T cell therapy towards solid tumors: antigen and T cell recruitment

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No...we have no financial or commercial interest in a company or otherwise that may have biased our data

http://www.erasmusmc.nl/interne_oncologie/research/research_groups/tumor.immunology1/
Presence and type of intra-tumoral T cells have both prognostic and therapy predictive value

Adoptive T cell therapy as well as checkpoint inhibitors demonstrate clear objective response rates

However, complete response rates of immune therapies are low
T cell therapy: 10-20%; Ipilimumab: 2-10%, reviewed in Kunert, Front Immunol, 2013
Tumor relapse following T cell therapy

Research aims:

1. **Understand the mechanism(s) that contribute to tumor relapse**
   Q1: Is tumor relapse due to loss or down-regulated expression of antigen after T cell therapy? 
   Q2: Is T cell recruitment decreased in relapsed tumor after T cell therapy?

2. **Design and test strategies to intervene with tumor relapse**

3. **Translate findings to clinical T cell therapy**
T cell receptor (TCR) gene therapy in an immune competent mouse model

Mouse melanoma tumor (B16)

Transduced with human gp100_{280-288} (YLE)/HLA-A2

sc

Mouse T cells

Transduced with human gp100_{280-288} (YLE)/HLA-A2 TCR

Schaft, J Immunol, 2003

Pre-conditioning with chemotherapy (busulfan/cyclophosphamide)

Pre-connected with chemotherapy

iv

Before T cells

14 days after T cells

HLA-A2 tgm (HHD: A2^{+/-}, \beta2m^{-/-}; H2-D^{b/-})
Outcome of TCR T cell treatment in immune-competent mice is similar to clinical studies: tumors initially regress but in most cases relapse again.

**Tumor growth**

- TCR T cells (n=9)
- Mock T cells (n=12)

**Persistence of TCR T cells in blood**

| gp100/A2 pMHC-binding CD8+ T cells (number/μl blood) |
|-----------------|-----------------|
| 4 | 11 | 17 | 24 |

- *p < 0.05
- **p < 0.01
Tumors that relapse following T cell treatment functionally express antigen

**Relapsed tumors:**
- harbor *intact* and *non-mutated antigen* genes
- genes are not silenced by promotor methylation
- functionally express surface antigen at levels equal to non-treated tumors

**Antigen expression**

**Killing of tumor cells**

**T cell IFN\(_\gamma\) production**
Relapsed tumors show continued expression of tumor antigen in a 2nd mouse model

Mouse melanoma tumor (B16)
Endogenously express TRP2/H2-K^b
SC

C57Bl/6 wt mice

Mouse T cells
Tranduced with TRP2_{180-188} (SVY)/H2-K^b TCR

Bendle, Nat Med, 2010

Pre-conditioning with chemotherapy (busulfan/cyclophosphamide)

Response rate and extent of regression were less

TRP2 expression

H2-K^b expression

T cell IFN_γ production

H2-K^b expression

TRP2 positive cells (%) progression

H2-K^b positive cells (%) progression

T cell IFN_γ production (pg/ml) progression
Tumor relapse following T cell therapy

Research aims:

1. Understand the mechanism(s) that contribute to tumor relapse

Q1: Is tumor relapse due to loss or down-regulated expression of antigen after T cell therapy?

Relapsed tumors show
- continued expression of tumor antigen
- resist a 2nd T cell therapy resistance
- T cell therapy resistance is not related to intrinsic changes of tumor cells, but rather to cues from established micro-environment

Q2: Is T cell recruitment decreased in relapsed tumor after T cell therapy?
Relapsed tumors show decreased signals for infiltrating CD8 T cells

Genome-wide expression analysis

Signals not altered for:
- B cells
- Natural Killer cells
- Dendritic cells
- Granulocytes
- T regulatory cells
- Myeloid-derived Suppressor Cells

Quantitative PCR analysis

CD8β1

IFNγ

PDL1
Relapsed tumors show decreased frequencies of tumor-specific CD8+ T cells

**Human gp100<sub>280-288</sub>/HLA-A2**

- Tumors: Regressed, Relapsed
- pMHC-binding T cells (%)
- p<0.01

**Mouse TRP2<sub>180-188</sub>/H2-K<sup>b</sup>**

- Tumors: Regressed, Relapsed
- Number of CD8<sup>+</sup> cells/1x10<sup>6</sup> tumor cells
- TCR-Vα12N-3 and Vβ26 T cells
- p<0.05
Critical is recognition of:

1. tumor antigen (TCR) continued expression
2. chemoattractants and adhesion ligands (chemoattractant receptors and integrins)
3. co-stimulatory ligands (co-stimulatory receptors)
Relapsed tumors show decreased expression of chemoattractants and adhesion ligands.
Relapsed tumors show altered T cell co-stimulation (ongoing studies)

2 signals needed for optimal T cell function:

1\textsuperscript{st} signal: TCR binds to pMHC,
2\textsuperscript{nd} signal: CD28 binds to CD80/86
TILs derived from relapsed tumors show enhanced expression of PD1

**PD1 expression (MFI)**

**Tumor type:**

- REG
- REL

**CD6+ TILs**

- REG: p<0.05
- REL: p<0.05

**CD4+ TILs**

- REG
- REL

**Erasmus MC**
Tumor relapse following T cell therapy

Research aims:

1. Understand the mechanism(s) that contribute to tumor relapse
   Q1: Is tumor relapse due to loss or down-regulated expression of antigen after T cell therapy?
       NO

   Q2: Is T cell recruitment decreased in relapsed tumor after T cell therapy?

   Relapsed tumors show
   - decreased numbers of T cells
   - decreased expression of chemoattractants and adhesion ligand
   - enhanced expression of T cell PD-1

2. Design and test strategies to intervene with tumor relapse
T cells gene-engineered with a co-stimulatory TCR

**TCR**

*wild-type TCR*

**TCR:**

1st generation TCR

2nd generation TCR

CD3ζ CD3ζζ

CD3ε CD3δ

CD3γ CD3ε

Vα Vβ

Cα Cβ

TCR:ζ

TCR:28ε

Willemsen, Gene Ther, 2000
Schaft, J Immunol Methods, 2003
Schaft, Int Immunol, 2006
Yang, Int Immunol, 2007
Sebestyén, J Immunol, 2008
Roszik, Eur J Immunol, 2011

Schaft, Int Immunol, 2006
Govers, J Immunol, 2014
T cells expressing a co-stimulatory TCR decrease the chance of tumor relapse

Tumor growth

**TCR: 28ε**

Tumor-free until day 40: 4/8 (50%)

**TCR**

Tumor-free until day 40: 0/5 (0%)
T cells expressing a co-stimulatory TCR show prolonged persistence in blood
T cells expressing a co-stimulatory TCR are better able to infiltrate tumor tissue

Ex vivo cultures of TILs from relapsed tumors

Antigen specific CD8+ T cells

- pMHC binding T cells (%)
- p<0.01
- TCR:28e
- TCR
Tumor relapse following T cell therapy

Research aims:

1. Understand the mechanism(s) that contribute to tumor relapse
   Q1: Is tumor relapse due to loss or down-regulated expression of antigen after T cell therapy? **NO**
   Q2: Is T cell recruitment decreased in relapsed tumor after T cell therapy? **YES**
   *Straetemans, Mol Ther, in press*

2. Design and test strategies to intervene with tumor relapse
   - **T cells expressing a co-stimulatory TCR**
     - decrease chance of tumor relapse
     - enhance T cell persistence and T cell infiltration
   *Govers, J Immunol, 2014*

3. Translate findings to clinical T cell therapy
Choice of T cell target antigen
critical to the further development of T cell therapy

Toxicities related to T cell therapy

TILs: Vitiligo and uveitis in 35 and 15% of pts, respectively
Dudley, Science, 2002; Dudley, JCO, 2005

CAR T cells: Liver enzyme abnormalities in 50% of pts (CAIX – Rotterdam study)
B-cell anaplasia and cytokine release syndrome in responding pts (CD19 target)
Respiratory distress and death of pt (ERBB2)
Lamers, JCO, 2006; Lamers, Mol Ther, 2013; Porter, NEJM, 2011; Morgan, Mol Ther 2010

TCR T cells: Severe melanocyte destruction of skin, eyes and ears in 25% of pts (MARTI/HLA-A2; gp100/HLA-A2)
Inflammation of colon in responding pts (CEA/HLA-A2)
Neurological toxicity in 33% of pts (2 out of 3 pts died) (MAGE-A3/HLA-A2, shared epitope)
Cardiac toxicity and death in 2 pts (MAGE-A3/HLA-A1, recognition of similar epitope)
MAGE (Melanoma Associated antiGENs) C2 is a candidate T cell target antigen

**MAGE-C2:**
- shows a high tumor-specific expression and is silenced in most healthy tissues  
  Hofmann, PNAS, 2008
- is expressed by tumors of different histological origins and its expression is related to poor clinical outcome  
- has been shown to actively contribute to oncogenesis  
  Yang, Cancer Res, 2007
- induces a T cell response in patients without signs of toxicity  

**Status:** We sequence characterized and performed initial functional assays with 10 patient-derived MAGE-C2-specific TCRs  
Straetemans, Clin Dev Immunol, 2012; Kunert, Manuscript in preparation

**Planning:**

- Preclinical phase (nearly complete)
- Translational/GMP phase (nearly complete)
- Phase I trial (in preparation)
Design of TCR gene therapy trial

- Lymphodepletion
- Novel targets (i.e., MAGE-C2)
- Modified TCR (i.e., TCR28:ε)
- Cytokine pre-treatment (i.e., IL15+IL21)
- Azacytidine and Valproate treatment
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