How to deal with the needle in the haystack??

**Heavy hitters**
- Romidepsin
- Vorinostat

**Selective activators**
- autologous vectors

“Jimmy Hoffa” approach
Encasing the latent HIV in “cement” or the “Jimmy Hoffa approach”

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Davidson et al., PNAS 2009; Lalonde et al., PLoS Path 2011
Encasing the latent HIV in “cement”

Inhibition of NL4-3 in U87.CD4.CXCR4 cells

Cellular uptake of fluorescein labelled L50

Inhibition in human PBMCs

IC₅₀ (µM)

<table>
<thead>
<tr>
<th>Drug</th>
<th>IC₅₀ (µM)</th>
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</thead>
<tbody>
<tr>
<td>3TC</td>
<td>0.1</td>
</tr>
<tr>
<td>L-50</td>
<td>0.25</td>
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<tr>
<td>L-51</td>
<td>0.01</td>
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<tr>
<td>L-22</td>
<td>0.065</td>
</tr>
</tbody>
</table>

Relative RT activity

[drug] (µM)

0.1  1  10  100  1000

Relative Infection (%) vs [drug] (µM)
Justification for selective activators?
Douek et al. Nature 2002

Would you see higher HIV-infected, HIV-specific CD4+ T cells if autologous peptide pools were used in this study?
EARLY HIV INFECTION

HIV-1

A
B
C
D

C-specific CD4+ T cells

B-specific CD4+ T cells

A-specific CD4+ T cells

D-specific CD4+ T cells

TT-specific CD4+ T cells

CMV-specific CD4+ T cells

HIV-specific TCR

flu-specific CD4+ T cells
EFFECTIVE HAART

C-specific memory CD4+ T cells
B-specific memory CD4+ T cells
D-specific memory CD4+ T cells
A-specific memory CD4+ T cells
CMV-specific CD4+ T cells
flu-specific CD4+ T cells
TT-specific CD4+ T cells
Cloning of near full length HIV-1 genomes from a patient into a defective virus vaccine construct

1. Extract RNA from plasma
2. PCR amplify the genome in overlapping thirds - recombine in yeast
3. ENV Amplicon
   - Mixed
   - No recombination
   - Recombination

Vectors selected by the indicated media:
Ex vivo testing of the autologous activating vector

1. Transfected 293T cells
2. Dendritic cells
3. DC-T cell co-cultures
4. Harvest defective virus vaccine
5. Induced virus from T cells
6. Vaccine vector
7. Harvest cell-free supernatant and extract RNA
8. Preferentially PCR amplify induced HIV-1 in T cells for viral load determination and 454 sequence analyses
Autologous activating vector production

Patient CH1

1. Sample for vaccine prep day 1221
2. Plasma: full genome amplification in overlapping thirds
3. Initiation of HAART day 2658
4. Collection of PBMCs day 3557

Days post infection

Viral load (RNA copies/ml)

Patient CH2

1. Samples for vaccine prep day 103, day 322
2. Collection of PBMCs day 2981
3. Initiation of HAART day 2152

Days post infection

Viral load (RNA copies/ml)

Diversity of HIV in:

- day 1221 plasma
  - n = 835
- Vaccine from day 1221 plasma
  - n = 1225

Diversity of HIV in:

- day 103/322 plasma
  - n = 1888
- Vaccine from day 103/322 plasma
  - n = 1225

454 sequence analyses to confirm that the autologous activating vector has a similar HIV-1 population as found in the patient
Protocol for priming DCs and co-cult with T cells

1. Sample for vaccine prep day 1221
2. Initiation of HAART day 2658
3. Collection of PBMCs day 3557
4. Maturation of DC from monocytes/macrophages
5. Isolation of memory CD4+ T cells by negative selection
6. ADD autologous vaccine NL4-3 vaccine flu/tetanus/CMV
7. Mix loaded DC with T cells
Activation of HIV production from memory T cell/DC co-culture

**CH1 autologous vaccine**

**NL4-3 vaccine**

**flu/tetanus/CMV**

**30-fold difference**

**20-fold increase**

**viral RNA levels (copies/ml)**

Genetic analyses of the stimulated virus population

vaccine day 1221 plasma

flu tetanus CMV

n = 1474

n = 3964

n = 1858

0.0050 sub/nt

vaccine day 103/322 plasma

flu tetanus CMV

n = 1474

n = 1858

NL4-3 vaccine

n = 3964

n = 1858
Could this vector be safe for HAART treated patient?

1. The patient is already infected with the specific HIV-1 population used to make the “dead” autologous vaccine/activator

2. The gRNA packaging element is mutated to prevent packaging

3. This therapeutic/activator autologous viral vector would only be used in patients on stable, ongoing HAART

4. Alternatively, we could use the third generation system, three vector system with the “transgene” vector that is defective for gRNA packaging
• The HIV-1 population from plasma can be efficiently sampled and amplified as overlapping genomic fragments and cloned into a vector by yeast recombination.

• An “activating” vector derived from the HIV-1 population prior to HAART efficiently activates HIV-1 in latently infected CD4 T cells from patient on stable HAART.

• An “activator” based on NL4-3 or a common antigens (Flu/Tetanus/CMV) are less efficient at activating HIV-1 production in latently infected CD4 T cells via DC presentation.

• Question: Will this activating vector also serve as therapeutic vaccine?

• Question: What is the fate of the HIV-specific activated T cells? The uninfected HIV-specific CD4 T cells may “help” or boost the CTL response
Autologous Activator Consortium

10 patients identified at acute infection, immediately treated, and stable HAART

10 chronic patient on stable HAART

5 peds patients infected perinatally, treated early, and on stable HAART

Sample for autologous vector

Large volume blood draw for ex vivo studies
- Autologous activator (multivalent)
- Heterologous activator (multivalent)
- HDAC inhibitors
- CD3/CD28 and PHA stimulation
- Viral RNA and DNA assessment in before and after stimulation
- Analyses of the fate of activated T cells

GMP grade production for phase I trials in these 25 patients

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Imperial/Oxford
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VGTI
Nicolas Chomont, Rafick Sekaly

Los Alamos National Laboratory
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Development of autologous, multivalent, therapeutic (activating) vaccine for activation of the latent HIV pool

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