Translation of TCRs to the clinic –

MAGE-A1 TCR gene therapy of Multiple Myeloma as an example

GERALD WILLIMSKY
Experimental and Translational Cancer Immunology
Institute of Immunology (Charité - Universitätsmedizin Berlin) and German Cancer Research Center (DKFZ)
MAGE-A1 TCR gene therapy of Multiple Myeloma

BMBF program „Personalized Medicine“
Redirected T cell therapy

Blood T cells

Gene transfer

Grafting of new antigen specificity
Interaction between T cells and target cells is a three body problem: TCR - peptide - MHC

TCR: pMHC affinity (μm)

- TCR:pMHC affinity (μm)
  - Tolerant repertoire
  - Non-tolerant repertoire

- Tumor rejection can be achieved
  - 10 nM
  - 1 nM

- Therapeutic efficacy
  - 20 000
  - 10 000
  - 5 000
  - 2 500
  - 1

POINTS TO CONSIDER FOR ADOPTIVE T CELL THERAPY

• best possible risk-benefit ratio for target antigen:
  - TSA (e.g. neoantigens) > CT > differentiation Ag

• most efficient rejection will occur when cancer cells are recognized as foreign:
  - TCRs with optimal-affinity
How to obtain therapeutic TCRs?

- Human with cancer
  - TIL
  - PBMC

- Cancer-free environment
  - humanized mouse
Transgenic mice with a diverse human T cell antigen receptor repertoire

- Mice are not tolerant for most human tumor antigens
- Human TCRs from non-tolerant repertoire in cancer free mice

huTCR locus-Tg mice

Li et al., Nat Med 16: 10129 (2010)
Li et al., Nat Prot 8: 1567 (2013)
Peripheral T cells in huTCR-locus Tg mice

huTCRαβ-Tg
HLA-A2-Tg
muTCRαβ-KO
H2-KO

huTCR locus-Tg mice

MHC I: human
TCR: human

→ CTL to various human TAA (>10)
→ CTL to cancer viruses (>10)
→ CTL to various human TSA (>30)
pMHC affinity is important

MAGE-A1

IC_{50} nM

270

300

| 22975 |

| 454 |

| 186 |

| 4.2 |

| 0.9 |

Human – ALAETSYVKLEYVKVSARVRFPSLREA-

Mouse – AFAETSKMKVLQFFASINKTHPRAYPEKYAE-

* **** *** *
Humanized mouse model for TCR generation

Antigen immunization

Mouse T cell carrying human TCR

Isolation of TCR and transfer into human T cells

Clonal expansion
Mouse-derived MAGE-A1 TCR has higher functional affinity

CANCER-ASSOCIATED ANTIGENS AS TARGET

Peptide pulsed T2 cells

Melanoma cells

* Ottaviani et al., 2005, Cancer Immunol Immunother 54, 1214
Timeline MAGE A1 TCR gene therapy of Multiple Myeloma

1. SA
   Oct. 2013
   Data summaries
   Study concept

2. SA
   Oct. 2015
   IMPD submission
   Study synopsis
   Manufacturing licence

CTA (planned)
   Q1 2017

R&D

Pre-clinical

Clinical
Potential safety concerns

- MAGE-A1 expression in healthy tissues
- Allo-reactivity
  - T1367 was generated in mice expressing a single HLA class I molecule (in part applies also for an allogeneic human environment)
- Cross-reactivity
  - Promiscuous TCR recognition has been reported
  - T1367 was positively and negatively selected based on a mouse peptide repertoire
• Limited expression in normal tissues (testis, placenta, embryonic tissue, mTECs)

Expression in tumors:
• Multiple myeloma
• Lung cancer (NSCLC)
• Melanoma
• Breast cancer
• Colon cancer
• Hepatocellular carcinoma
• Cholangiocellular carcinoma
CANCER-ASSOCIATED ANTIGENS AS TARGET

Restricted MAGE-A1 expression

[Image showing a diagram with various tissues and corresponding expression levels of MAGE-A1 and GAPDH]
**CANCER-ASSOCIATED ANTIGENS AS TARGET**

No evidence for off-target recognition

More than 100 HLA-A2 peptide ligands

<table>
<thead>
<tr>
<th>Peptide</th>
<th>Sequence</th>
<th>IC₅₀ (nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAGE-A1</td>
<td>KVLEYVIKV</td>
<td>4.69</td>
</tr>
<tr>
<td>MAGE-B5</td>
<td>KVLEYLAKV</td>
<td>4.11</td>
</tr>
<tr>
<td>MAGE-B16</td>
<td>KVLEFVAKV</td>
<td>4.97</td>
</tr>
</tbody>
</table>
No evidence for allo-recognition

LCL panel

<table>
<thead>
<tr>
<th></th>
<th>HLA-A*</th>
<th>HLA-B*</th>
<th>HLA-C*</th>
</tr>
</thead>
<tbody>
<tr>
<td>AMAI</td>
<td>68:02</td>
<td>53:01</td>
<td>04:01</td>
</tr>
<tr>
<td>AMALA</td>
<td>02:17:01</td>
<td>15:01:01</td>
<td>03:03:01</td>
</tr>
<tr>
<td>Bello</td>
<td>02:02</td>
<td>11:01</td>
<td>41:01</td>
</tr>
<tr>
<td>BM14</td>
<td>03:01</td>
<td>07:02</td>
<td>07:02</td>
</tr>
<tr>
<td>BSM</td>
<td>02:0101</td>
<td>15:010101</td>
<td>03:0401</td>
</tr>
<tr>
<td>CLA</td>
<td>02:06:01</td>
<td>24:02</td>
<td>08:01</td>
</tr>
<tr>
<td>DUCAF</td>
<td>30:02</td>
<td>18:01</td>
<td>05:01</td>
</tr>
<tr>
<td>HOR</td>
<td>33:0301</td>
<td>44:0301</td>
<td>14:03</td>
</tr>
<tr>
<td>KAS011</td>
<td>01:0101</td>
<td>37:01</td>
<td>06:02</td>
</tr>
<tr>
<td>KAS116</td>
<td>24:020101</td>
<td>51:01</td>
<td>12:03</td>
</tr>
<tr>
<td>KE</td>
<td>02:01</td>
<td>29:02</td>
<td>44:03</td>
</tr>
<tr>
<td>KLO</td>
<td>02:08</td>
<td>01:01:01</td>
<td>01:01:01</td>
</tr>
<tr>
<td>LCLW01</td>
<td>03:01</td>
<td>24:02</td>
<td>15:01</td>
</tr>
<tr>
<td>LCLW02</td>
<td>02:01</td>
<td>26:01</td>
<td>38:01</td>
</tr>
<tr>
<td>LCLW03</td>
<td>02:01</td>
<td>23:01</td>
<td>15:01</td>
</tr>
<tr>
<td>MT14B</td>
<td>31:01</td>
<td>40:01</td>
<td>03:04</td>
</tr>
<tr>
<td>OZB</td>
<td>02:09</td>
<td>03:01:01</td>
<td>38:01</td>
</tr>
<tr>
<td>RML</td>
<td>02:04</td>
<td>51:0101</td>
<td>15:02</td>
</tr>
<tr>
<td>SA</td>
<td>24:020101</td>
<td>07:0201</td>
<td>07:02</td>
</tr>
<tr>
<td>SPO</td>
<td>02:01</td>
<td>44:02</td>
<td>05:01</td>
</tr>
<tr>
<td>TAB089</td>
<td>02:07</td>
<td>46:01</td>
<td>01:02</td>
</tr>
<tr>
<td>TISI</td>
<td>24:020101</td>
<td>35:08</td>
<td>04:01</td>
</tr>
<tr>
<td>VAVY</td>
<td>01:01</td>
<td>08:01</td>
<td>07:01</td>
</tr>
<tr>
<td>WIN</td>
<td>01:01</td>
<td>57:0101</td>
<td>06:02</td>
</tr>
<tr>
<td>WT24</td>
<td>02:0101</td>
<td>27:0502</td>
<td>02:0202</td>
</tr>
<tr>
<td>WT49</td>
<td>02:05:01</td>
<td>58:01:01</td>
<td>07:18</td>
</tr>
<tr>
<td>XLI-ND</td>
<td>02:10</td>
<td>30:01</td>
<td>13:02</td>
</tr>
</tbody>
</table>

LCLs representing more than 75% for HLA-A*, 60% for HLA-B* and 78% for HLA-C* of the alleles within the German population
CANCER-ASSOCIATED ANTIGENS AS TARGET

MAGE-A1 recognition motif-related human self-peptides

Alanine scan

<table>
<thead>
<tr>
<th>Sequence</th>
<th>Gene Symbol</th>
<th>IC&lt;sub&gt;50&lt;/sub&gt; (nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>X X X E Y X I K X</td>
<td>T1367 motif</td>
<td></td>
</tr>
<tr>
<td>K V L E Y V I K V</td>
<td>MAGEA1</td>
<td>4.11</td>
</tr>
<tr>
<td>E L L E Y Y I K V</td>
<td>TRI25</td>
<td>23.34</td>
</tr>
<tr>
<td>G L L E Y L I K S</td>
<td>SAMD9</td>
<td>73.23</td>
</tr>
<tr>
<td>K Q F E Y D I K T</td>
<td>MMP27</td>
<td>834.74</td>
</tr>
<tr>
<td>R S L E Y D I K L</td>
<td>COSA1</td>
<td>4258.75</td>
</tr>
<tr>
<td>R L K E Y T I K S</td>
<td>DAPK3</td>
<td>5212.32</td>
</tr>
</tbody>
</table>
SAMD9 is recognized at high peptide concentration

SAMD9: Sterile Alpha Motif Domain Containing Protein 9
CANCER-ASSOCIATED ANTIGENS AS TARGET

SAMD9 is not naturally presented

T cells

SAMD9: Sterile Alpha Motif Domain Containing Protein 9
Limitations in xenograft cancer models

Biodistribution and pharmacokinetics of T1367-transduced T cells can be addressed only in the autologous host, the MM patient

- human T cells in mice poorly expand or survive (species-specific factors)
- human T cells in NSG mice acquire functional activity, but may elicit lethal graft-versus-host disease (GvHD).
Does TCR gene therapy lead to rejection or relapse of tumor?

Leisegang et al., JCI 126:854 (2016)
In vivo function of TCR modified T cells

The TCR repertoire against MAGE-A1_{278} is likely skewed towards low affinity in humans

MAGE-A1 TCR gene therapy: Study Information

- Chimeric mouse/human TCR
- Target: Cancer-germline antigen
- Vector: Gamma retroviral MP71
- Indication: Relapsed/refractory Multiple Myeloma
TCR $\alpha$- and $\beta$-chain gene expression cassettes

WT-1 TCR

MP71

Single chain

$\alpha + \beta$

IRES

$\alpha i\beta$

$\beta i\alpha$

P2A

$\alpha p\beta$

$\beta p\alpha$

Transgene cassette determines TCR expression level

- **neg.**
- **α+β**
- **αiβ**
- **βiα**
- **αpβ**
- **βpα**

<table>
<thead>
<tr>
<th>Single</th>
<th>Counts</th>
<th>WT-1 tetramer</th>
<th>IFN-γ (pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>neg.</td>
<td>7</td>
<td>0</td>
<td>71</td>
</tr>
<tr>
<td>α+β</td>
<td>28</td>
<td>7</td>
<td>619</td>
</tr>
<tr>
<td>αiβ</td>
<td>17</td>
<td>1</td>
<td>94</td>
</tr>
<tr>
<td>βiα</td>
<td>22</td>
<td>1</td>
<td>128</td>
</tr>
<tr>
<td>αpβ</td>
<td>44</td>
<td>12</td>
<td>706</td>
</tr>
<tr>
<td>βpα</td>
<td>52</td>
<td>29</td>
<td>2095</td>
</tr>
</tbody>
</table>

Vβ2

IFN-γ (pg/ml)
Optimization of TCR genes improves T cell functionality

- Human TCR (wild type)
- Murinization (mu)
  Preferential pairing of tg TCR chains
  (Cohen, 2006)
- Disulfide bond (cys)
  Preferential pairing of tg TCR chains
  (Kuball, 2007)
- Codon optimization (co)
  Enhanced tg TCR expression level
  (Scholten, 2006)

Others

- increase the avidity of therapeutic TCR
- replace wild type TCR
9 amino acids of the mouse TCR C-regions enhance human TCR expression

High-performance $\gamma$-retrovirus vector for TCR gene therapy

- Modification of retrovirus vector
- Optimization of TCR gene cassette
- Engineering of TCR genes

TCR gene-modified T cells:
- High expression level of tg TCR
- Homogeneous population
- High functional activity
MAGE-A1 TCR T1367 is primarily active in CD8\(^+\) T cells
GMP Transduction Protocol

**Starting material**

- CD4+ cells

**Adherence**

depletion of monocytes

**Activation**

bead bound antibodies + Selection

**Transduction**

1x 90 min, 800g, 32°C
100 ml pure Virus

**Cultivation**

Up to 12 days in Wave Reactor

**Spectra Optia® Apheresis System**

CD4+ cell-depletion with CliniMACS®, Miltenyi

PBMCs
Mean pMP71 vector copy number per cell
MAGE-A1 TCR T1367 transduced T cells have stem cell–like memory phenotype

Buffy coat
(control)

Leukapheresis product, RV transduced

CD4
CCR7
CD62L

CD3+ CD8+ CD45RA+ CD45RO- CD62L+ CCR7+
Phase I trial of MAGE-A1⁺ Multiple Myeloma

<table>
<thead>
<tr>
<th>Cohort</th>
<th>n</th>
<th>T cells / kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3</td>
<td>1 x 10⁵</td>
</tr>
<tr>
<td>2</td>
<td>3</td>
<td>1 x 10⁶</td>
</tr>
<tr>
<td>3</td>
<td>3</td>
<td>1 x 10⁷</td>
</tr>
<tr>
<td>4</td>
<td>3</td>
<td>1 x 10⁸</td>
</tr>
<tr>
<td>5</td>
<td>3</td>
<td>1 x 10⁸</td>
</tr>
</tbody>
</table>
Identifying MAGE-A1+ myeloma

CANCER-ASSOCIATED ANTIGENS AS TARGET

MAGE-A1 positive samples

(n = 99)
Heterogenous expression pattern of MAGE-A1 within a tumor

Correlation between extramedullary disease and homogenous MAGE-A1 expression

Many patients with MAGE-A1 expressing myeloma are primary refractory to either a bortezomib or lenalidomide based regimen
Standardization of the manufacturing process: 
“Master Processes”

- technology platforms (GMP facility, medical device status)

- generic vectors documented in a dossier
  (solution for bottlenecks for academia as a small customer)

- establishment of INDs for established products that can be used as a blueprint to develop processes and products that are derived from the existing process
Principles and Applications of Adoptive T Cell Therapy

BMBF program „Personalized Medicine“

German Cancer Aid Priority Program ‘Translational Oncology’
*Exploring mutant immunogenic epitopes for T cell therapy of cancer*

German Cancer Consortium (DKTK)

Berlin School of Integrative Oncology
THANK YOU!