Effects of rhIL-7 administration in humans on in vivo expansion of naïve, memory and effector subsets of CD4$^+$ & CD8$^+$ T-cells

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Experimental Transplantation & Immunology Branch, National Cancer Institute, NIH, DHHS

Cytheris Inc., Vanves, France
This study was performed as a collaboration between

- National Cancer Institute, NIH, DHSS
- Cytheris, Inc. (Rockville, MD)

Under a Cooperative Research and Development Agreement (CRADA # 01649)

- Some of the co-investigators have financial interest in and / or are employees on Cytheris Inc.
- The other co-investigators (including the presenter / Principal Investigator) are federal employees and have no conflict on interest
IL-7 is a non redundant cytokine

IL-7 is critical in lymphoid development

IL-7 is critical in post development lymphocyte homeostasis

IL-7 multitude of immune properties may have important clinical applications
Possible IL-7 use in cancer vaccine / immuno-therapy

- **Lymphocyte count**
  - Expansion of naïve / memory T-cell pools
  - Anti-apoptotic effect during immune reconstitution following lymphodepleting therapies
  - ↑ T-cell proliferation upon engagement of the TCR

- **Widening the immune response**
  - Expansion of naïve T-cell pool increasing the repertoire of T-cell specificities
  - ↓ Threshold of immune response
  - Recruitment of sub-dominant immune responses

- **Generation of better effectors**
  - ↑ Cytotoxicity of sensitized lymphocytes
  - ↓ T-cell apoptosis following antigenic exposure
  - ↑ DC function (?)
  - *In vitro* or *in vivo*; in autologous or allogeneic settings
Phase I study of IL-7 (1)
NCI protocol 03-C-0152

Inter-subject dose escalation study

• Recombinant (E. Coli) human IL-7, “CYT 99 007”

• Provided by Cytheris Inc. (Rockville, MD)

• 4 cohorts of 3-6 subjects

• Doses: 3, 10, 30, 60 μg/Kg/ dose

• Given sub-cutaneously every other day for 2 weeks (8 doses)
Phase I study of IL-7 (2)
NCI protocol 03-C-0152

- **Primary end points**
  - Dose Limiting Toxicity (DLT)
  - Maximum Tolerated Dose (MTD)

- **Secondary end points**
  - Determine a range of biologically active doses
  - Pharmacokinetics and Pharmacodynamics
  - Possible anti-tumor activity
Phase I study of IL-7 (3)
NCI protocol 03-C-0152

Inclusion Criteria

• Diagnosis of incurable malignancy

• Measurable or evaluable disease

• **Stable peripheral CD3+ count > 300/mm³**
  • 4 determinations over 2 weeks prior to entry
  • No systemic steroids 2 weeks prior to CD3 determinations

• No therapy in previous 4 weeks with:
  • chemotherapy, cytokine immunotherapy,
  • anti-tumor vaccines or MoAb
Phase I study of IL-7 (4)
NCI protocol 03-C-0152

Exclusion Criteria

• Hematopoietic malignancies
• Primary carcinoma of the lung
• Life expectancy < 3 months
• HIV, hepatitis B, or hepatitis C
• Need for full anticoagulation or systemic steroids
• Hypertension uncontrolled with standard Rx
Phase I study of IL-7
Preliminary results (1)

- 11 men, 3 women,
- Age from 20 to 71 years (median: 48.5)
- With the following metastatic diseases:
  - renal cell carcinoma (2)
  - malignant hemangiopericytoma (1)
  - melanoma (4)
  - Adenocarcinoma: colon (1), duodenal (1), unknown primary (1)
  - Sarcomas: osteogenic (1), alveolar rhabdomyosarcoma (1), synovial cell (1)
  - Pheochromocytoma (1)
Phase I study of IL-7
Preliminary results (2)

- **Toxicity**
  - Grade 1-2 constitutional symptoms & local reaction
    - Chills, fever, malaise
    - 6-8 hours following injections
    - After most injections, in most subjects receiving $>3 \mu g /Kg/\text{dose}$
  - Grade 3 LFT elevation following first injection (DLT)
    - In 1 subject (Rx stopped, normalized within 5 days; possibly related)
  - Grade 3 chest pain, hypertension with *mild* Troponin elevation
    - Patient with Pheochromocytoma
    - After 3 doses (Rx stopped, normalized within 1 day; probably related)

- **Immunogenicity**
  - Non neutralizing anti-IL-7 antibodies (low titers) in 3 subjects
  - No neutralizing antibodies (DLT)
Total circulating lymphocytes

Day 14 = End of treatment

--- cohort 1 (3μg)  --- cohort 2 (10μg)  --- cohort 3 (30μg)  --- cohort 4 (60 μg)
----- cohort 1 (%)  ----- cohort 2 (%)  ----- cohort 3 (%)  ----- cohort 4 (%)
CD3⁺ / CD4⁺

CD3⁺ / CD8⁺

Day 14 = End of treatment

- cohort 1 (3μg)
- cohort 2 (10μg)
- cohort 3 (30μg)
- cohort 4 (60μg)

- cohort 1 (%)
- cohort 2 (%)
- cohort 3 (%)
- cohort 4 (%)

Mean % over baseline
T-cell subsets were defined and analyzed by multicolor Flow Cytometry

- after cell sorting of peripheral blood CD4+ & CD8+ cells
- at several time-points before, during and after IL-7 administration

\[
\begin{align*}
\text{CD}4^+ / \text{CD}45\text{RA}^+ / \text{CD}27^+ (\text{_________}) \\
\text{CD}4^+ / \text{CD}45\text{RA}^+ / \text{CD}31^+ (\text{_________} \text{Recent Thymic Emigrants}) \\
\text{CD}4^+ / \text{CD}45\text{RA}^- / \text{CD}27^+ (\text{_________}) \\
\text{CD}4^+ / \text{CD}45\text{RA}^- / \text{CD}27^- (\text{_________}) \\
\text{CD}8^+ / \text{CD}45\text{RA}^+ / \text{CD}27^+ (\text{_____}) \\
\text{CD}8^+ / \text{CD}45\text{RA}^- / \text{CD}27^+ (\text{_________}) \\
\text{CD}8^+ / \text{CD}45\text{RA}^- / \text{CD}27^- (\text{_________}) 
\end{align*}
\]
CD4⁺ / CD45RA⁺ / CD31⁺ (Most Naïve)

Day 14 = End of treatment
— Mean “1” — Mean “2” — Mean “3” — Mean “4”

CD4⁺ / CD45RA⁺ / CD27⁺ (Naïve)
Correlation of age with Most Naïve CD4

\[ R^2 = 0.0186 \]
CD4⁺/CD45RA⁻/CD27⁺ (Memory)

Day 14 = End of treatment
— Mean “1” — Mean “2” — Mean “3” — Mean “4”
**Kinetics of CD4+ Cell Cycling: Most naive**

CD4⁺ / CD45RA⁺ / CD31⁺ & Ki67⁺

**Percent of Most Naive cells in cycle**

<table>
<thead>
<tr>
<th>Time</th>
<th>Pre</th>
<th>D7</th>
<th>D14</th>
<th>D21</th>
<th>D55</th>
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<tbody>
<tr>
<td>IL7-R mRNA / 10⁴</td>
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<td>Bcl-2: Mean Fluorescence Intensity</td>
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</table>
Kinetics of CD4⁺ Cell Cycling: Naive

CD4⁺ / CD45RA⁺ / CD27⁺ & Ki67⁺

Percent of Naive cells in cycle

**IL7-R** mRNA / 10⁴
Actin mRNA

Bcl-2: Mean Fluorescence Intensity
Kinetics of CD4$^+$ Cell Cycling: Memory

CD4$^+$ / CD45RA$^-$ / CD27$^+$ & Ki67$^+$

Percent of Memory cells in cycle

IL7-R mRNA / 10^4 Actin mRNA

Bcl-2: Mean Fluorescence Intensity
Kinetics of CD4⁺ Cell Cycling: Effectors

CD4⁺ / CD45RA⁻ / CD27⁻ & Ki67⁺

**Percent of Effectors in cycle**

**Bcl-2**: Mean Fluorescence Intensity

**IL7-R** mRNA / 10⁴

Actin mRNA

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<td><strong>Bcl-2 Fluorescence Intensity</strong></td>
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<td><strong>IL7-R mRNA</strong></td>
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<td><strong>Actin mRNA</strong></td>
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MFI

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<td>2000</td>
<td>12000</td>
<td>22000</td>
<td>32000</td>
<td>42000</td>
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National Cancer Institute

Cytheris
CD8⁺ / CD45RA⁺ / CD27⁺ (Naïve)

Day 14 = End of treatment
— Mean “1” — Mean “2” — Mean “3” — Mean “4”
**CD8⁺ / CD45RA⁻ / CD27⁺ (Memory)**

- **CD8⁺ / CD45RA⁻ / CD27⁻ (Effector)**

**Day 14 = End of treatment**

- Mean “1”
- Mean “2”
- Mean “3”
- Mean “4”
Kinetics of CD8+ Cell Cycling: Naive

CD8+ / CD45RA+ / CD27+ & Ki67+

IL7-R mRNA / 10^4
Actin mRNA

Bcl-2: Mean Fluorescence Intensity

Percent of Naive cells in cycle
**Kinetics of CD8⁺ Cell Cycling: Memory**

CD8⁺ / CD45RA⁻ / CD27⁺ & Ki67⁺

**Percent of Memory cells in cycle**

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<tbody>
<tr>
<td>3%</td>
<td>9%</td>
<td>13%</td>
<td>5%</td>
<td>4%</td>
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**Bcl-2: Mean Fluorescence Intensity**

**IL7-R mRNA / 10⁴ Actin mRNA**

<table>
<thead>
<tr>
<th>Pre</th>
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<tbody>
<tr>
<td>Pre</td>
<td>D7</td>
<td>D14</td>
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<td>D55</td>
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<tr>
<td>-80%</td>
<td>-40%</td>
<td>0%</td>
<td>40%</td>
<td>80%</td>
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</tbody>
</table>
Kinetics of CD8⁺ Cell Cycling: Effectors

CD8⁺ / CD45RA⁻ / CD27⁻ & Ki67⁺

Percent of Memory cells in cycle

Bcl-2: Mean Fluorescence Intensity

IL7-R mRNA / 10⁴ Actin mRNA

Pre D7 D14 D21 D55

Percent of "Memory"

Pre D7 D14 D21 D55

2000 32000 42000 52000 62000
Mean % Increase over baseline of spleen size
(bi-dimensional product by CT)
TREC analysis: CD4⁺

Circulating CD4+ TREC

*Absolute number / mm³*

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<td>500,000</td>
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Circulating CD4+ TREC

*Percent Change*

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CD4⁺/CD45RA⁺/CD31⁺ (Most Naïve)

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Day 14 = End of treatment

% increase over baseline
CD4 T-regs / Fox-P3

Percent change over baseline in FoxP3 mRNA copies per 10^4 Actin copies

Pre  D7  D14  D21

-100% -80% -60% -40% -20%  0%  20%  40%  60%  80%  100%
Conclusions (1)

IL-7 appears to have, in humans, the range of biologic activity foreseen from animal data:

- Initial (Day 1) tissue redistribution of circulating T-lymphocytes
- Characteristic down-regulation of the IL-7 Rα and up-regulation of Bcl-2
- Reversible lymphoid organ enlargement: spleen, LN

- Induction of massive proliferation and expansion of T-lymphocytes subsets
  - In most naïve CD4 (Recent Thymic Emigrants)
  - In naïve, memory & effector subsets (CD4 & CD8)
  - Regardless of the subjects age

- These effects are:
  - Dose-dependent
  - Maximum within 1st week
  - Sustained several weeks after the end of IL-7 exposure
  - More pronounced in the naïve subsets
**Conclusions (2)**

- These findings set the stage for the design of clinical studies evaluating the possible role of IL-7 in augmenting immune responses in the context of anti-tumor vaccines and immunotherapy.
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