Studies of Lymphocyte Kinetics in HIV Infection

• To understand the effects on lymphocyte turnover of:
  – HIV infection
  – HAART therapy
  – Interleukin-2 therapy
• Methods-in vivo labeling
  – BrdU
  – Deuterated glucose
Bromodeoxyuridine (BrdU): Current Status

- DSMB review secondary to malignancies
  - Not definitively associated, but cannot be ruled out
  - Recommendation: No additional patients be enrolled and no additional BrdU be administered
  - Further utilization only in well-defined studies
Flow Cytometry Analysis of Cells Incorporating BrdU

Day 3

CD4 Cells

Monocytes

3.5%

1.9%

34.2%
Kinetics of BrdU Incorporation by Lymphocytes and Monocytes

- CD4+ T Cells
- CD8+ T Cells
- Monocytes
- B Cells
9 pts. underwent LN biopsy 8 hrs to 3 days after BrdU infusion (2 rpt bx)
Comparison of Ki67 and BrdU Labeling of Cells in Lymph Nodes

Ki67

BrdU

Secondary Follicle

CD4=1,146 cells/mm³
Kinetics of BrdU Decay Differ by Cell Type

- Semi-empiric modeling to describe delabeling (Dimitrov, Sidorov)
  - Parameters: decay rate; source ≈ size
- Single exponential decay for monocytes
- Biphasic exponential decay for CD4+ and CD8+ T lymphocytes:
  - Two populations of cells: rapid turnover and slow turnover
Incorporation of BrdU by Lymphocytes: Comparison of the Data to the Model

Fraction BrdU Incorporation

CD4 T Cells

CD8 T Cells

CD14 (Monocytes)

0.001 0.01 0.1

Time (days)

Diamonds, measured data
Line, modeling
Correlations Between the Source of the Rapidly Dividing Pool of Cells and Viral Load

**CD4 T Cells**

- \( R = 0.77, P < 0.001 \)

**CD8 T Cells**

- \( R = 0.81, P < 0.001 \)

**CD14 (Monocytes)**

- \( R = 0.56, P = 0.003 \)

No relationship between viral load and \( d_1 \), \( d_2 \), or \( s_2 \).
Changes in CD4 T Cell Production Following Initiation of Antiretroviral Therapy

Percent BrdU Incorporation

Time (days)

Before treatment
After treatment

8 hr Lymph Node Labeling

% BrdU

0.5
1.0
1.5
2.0

0
10
20
30
40
50
60

Time (days)
### Changes in CD8 T Cell Production Following Initiation of Antiretroviral Therapy

<table>
<thead>
<tr>
<th>Time (days)</th>
<th>Before treatment</th>
<th>After treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>4.0</td>
<td>2.0</td>
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<tr>
<td>10</td>
<td>3.0</td>
<td>1.5</td>
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<td>20</td>
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<tr>
<td>50</td>
<td>0.5</td>
<td></td>
</tr>
<tr>
<td>60</td>
<td>0.0</td>
<td></td>
</tr>
</tbody>
</table>

#### 8 hr Lymph Node Labeling

- **% BrdU**
  - 0.0%
  - 0.5%
  - 1.0%
  - 1.5%
  - 2.0%
Schematic of CD4 or CD8 T Lymphocyte Turnover: Low Levels of HIV Replication

Slowly proliferating pool: $T_{1/2} > 20$ days

Rapidly proliferating pool: $T_{1/2} \approx 2$ days

Periphery

Thymus

Progenitor cell

Death
Schematic of CD4 or CD8 T Lymphocyte Turnover: High Levels of HIV Replication

- Progenitor cell
- Slowly proliferating pool: $T_{1/2} > 20$ days, 60-90%
- Rapidly proliferating pool: $T_{1/2} \approx 2$ days, 10-40%
- Thymus
- Periphery
- Activation
- Death
Correlations Between the Source of the Rapidly Dividing Pool of Cells and Endotoxin Levels

**CD4**

\[ R = 0.28, P = 0.21 \]

**CD8**

\[ R = 0.40, P = 0.09 \]

**HIV**

\[ R = 0.52, P = 0.016 \]
Peak BrdU Labeling in Naïve and Memory Subsets

**CD4**

- Naïve
- Central Memory
- Effector Memory

**CD8**

- Naïve
- Central Memory
- Effector Memory
Peak BrdU Labeling in Activated Subsets

CD4

CD8

Percent BrdU Positive

Activated

Activated

= Median

= Median
CD4 vs. CD8 BrdU Incorporation

Initial Cohort of Patients

Poor Immunologic Responder Patients

- CD4 vs. CD8
  - r=0.89

- CD4<300 HIV<50

- CD4 vs. CD8
  - r=0.18

Controls
Peak BrdU Labeling in Naïve and Memory Subsets

Poor Immunologic Responder Patients

CD4

CD8

= Median

Naive, Central Memory, Effector Memory

Percent BrdU Positive

0 1 2 3 4 5

4+/RA+/27+, 4+/RA-/27+, 4+/RA+/27-, 4+/RA-/27-

8+/RA+/27+, 8+/RA-/27+, 8+/RA+/27-, 8+/RA-/27-
Peak BrdU Labeling in Activated Subsets

Poor Immunologic Responder Patients

- CD4
- CD8

Activated subsets with different co-stimulatory markers and their corresponding BrdU labeling percentages.
Intermittent IL-2 therapy increases CD4 counts in HIV-infected patients.

US Government has been granted a use patent for intermittent IL-2 therapy; J. Kovacs included as inventor.
CD4 Cells Show an Increase in Survival with Ongoing IL-2 Cycles

Percent Deuterium Incorporation

Weeks

IL-2 + Deuterium

No IL-2

T_{1/2}

No IL-2 2.5 wks
CD4 Cells Show an Increase in Survival with Ongoing IL-2 Cycles

JCI, 2005
CD4 Cells Show an Increase in Survival with Ongoing IL-2 Cycles

- Cycle 1: T_{1/2} = 1.5 wks
- Cycle 6: T_{1/2} = 42.7 wks

No IL-2

IL-2 + Deuterium

Percent Deuterium Incorporation

Weeks
CD8 Cells Show No Change in Survival with Ongoing IL-2 Cycles
The overall decay can be viewed as the decay of multiple populations.

The kinetics of labeled cells can be described by three parameters:

- $da$: average log decay
- $ds$: standard deviation of $da$
- $S$: sources
CD4 Cells Show an Increase in Survival with Ongoing IL-2 Cycles

T₁/₂:
- Pre-IL-2: 2.5 wks
- Cycle 1: 1.5 wks
- Cycle 6: 42.7 wks

PD = probability density function

log(Rate of turnover, week⁻¹)

PD*S, week⁻¹
CD8 Cells Show No Change in Survival with Ongoing IL-2 Cycles

**T $1/2$:**
- Pre-IL-2: 0.5 wks
- Cycle 1: 5.6 wks
- Cycle 6: 3.3 wks

$T = \frac{1}{2}$

**PD** = probability density function

**Pre-IL-2**

**Cycle 1**

**Cycle 6**

**log(Rate of turnover, week$^{-1}$)**

**PD*$S$, week$^{-1}$**
# Intermittent IL-2 Prolongs Survival of CD4 but not CD8 cells

<table>
<thead>
<tr>
<th>Cohort</th>
<th>CD4 cells</th>
<th></th>
<th>CD8 cells</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>Size</td>
<td>$t_{1/2}$</td>
<td>n</td>
</tr>
<tr>
<td>non-HIV</td>
<td>8</td>
<td>3.3</td>
<td>4.1</td>
<td>7</td>
</tr>
<tr>
<td>HIV, no IL-2</td>
<td>9</td>
<td>15.1</td>
<td>0.5</td>
<td>9</td>
</tr>
<tr>
<td>IL-2, cycles 3-28</td>
<td>15</td>
<td>70.3</td>
<td>37.6</td>
<td>15</td>
</tr>
</tbody>
</table>

$p$, non-HIV vs. HIV, no IL-2  0.04  0.01  0.02  0.06
$p$, HIV, IL-2 vs. HIV, no IL-2 0.002 <0.001 <0.001 0.13
CD4 Cell Increase Following an IL-2 Cycle Correlates with Turnover Rate

\[ R = -0.67, \ p < 0.0001 \]
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Special thanks to the patients for their willingness to participate in these studies.