Reservoirs and HIV cure in Adults and Children: Insights and Challenges

Caroline T. Tiemessen
DST/NRF Research Chair of HIV Vaccine Translational Research, University of the Witwatersrand
Centre for HIV and STIs,
National Institute for Communicable Diseases
The need for an HIV cure

- Combination antiretroviral therapy (cART) has been instrumental in transforming an otherwise fatal disease into a chronic condition BUT cART cannot eradicate HIV

- Mathematical models have shown that most patients would need HAART for 60-80 years for reservoirs to be depleted

- Factors such as cost, availability, adherence and the development of drug resistance, consequences of residual elevated inflammation, long-term toxicities, stigma and discrimination highlight the need for curative interventions
HIV cure - definitions

More feasible

- **Functional cure**
  - Long term viral suppression in the absence of HAART
  - Transmission is possible, but very rare
  - HIV RNA < 50 copies/ml

- **Remission**
  - Transient
  - Sustained
  - Early ART, ?

Less feasible

- **Eradicating cure**
  - Elimination of all HIV-infected cells/replication-competent virus, no need for treatment
  - HIV RNA < 1 copy/ml

- **Cure**

Elite controllers:
study model for “functional cure”

The Berlin patient

Post-treatment controllers (PTCs)

Mississippi baby
"The Berlin patient"

Eradication of HIV-1?

First proof-of-concept that HIV-1 infection can be cured, and although not practical in approach, has injected renewed interest in the possibility of HIV-1 eradication.

Bone-marrow transplantation with cells from HLA-matched CCR5Δ32 donor

Timothy Ray Brown

CURED
Transient “remission” is possible

How can sustained “remission” be achieved?

Biomarkers?

Remission off ART – 3 and 7 months

Remission off ART – 27 months

Undetectable

only one person cured

VISCONTI post-treatment controllers - 7+ years remission

Why HIV cannot be cured with current therapies

- **HIV persists in a latent form in different cellular and anatomical reservoirs** (blood and tissue: GIT, genital tract, CNS)

  ART and the host immune response are ineffective in eliminating reservoirs

- The major reservoir of cells that harbour latency in vivo are **resting memory CD4+ T cells**
  - Central memory CD4+ T-cells (CD45RA- CCR7+ CD27+)
  - Transitional memory CD4+ T-cells (CD45RA- CCR7- CD27+)

- Latent infection can be established in **other long lived cells** including
  - Naive T-cells
  - Bone marrow progenitor cells
  - Thymocytes
  - Astrocytes
  - Other cells such as monocyte/macrophages can support long lived low level productive infection
Memory T cell frequency, pathogen susceptibility and mortality throughout human life

Memory T cells
- most abundant lymphocyte population
- predominantly quiescent
- capable of intermittent self-renewal
- long-term survival
- heterogeneous

Peripheral blood only contains 2-2.5% (5-10 x 10⁹ cells) of the total T cell complement in the body

Blood, intestines, skin, liver, brain and lymphoid tissue

Farber et al, 2014
The pathway of T cell differentiation is not well understood in humans.

- Functionally different
- Serve as reservoirs for HIV
Memory T cell heterogeneity in the blood and in tissues

Viral processes
- replication
- latency
- reactivation from latency

are dependent on:

Cellular composition
- cell type/subset
- cell state
- nature of latency

Extracellular environment
- type of stimuli
- exposure time

Farber et al, 2014
Mohammadi et al, www.co-hivandaids.com
Persistent HIV Infection

4 Phases of viral decay: correspond to half-lives of different cell populations

Residual viremia

Phase | Half-life | Cell type
--- | --- | ---
1 | 1 - 2 days | Activated CD4+ T-cells
2 | 2 weeks | Partially activated CD4+ T-cells, macrophages, dendritic cells
3 | 39 weeks | Resting memory CD4+ T-cells
4 | ∞ | Resting memory CD4+ T-cells

The IAS Scientific Working Group on HIV Cure: Nature Reviews Immunology 2012
Palmer et al. JIM 2011
Palmer, AIDS 2014
Measuring Persistent HIV

Latent infection

Productive infection

Co-culture methods: IUPM
Replication-competent virus

External HIV RNA
HIV DNA*
2-LTR circles
Integrated DNA Infectious Units (IUPM)
Cell associated RNA
US RNA and MS RNA
HIV RNA (SCA)

Lewin & Rouzioux, AIDS 2011
Rouzioux & Richman, 2012

From Palmer, AIDS 2014
Current and future cure strategies

- Intensification of HAART (+/- other interventions)
- Early initiation of HAART (limit viral reservoirs)
- Improved Drugs (penetrating anatomical compartments)
- Reactivation of HIV replication from latent reservoirs: ”Kick and Kill” (LRA: latency reversing agents)
- Gene-editing therapy (Zinc fingers to disrupt CCR5)
- Interfering with immunological mechanism that contribute to HIV persistence (reactivation with IL-7)
- Therapeutic vaccines (enhance HIV-specific immunity)
- Broadly neutralizing/other antibodies, bispecific antibodies
Timing of ART initiation in acute infection and HIV reservoirs

- HIV persistence is established early in acute HIV infection (AHI) - in long-lived memory CD4+ T cells

- ART instituted during AHI can reduce the HIV reservoir size to a greater extent than when treatment is given in chronic HIV
  BUT HIV persists in memory CD4+ T cells in most early treated individuals

- Recent data suggest that treatment in the earliest AHI stage (Fiebig I) may protect central memory CD4+ T cells from infection and skew the distribution of latently infected cells to the shorter lived memory CD4+ T cells as is the case in individuals who control HIV without ART (elite controllers and PTCs)

- ART in AHI may be the first critical step in the path to achieve HIV remission by containing HIV reservoir seeding prior to instituting additional interventions to eliminate all latently infected cells

Ananworanich et al, Curr Opin HIV/AIDS 2015; Buzon et al, JVI 2014
Size and composition of the latent HIV reservoir

Ananworanich et al, Curr Opin HIV/AIDS 2015
Post-treatment controllers (PTCs)

**VISCONTI cohort**

*(Viro-Immunologic Sustained CONtrol after Treatment Interruption)*

<table>
<thead>
<tr>
<th>Médian (IQR)</th>
<th>PHI</th>
<th>Before interruption</th>
<th>After interruption</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD4/mm³</td>
<td>544</td>
<td>915</td>
<td>855</td>
</tr>
<tr>
<td>Ratio CD4/CD8</td>
<td>0.70</td>
<td>1.51</td>
<td>1.48</td>
</tr>
<tr>
<td>Viral loads, Log cp/mL</td>
<td>5.2</td>
<td>&lt;1.7</td>
<td>&lt;1.7</td>
</tr>
</tbody>
</table>

Therapy started within 10 weeks following primary Infection
36 months on therapy followed by a median of 89 months (>7 years) of control off therapy

Sáez-Cirión et al, PLoS Pathogens 2013

Published reports of PTCs: Lisziewicz et al, NEJM 1999 (N=1); Lafeuillade et al, J Infect Dis 2003 (N=2); Steingrover et al, AIDS 2008 (N=2); Hocqueloux et al, AIDS 2010 (N=5); Salgado et al, Retrovirology 2011 (N=1); Goujard et al, Antiv Therapy 2012 (N=14); Lodi et al, Arch Intern Med 2012 (N=11); Sáez-Cirión et al, PLoS Pathogens 2013 (N=14) [VISCONTI study]
VISCONTI Post-Treatment Controllers Characteristics

- Lack protective HLA-B alleles that are overrepresented in HIV controllers (HICs)

- Poor CD8 T cell responses

- More severe primary infections than HICs

- Incidence of viral control after interruption of early ART = higher among PTCs than for spontaneous control

- Maintain low reservoir, and in some reduce this reservoir to very low

- Low levels of T cell activation

- The mechanism of the viral control is different from that observed for HIV controllers

Saez-Cirion et al. PLoS Path 2013
PTCs have specific NK cell phenotypes with high anti-HIV capability

Compared to ECs and healthy donors:
- Higher expression of KIR2DL1, KIR2DL2 and NKG2A
- Higher expression of KIR3DL1 (vs HD)
- Lack of expression of CD160
- Lower expression of NKp46
- Lower activation as measured by CD69 marker
- Higher production of IFN-γ (normal degranulation)

- High capacity to control in vitro HIV infection of autologous CD4 T cells

Suggests early treatment may protect NK cells, vulnerable subset/s?
Other cytokines?
Genetic characteristics?

Scott-Algara D. et al CROI 2014
Elite controllers: model for functional cure

- Virus fitness not a major factor (mutations/defective virus)
- Small reservoirs

- Strong HIV-specific T-cell responses
- Favourable HLA types
- Neutralizing antibodies not a major factor
- ADCC responses elevated
- Enhanced activity of myeloid dendritic cells
- Increased production of IL-21

- Long term effects
  - Loss of CD4 (7%)
  - Ongoing virus replication and evolution
  - Immune activation increased

Long term nonprogressors

Best candidates for testing sterilizing cure strategies?

Deeks SG and Walker BD, Immunity, 2007
In general, HIV-specific T cell responses are strong—a spectrum of magnitudes and breadth of CD4 and CD8 T cell responses to pools of HIV-1 peptides ranged from absent to substantial.
Other characteristics of LTNPs/ECs

**LTNPs/ECs vs Healthy controls?**

- CD4 and CD8 **T cell activation** levels are higher

- **CCR5 density** on T cells and monocytes significantly decreased – a more “quiescent” T cell phenotype, but % CCR5+ CD8T cells significantly elevated

- **Soluble CD14** plasma levels similar – suggests no major activation of monocytes

- **PHA-induced cytokines**
  - “Blunted” production of proinflammatory chemokines: CCL3, CCL4, IL8
  - Th1>Th2 profile: IL2, IFNγ, G-CSF, GM-CSF, TNF = not different, **IL4 and IL10 decreased, IL12 increased**
Immunity in early life: Factors that may limit or favor HIV persistence

**Persistence**

- Few memory CD4+ T cells
- Fewer activated CD4+ T cells
- Higher levels CC chemokines, neonatal NK and CD8+ T cells suppression of HIV > than adults

**Persistence**

- Abundance of CD4+CCR5+ T cells in gut
- Immature innate immune responses
- Immature adaptive immune responses
- High viremia
- Memory-like T cells in cord blood


Shalekoff et al, ARHR 2004
Early ART in children

• Early vs deferred ART improves **survival**

• Influences **viral control** on ART

• Reduces **reservoir size** (integrated DNA, Total HIV DNA, 2-LTR circles, RNA)

• Reduces detection of **HIV-specific immune responses** (antibodies and CD4/CD8 T cell responses), **immune activation**

Cases of early treated infants - transient remission

Mississippi baby
ART at 30 hours
HIV RNA 19,812 c/ml DNA+
18 months
HIV RNA 16,750 c/ml
Viral rebound after 27 months off ART

Canadian baby
ART at <24 hours
HIV RNA 152,560 c/ml
Viral rebound after 2-3 weeks off ART

Milan baby
ART at 12 hours
HIV RNA 808 c/ml
1 month
3 months
3 years
High % activated T cells
HIV-specific T cell responses

All 3 had undetectable: HIV DNA, Replication-competent virus, HIV Ab

Persaud, NEJM 2013; Brophy, IAS 2014; Giacomet, Lancet 2014
Early ART Clinical Trials in children

- **4 NIH-funded clinical trials:** IMPAACT P1115 (9 countries – Argentina, Brazil, Haiti, Malawi, South Africa, Uganda, US, Zambia and Zimbabwe); EIT Botswana; LEOPARD Johannesburg South Africa; Thailand (J Ananworanich)

**The question:** Can early ART initiation soon after birth lead to remission of HIV such that children can stop treatment for an extended period of time?

**Markers to monitor HIV persistence:**

**Viral:** HIV RNA (VL and cell-associated), DNA (total and 2-LTR circles), Replication-competent virus

**Host:** HIV Ab, HIV-specific T cell responses, immune activation, B and T cell subset maturation
Challenges: pediatric early treatment

- Identifying HIV-positive babies at birth and starting treatment ASAP (POC testing), PMTCT has reduced TM rates to <2%

- In utero infection – when does transmission occur, duration of infection prior to birth/treatment?

- Dynamic changes in cell subsets, immune markers, immune capability with age

- Small sample volumes from neonates and young infants

Rouzioux et al.  American Journal of Epidemiology 1995
Host genetic variability and phenotypic outcomes

The human host: “Unprecedented genetic diversity” in Africa

Tishkoff et al, Science 2009

- This also predicts that within Africa in particular, the many genotypes will produce many different phenotypes
  - susceptibility to microbe infection
  - rate of disease progression/disease severity
  - response to adjuvants and to vaccinations
  - response to treatments
  - response to cure interventions

Sex differences

- Women have a **lower burden of bacterial, viral and parasitic infections** BUT a **higher prevalence of autoimmune diseases, stronger vaccine responses**

- Following seroconversion **women** have up to 40% lower VLs and higher CD4 counts than men

- **BUT** at same level of viraemia, women **progress to AIDS faster** (1.6-fold higher risk) – ultimately similar times to AIDS

- **Higher T cell activation in women** than men when adjust for VL (same level of CD8 T cell activation immune as men at 1 Log₁₀ lower VL)

- Women have **more frequent ART side effects** and discontinuation of treatment

In summary – some gaps and considerations.....

• There is very little known about the potential for **demographic factors** to affect safety or efficacy of curative interventions.

• A systematic review (1991-2011): **participation in cure studies does not reflect the burdens of infection in women, older adults and nonwhites** – studies largely conducted in young white males; only 23% of 151 publications reported demographic data and only 6% conducted efficacy analyses.

Johnston and Heitzeg, ARHR 2015

• Limited information on establishment of **reservoirs in tissue compartments**

• Need continued development of **assays for measuring cellular and tissue reservoirs** – accurate, sensitive, reproducible, standardized

• Continue to identify **additional biomarkers** that help predict cure outcomes
In conclusion....

- Resource-limited settings – additional challenges compared to high-income settings:
  - Responses to interventions are likely different (host and environmental factors)
  - Virus clades are different
  - Other coinfections

- Treatment in early and acute infection and pediatric studies are feasible (Limiting seeding of the HIV reservoir) - requires early diagnosis and early treatment, and subsequent monitoring of sustained viral suppression – technically possible but each represent a challenge

- Need to consider the large numbers of HIV-infected individuals who have started ART beyond the acute infection stage – an even greater challenge for cure strategies

The ideal of controlling HIV-1 in the absence of ART (remission) or eradicating HIV-1 (elimination) will have enormous individual and public health benefits
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