Anti-proliferative therapy for HIV Cure

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Overview

• Mechanisms of HIV reservoir persistence despite ART
• Anti-proliferative strategy for decreasing the HIV reservoir
• Clinical trial of MMF to reduce the HIV reservoir
HIV dynamics during ART

The HIV reservoir

• The body:
  • 1-10 million latently infected cells
  • One cell per $10^6$ resting memory CD4+ T-cells
  • Cells disseminated throughout the body

• The infected cell:
  • HIV is integrated into human chromosomal DNA
  • One HIV DNA molecule per infected cell
  • Low protein expression & low immunogenicity
The HIV reservoir is remarkably stable

Siliciano, Nat Med, 2003
Crooks, JID, 2015
The HIV reservoir is challenging to measure
Possible mechanisms of HIV persistence during ART

- Ongoing replication
- Longevity
- Proliferation
## Therapeutic implications

<table>
<thead>
<tr>
<th>Mechanism</th>
<th>Therapeutic solution</th>
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<tbody>
<tr>
<td><strong>HIV replication</strong></td>
<td>• Micro-anatomic ART sanctuary</td>
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<td></td>
<td>• Improved delivery of ART to micro-anatomic sanctuaries (nanoparticles, new agents)</td>
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<tr>
<td><strong>Infected cell longevity</strong></td>
<td>• Lack of HIV epitope expression</td>
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<td></td>
<td>• Lack of HIV replication</td>
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<td>• HIV latency reactivating agents</td>
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<td></td>
<td>• Vaccines / CAR T cells / antibody infusions / stem cell transplant with CCR5</td>
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<td></td>
<td>deleted cells</td>
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<td><strong>Infected cell proliferation</strong></td>
<td>• Homeostatic proliferation</td>
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<td>• Antigen driven proliferation</td>
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<td></td>
<td>• Lymphocyte anti-proliferation therapies (MMF, azathioprine)</td>
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</tbody>
</table>

**Diagram:**

- **ongoing replication**
- **longevity**
- **proliferation**
Evidence for cellular proliferation during ART

Von Stockenstrom, JID, 2015.
Replication competent sequences exhibit clonal proliferation

Clonal proliferation is confirmed by human chromosomal integration site data

Schroder, Cell, 2002.

Mathematical modeling
What is the true percentage of HIV reservoir cells generated via cellular proliferation?

The experiment is the equivalent of randomly sampling 100 Americans & assigning clonality according to city of residence.
Computationally recreate the entire iceberg

Generate 1000s of theoretical clonal distributions of all HIV infected cells in the reservoir

- $10^9$ total HIV DNA sequences
- $10^7$ replication competent HIV sequences
- Free parameter: Power law slope
- Free parameter: Sequence richness

Model fit to data

Generate 1000s of theoretical clonal distributions of all HIV infected cells

Randomly sample 100 sequences from each distribution & fit to proportional abundance curves from actual data

Model prediction: a highly organized clonal structure of the HIV reservoir

Total HIV DNA

Replication competent HIV DNA

A small number of massive clones

A massive number of small clones

A small number of massive clones

A massive number of small clones

HIV DNA in the reservoir has a remarkable similarity to the distribution size of towns & cities in the USA

Reality:
• 10⁹ total HIV DNA sequences
• 2.25x10⁸ Americans
• 10⁴-10⁵ unique HIV DNA sequence clones
• 19354 American towns / cities
Conclusion: HIV persistence during ART is primarily due to cellular proliferation

- >99% of cells with HIV DNA were generated via cellular proliferation
- >98% of cells with replication competent HIV DNA were generated via cellular proliferation
Anti-proliferative therapy to reduce the HIV reservoir

\[
\begin{align*}
\dot{S} &= \alpha_S - \delta_S S - \beta_e S V \\
\dot{L} &= \theta_L L + \tau \beta_e S V \\
\dot{A} &= (1 - \tau) \beta_e S V - \delta_A A + \xi L \\
\dot{V} &= \pi A - \gamma V
\end{align*}
\]
Mathematical model of the HIV reservoir

- A central memory CD4+ T cell proliferates ($\alpha_L$) once every 45 days
- HIV reactivates ($\xi$) from a latently infected memory CD4+ T cell once every 138 years
Anti-proliferative therapy requires far less potency than latency reactivating agents for therapeutic success

\[ \dot{L} = \alpha L - \delta L - \xi L \]

Potency (multiple of \( \xi \))

Potency (divisor of \( \alpha \))

functional cure suppression for 1 yr in 50% of patients
Effect of reservoir heterogeneity on time to eradication

Chomont et al. Nat Med 2009
Mycophenolate mofetil (MMF)

- Specifically targets proliferation of B and T lymphocytes
- Prevents organ rejection following solid organ transplantation
- Prevents graft versus host disease following hematopoietic cell transplantation
- Steroid-sparing agent for autoimmune diseases
- Well tolerated & safe in hundreds of persons with HIV
- Associated risk of opportunistic infection (when co-dosed with prednisone)
- Teratogenic
Decreased numbers of HIV infected cells following 24 weeks of mycophenolate mofetil (MMF) in 3 of 6 treated people.

Chapuis A et al. Nat Med 2000
MMF clinical trial
MMF clinical trial

- **Hypothesis**: Prolonged anti-proliferative therapy for 2 years will lower reservoir volume of HIV DNA and replication competent HIV
- **5 participants fully suppressed on ART with CD4 nadir >350/uL** will receive MMF twice daily
- Historical controls
- Frequent evaluation for neutropenia, lymphopenia, infections
- Inclusion criteria include documented *in vivo* response to MMF using serum & PBMCs from participants
MMF clinical trial

- Optional GI biopsy
- Frequent safety monitoring / blood draws early during study
- No ATI planned
- Emphasis to participants that cure is unlikely
Study progress

- 4 enrolled
  - 6 month analysis pending
  - Drug well tolerated

- Results CROI 2020!
HIV DNA as a primary outcome

• Pros:
  • HIV DNA will remain positive for longer than QVOA if there is a therapeutic effect
  • The therapeutic effect could theoretically be equal for HIV DNA & QVOA
  • Less sample to sample variability
    • HIV DNA median clearance rate: -0.017 log / year (IQR: -0.061 – 0.02, range: -0.195 – 0.166)
    • QVOA: 0.4 log changes on samples separated by 3 months occur 7% of the time

• Cons:
  • Pre-treatment HIV DNA does not correlate with QVOA
  • Optimal timing of ART treatment interruption unknown

Crooks, JID, 2015
Plans for a negative result:

- A lack of reduction in reservoir volume would **not** reject the hypothesis that cellular proliferation sustains the HIV reservoir

<table>
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<tr>
<th>Possible cause</th>
<th>Assessment</th>
<th>Solution</th>
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<tbody>
<tr>
<td>Poor drug delivery</td>
<td>• Drug levels</td>
<td>• Higher dose</td>
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<tr>
<td>MMF resistance</td>
<td>• Anti-proliferative assay</td>
<td>• Screen participants</td>
</tr>
<tr>
<td>Daughter cell death may be functionally linked to proliferation</td>
<td>• IL-7 levels pre &amp; during MMF&lt;br&gt;• Radiolabeling study (future)</td>
<td>• Limit IL-7 effects</td>
</tr>
<tr>
<td>Reduction of infected CD4+ memory cells with persistence of infected macrophages</td>
<td>• ??</td>
<td>• ??</td>
</tr>
<tr>
<td>Toxicity</td>
<td>• Trial</td>
<td>• Lower dose</td>
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Plans for a positive result

- A meaningful reduction in reservoir volume would strongly support the hypothesis that proliferation sustains the HIV reservoir
- Further trials in Africa, Asia
- Trials in persons with primary HIV
- Combination trials
- Specific targeting to CD4 T cells
10,000 simulated trial participants

Suppression for 1 yr in 50% of patients
Functional cure

ART alone

stress (years on ART + anti-proliferative)
MMF as a combination therapy

• May antagonize:
  – Latency reversal agents
  – CAR T cells / immunotherapies
  – Therapeutic vaccines

• May synergize:
  – “Block & lock” approaches

• May be additive:
  – Passive neutralizing antibody
Conclusions

• The HIV reservoir is sustained by clonal proliferation of infected cells
• Anti-proliferative therapy may reduce HIV reservoir volume & achieve functional cure
• Results from MMF study coming soon!
Thank you!

• Study participants
  – Mel Padullo
  – Eric Helgeson
  – Bob Harrington
  – Katrina Puckett

• UW ACTU
  – Mel Padullo
  – Eric Helgeson
  – Bob Harrington
  – Katrina Puckett

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• Schiffer group
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  – Dave Swan
  – Pavi Roychoudhury
  – Fabian Cardozo Ojeda
Possible evidence for ongoing HIV replication


Possible evidence for longevity of infected cells