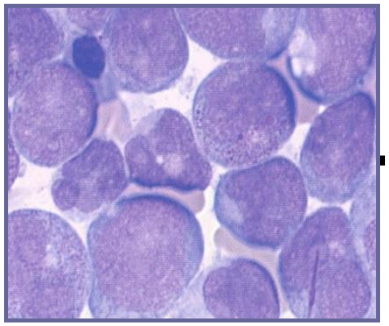


CD56^{bright}CD16$^{dim\&neg}$ (immune regulatory)			CD56^{dim}CD16^{pos} (cytotoxic)	
E/T			E/T	
1:1	5:1		1:1	5:1
++	0	anti-inflammatory:	+	+
+	+	IL-4	+	+
		IL-10		
		pro-inflammatory:		
++	+	IL-6	+	+
++	+	IL-17A	+	-
+	+	IFNγ	-	-
++	++	TNFα	+	+
		pro-apoptotic:		
+	0	GrA	++	+
0	0	GrB	++	++
-	-	Perforin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

Primary CAR expressing NK cells

redirected against AML cell lines and

patients own leukemic cells



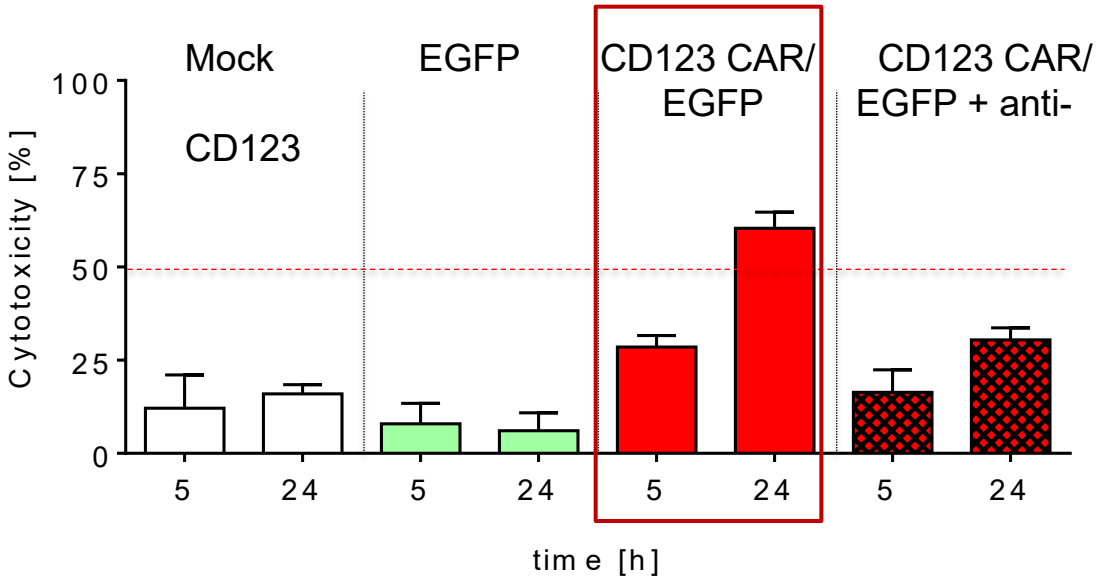
Coop.: M. Morgan, A. Schambach, M. Heuser, M. Sauer

MHH

Fraunhofer IZI

CAR NK cells against patient´s CD123⁺AML

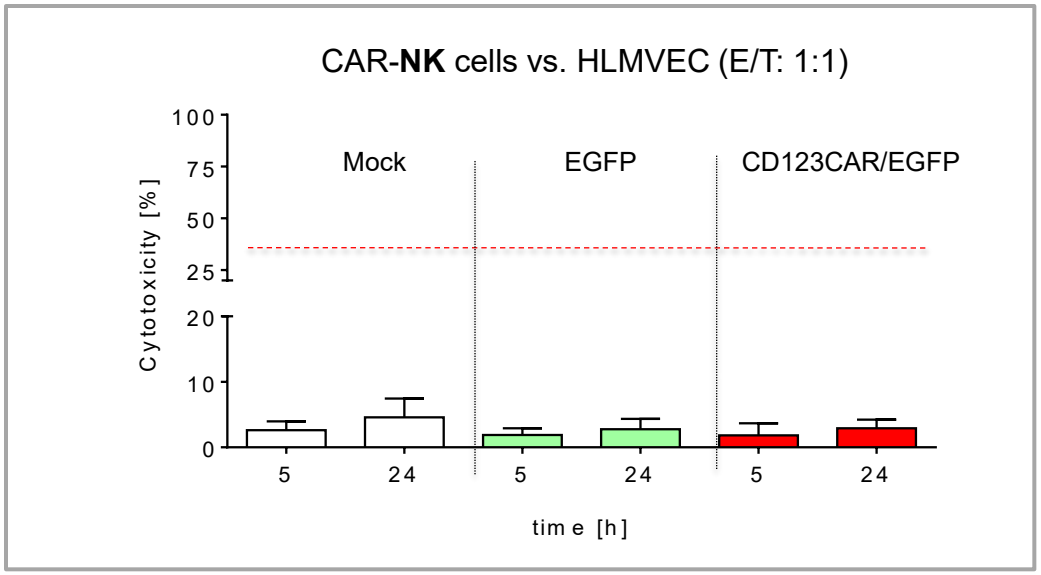
Cytotoxicity



MOI1

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

Side effects

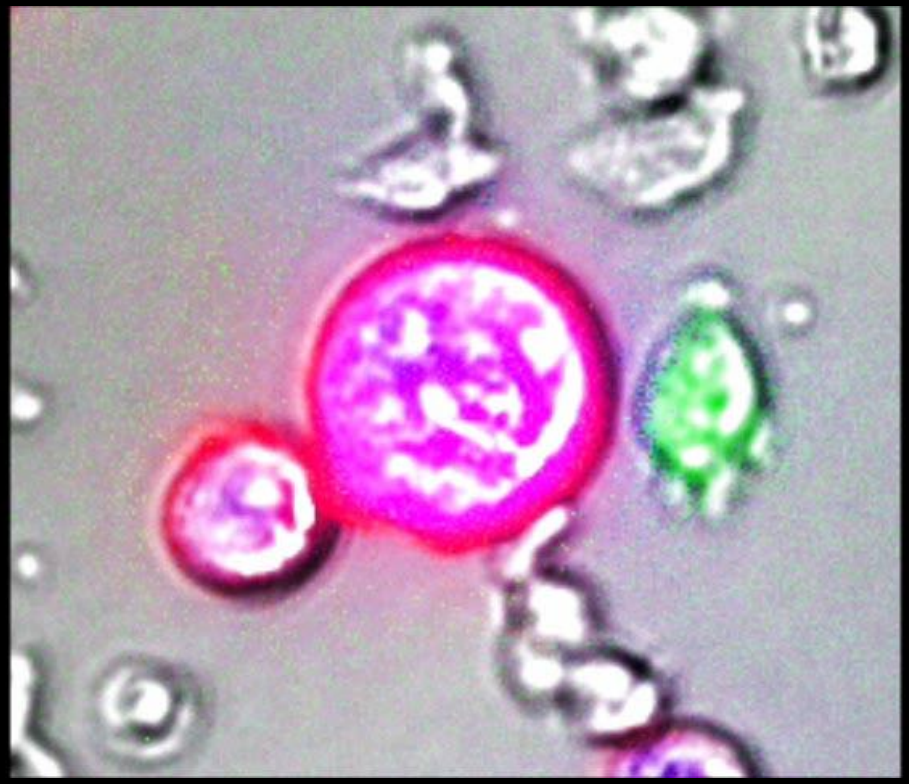


Coop.:
M. Heuser,
A. Schambach MHH

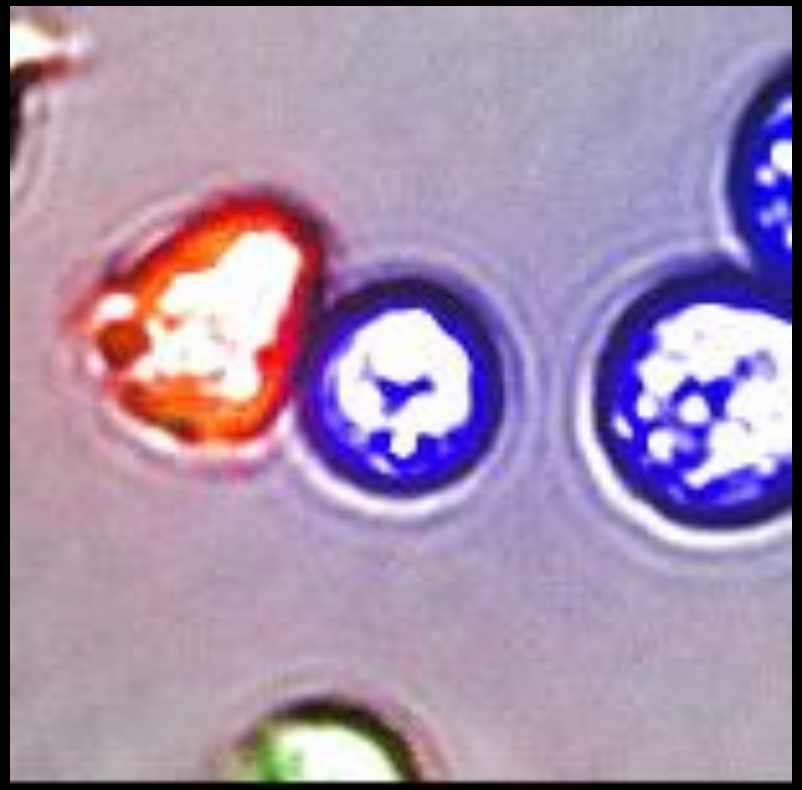
Human Gene Therapy 2017

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

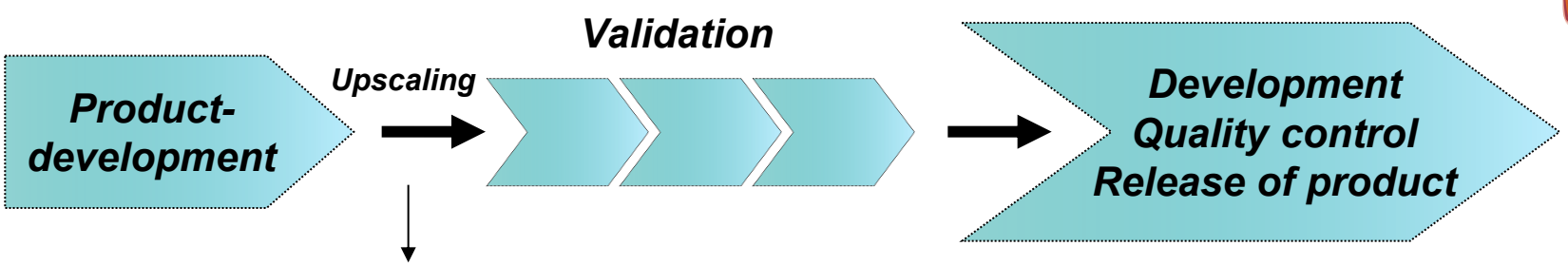


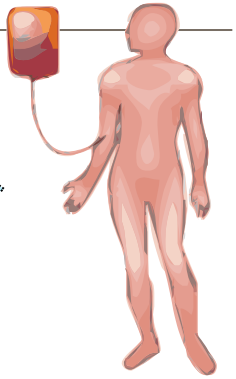
CD123$^+$KG1α cell proliferation dye: eFluor®450, anti-CD34-PE

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

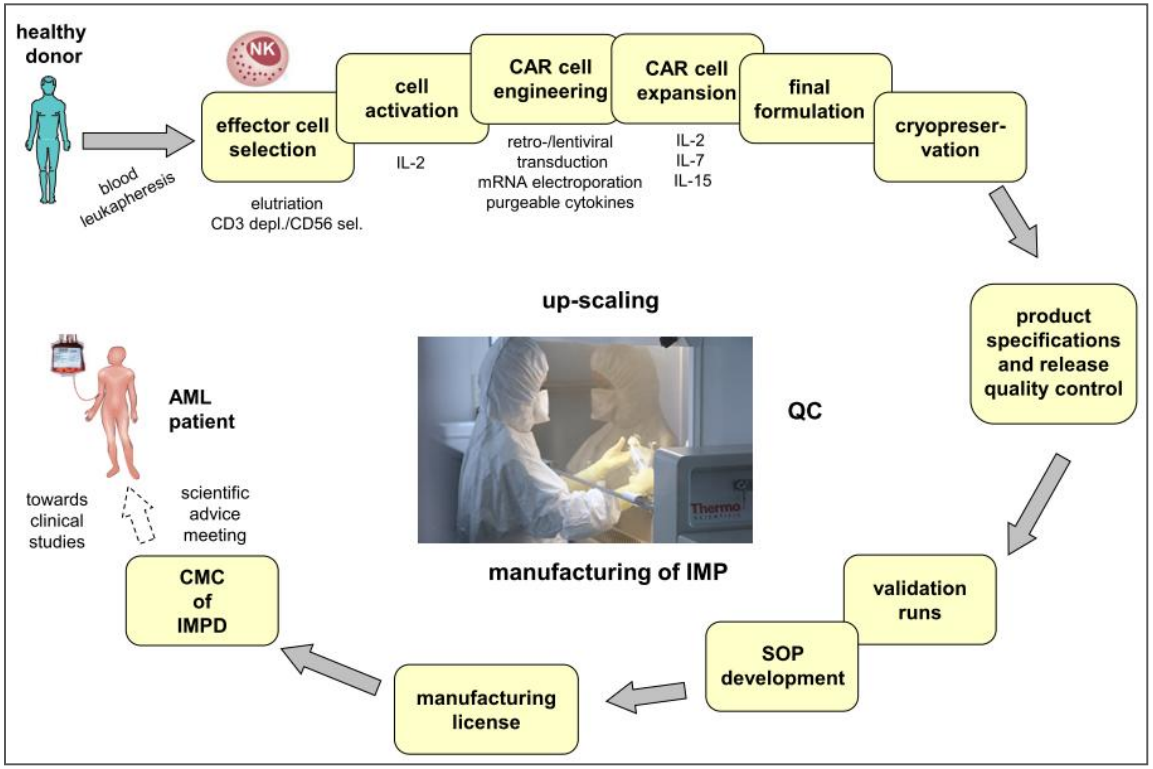
Clinical scale – CAR expressing NK cells

results





GMP-compliant protocol

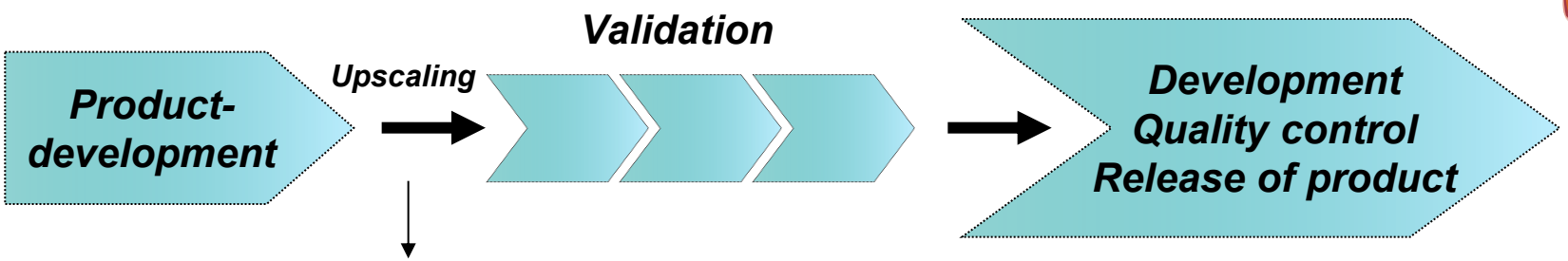


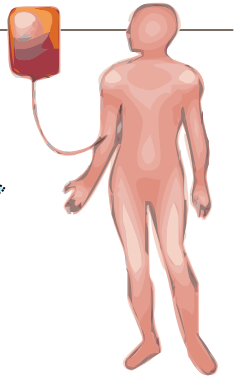
„Off the shelf product“ Advanced Therapy Medicinal Product

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

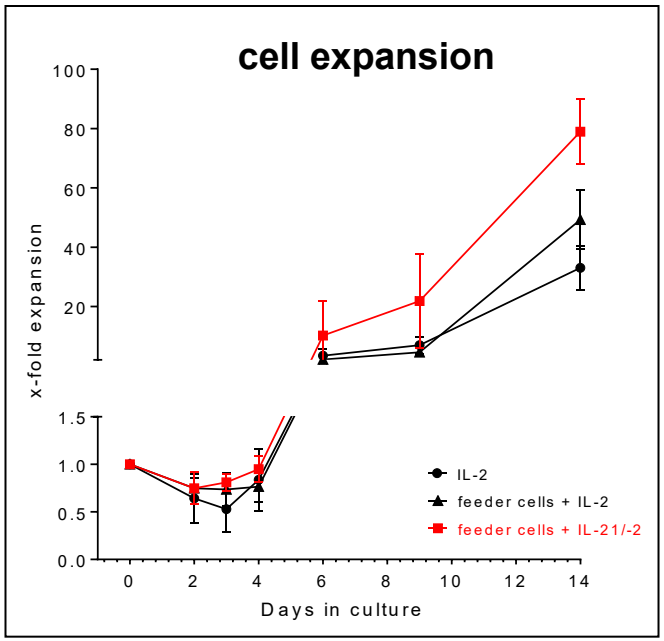
Clinical scale – CAR expressing NK cells

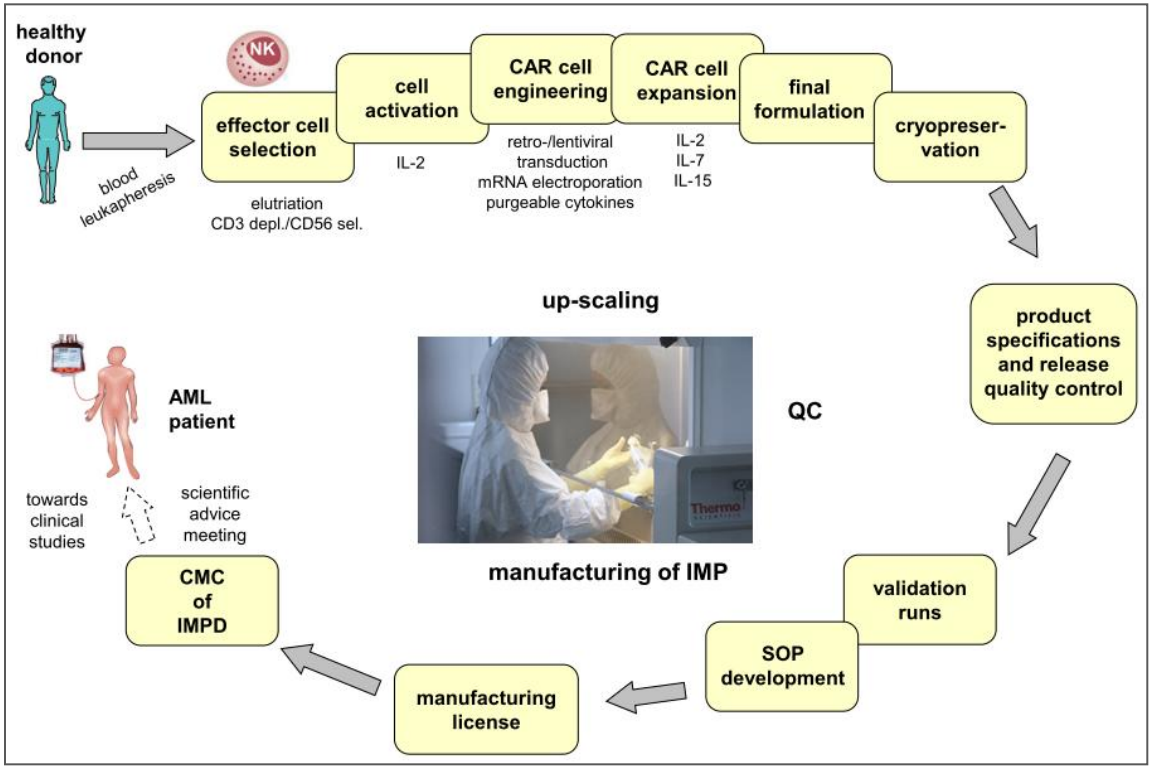
results





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:

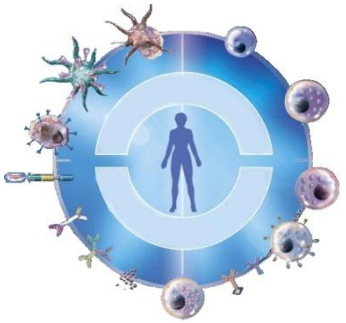
- Successful clinical CAR T cells studies (385 documented world wide)
- Our data about automated manufacturing of CAR expressing T cells gives rise for harmonized protocols in future clinical studies
- Manufacturing failure of autologous CAR T cells needs complementary concepts

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



ulrike.koehl@izi.fraunhofer.de
koehl.ulrike@mh-hannover.de
www.mh-hannover.de/zelltherapeutika.html
www.izi.fraunhofer.de

Fraunhofer Cell. Therapy IZI, Leipzig, D

F. Emmrich
G. Schmiedeknecht
K. Kebbel
J. Lehmann
A. Bochmann-Seidel
S. Mitzner
U. Demuth
S. Ulbert
F. Horn
T. Grunwald
T. Tradler
S. Fricke

Clinic. Immunol., University Leipzig, D

U. Sack
A. Boldt

Inst. Cellular Therap., MHH Hannover, D

R. Esser
S. Klöss
W. Glienke
O. Oberschmidt
L. Arseniev
K. Aleksandrova
C. Priesner
J. Leise

Pediatric Hematol/ Oncol., Frankfurt, D

T. Klingebiel D. Schwabe P. Bader S. Hünecke

Kantons-Spital Basel, CH

J. Passweg M. Stern C. Kalberer

Experimental Haematology, MHH, D

A. Schambach M. Morgan H. Büning T. Moritz

Tumorimmunology, Regensburg

H. Abken

BSD Hessen/BW und BSD Ost, D

E. Seifried C. Seidl T. Tonn

Pediatric Hematol/ Oncol., MHH, D

C. Kratz M Sauer B Maecker-Kolhoff

Transfusion Medicine, MHH, D

R. Blasczyk L. Goudėva B. Eiz-Vesper

Georg Speyer House Frankfurt, D

W. Wels

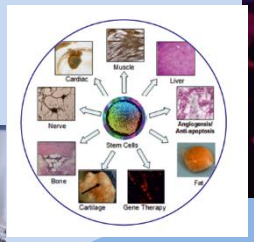
Haematol. /Oncol./ SCT, MHH, D

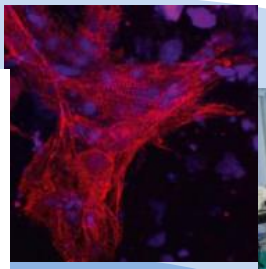
A. Ganser M. Heuser M. Eder C. Könecke

Internal Medicine, Würzburg, D

H. Einsele M Hudecek

MACS Miltenyi Biotec
SEVENTH FRAMEWORK PROGRAMME MARIE CURIE ACTIONS
Bundesministerium für Bildung und Forschung

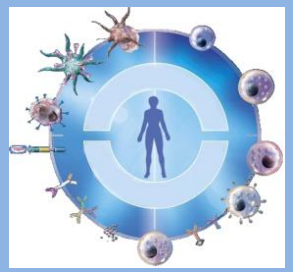




Diagnostic - devices
cell-based therapies

Havard Medical School * Boston
Gene Therapy

International partners

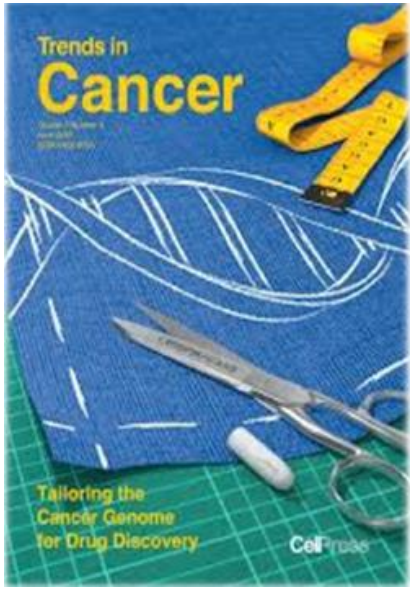


Technologies
Automation

72 Institutes

> 25.000 staff members

Network for individualised stratified medicine using cell-based therapies



…. and thanks for listening

MHH

Universitätsklinikum Leipzig
Medizin ist unsere Berufung.
UNIVERSITÄT LEIPZIG
Medizinische Fakultät

Fraunhofer IZI