Cancer immunotherapy with TCR gene modified T cells

Mirjam Heemskerk
Associate professor
Department of Hematology
LUMC
Adoptive cell transfer (ACT) of T cells engineered with CAR/TCR

Target antigen has to be tumor specific
Engineering T cells with CAR/TCR

Lymphodepletion and infusion of CAR/TCR T cells

T cell activation and gene transfer

T cells or selected T cell subsets from blood

Expansion

Lymphodepletion and infusion of CAR/TCR T cells
THE PRODUCTION OF ENGINEERED T CELLS

T-cells isolated from patient

Introduction of CAR/TCR via virus in T-cells

CAR/TCR T-cells

Expansion

10 days
Difference between CAR and TCR

**TCR**
- α-chain
- β-chain
- LTR

**CAR**
- F<sub>ab</sub>
- Stalk
- TM
- Signaling domains
- LTR

**T cell**
- Intracellular or surface antigen
- HLA

**Cancer cell**
- Mutated antigen
- HLA

**Cell surface antigen**
- Antibody (same F<sub>ab</sub> as CAR)

Current Opinion in Pharmacology
Difference between CAR and TCR

- CAR (Chimeric antigen receptor)
  - Cell-surface expressed target antigen

- TCR (T cell receptor)
  - Antigen in context of HLA molecule
  - Intracellular and extracellular antigens (broader spectrum)
TCR gene therapy: The TCR solely determines the specificity of the T cell

- Anti-tumor T-cell
- Introduced TCR
- Endogenous TCR
- T-cell
- Target cell
- HLA-peptide
- Retroviral/lentiviral vector

The TCR gene therapy involves introducing a tumor-specific TCR into a T-cell using a retroviral/lentiviral vector. The introduced TCR allows the T-cell to recognize and target the specific tumor cell.
Aim: Identify new tumor specific TCRs
Identification of tumor specific HLA binding peptides

- Isolation of HLA molecules from tumors
- Identify HLA binding peptides by Mass Spectrometry
- Select peptides of tumor specific genes
- Generate pMHC tetramers-PE

<table>
<thead>
<tr>
<th>Protein</th>
<th>Pep Nr.</th>
<th>Sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>BOB1</td>
<td>127</td>
<td>YALNHTLSV</td>
</tr>
<tr>
<td>TLR10</td>
<td>128</td>
<td>YLDLSNNRL</td>
</tr>
<tr>
<td>FCRL2</td>
<td>132</td>
<td>AVGDLLEL</td>
</tr>
<tr>
<td>RALGPS2</td>
<td>245</td>
<td>ALMAVVSGL</td>
</tr>
<tr>
<td>RALGPS2</td>
<td>240</td>
<td>KLYELNNLHA</td>
</tr>
<tr>
<td>PAX5</td>
<td>249</td>
<td>GLDDMKANL</td>
</tr>
<tr>
<td>FAM 129C</td>
<td>250</td>
<td>GLSHSLETV</td>
</tr>
<tr>
<td>KLHL14</td>
<td>254</td>
<td>LLDAMNYHL</td>
</tr>
<tr>
<td>KLHL14</td>
<td>264</td>
<td>VMNDRLYAI</td>
</tr>
</tbody>
</table>
Identify tumor specific TCRs

FACS analysis/
single peptide stim.

T cell clone

ca. 17 days

FACSorting
CD8+/pMHC-Tet+

Cytokine Production

FACSorting

A2
B7
A2 + pep pool
B7 + pep pool

CD8+/pMHC-Tet+
Identification of tumor specific TCRs
High throughput screening

Affinity and Functional avidity

Recognition of B-cell malignancies

Recognition of healthy Cell subsets

Affinity and Functional avidity

Recognition of B-cell malignancies

Recognition of healthy Cell subsets
Multiple myeloma *in vivo* model – BOB1-TCR

NSG xenograft model (n=10 per group)

Day 0  
U266-Luc i.v.

Day 21  
T cells i.v.

Tumor growth (BLI)

Day 21

Injection of T cells

Day 23

Day 27

Day 30

Day 33

Control

BOB1-TCR
Identification of tumor specific TCRs

- BOB1-TCR for the treatment of Multiple Myeloma
- PRAME-TCR for the treatment of acute myeloid leukemia (AML), neuroblastoma, uveal melanoma
Acknowledgements

Hematology, LUMC
Lorenz Jahn
Marleen van Loenen
Miranda Meeuwsen
Renate de Boer
Marije de Rooij
Rosa van Amerongen
Laura Morton
Rogier Reijmers
Sander Tuit
Dirk van der Steen
Renate Hagedoorn
Michel Kester
Marian van de Meent
Anne Wouters
Dennis Remst
Chris Kweekel
Marieke Griffioen
Fred Falkenburg

CPM, LUMC
Arnoud de Ru
Peter van Veelen

FACS facility, LUMC
Sabrina Veld
Guido de Roo

Bellicum Pharmaceuticals, Houston, USA
Tsvetelina Hoang
Tania Rodrigues
Aaron Foster
Annemarie Moseley
Ken Moseley