Clinical trials in HIV Cure

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Pitié-Salpêtrière Hospital, Paris
- Decrease reservoir
  Drug free remission: Functional cure

- Eradicate reservoir
  Sterilizing cure

Drug burden decrease: Reduce ARV
Potential strategies to reduce HIV reservoirs

Maraviroc
- Anti-inflammatory drugs
  - Statins
  - Gold salts
  - OH-Chlorochin

Systemic Inflammation
- Massive CD4 T-cell depletion
- Bacterial translocation

Pre-Probiotics

Viral Co-Infections
- Antiviral drugs

Immune Activation

HIV Reservoirs Latency

Residual Replication

ARV Intervention
- Intensification
- Nevirapine

Cellular Immunity
- Anti-HIV vaccine
- IL7

Immune Intervention
- Anti-HIV vaccine
- IL7

Pre-Probiotics

Gene therapy

Quiescent T cells activation
- IL7

Pre/post-transcriptional factors disruption
- HDACi
- HMBA methylation inhibitors

Anti-co-stimulatory molecules
- anti PD1 / anti PDL1
- anti-CTLA4
- anti-CD137
Cure-related clinical pilot trials in progress in 2012/13

<table>
<thead>
<tr>
<th>Study</th>
<th>Intervention</th>
</tr>
</thead>
<tbody>
<tr>
<td>ERAMUNE-01</td>
<td>Interleukin-7 + ART intensification (RAL/MVC)</td>
</tr>
<tr>
<td>ERAMUNE-02</td>
<td>HIV-rAd5 vaccine + AT intensification (RAL/MVC)</td>
</tr>
<tr>
<td>Disulfiram</td>
<td>Disulfiram 500 mg/day for 14 days</td>
</tr>
<tr>
<td>Vorinostat</td>
<td>Vorinostat 200-600 mg/day</td>
</tr>
<tr>
<td>Vorinostat</td>
<td>Vorinostat 400 mg/day</td>
</tr>
<tr>
<td>Panobinostat</td>
<td>Panabinostat 20 mg 3 times/week for 8 weeks</td>
</tr>
<tr>
<td>CD4 T cells with modified CCR5 by zinc-finger nuclease</td>
<td>Autologous CD4 T cells with modified CCR5 gene by zinc finger nuclease SB-728-T</td>
</tr>
<tr>
<td>Lentivirus vector rHIV7-shl-TAR-CCR5RZ- transduced haematopoietic progenitor cells</td>
<td>Autologous CD34+ haematopoietic cells modified by lentivirus-transduced non-functional CCR5RZ gene</td>
</tr>
<tr>
<td>Interferon alfa-2b</td>
<td>Interferon alfa-2b intensification</td>
</tr>
</tbody>
</table>

Katlama et al. Lancet 2013
International, multicenter, randomized, non-comparative controlled study of therapeutic intensification with raltegravir /maraviroc with or without Interleukin 7 in HIV-infected patients with suppressed viremia

The ERAMUNE 01 Study

Christine Katlama, Sidonie Lambert-Niclot, François Lecardonnel, Laura Papagno, Lambert Assoumou, Giuseppe Tambussi, Bonaventura Clotet, Mike Youle, Dominique Costagliola, Brigitte Autran and the EraMune-01 Study Group
Rationale for using IL-7 to target latently infected cells

IL-7 is a potent and proviral strain–specific inducer of latent HIV-1 cellular reservoirs of infected individuals on virally suppressive HAART


IL-7 induces HIV production in vitro from resting CD4 T cell subsets, independently of cell proliferation

Bacchus et al. PlosOne 2013

The Challenge of Finding a Cure for HIV Infection

Douglas B. Richman,1,2 David M. Margolis,3 Martin Delaney,4† Warner C. Greene,4 Darla Hanusa,5 Roger J. Pomerantz3

6 March 2009 Vol 323 Science

Viral Inducibility

Cell Proliferation

Bacchus et al. PlosOne 2013
Eramune 01 : Milestones

★ EraMune Concept and identification of therapeutic strategies for controlling the reservoirs

★ March: Finalization of EraMune Studies Design

★ November: Initial IRB/IEC submission (France)

★ May/June: Complete IRB/IEC authorizations for the 4 countries

★ September: 1st patient included

★ May: Last patient included (SP)

★ July: Last patient W56 visit

★ February: last patient W80 visit

★ March: CROI 2013: W56 results

★ July: IAS 2013: W80 and secondary endpoints results
The EraMune-01 Study Design

**Screening**
- W -4

**Therapeutic Intervention**
- D0
- W8
- W24
- W56
- W80

**cART intensification + IL7 (RAL/MVC/IL7)**
- IL-7: 3 weekly injections (W8, 9, 10)
- Dose: 20 μg/kg/week

**Randomization**

**Primary Endpoint**

**Post-intervention interruption follow-up**

**cART intensification (RAL/MVC*)**

*RAL/MVC doses*
- RAL: 400 mg BID
- MVC: 150/300/600 mg BID depending on concomitant cART
Study Objectives

- **Primary endpoint**
  - Decrease from baseline in HIV proviral DNA in the PBMCs at week 56 of at least 0.5 log copies per million PBMCs

- **Secondary endpoints**
  - Change in HIV total proviral DNA and in CD4 T cell subsets,
  - Changes in CD4 and CD8 lymphocyte count and CD4/CD8 ratio
  - Changes in activation and differentiation markers of the CD4 and CD8 peripheral blood T cells
  - Safety
Main Eligibility Criteria

- HIV-1 infected adult between 18 and 70 years
- At least 2 years of suppressive ART
- CD4+ count ≥ 350 cells/mm³
- $10 \leq \text{Proviral DNA} \leq 1000 \text{ copies/10}^6 \text{ PBMCs}$ within 60 days of entry
- No active hepatitis B or C
- No prior exposition to immune modulators
Biological Samples Lab Processing

**Centralized**
- HIV-DNA ultrasensitive real-time PCR (Biocentric® Kit)
- Immunological investigations

**Locally**
- Screening HIV-DNA HIV-RNA plasma VL CD4/CD8 counts
## Immunological assessments

<table>
<thead>
<tr>
<th></th>
<th></th>
<th>All Visits</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><strong>CD4 T Lymphocytes count (fresh blood – local labs)</strong></td>
<td></td>
</tr>
</tbody>
</table>
| 2 | Quantitative and phenotypic characterization of the different CD4 and CD8 T cells subsets (blood and rectal mucosa):  
   - **Naïve**: CD27⁺, CCR7⁺ and CD45RA⁺  
   - **Central Memory**: CD27⁺, CCR7⁺ and CD45RA⁻  
   - **Transitional Effector Memory**: CD27⁺, CCR7⁻ and CD45RA⁻  
   - **Effector Memory**: CD27⁻, CCR7⁻ and CD45RA⁻  
   - **Effector**: CD27⁻, CCR7⁻ and CD45RA⁺  
   **T cells activation** (CD38⁺, CD25⁺, CD69⁺, HLA-DR⁺) and IL7R (CD127) expression | Day 0, Weeks 8, 12, 28, 32 and 56                                           |
| 3 | Monitoring of the effects of rhIL7 therapy on **cell cycling** and **apoptosis** (Ki67, Bcl-2, and other possible markers) | Day 0, Weeks 8, 12, 28, 32 and 56                                           |
| 4 | Peripheral Blood Mononuclear Cells (PBMCs) storage for future additional immunologic testing | Day 0, Weeks 36 and 56                                                     |

*Fresh rectal mucosa cells and cryopreserved PBMCs were stained and analyzed in flow cytometry on a 5-laser LSRFortessa cell analyser (Becton Dickinson) on the CyPS platform (UPMC)*
Eramune 01: Flowchart

Screened (n=57)

Eligible (n=31)

- Screen failure (n=26)
  - DNA >1,000 (n=17)
  - DNA < 10 (n=2)
  - VL too high at screening (n=1)
  - Consent Withdrawals before inclusion (n=4)
  - CD4 < 350 (n=1)
  - No 3 years ART (n=1)

Randomized (n=29)

- Not randomized (n=2)
  - Consent Withdrawals (n=1)
  - Serious adverse event (n=1)

RAL/MVC (n=14)

RAL/MVC+IL-7 (n=15)

Analysed (n=14)

Analyses (n=15)
### Patients baseline characteristics

<table>
<thead>
<tr>
<th></th>
<th>RAL/MVC n=14</th>
<th>RAL/MVC/IL7 n=15</th>
<th>Total n=29</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age (years)</strong></td>
<td>43 (36 – 48)</td>
<td>49 (42 – 56)</td>
<td>47 (41 – 53)</td>
</tr>
<tr>
<td><strong>Sex (% Male)</strong></td>
<td>13 (93 %)</td>
<td>14 (93 %)</td>
<td>27 (93 %)</td>
</tr>
<tr>
<td><strong>Time on cART (years)</strong></td>
<td>7 (5 – 13)</td>
<td>13 (11 – 15)</td>
<td>12 (8 – 14)</td>
</tr>
<tr>
<td><strong>CD4 nadir (cells/mm³)</strong></td>
<td>267 (216 – 579)</td>
<td>248 (125 – 309)</td>
<td>252 (171 – 351)</td>
</tr>
<tr>
<td><strong>CD4 count (cells/mm³)</strong></td>
<td>549 (416 – 769)</td>
<td>561 (462 – 701)</td>
<td>558 (452 – 726)</td>
</tr>
<tr>
<td><strong>CD8 count (cells/mm³)</strong></td>
<td>719 (642 – 954)</td>
<td>680 (540 – 881)</td>
<td>703 (603 – 900)</td>
</tr>
<tr>
<td><strong>Ratio CD4/CD8</strong></td>
<td>0.79 (0.56 – 0.93)</td>
<td>0.83 (0.54 – 1.12)</td>
<td>0.81 (0.56 – 0.99)</td>
</tr>
<tr>
<td><strong>Duration of VL &lt; 50 cp/mL (years)</strong></td>
<td>2.2 (2.1 – 2.4)</td>
<td>2.4 (2.3 – 2.9)</td>
<td>2.3 (2.1 – 2.6)</td>
</tr>
<tr>
<td><strong>HIV-DNA copies/10⁶ PBMCs</strong></td>
<td>287 (106 – 822)</td>
<td>564 (320 – 869)</td>
<td>360 (228 – 828)</td>
</tr>
</tbody>
</table>
Massive Amplification of CD4 counts with IL7: W56 Results

<table>
<thead>
<tr>
<th></th>
<th>BL vs W56</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>RAL/MVC</td>
<td>0.017</td>
<td></td>
</tr>
<tr>
<td>IL-7</td>
<td>0.001</td>
<td></td>
</tr>
</tbody>
</table>

The chart shows a box plot with median change from baseline in CD4 count cells/mm3 over weeks 4 to 56 for RAL/MVC and RAL/MVC+IL-7 groups. The IL-7 intervention significantly amplifies CD4 count changes compared to baseline and RAL/MVC alone.
Eramune 01 : Primary endpoint

No patient reached the 0.5 log copies decrease (3-fold change) in total HIV proviral DNA /10^6 PBMCs at Week 56

<table>
<thead>
<tr>
<th>Treatment</th>
<th>P-value BL vs W56</th>
</tr>
</thead>
<tbody>
<tr>
<td>RAL/MVC</td>
<td>0.875</td>
</tr>
<tr>
<td>RAL/MVC+C/IL-7</td>
<td>0.088</td>
</tr>
</tbody>
</table>
Median changes in blood HIV DNA from Baseline to W56 (Nb copies)

<table>
<thead>
<tr>
<th>HIV-DNA</th>
<th>Baseline (n=27)</th>
<th>Delta W56-BL (n=27)</th>
<th>p-value (BL vs W56)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Per 10⁶ PBMCs</td>
<td><strong>RAL/MVC</strong> 287 (108 - 822)</td>
<td>-7 (-66 - 96)</td>
<td>0.875</td>
</tr>
<tr>
<td>Per 10⁶ CD4 cells</td>
<td><strong>RAL/MVC</strong> 586 (299 - 1912)</td>
<td>+11 (-129 - 470)</td>
<td>0.463</td>
</tr>
<tr>
<td>Per mL whole blood</td>
<td><strong>RAL/MVC</strong> 666 (369 - 1474)</td>
<td>+156 (-117 - 446)</td>
<td>0.116</td>
</tr>
<tr>
<td>HIV-DNA copies</td>
<td>BL n=27</td>
<td>Δ W56-BL n=27</td>
<td>p-value (BL vs W56)</td>
</tr>
<tr>
<td>----------------</td>
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<td>---------------</td>
<td>---------------------</td>
</tr>
<tr>
<td>Per 10⁶ PBMCs</td>
<td>RAL/MVC/IL-7</td>
<td>570 (305 - 964)</td>
<td>+92 (-41 - 353)</td>
</tr>
<tr>
<td>Per 10⁶ CD4 cells</td>
<td>RAL/MVC/IL-7</td>
<td>1315 (651 - 2094)</td>
<td>+41 (-87 - 656)</td>
</tr>
<tr>
<td>Per mL whole blood</td>
<td>RAL/MVC/IL-7</td>
<td>1321 (548 - 2004)</td>
<td>+909 (143 - 3028)</td>
</tr>
</tbody>
</table>
Median change of total HIV DNA in rectal mucosa

### Median values in rectal mucosa HIV DNA log_{10}/10^6 cells

<table>
<thead>
<tr>
<th>HIV-DNA log_{10}/10^6 cells</th>
<th>Baseline n=18</th>
<th>W56 n=18</th>
<th>ΔW56-BL n=18</th>
<th>p-value (BL vs W56)</th>
</tr>
</thead>
<tbody>
<tr>
<td>RAL/MVC n=9</td>
<td>2.26 (2.04-2.96)</td>
<td>2.47 (2.27-2.82)</td>
<td>0.04 (-0.09-0.23)</td>
<td>0.374</td>
</tr>
<tr>
<td>RAL/MVC/IL-7 n=9</td>
<td>2.57 (2.35-2.88)</td>
<td>2.83 (2.42-2.94)</td>
<td>0.14 (-0.14-0.46)</td>
<td>0.314</td>
</tr>
</tbody>
</table>

ΔW56 – BL = 0.14 (-0.14 – 0.46) \( p = 0.314 \)

ΔW56 – BL = 0.04 (-0.09 – 0.23) \( p = 0.374 \)
Median change in CD8 count at W56

<table>
<thead>
<tr>
<th></th>
<th>P-value BL vs W56</th>
</tr>
</thead>
<tbody>
<tr>
<td>No IL-7</td>
<td>0.300</td>
</tr>
<tr>
<td>IL-7</td>
<td>0.001</td>
</tr>
</tbody>
</table>
Median changes in blood CD4 subsets

<table>
<thead>
<tr>
<th></th>
<th>Results in Percentages</th>
<th>Results per mm3 of blood</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Median changes in blood CD4 subsets (%)</td>
<td>Median changes in blood CD4 subsets (absolute values)</td>
</tr>
<tr>
<td></td>
<td>p=0.001</td>
<td>p=0.001</td>
</tr>
<tr>
<td>CD4 Naive</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CD4 Central Memory</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CD4 Transitional</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Memory</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CD4 Effector</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DR+ (Activated)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Graphs:**
- **Results in Percentages**
  - RAL/MVC
  - RAL/MVC/IL7
- **Results per mm3 of blood**
  - RAL/MVC
  - RAL/MVC/IL7
The HIV reservoirs in sorted CD4 T cell subsets do not significantly change from baseline with or without IL-7.

- Cell sorting of resting (DR-, CD25-, 69-) CD4 T cell subsets:
  - Naive
  - Central-Memory
  - Transitional-Memory
  - Effector-Memory
- Quantification of total HIV-DNA in each subset ex-vivo
Durable amplification of CD4 cells from Baseline to W80

- P-value
  - BL vs W80: 0.035
  - RAL/MVC: 0.035
  - IL-7: 0.015

Change from baseline in CD4 count cells/mm3

Week:
- 4
- 8
- 12
- 16
- 20
- 24
- 28
- 32
- 36
- 40
- 48
- 56
- 80
Changes in Total HIV DNA in PBMCs
Results at W80

HIV DNA back to baseline in IL7 arm
Trends towards a decrease in HIV DNA in RAL/MVC arm at W80
<table>
<thead>
<tr>
<th>HIV-DNA log₁₀ copies</th>
<th>Basline n=27</th>
<th>Δ W56-BL n=27</th>
<th>p-value (BL vs W56)</th>
<th>Δ W80-BL n=28</th>
<th>p-value (BL vs W80)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Per 10⁶ PBMCs</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RAL/MVC</td>
<td>2.45 (2.03-2.91)</td>
<td>-0.02 (-0.10-0.09)</td>
<td>0.875</td>
<td>-0.18 (-0.35-0.04)</td>
<td>0.064</td>
</tr>
<tr>
<td>RAL/MVC/IL-7</td>
<td>2.75 (2.50-2.94)</td>
<td>0.02 (-0.04-0.24)</td>
<td>0.088</td>
<td>0.03 (-0.07-0.27)</td>
<td>0.331</td>
</tr>
<tr>
<td><strong>Per 10⁶ CD4 cells</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RAL/MVC</td>
<td>2.78 (2.50-3.29)</td>
<td>0.02 (-0.08-0.19)</td>
<td>0.463</td>
<td>-0.21 (-0.31-0.03)</td>
<td>0.096</td>
</tr>
<tr>
<td>RAL/MVC/IL-7</td>
<td>3.12 (2.83-3.30)</td>
<td>0.00 (-0.04-0.23)</td>
<td>0.198</td>
<td>0.07 (-0.05-0.29)</td>
<td>0.397</td>
</tr>
<tr>
<td><strong>Per mL whole blood</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RAL/MVC</td>
<td>2.84 (2.66-3.19)</td>
<td>0.04 (-0.12-0.26)</td>
<td>0.116</td>
<td>-0.21 (-0.31-0.04)</td>
<td>0.177</td>
</tr>
<tr>
<td>RAL/MVC/IL-7</td>
<td>3.12 (2.93-3.29)</td>
<td>0.19 (0.07-0.36)</td>
<td><strong>0.001</strong></td>
<td>0.22 (0.02-0.35)</td>
<td><strong>0.022</strong></td>
</tr>
</tbody>
</table>
No patient neither in the RAL/MVC Intensification nor in the RAL/MVC/IL7 arm was able to reach at W56 the primary end point of a 0.5 log\(_{10}\) decrease in cell associated DNA/10\(^6\) PBMC from baseline.

IL-7 combined to ARV intensification induces:

- A massive peripheral blood CD4 (and CD8) T cell expansion:
  - persisting at W56 and W80
  - involving primarily the blood central-memory T cells (T\(_{CM}\)), but not the rectal ones,
  - contrasting with a relative decline in:
    - Naïve CD4 T cells, and senescent Effector CD4 T cells
    - Activated CD4 T cells

As a consequence a transient increase is observed in the HIV reservoir (HIV-DNA copy numbers) within the 6-8 months post-infusion:
- back to baseline values at W56 and W80 when calculated per million PBMC,
- but not when calculated /mm\(^3\) of blood, due to the persisting CD4 T cell increase
The transient increase in the HIV reservoir induced by IL7

- does not reflect an increase of the reservoir within sorted TCM,
- but reflects the $T_{CM}$ expansion, the main contributor to HIV reservoirs,
  - which may mask the HIV reactivation effect of IL-7:
    - although HIV mRNA transcripts were detectable in 90% enrolled patients and before and 2-12 months after IL-7 infusions

- precludes the use of IL-7 as a strategy to purge the HIV reservoirs
- Dual RAL/MVC intensification induces
  - No change in the total peripheral blood HIV-DNA reservoirs over 56 weeks
    - Trend towards a decrease at W80

- This effect suggests a continuous low level HIV production persists during fully suppressive ARV
  - As assessed by detection of HIV mRNA transcripts in CD4 T cells from 90% patients
  - Need for evaluation of long term suppressive therapies with optimal intracellular drug penetration
**Rationale:**
To target Residual virus production with anti-HIV immune effectors induced by anti-HIV vaccine

**International, multicenter, randomized, non-comparative controlled study of therapeutic intensification plus vaccine in HIV-infected patients with long-term viral suppression**

**ERAMUNE-02 (USA, PI: R Murphy)**

**ARM A**
- W-4
- Randomization
- W0
- 3 ARV + Intensification (raltegravir + maraviroc)

**ARM B (n=14)**
- W8
- 3 ARV + Intensification (raltegravir + maraviroc) + VRC Vaccine:
  - 3x HIVDNA016-00-VP (W8, 12, 16)
  - 1x rAd5 (W32)
  - VRC-HIVADV014-00-V

**Primary Evaluation**
- W56

**Primary objective:**
- Decrease in the HIV-1 viral reservoir
- Results end Q2 2013

Potential long term ART strategies based on HIV reservoir.

- Can we decrease HIV DNA?
- Can we stop ART in patients with undetectable HIV DNA?
- Can we reduce drug burden?
**Rationale:** Functionnal cure possible

- **Mississippi baby**
- **Visconti cohort**
- **Salto patients**

All have been early (very) ART patients
All have

- Low HIV reservoir
- High CD4; normal CD4/CD8 ratio

**Objectives**

- **To stop ARV** in patients with undetectable HIV Reservoirs « good » immune profile
  - To test the concept of Functional Cure

- **To study the mechanisms and correlates of cure**

**Agenda:** 2013 - Q4
ULTRASTOP Design

Eligibility criteria
Group I, n = 5 patients
- CD4 count ≥ 500 cells/mm³
- Ratio CD4/CD8 ≥ 0.9
- Nadir CD4 ≥ 300 cells/mm³
- VL HIV-RNA < 50 copies/mL under ART > 2 years
- VL HIV-RNA < 20 copies/mL at baseline
- HIV-DNA < limit of detection

STOP ART

HIV-RNA VL and CD4 at W2 and W4 and then every 4 weeks till W24, and every 8 weeks till W48

W8 of the last patient of Group I: if at least 1 patient out 5 without failure*

Inclusion of Group II
n = 5 patients

Principal Evaluation Criteria: W24

In case of failure
- virologic
- or immunologic
- or clinic

Immediate ART re-introduction

W8 of the last patient of Group II: if at least 2 patients out 10 without

Inclusion of Group III
n = 5 patients

*FAILURE
VL > 400 copies/mL confirmed or CD4 < 400/mm³ confirmed or AIDS-related event (CDC B or C)
Big Bang 13.82 billion years ago

Necessitates international partnerships
Thanks to our partners in research
The Patients