Manufacturing and application of CAR T cells to treat cancer: chances and safety matters

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

Fraunhofer IZI

Disclosures

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“.

- CTL019 European study trial 

Kymriah®
- Consulting: AstraZeneca, Affimed, Glycostem

The Era of Advanced Therapy Medicinal Products

2009 2010 2011 2012 2013 2014 2015 2016 2017 2018 2019

Imlygic®
Holoclar®

Zalmoxis®
Strimvelis®

Spherox®

Yescarta® (FDA)
Kymriah® (FDA)

Kymriah® (EMA)
Yescarta® (EMA)

Alofisel®
Luxturna®

Zolgensma®
Zynteglo ®

Chondrocelect®
(withdrawn in 2017)

Glybera®
(expired in 2017)

Provenge® and MACI®
(withdrawn in 2015 and 2018)

centralized

Fraunhofer IZI

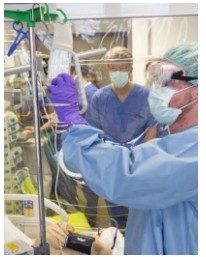
decentralized

UKL UL

GMP facility
21 clean rooms
„A in B“ safety level S2
> 130 staff members
(IZI overall > 400)

CTL019 trial/
Kymriah Europe
Press conference
NOVARTIS and
Fraunhofer IZI
8/2018

CAR T cell
Melanoma trial
Miltenyi Biotec,
H. Abken
U. Köhl



CAR T cells
first patient
treated
at UKL
U. Platzbecker
5/2019

F. Lordick F, Platzbecker U, Köhl U. Molekulare und zellbasierte Krebstherapie – Quo vadis? Onkologie 2019

Manufacturing of cell-based therapies

MHH UKL UL Fraunhofer IZI

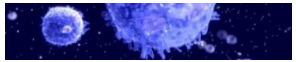
Pre-clinical → Manufacturing and proof „AMG“ → „First in man“ study → Late clinical studies → Routine

Partner: Universities Industry

- 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

- Anti-CD19 CAR T cells
- Anti-CD20 CAR T cells
- Anti-ROR1 CAR T cells
- CAR NK cells
- Haploidentical donor NK cells
- Dendritic cells
- Gene-transduced stem cells
- CRISPR-Cas9--modified stem cells
- iPS-cardiomyocytes



Virus-free *Sleeping Beauty* gene-transfer for immunotheray of ROR1⁺ cancers

HELMHOLTZ Universit Fraunhofer

HORIZON 2020

EU consortium Mature-NK: 2018-2022: Manufacturing tumor reactive CAR NK cells

Speaker: U. Koehl

EU IMI ImSAVAR 2020-2025

Safety assessment

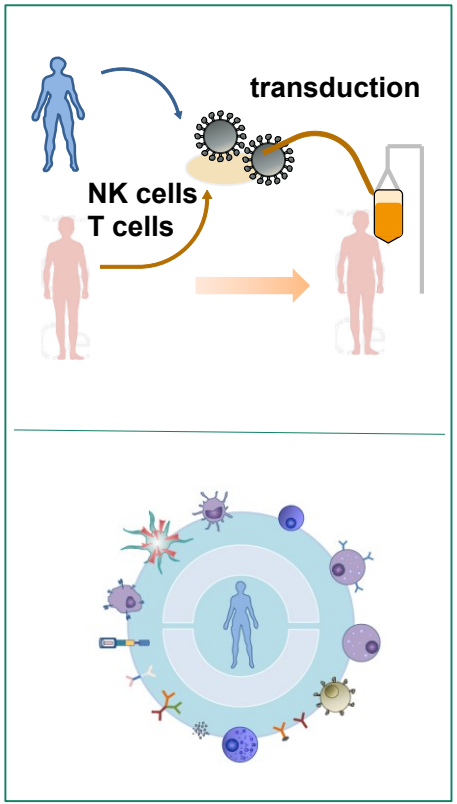
Speaker: U. Koehl

> 450 cellular products/ year

Overview

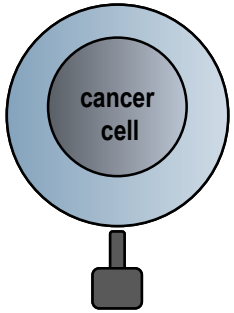
CAR expressing T cells

- Clinical trials
- Risk and benefit
- Manufacturing challenge:
  From manual to automation and digitalization
- From personalized medicine to allogeneic
  “off the shelf” CAR effector cells

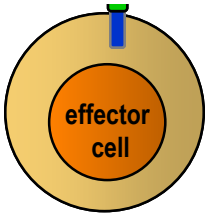


CAR = chimeric antigen receptor

CAR-expressing effector cells - mechanism



- MHC independent
- of high affinity / specificity
- not requiring co-receptors

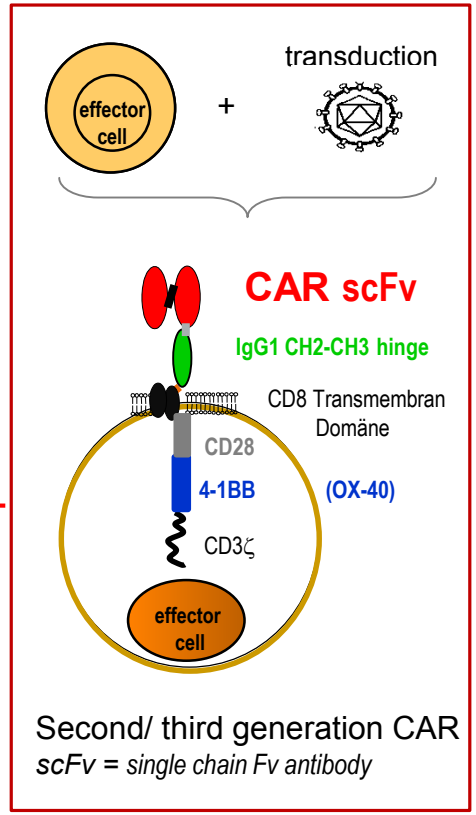


activation of effector cells

specific attack and killing of cancer cells

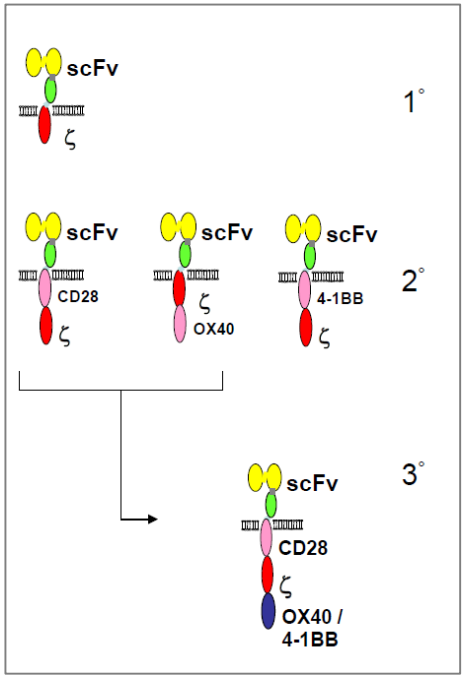
CAR expressing T cell

CAR expressing NK cell



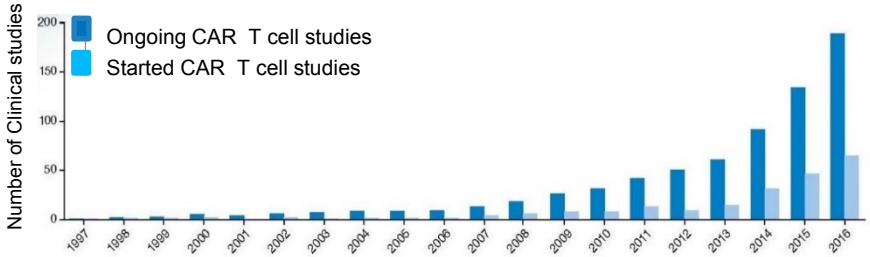
Second/ third generation CAR

scFv = single chain Fv antibody

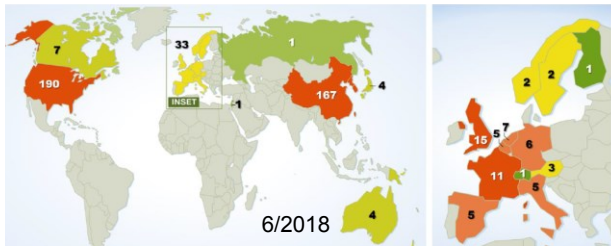


Adopted from: Hombach et al., Curr. Mol. Med. 13, 1079-1088 (2013)

Clinical CAR T cell studies



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



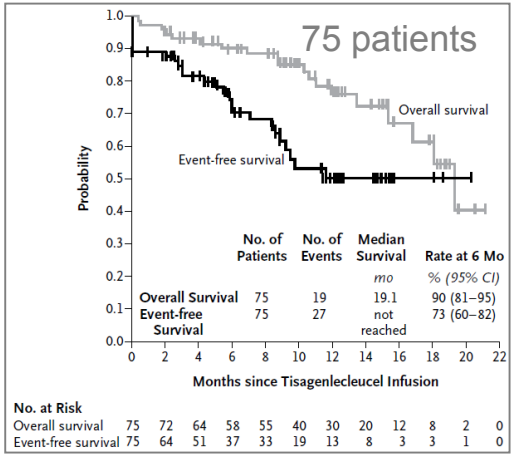
Clinicaltrials.gov

- > 500 (1000) clinical trials (10/2019)
- 10% of the studies in Europe, only
- Benefit in patients with Acute Lymphoblastic Leukaemia (ALL)

r/r = relapsed/ refractary

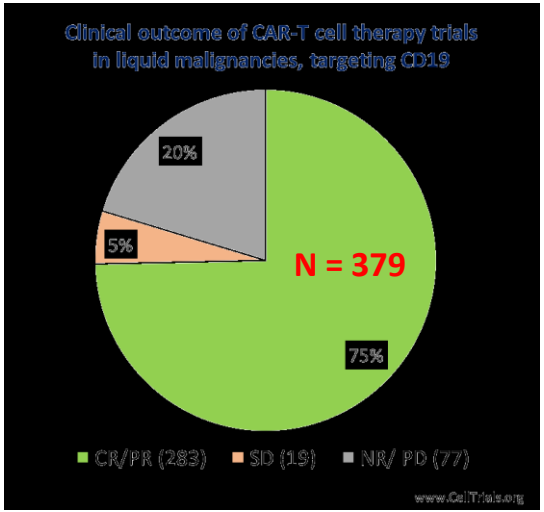
- r/r CD19+ Leukaemia: cure rate ~ 60-75%

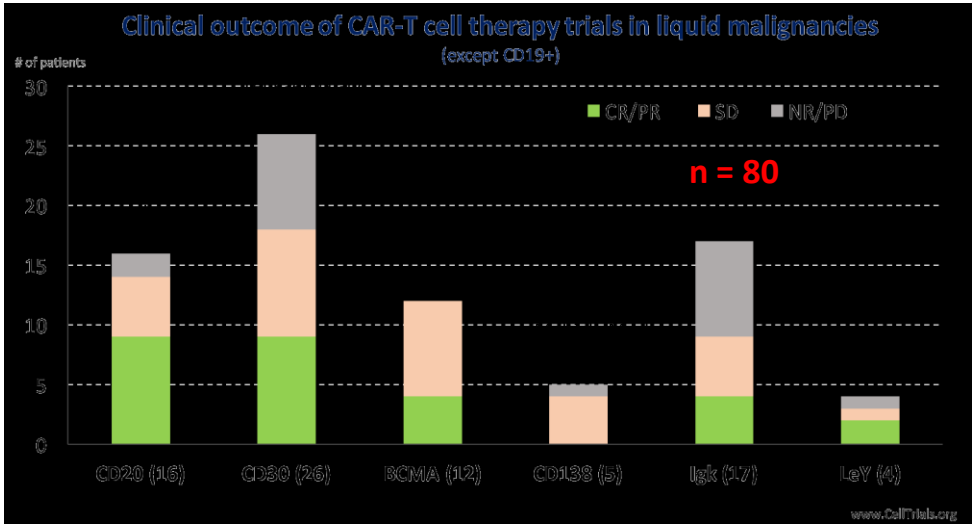
Paediatric r/r ALL – ELIANA



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

CAR T cells – Update clinical studies (haematology)





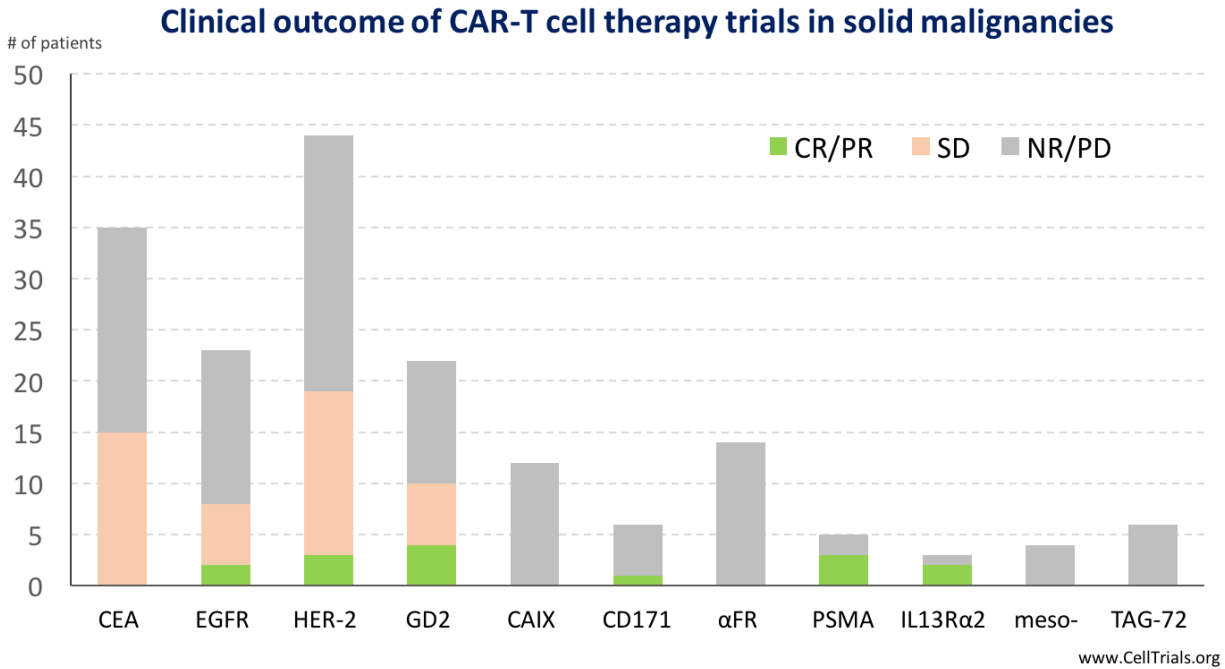
- Successful results in CD19+ ALL and DLBCL
- However failure of complete resmission in some diseases

CLL = Chronic Lymphatic Leukaemia

EMBO Mol Med. 2017 Sep;9(9):1183-1197. doi: 10.15252/emmm.201607485.

Fraunhofer IZI

CAR T cells – Update clinical studies (oncology)

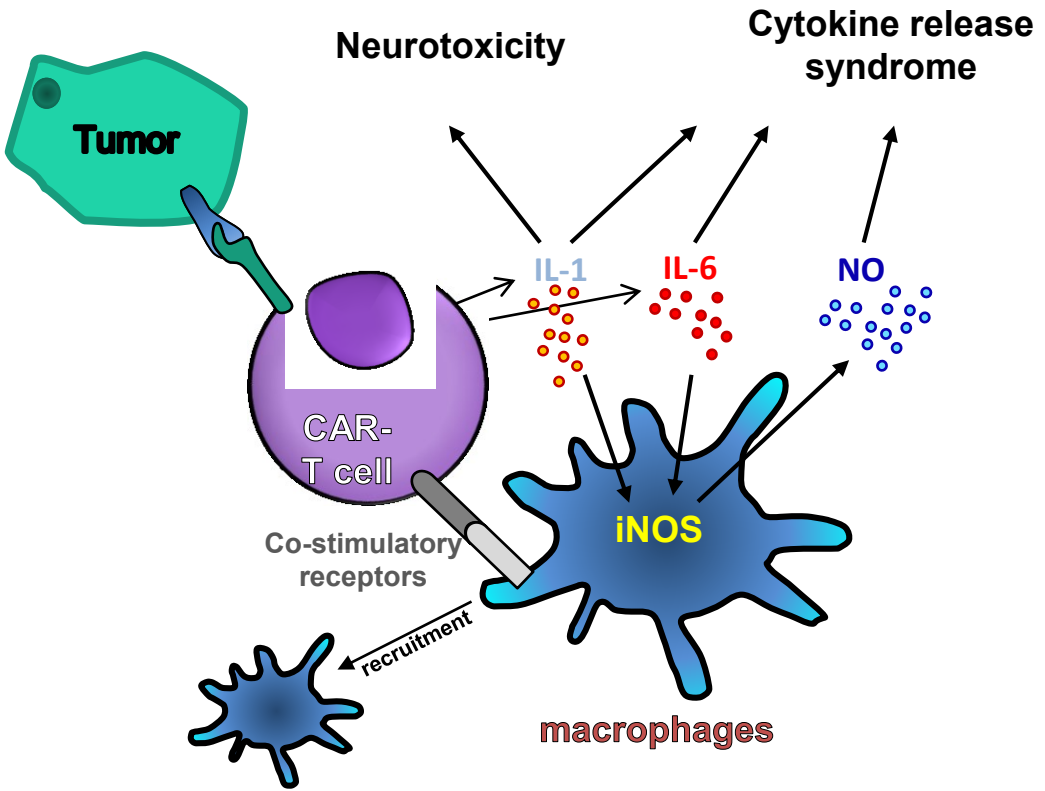


- Breast cancer, glioblastoma, neuroblastoma, prostata cancer ….
- Very few complete or partial remissions! To date, less successful in tumours

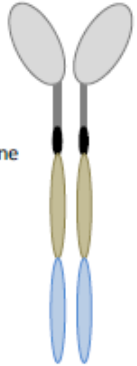
EMBO Mol Med. 2017 Sep;9(9):1183-1197. doi: 10.15252/emmm.201607485.

Fraunhofer IZI

Side effects of CAR T cells



Adapted from Rooney C & Sauer T, Nat Med. 2018 Jun;24(6):705-706

		Yescarta[a] Kite/Gilead	Kymriah[b] Novartis	JCAR017[c] Celgene	UCART-19[d] Cellectis/Servier	bb21217 (BCMA)[5] BlueBird/Celgene
scFv		anti-CD19 / FMC63	anti-CD19/ FMC63	anti-CD19/ FMC63	anti-CD19/ FMC63	anti-BMCA
Hinge						
Transmembrane		IgG1	CD8A	IgG4	CD8A	nk
Costimulatory domain		CD28	4-1BB	4-1BB	4-1BB	4-1BB
Signalling domain		CD3ζ	CD3ζ	CD3ζ	CD3ζ	CD3ζ
Cell population		PBMC	PBMC	$CD4^+ + CD8^+$	PBMC	PBMC
Ablation/safety module		None	None	EGFR cetuximab	RQR8 rituximab	None
Other modification		None	None	None	TCRα/CD52 ko	None
Vector		Retrovirus	Lentivirus	Lentivirus	GE/Talen	Lentivirus
T-cell activation		CD3/IL-2	CD3/CD28	nk	nk	nk
Donor		Autologous	Autologous	Autologous	Allogeneic	Autologous
Dose		2×10^6 – 2×10^8/kg $CD3^+/CAR^+$	0.2×10^6- 2.5×10^8/kg $CD3^+/CAR^+$	5×10^7 or 1×10^8 cells (total)	$6 \times 10^{6*}$ CAR^+ cells total	$50 – 800 \times 10^6$ CAR^+ T cells total
Indication		ALL	DLBCL	ALL	ALL	MM
Phase		MA/US	MA/US	Post PhI	PhI	PhI

Salmikangas P et al. Pharm Res 2018

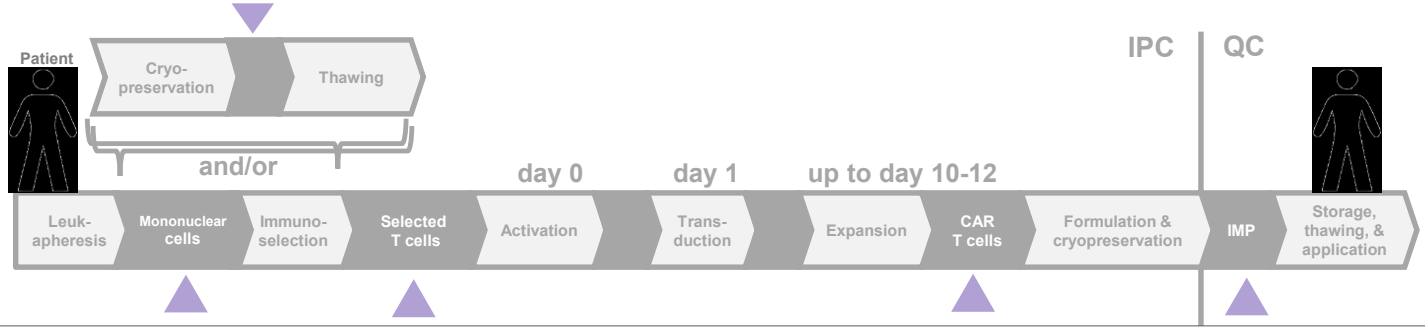
Manual manufacturing of peronalized CAR T cells is complex

Manufacturing of CAR T cells

- Coop. Fraunhofer IZI - NOVARTIS
- Manual process well established
- Currently: > 150 products
- European CTL019 trial as well as Kymriah®

Autologous CAR-T cell manufacturing and quality control is complex

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



Bottle neck:

- No harmonized rules for QC
- Patient selection for leuka-pheresis unclear (WBC? CD3? ..?)
- Surrogate marker still missing to predict production failure

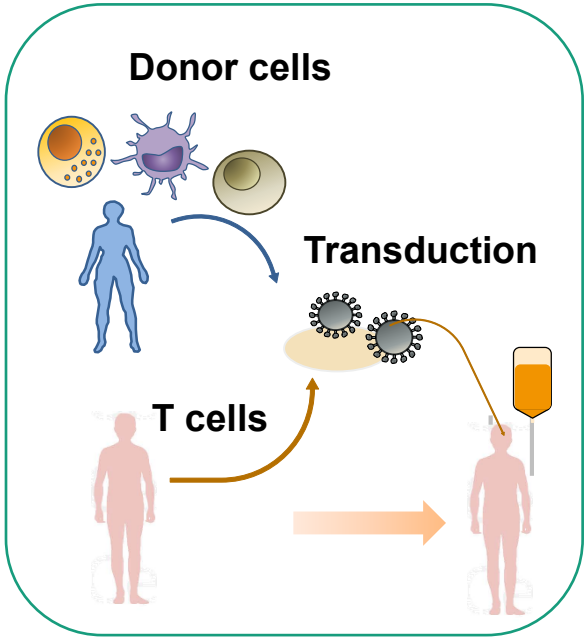
- Appearance & description
- Identity
- Safety
- Purity
- Impurities
- Quantity
- Potency

exemplarily:

- EP 2.7.23 Flowcytometric analysis
- EP 2.6.21 Realtime PCR for VCN (vector copy number)
- EP 2.6.14 Bacterial Endotoxin test
- EP 2.6.27 Microbiological control
- EP 2.6.21 Mycoplasmas

Limitation in autologous CAR T cells

- Identification of tumor-specific targets still not successful
- Concepts are missing to address 100 fold more patients
- Manufacturing of autologous cells is time consuming and expensive (> ¼ Mill €/ product)
- In some cases failure in manufacturing (heavily pre-treated patients): around 5-15% failure
- Relapse due to contaminating transduced leukemic clone



→ Technical challenge – Purification, automation, digitalization and non viral strategies to minimize costs

→ Allogeneic „off the shelf“ CAR effector cells (e.g. NK cells)

CD19-negative B-ALL relapse post CD19 CAR T cell therapy

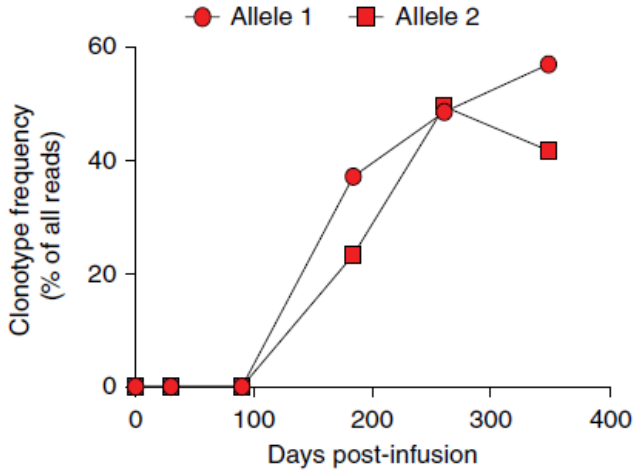
nature medicine
BRIEF COMMUNICATION
https://doi.org/10.1038/s41591-018-0201-9

Induction of resistance to chimeric antigen receptor T cell therapy by transduction of a single leukemic B cell

Marco Ruella[1,2,3,4,5,11], Jun Xu[1,2,3,11], David M. Barrett[6,11], Joseph A. Fraietta[1,2,3,4], Tyler J. Reich[1], David E. Ambrose[1], Michael Klichinsky[1,7], Olga Shestova[1], Prachi R. Patel[1], Irina Kulikovskaya[1], Farzana Nazimuddin[1], Vijay G. Bhoj[1,2,3], Elena J. Orlando[8], Terry J. Fry[9], Hans Bitter[8], Shannon L. Maude[6], Bruce L. Levine[1,2,3], Christopher L. Nobles[10], Frederic D. Bushman[10], Regina M. Young[1], John Scholler[1], Saar I. Gill[1,3,5], Carl H. June[1,2,3,4*], Stephan A. Grupp[6], Simon F. Lacey[1,2,3,12] and J. Joseph Melenhorst[1,2,3,12*]

- Leukaemia – transduction of a single clone
- No CD19 expression via FACS
  but CD19 mRNA detectable
- CAR-19 binding in *cis*-conformation to CD19 on the cell surface – target epitope masked

Serial monitoring of IgH clonotypes over time in the bone marrow.



Improved manufacturing necessary?

Ruella M. et al, Nature Medicine volume 24, pages1499–1503 (2018)

Automated manufacturing of selected CAR T cells

Manufacturing of CAR T cells

hands on → step → by → step → time consuming

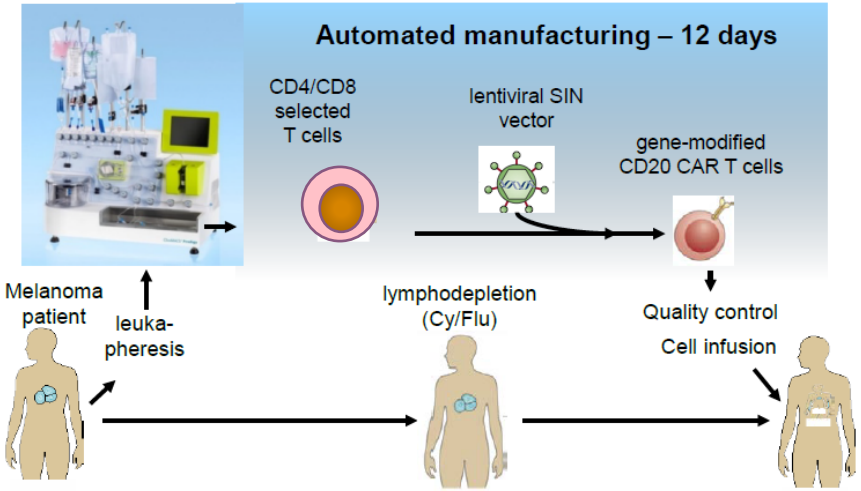
day 0 — automation/ optimization — day 12

automation/ shortening

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

Ulrike Köhl,[1–3] Stanislava Arsenieva,[1–3] Astrid Holzinger,[4,5] and Hinrich Abken[4,5,*]

Human Gene Therapy, Vol 29, No 5, 2018



Multicenter trial (BMBF):

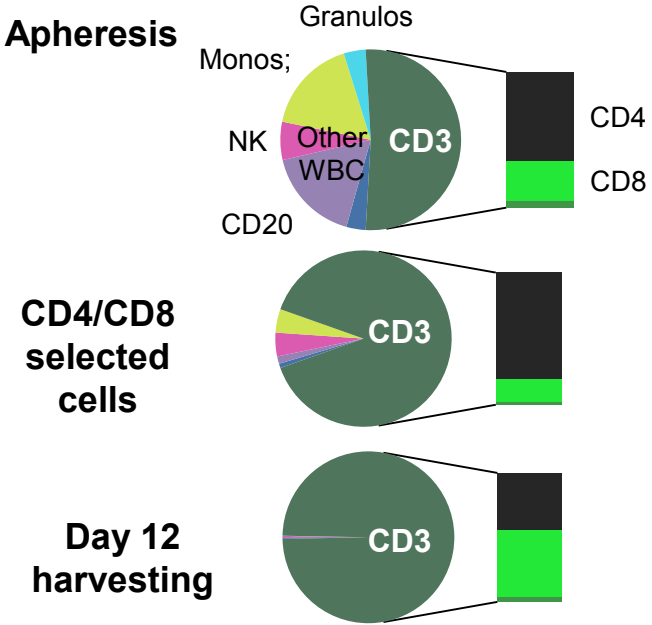
Refractory metastatic Melanoma stage III/IV

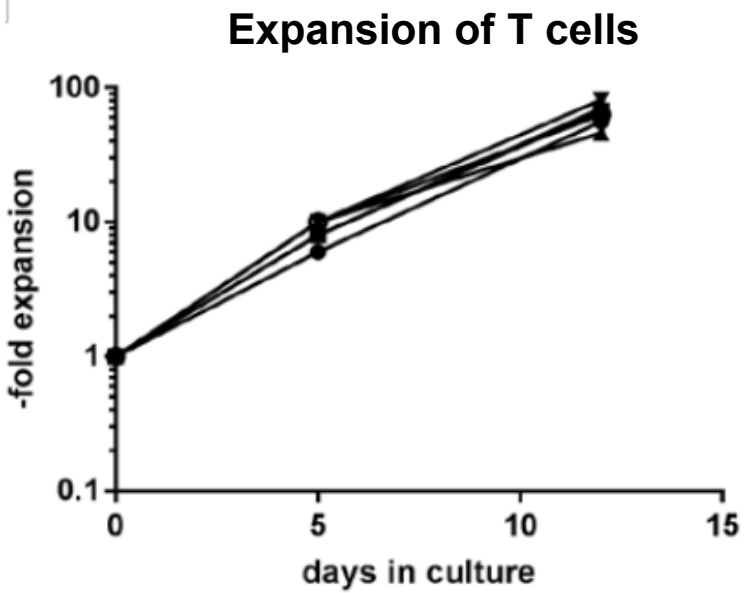
- Coop.:Miltenyi Biotec (Sponser)
  H. Abken (FE)/ U. Köhl (Manufacturing)
- Clinical trial „3+3“ design - Cologne, Munich …
  CD20 CAR T cells: 10e5 / 10e6 / 10e7/ kg BW

First three patients treated

Priesner C … Koehl U. HGT 2016 Aleksandrova K … Koehl U. Transfusion Medicine and Hemotherapy 2019

Automated manufacturing of CAR T cells

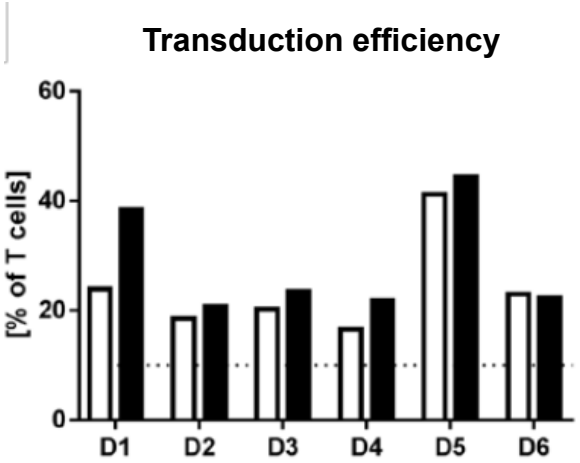


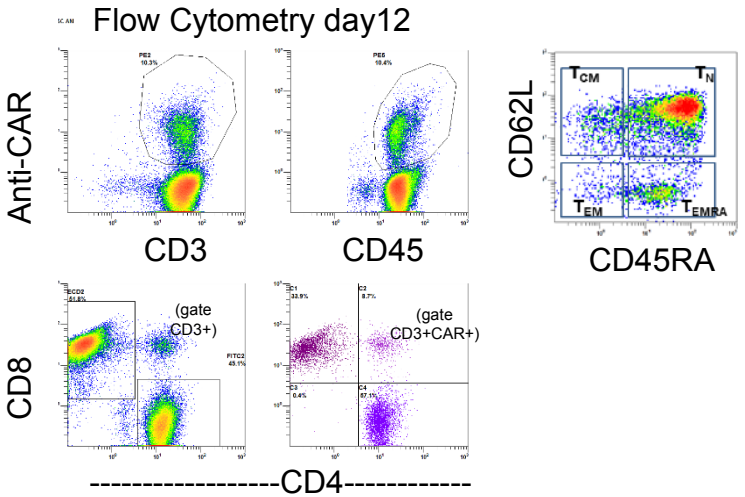


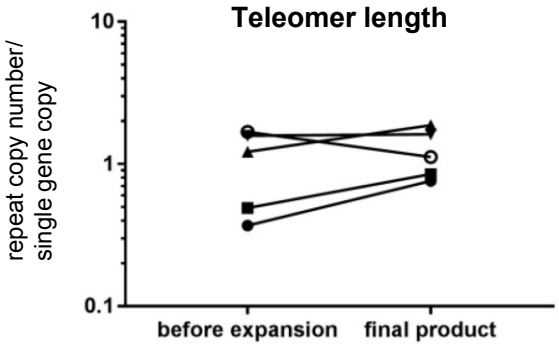
- Pure T cells at harvesting
- Homogenious expansion rate: median 30-fold (yield: 6x10e9 total T cells)

Aleksandrova K … Koehl U. Transfusion Medicine and Hemotherapy 2019, Vol.46, No. 1

Final end product at harvesting







- Successful transduction efficiency (differences between CD4+ and CD8+ cells)
- Naive (T_N) & central memory (T_{CM}) T cells
- However: Differences in Senescence

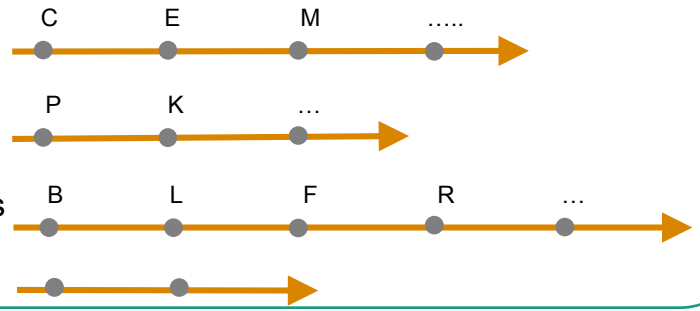
Aleksandrova K … Koehl U. Transfusion Medicine and Hemotherapy 2019, Vol.46, No. 1

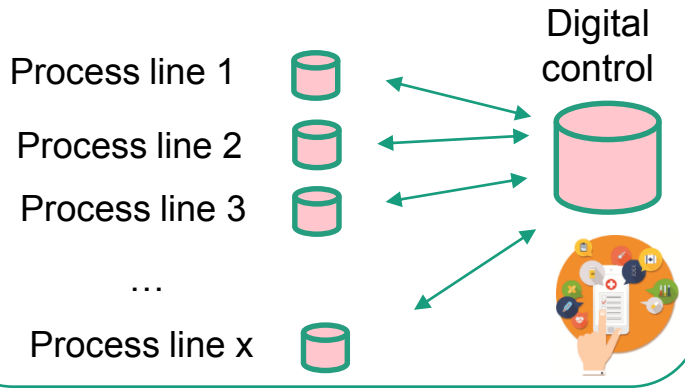
Automation and Digitalisation for manufacturing

Process

Equipment
Market analysis
Manufacturing

Product — Process line * Automation of interfaces





Analoge Umsetzung für (i) Herstellung und (ii) Qualitäts-/ in-Prozess Kontrollen

Fraunhofer IZI

Clinical Trial: Allogeneic „off the shelf“ NK cells

NK-DLI = NK donor lymphocyte infusion
coop.: J. Passweg, CH

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

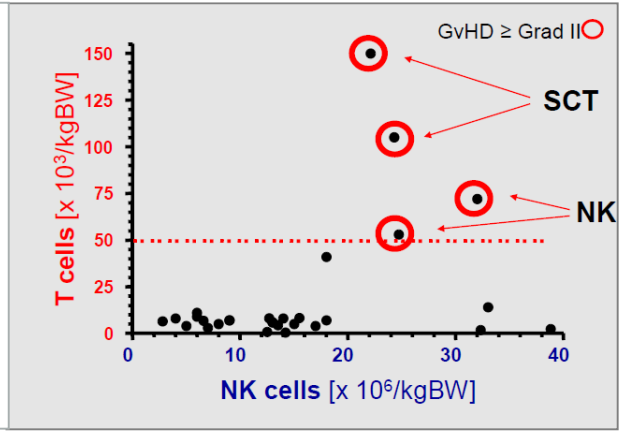
Advantage

- 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

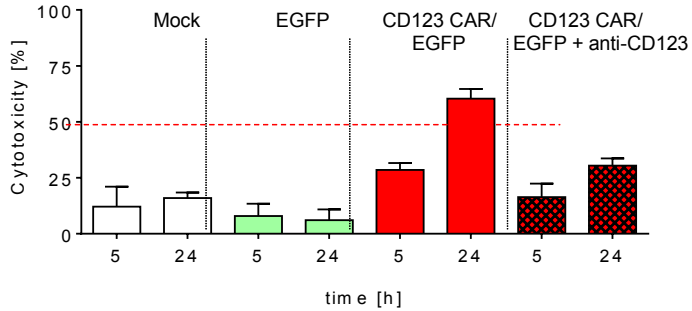


Koehl U..BCMD 2004 Koehl U..KlinPad 2005 Koehl U..Front Oncol 2013 Stern M..BMT 2013 Koehl U.. OncoImmunol 2016

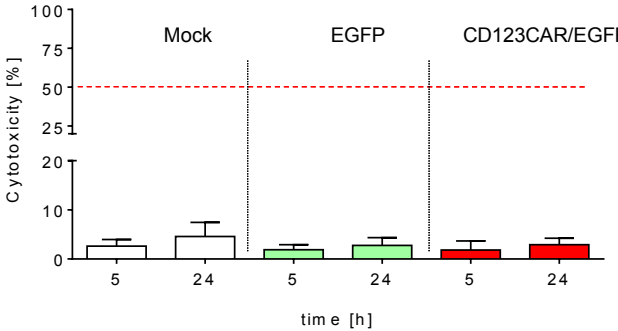
To overcome those hurdles: CAR NK cells?

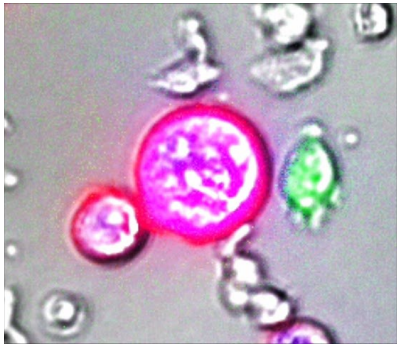
Retargeted CAR NK cells

Cytotoxicity: „CAR NK“ vs. CD123 AML
(patient cells)



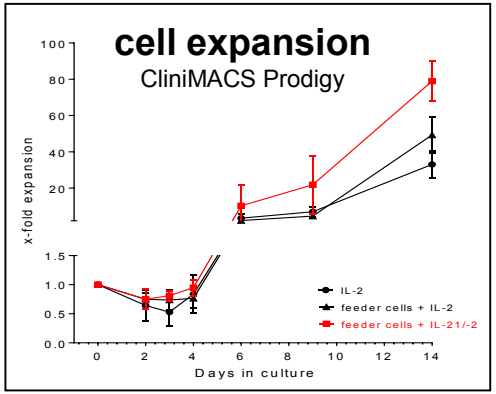
Side effects: “CAR **NK**” vs. HLMVEC (E/T: 1:1)





CD123+ AML

CAR NK cells



Kloess … Koehl. HGT 2017 and 2019

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

Clinical trials using CAR NK cells

K. Rezvani

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

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 Houston, USA**
NCT03656705	**CCCR**	**Non-small Cell Lung**	**NK92**	**I**	**recruiting**	**Hospital of Xinxiang Henan, China**

7/9 patients CR/PR
no CRS
(EBMT 03/2019)

CCCR: Chimeric Costimulatory Converting Receptor

Kloess S … Koehl U. Transfusion Medicine and Hemotherapy 2019

Conclusion and outlook

CAR T cells:

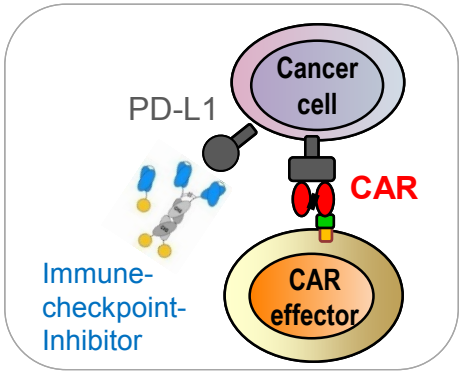
- > 500 (1000) clinical trials worldwide - with benefit in CD19+ malignancies
- Manufacturing is complex – results in automation are promising, however needs improvement
- Predictive markers are still missing: Leukapheresis → quality of the final end product

Allogeneic „off the shelf CAR products“:

- Allogeneic haploidentical/ „third party NK cells“→ first successful trial
- CAR NK cells → e.g. elimination of CD123+ leukemic cells

Improvement in future studies:

- “CAR cells” & checkpoint inhibitors → combination
- CAR effector cells with transient cytokine secretion
- Technical side: Digital control of automated process lines to address tumor patients



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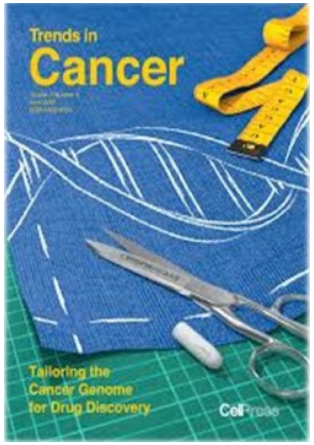
Network for individualised stratified medicine using cell-based therapies



…. and thanks for listening

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