CAR NK cell therapy

Ulrike Koehl
Disclosure

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

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➢ CTL019 European study trial

➢ 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®


- CAR T cells
- CAR T Melanoma trial
- Coop.; Miltenyi Biotec, H. Abken (Regensburg), U. Köhl (MHH)
- CORS, USA, Europe
- CTL019 trial
- Kymriah Europe

Press conference NOVARTIS

USA Europe

CAR = chimeric antigen receptor
Clinical CAR T cell studies

- Ongoing CAR T cell studies
- Started CAR T cell studies

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

Paediatric r/r ALL – ELIANA

- > 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

Maude SL et al. New Engl J Med 2018

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
Natural Killer (NK) cells

- CD56⁺CD3⁻ NK cells comprise 2-18% of lymphocytes in the peripheral blood
  - CD56^{bright}CD16^{negative} (immunoregulatory)
  - 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 cytotoxicity receptors)
Mechanisms of immune escape in the tumor microenvironment

- Induction of T<sub>reg</sub> and MDSC
- Inhibition of CD8<sup>+</sup> and NK Cells
- Inhibition of DC Maturation
- Reduced Expression of MHC class I
- Disrupted expression of immune checkpoint ligands
- Induction of Immune Cell Apoptosis

However: Improved NK cell attack

Signaling and CARs in primary human NK cells

Oberschmidt O, Kloess S, Koehl U. Front Immunol 2017
Advances in clinical NK cell studies: Donor selection, manufacturing and quality control


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)

NK-DLI = NK donor lymphocyte infusion

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

IL-2 activated NK cells improve NKG2D mediated cytotoxicity via scavanging of solMICA in plasma
Impaired NK cell cytotoxicity in patients with head and neck cancer (HNSCC)

n=67 patients with HNSCC peripheral blood screening

Dysregulation of autologous NK cells in patients and inhibition of autologous and allogeneic NK cells via soluble plasma MICA/TGF-β

To overcome those hurdles: CAR NK cells?

Kloess S et al. Oncoimmunology 2016
# Clinical trials with CAR expressing NK cells

<table>
<thead>
<tr>
<th>Clinical identifier</th>
<th>Target</th>
<th>Condition/disease</th>
<th>Origin of NK cells</th>
<th>Phase</th>
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**CCCR:** Chimeric Costimulatory Converting Receptor  

**CAR:** CD19-CD28-zeta-2A-iCasp9-IL15 (K. Rezvani)
Redirected “CAR” NK-92 cell line

**anti-GD2**

- SFFV
- SP
- scFv
- CD8α
- CD28
- CD3ζ
- IRES
- EGFP

**Neuroblastoma**

- UKF-NB-3 (NB) tumor

**GD2 Expression**

- Events

**fluorescence**

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

**CAR Expression**

**Specific lysis (%)**

breast cancer cell line MDA-MB453
Chimeric Antigen Receptor Vector Design for primary human NK cells

- **Endodomain**: FMC63 → CD19
- **Endodomain**: CD28 + 4-1BB(CD137) + CD3ζ
- **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
CAR expressing NK cells redirected against CD19

**Alpha SIN vector**
Transduction of mature primary human dNK cells feasible

Mock  alpha

MOI

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

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<tr>
<th>E/T</th>
<th>CD56&lt;sup&gt;bright&lt;/sup&gt;CD16&lt;sup&gt;dim&amp;neg&lt;/sup&gt; (immune regulatory)</th>
<th>CD56&lt;sup&gt;dim&lt;/sup&gt;CD16&lt;sup&gt;pos&lt;/sup&gt; (cytotoxic)</th>
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<tr>
<td>1:1</td>
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<tr>
<td>5:1</td>
<td>0</td>
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**anti-inflammatory:**
- IL-4
- IL-10

**pro-inflammatory:**
- IL-6
- IL-17A
- IFN<sub>γ</sub>
- TNF<sub>α</sub>

**pro-apoptotic:**
- GrA
- GrB
- Perforin
- Granulysin
Primary CAR expressing NK cells
redirected against AML cell lines and
patients own leukemic cells
CAR expressing NK cells redirected against CD123+

IL-2 activated NK cells against KG1a

CD123-CAR-NK cells against KG1a

CD123+ leukemia blasts [cells/µl]

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

Cytotoxicity

|                | Mock | EGFP | CD123 CAR/EGFP | CD123 CAR/EGFP + anti-
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<td>CD123 CAR/EGFP</td>
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<td>CD123 CAR/EGFP + anti-</td>
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Side effects

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

MOI1

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 against 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

Product-development → Upscaling → Validation → Development

Quality control → Release of product

GMP-compliant protocol

Cell expansion

X-fold expansion vs. Days in culture

IL-2, feeder cells + IL-2, feeder cells + IL-21/2

healthy donor

blood

leukapheresis

NK cell selection

elutriation

CD3 depl./CD56 sel.

cell activation

CAR cell engineering

retro-/lentiviral transduction

mRNA electroporation

purgable cytokines

CAR cell expansion

IL-2

IL-7

IL-15

final formulation

cryopreservation

up-scaling

AML patient

manufacturing of IMP

QC

SOP

development

validation runs

manufacturing license

CMC of IMPD

investigational medicinal product dossier (IMPD)

standard operation protocol (SOP)

chemical manufacturing and control (CMC)

GMP-compliant protocol

"Off the shelf product" Advanced Therapy Medicinal Product

Results
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

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... and thanks
for listening

16 & 17 September 2019

CAR-T cells
and beyond

TOPICS & SESSIONS
Pre-clinical development
Vector production & manufacturing
Regulation
Ethics of CAR-T cells
Clinical studies

CONFIRMED SPEAKERS
Hinrich Abken
Michael Bachmann
Chiara Bonini
Boris Fehse
Ulrike Köhl
John Maher
Stephan Mielke
Kai Pinkernell

Hans Stauss
Winfried Wels
Christiane Woopen
Nina Worel

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