Genetically modified T cells to rebuild the adaptive immunity

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T-cell production

- T-cell generation, a slow process even in healthy context.

Diagram:
- Bone Marrow
  - Hematopoietic Progenitors CD34+
  - Lymphoid Progenitors
- Thymus
  - Lymphoid Progenitors
  - CD7+ T-cell precursors
  - T lymphocytes
- Periphery
  - TCR β recombination
  - TCR recombination
  - Positive Selection
  - Negative Selection
  - naive T lymphocytes

Commitment

TCR β recombination
• T-cell generation, a slow process even in healthy context.

half of the thymopoiesis process is devoted to the DN stage
T-cell production: pitfalls and how to speed it up

• Thymopoiesis is altered by:
  – Age of the recipient (thymic involution after puberty)
  – Disease, inflammation, treatments, infections
  – Limited thymic seeding progenitor supply

• Strategy: supply the recipient with:
  – large amounts of thymus seeding progenitors, to compensate the limited thymic colonization
  – Progenitors already engaged in the thymic process to skip the first steps of thymopoiesis
T-cell precursors in the thymus

Notch1/DL-4
IL-7, SCF, TPO and FL
Retronectin
Implementation of an artificial thymus mimicking the first steps of thymopoiesis

hDL-4/Fc=DL-4
Recombinant Fusion protein

CD34+
Hematopoietic stem and progenitor cells

DL-4
IL-7, SCF, TPO and FL
Retronectin

- Phenotype
- Molecular profile
- *In vitro* and *in vivo* potential
Production of CD7+ cells within 7 days

Day 3

Neonatal CB

Day 7

Adult mPB

→ Production of CD7+ cells within 7 days
Day 7 adult CD7+ cells are true T cell precursors

500 fold increase in the T-cell precursor frequency

Molecular profile of T-cell precursors

Early Lymphoid commitment

T lineage commitment

Thymus homing and crosstalk
Day 7 adult CD7+ cells are true T cell precursors

Irradiated or Busulfan conditioned adults
Day 0-3 newborns

→ Accelerated thymic engraftment

<table>
<thead>
<tr>
<th>Transplant (number of recipients)</th>
<th>Weeks post transplant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 0 CD34+ cells (n=9)</td>
<td>4-5 6-7 8-10 11-16</td>
</tr>
<tr>
<td>Day 3 DL-4 precursors (n=5)</td>
<td>0/1 0/2 1/2 4/4</td>
</tr>
<tr>
<td>Day 7 DL-4 precursors (n=7)</td>
<td>nd 1/2 0/1 2/2</td>
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<tr>
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<td>3/3 1/2 1/1 1/1</td>
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</tbody>
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Presence of human cells in the thymus

Day 0

Day 3

Day 7

0 4-5 6-7 8-10 11-16

Weeks post transplantation
Day 7 adult CD7+ cells are true T cell precursors

→ Improved T-cell recovery in the thymus and the periphery

Reimann, Stem Cells 2012
Simons-Debatin, Ma, submitted
Perspectives

• Phase I/II clinical trial based on the infusion of ex vivo generated T-cell precursors (January 2018)
  • Adults with malignant hemopathies transplanted with dCB units
  • Children with PIDs transplanted with haplo-identical adult CD34+ cells
• Objectives: toxicity, reconstitution of the naïve T cell pool, infections, relapses, survival

• Other applications of DL-4 culture: Production of genetically modified T-cell precursors
Can we combine DL-4 culture and lentiviral transduction?

• Advantages of T-cell precursors in gene therapy treatment of HIV
  
  • As compared to T cells: production of a pool of naïve T cells with a long life expectancy
  
  • as compared to HSC:
    • Fast production of T cells
    • No or reduced conditioning required
Production of genetically modified T-cell precursors

- Are DL-4 culture conditions compatible with transduction?
- Proof of concept with CD34+ cells from a SCID-X1 patient
Production of genetically modified T-cell precursors: POC

Generation of gene-corrected T-cell precursors (day 4)

GT

GT + DL4

2%

13.7%
Production of genetically modified T-cell precursors: POC

Generation of gene-corrected T-cell precursors (day 4)

Exposure to DL-4 accelerates the production of T cells *in vitro* with a diverse repertoire

*OP9/DL1 culture*

<table>
<thead>
<tr>
<th>Day 18</th>
<th>Day 25</th>
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<tbody>
<tr>
<td>Gene correction</td>
<td>Gene correction /DL-4</td>
</tr>
<tr>
<td>CD4</td>
<td>CD3</td>
</tr>
<tr>
<td>Comp-APC: CD8</td>
<td>Comp-APC: CD8</td>
</tr>
<tr>
<td>Comp-APC: CD3</td>
<td>Comp-APC: CD3</td>
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<tr>
<td>Comp-PEA: TCRγδ</td>
<td>Comp-PEA: TCRγδ</td>
</tr>
<tr>
<td>Comp-PEA: TCRαβ</td>
<td>Comp-PEA: TCRαβ</td>
</tr>
<tr>
<td>1.1%</td>
<td>1.1%</td>
</tr>
<tr>
<td>2.6%</td>
<td>8%</td>
</tr>
<tr>
<td>8%</td>
<td>8.1%</td>
</tr>
<tr>
<td>1.9%</td>
<td>0.15%</td>
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</tbody>
</table>
Conclusions and perspectives

HIV

• Transduction of CD34+ cells from HIV-1 infected patients with LV-c46-shCCR5

• *In vitro* and *in vivo* T-cell differentiation

• *In vitro* testing of the capacity of differentiated T cells to resist to HIV infection

• Use of a humanised murine model to test

  1) the capacity of the produced T cells to resist to HIV infection

  2) the survival advantage of gene corrected cells

• Toxicity studies (IS analysis, IVIM and SAGA assays)
Key points on T cell gene modification

- Activation of T-cell remains a prerequisite for T cell gene-modification via lentiviral vector
- Alternative: measles envelopes / targeted enveloped

- Different activation methods for different purposes:
  - Preservation of cell phenotype and plasticity properties
  - Expansion of gene-modified cells

- Remaining questions:
  - Determination of number of T-cell to be infused
  - Conditioning
  - Fate of transduced cells

- Progress acquired in T cell transduction can be applied to other immunodeficiencies: the example of FHL and IPEX
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