Engineered T cells: Next-generation cancer immunotherapy

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Outline

- Introduction
  - Adoptive T cell therapy: Engineered CAR-T cells
  - Review of recent clinical trials
  - Challenges of CAR T cell therapy

- Results
  - T cell subsets: Th17 and Th9 cells in cancer immunotherapy

- Summary
  - Adoptive T cell therapy: what we learnt and what we should do next
Overview: Adoptive T cell therapy

1. Isolation of TILs or tumor specific T-cells from blood
2. Expand and activate T-cells *ex vivo*
3. Infuse the "boosted" T-cells into the patient.

- **Target therapy with Tumor specific T cells**
  - Cancer: Melanoma
  - Autologous tumor infiltrating lymphocytes (TILs); “Live drug”

- **Advantages**
  - High response rate (>50%),
  - Long-term remission,
  - Less toxic & gentler to the patient

- **Limitation:**
  - Extraction of TILs,
  - Cell manufacturing

- **Possible alternate**
  - T cell Engineering (CAR-T cells)

*Rosenberg SA & Dudley ME 2009 Current Opinion of Immunology*
Adoptive T cell therapy: CAR-T cells

- **CAR-T cells (Chimeric antigen receptor-T cells)**
  - T cells transduced with tumor-specific CAR
  - CAR: Single fusion molecule with antigen specificity plus signaling domain
  - Three types of CAR: First/second/generations
    - Based on co-stimulatory receptors
    - Cancer: Solid tumor & hematological malignancies

**Advantages of CAR T cells**

- “Live drug”
- Tumor recognition independent of HLA (no HLA typing needed)
- Multiple anti-tumor immuno-modulators can be engineered
- Target variety of antigens (protein, carbohydrate, glycolipid)

## Clinical significance of CAR-T cells

<table>
<thead>
<tr>
<th>Target</th>
<th>CAR</th>
<th>Cancer</th>
<th>Objective response</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD19</td>
<td>CAR:CD28-CD3ζ</td>
<td>Lymphoma and CLL</td>
<td>N=7: 1CR, 5 PR &amp; 1SD</td>
</tr>
<tr>
<td></td>
<td>CAR:CD137-CD3ζ</td>
<td>ALL</td>
<td>2CR</td>
</tr>
<tr>
<td></td>
<td>CAR:CD28-CD3ζ</td>
<td>ALL</td>
<td>5CR</td>
</tr>
<tr>
<td>CD20</td>
<td>CAR:CD137-CD28-CD3ζ</td>
<td>NHL</td>
<td>N=3: 1PR, 2NED</td>
</tr>
<tr>
<td>CEA</td>
<td>CAR-CD3ζ (1st gen)</td>
<td>Colorectal &amp; breast</td>
<td>N=7: minor responses in two patients</td>
</tr>
<tr>
<td>GD2</td>
<td>CAR-CD3ζ (1st gen)</td>
<td>Neuroblastoma</td>
<td>N=19: 3CR</td>
</tr>
<tr>
<td>ERBB2</td>
<td>CAR:CD28-CD137-CD3ζ</td>
<td>Colorectal cancer</td>
<td>N=1, patient died</td>
</tr>
</tbody>
</table>

Kershaw et. al. 2013 Nature Reviews cancer
Challenges of CAR-T cells

- **Toxicities**
  - **On target/off tumor toxicities**
    - Metastatic colon cancer patient died after 5 days of infusion of ERBB2+CAR-T cells
      - Low levels of ERBB2 express on lung epithelium (lung tox)
    - Renal cell carcinoma: 5/11 patients developed liver toxicity
  - **Cytokine syndrome**
    - Elevated levels of pro-inflammatory cytokines
      - Treatable by anti-IL-6mAb and steroids
Determinants of successful ACT: CAR-T cells

- **Tumor target**
  - Target antigen is critical determinant for efficacy & safety
  - Ideal target uniquely express on tumor cells or on cells which are not essential for survival

- **Efficacy & Long-term persistence**
  - Subtypes of CD4+T cells (Th1, Th2, Th17, Th9 cells),
  - CD8+T cells
    - naïve, central memory; long-term
    - effector; active but short lived

- **Trafficking of CAR T cells to tumor**
  - Expression of addressins
  - Route of CAR-T cell infusion
    - Intra-tumoral/intravenous
  - Optimal co-stimulation of T cells

- **Patient conditioning before ACT**
  - Reduced-intensity or non-myeloablative
  - Increased intensity myelo ablative
Outline

- **Introduction**
  - Adoptive T cell therapy: focus on engineered CAR-T cells
  - Overview of current & investigational CAR therapies
  - Challenges of CAR T cell therapy

- **Results**
  - Roles of T-helper cell subsets in cancer immunotherapy

- **Summary**
  - Adoptive T cell therapy: what we learnt and what we should do next
Adoptive T cell therapy: Right T cell population?

CD4+ T cells

Zou W & Restifo NP Nature Reviews Immunology 2010
Role of Th17 cells in tumor immunity is controversial

**Pro-tumor:**

- enhances vascularization/angiogenesis
- promotes metastasis
- promotes growth


**Anti-tumor:**

- enhances tumor immunity by promoting CD8+ T cell and DC function

Martin-Orcazzo et al Immunity 2010
Tumor growth suppression in RORγ-/- mice (Th17 cell deficient)

Melanoma tumor growth

Survival of tumor bearing host

Abrogation of Th17 pathways promotes anti-tumor immune responses
Gene-expression analysis: RORγ−/− CD4+T cells

Increased IL-9 expression in RORγ−/− CD4+T cells

Th17 genes

IL-17f
IL-17a
IL-23r

CD4+T cells differentiated under Th17 conditions (RORγ−/− / RORγ+/+ Fold change)
Treatment with exogenous rIL-9 suppresses tumor growth

**Efficacy study plan**

Day 0

Subq
B16F10 (1x10^5/100 μl)
LLC (1x10^5/100 μl)

Day 0 onwards

Follow tumor development (3x/week)

Results

Delayed tumor growth in IL-9 treated group

**IL-9 mediated anti-tumor effects are not limited to melanoma tumor model**
Effects of rIL-9 on melanoma tumor growth in Rag1-/- mice (T cell and B cell deficient host)

IL-9 mediated tumor growth suppression is independent of T cells and B cells
Effects of rIL-9 on tumor tumor growth in mast cell-/- mice

IL-9 mediated tumor growth suppression is dependent of mast cells
Engineering Th9 cells: TAA specific Tumor model

OT2 TCR transgenic mice (CD4 cells recognize ova)

Naïve OT2

CD4+CD25- CD62L+

TGFβ + IL-4

Th9

TGFβ + IL-6

Th17

Normal WT or Rag1-/-

i.v.

s.c.

B16F10-Ova cells

Follow tumor development

Generation of Ovalbumin expressing B16 tumor cells (Lentiviral method)
Treatment with Th9 cells suppresses tumor growth

Immunocompetent host (Wild type)

Treatment with Th9 cells suppresses tumor growth.

Graph showing tumor volume (mm$^3$) over days after tumor induction. The graph compares no T cells, Th17 cells, and Th9 cells. The Th9 cells show a significant suppression of tumor growth compared to the other groups.

Images showing tumors in different groups:
- No T cells
- Th17 cells
- Th9 cells
Th9 cell therapy: efficacy studies in immunodeficient host

Rag1-/- (T cells and B cells deficient)

- no T cells
- Th0 cells
- Th9
- Th9 plus α-IL-9

Th9 cells suppresses tumor growth independent of T cell and B cell presence
Mechanism of anti-tumor effects of Th9 cells

Effects of Th9 cells on CD8+T cells (OT-1 cells) proliferation

Presence of Th9 cells promotes the OT1-CD8-T cells proliferation.

OT1 cells: ovalbumin specific CD8+T cells
CFSE: Carboxyfluorescein succinimidyester
Mechanism of anti-tumor effects of Th9 cells

Examining Cytotoxic activity of Th9 cells

5mM CFSE labeled B16F10-ova cells + 0.5 mM CFSE labeled EL-4 cells

± OT2-Th0 or OT2-Th9

36 h.

Tumor cell lysis was measured by flow cytometry.

Th9 cells lyse tumor cells in antigen/target dependent manner

Target cells: B16-Ova
B16 ova specific Th cells: OT2-Th cells
What is the relevance of these findings in human?
Th9 cells are present in human “skin” and “blood”

Th9 cells are not just a murine phenomenon
### Summary

#### CAR-T cells
- T cells transduced with tumor-specific Chimeric Antigen Receptor (CAR)
- Tumor recognition independent of HLA (no HLA typing needed)
- Target: variety of tumor antigens (protein, carbohydrate, glycolipid)
- High response rate (up to 88%): pre-clinical and clinical findings

#### Limitation of CAR-T cells
- Toxicities
  - On target/off tumor toxicities
  - Cytokine syndrome
- Tumor microenvironment
  - Presence of MDSCs & Treg in tumor
  - Immunosuppressive agents

### Results & Conclusion
- IL-9 is a novel anti-tumor cytokine and anti-tumor effects are mediated via mast cells
- Th9 cells are the most superior anti-tumor Th cells
- Th9 cells exists in human: not just murine phenomenon
- Strategies that promotes IL-9 production will be a critical for the development of robust treatment for melanoma and lung carcinoma.
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