A Collaborative Research Agenda Towards Curing HIV

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Presented at the 13th Int. Workshop on Clin. Pharmacology of HIV Pharmacology – 2012, Barcelona Spain
The Challenge of Finding a Cure for HIV Infection

DD Richman, DM Margolis, M Delaney, WC Greene, D Hazuda & RJ Pomerantz
Goal: “The goal of this initiative is to expand the knowledge base on HIV latency and persistence so that eradication strategies can be designed, developed, and evaluated.”

Requirements: Linked, multidisciplinary, collaborative projects with both basic research and translational activities, including a project led by a private sector entity
Why Can’t We Cure HIV with ARVs

Where is the virus and how is it maintained in the face of “suppressive” therapy?

Defining the problem…..
HIV-1 “Hides” in Resting Cells in a Replication Competent, Latent State

Presence of an Inducible HIV-1 Latent Reservoir During Highly Active Antiretroviral Therapy
Tae-Wook Chun, Lieven Stuyver, Stephanie B. Mizell, Linda A. Ehler, Jo Ann M. Mican, Michael Baseler, Alun L. Lloyd, Martin A. Nowak, and Anthony S. Fauci

Recovery of Replication-Competent HIV Despite Prolonged Suppression of Plasma Viremia
Joseph K. Wong, Marjan Hezareh, Huldrych F. Günthard, Diane V. Havlir, Caroline C. Ignacio, Celsa A. Spina, Douglas D. Richman

Identification of a Reservoir for HIV-1 in Patients on Highly Active Antiretroviral Therapy
Diana Finzi, Monika Hermankova, Theodore Pierson, Lucy M. Carruth, Christopher Buck, Richard E. Chaisson, Thomas C. Quinn, Karen Chadwick, Joseph Margolick, Ronald Brookmeyer, Joel Gallant, Martin Markowitz, David D. Ho, Douglas D. Richman, Robert F. Siliciano

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The Reservoir of HIV Latently Infected Cells Decays Slowly

Decay of Latent Reservoir in Patients with full HAART suppression for 3-7 years

- HIV Integrates into cells with extremely long biological half-life
- Integrated HIV persists in patients on HAART
- Latent reservoir makes HIV infection incurable
- Potential for clonal expansion of latently infected cells and proliferation


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The Reservoir of HIV Latently Infected Cells may be Replenished & Maintained

HIV reservoir size and persistence are driven by T cell survival and homeostatic proliferation

Chomont et al, Nature Medicine, 2010
HIV Latency may Result from Transcriptional Silencing during Memory Cell Differentiation

Antigen → Naïve T-cell → Activated T-cell → Proliferating effector cells → Resting memory T-cell

HIV infection

Resting memory T-cell with latent HIV provirus
Persistent low level viremia can be detected in nearly all patients on HAART.

Median 3.1 copies RNA/mL at week 60

Nelfinavir
Lopinavir/Ritonavir

Common Pathways may Affect T-cell Function and HIV-1 Latency

PD-1 (inhibitory receptor programmed death 1) is member of CD28/CTLA-4 family of T-cell regulators

- The interaction of PD-1 w/PDL-1 inhibits the effector function of HIV-specific CD8+ T cells
- Triggering PD-1 inhibits HIV-1 production; PD-1 blockade enhances HIV-1 production (Sekaly and Chomont, unpublished)

- PD-1 expressing CD4+ T cells are a preferential reservoir for HIV
  - PD-1high CD4+ T Cells are enriched for integrated HIV DNA (Chomont et al, Nat Med 2009)
  - Cell-associated RNA and proviral DNA levels are positively correlated with frequencies of CD4+ and CD8+ T cells expressing PD-1 (Hatano et al, IAS 2011)
T cell activation declines, but remains abnormal even after many years of HAART

The size of the HIV reservoir (defined by RNA/DNA ratio) is associated with frequency of activated CD4+ T cells in rectal tissues.

Spearman's rho: 0.65
P=0.012

Hunt, Yukl and Wong

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Residual HIV replication?

- Arguments against HIV replication
  - Lack of impact of intensification on plasma HIV RNA or plasma inflammatory markers
  - Lack of evolution in plasma and GALT

Multiple studies show RAL intensification does not impact residual plasma viremia

**Treatment intensification does not reduce residual HIV-1 viremia in patients on highly active antiretroviral therapy**


**Short-Course Raltegravir Intensification Does Not Reduce Persistent Low-Level Viremia in Patients with HIV-1 Suppression during Receipt of Combination Antiretroviral Therapy**

…but intensification may impact immune activation?

HIV-1 replication and immune dynamics are affected by raltegravir intensification of HAART-suppressed subjects

Maria J Buzón¹,9, Marta Massanella¹,9, Josep M Llibre², Anna Esteve³, Viktor Dahl⁴, Maria C Puertas¹, Josep M Gatell⁵, Pere Domingo⁶, Roger Paredes¹,², Mark Sharkey⁷, Sarah Palmer⁴, Mario Stevenson⁷, Bonaventura Clotet¹,², Julià Blanco¹ & Javier Martinez-Picado¹,⁸

Massanella et al., CROI 2011
Residual HIV replication?

- Arguments against HIV replication
  - Lack of impact of intensification on plasma HIV RNA or plasma inflammatory markers
  - Lack of evolution in plasma and GALT

- Arguments supporting HIV replication
  - Ex vivo evidence and theoretical modeling suggest replication may involve cell-to-cell transfer in T cell-rich zones
  - Intensification reduces HIV RNA in ileum
  - Antiretroviral drugs may not penetrate those tissues where HIV is known to persist at high levels (lymph nodes, gut)
  - Inflammation remains high during therapy and activated cells are enriched for HIV (compared to resting cells)
Is tissue the issue?

Effect of raltegravir-containing intensification on HIV burden and T-cell activation in multiple gut sites of HIV-positive adults on suppressive antiretroviral therapy.

Yukl, Steven; Shergill, Amandeep; McQuaid, Kenneth; Gianella, Sara; Lampiris, Harry; Hare, C; Pandori, Mark; Sinclair, Elizabeth; Gunthard, Huldrych; Fischer, Marek; Wong, Joseph; Havlir, Diane

DOI: 10.1097/QAD.0b013e32833ef7bb

Fig. 2. Change in cell-associated unspliced HIV RNA (a, b) and HIV DNA (c) per 106 CD4+ T cells. HIV copy numbers were measured by real-time PCR, normalized for the total cell input into the PCR (by μg RNA or DNA), and then normalized to the percentage of all cells that were CD4+ T cells (by flow cytometry). (b) Shows the HIV RNA values in the ileum for each participant at weeks 0 and 12. In (a) and (c), column heights indicate the average of the changes (week 12-week 0) in HIV copy number per 106 CD4+ T cells, as measured from peripheral blood mononuclear cells or total gut cells (obtained by collagenase digestion of endoscopic biopsies) from each of the four gut sites. Error bars indicate the standard error of measurement (SEM).
Multiple Potential Mechanisms of HIV Persistence

- Low-level (“cryptic”) viral replication, including cell-to-cell transfer of HIV and/or inadequate pharmacology
- Long-lived reservoir of resting CD4+ T cells that harbor transcriptionally silent, integrated (latent) HIV genomes
- Long-lived reservoir of non-T-cell populations (e.g., tissue macrophages)
- Homeostatic proliferation of CD4+ T cells that harbor latent genomes
- Lack of effective HIV-specific immunity

Not mutually exclusive but, in fact, may be interdependent mechanisms
Why can’t we cure HIV with ARVs
Where is the virus and how is it maintained in the face of “suppressive” therapy?

Virus Host Interactions

Latently infected cells

Homeostatic proliferation

Persistent viremia

Residual replication

Combinations will likely be needed to induce and clear the latent reservoir

Inflammation

Immune dysfunction
Goal: “The goal of this initiative is to expand the knowledge base on HIV latency and persistence so that eradication strategies can be designed, developed, and evaluated.”

Requirements: Linked, multidisciplinary, collaborative projects with both basic research and translational activities, including a project led by a private sector entity

Outcome: Three groups funded (total five year commitment of $70 million)
Steve Deeks, Mike McCune, & Rafick Sekaly, UCSF & VGTI

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Chronic Inflammation and/or immune dysfunction may drive HIV persistence through several non-mutually exclusive mechanisms (upregulation of “negative regulators”, homeostatic proliferation, increased target cells, lack of effective HIV-specific T cells)

Microbial translocation, ↑ co-pathogens (CMV), thymic dysfunction, loss of regulatory cells

HIV replication

T cell dysfunction

Myeloid Cell

Homeostasis

PD1/PD1L
Module 1: Innate Immunity

- Where does SIV reside during treated SIV infection?
- What is the role of macrophages in SIV persistence?
- Can modification of innate immune responses reduce SIV persistence (IDO or GMCSF inhibitors)?

Module 2: Inflammation and Persistence

- What is the role of negative regulators in persistence (PD-1, CTLA-4)?
- What is the role of chemokines and their receptors in HIV persistence?
- What is the role of homeostatic proliferation in SIV/HIV persistence?
- Can inhibitors of these pathways reverse latency in SIV-infected macaques?

Module 3: Clinical Studies

- Is HIV enriched in cells expressing specific markers associated with T cell activation, T cell memory, T cell function and T cell specificity?
- Can HIV be definitively identified in macrophages during long-term therapy?
- Are there anatomic differences in distribution of HIV in these cell types?
- Does HIV evolve under treatment and if so is this a tissue-dependent process?
HIV DNA transcription prevented by restricted access to needed host enzymes, chromatin remodeling and transcriptional interference

Chromatin and Associated Factors (HDACs, Histone Methyltransferases....)

Transcription Factors (NF-κB, NFAT....)
Objective 1: Identify the molecular mechanisms underlying viral persistence and latency
Leaders: Greene, Karn

Objective 2: Identify drug candidates and therapeutic strategies to reduce the latent viral pool
Leaders: Hazuda, Margolis

Objective 3: Establish informative animal model systems to evaluate latency and test therapeutic strategies
Leaders: Clements, Garcia-Martinez

Objective 4: Perform studies in humans to delineate the basis of viral persistence
Leaders: Richman, Siliciano

PK Core
Kashuba

Genomics Core
Woelk

In Situ Core
Haase
HIV Lives within Chromatin

*Latency restriction at transcription initiation*

**“Closed” Nucleosome**
- Hypo-Acetylated Histone tails
- Stable, Compact Chromatin
- Less accessible to Transcription Factors
  - Transcription Repressed

**“Open” Histones**
- Acetylated Histone tails
- Reduced Higher Order Structure
- Access to Transcription Factors
  - Transcription Enabled
Epigenetic Silencing of HIV Expression Leads to Latency

From J. Karn
Agents Which Induce Latent HIV Expression in vitro

- Histone deacetylase (HDAC) inhibitors
  - Class I-selective: Vorinostat (Vor, SAHA)
  - Non-selective: Trichostatin A (TSA), valproic acid (VPA)

- NF-κB activators
  - Prostratin
  - PMA
  - TNFα

- Akt/HEXIM-1 modulators
  - Hexamethylbisacetamide (HMBA)

- Histone/DNA methyltransferase inhibitors
  - BIX1294: targets G9a HMT – Imai et al, 2010
  - azaCdR: targets DNA MT – Kauder et al, 2009

- Jak/Stat pathway
  - IL-7
siRNA Screen for Inducers of Latent HIV Gene Expression

• siRNA Libraries
  – Genome wide library: >18,000 genes
  – Targeted library: “Establishment and/or maintenance of chromatin” GO definition: 286 genes

• Three siRNAs targeting each transfected into HeLa/LTR-βGAL cells.

• βGAL assayed 72h post-transfection.

• Confirm genes where 2+ siRNAs targeting the same gene activate LTR

Histone H3
Histone metabolism:
HDACs
SUPT7H transcription EF binds histone H3
TLK2 kinase assembly of histone octamers
Cell-based Screens for HIV-1 Inducers

~ 1.5 million compounds (Merck Chemical Library circa 2009)

↓ HeLa LTR-βGal screen

~ Confirmed 104 compounds (not known HDACIs)

↓ NFAT Jurkat cell assay

~ 92 compounds that did not activate T-cell

↓ HDAC activity assay (novel HDACis)

~ 83 compounds with unknown mechanism of action
Merck HTS Screen: Induction of HIV-1 Expression Correlates with HDACi Potency
Phase I Vor Proof-of-Concept: Effect on HIV expression in resting CD4+ T cells in vivo

Step 1: **Screening**: Increase in resting CD4+ T cell-associated HIV RNA can be detected after a physiological exposure to VOR ex vivo.

Step 2: 12 hour PK after 400 mg VOR: VOR plasma level, histone acetylation, p21 gene acetylation by ChIP.

Step 3: Compare resting CD4+ T cell-associated HIV RNA expression at baseline to that after single 400 mg doses.

✧ Correlate effect of HDACi on biomarkers with HIV RNA

✧ Define potential for VOR to disrupt latency
Pharmacokinetic validation of VOR levels and effect on biomarkers

Collect resting CD4+ cells:
- Acetylation at the human p21 gene in PBMCs
- Acetylation of total cellular histones in PBMCs
- Change in intracellular HIV RNA in resting CD4+ T cells

Margolis et al CROI 2012 Abstract 157LB
Clinical POC: HDAC inhibitor vorinostat can induce expression of HIV mRNA in ART-suppressed patients

- Patients with VL <50/ml on ART
- HIV gag RNA measured in PBMCs isolated before and after patients were treated with 400mg vorinostat
- Treatment with vorinostat triggered significant increases in HIV RNA
- Does HIV RNA expression lead to death of latently infected cells?

Archin, et al., 2012, CROI

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HIV Latency Therapy Requires Both Induction and Eradication

Will induction lead to eradication?

- Following removal of induction stimuli, latently cells can return to resting state in vitro (several groups)
- Resting CD4+ T-cells survive despite CPE even in the presence of autologous CD8+ T-cells from HIV patients

Shan et al CROI 2012 Abstract 153

The inability to eradicate HIV-1 infection in the face of suppressive ARV therapy is a consequence a dynamic, latently infected reservoir, persistent viremia and an anergic immune response.
Curative Approaches for HIV…

• Eradication of the virus, “sterilizing” cure

• Permanent suppression of HIV replication without eradication, “functional” cure
PIs: Keith Jerome & Hans-Peter Kiem, FHCRC
Long-Term Control of HIV by CCR5 Delta32/Delta32 Stem-Cell Transplantation

Gero Hütter, M.D., Daniel Nowak, M.D., Maximilian Mossner, B.S., Susanne Ganepola, M.D., Arne Müßig, M.D., Kristina Allers, Ph.D., Thomas Schneider, M.D., Ph.D., Jörg Hofmann, Ph.D., Claudia Kücherer, M.D., Olga Blau, M.D., Igor W. Blau, M.D., Wolf K. Hofmann, M.D., and Eckhard Thiel, M.D.

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“Targeted Modification of Host and Proviral DNA to Treat Latent HIV Infection”

1. Hematopoietic Cell Transplant: Use of an HIV-associated lymphoma cohort to define the impact of conditioning on HIV persistence (Ann E. Woolfrey)

2. ZFN-modified NHP stem cells for SHIV eradication (Philip Gregory)

3. CCR5 Targeting to control HIV/SHIV in nonhuman primates (Hans-Peter Kiem)

4. Targeted disruption of integrated SHIV by engineered homing endonucleases (Keith R. Jerome)

5. Aptamer and dendrimer delivery of Zn finger nuclease and homing endonuclease mRNA and cDNA (John Rossi)
Life Expectancy of Recently Diagnosed Asymptomatic HIV-infected Patients Approaches that of Uninfected Individuals

A. van Sighem et al. on behalf of the ATHENA National Observational Cohort Study

- **Life expectancy** for HIV-infected patients (without AIDS) aged 25 yrs at six months postinfection
  - **Men:** an additional 52.7 yrs (versus 53.1 yrs in general population)
  - **Women:** an additional 57.8 yrs (versus 58.1 yrs in general population)
The Role of the Cure in the Context of the HIV/AIDS Pandemic

- If we stopped all HIV transmissions today, we have a disease burden of 34M people living with HIV/AIDS.
- In order to ultimately control the epidemic we must convert our treatment mortgage from a variable rate loan to a fixed rate, which we can pay down.
- *Only by this combined approach of treatment at scale, prevention and cure will we succeed.*

Carl W. Dieffenbach, Ph.D.
Director, Division of AIDS
November 29, 2011
How Do We Define Success

Permanent remission in the absence of ARV therapy.

Will the same therapy or therapies be effective for all patients?
HCV SVR Rates: Progress Over Two Decades

SVR rates improve with:
- Longer therapy
- Addition of ribavirin
- PEG-IFN
- Weight-based dosing (WBD)
- Adherence
- Addition of Novel Agents (PI)

HCV Genotype 1

1 McHutchison, NEJM (339) 1998
2 Poynard, Lancet (352) 1998
3 Lindsay, Hepatology (34) 2001
4 Manns, Lancet (358) 2001
5 McHutchison, Gastro (123) 2002
6. SP Press Release-Phase 2, SVR 12 August 2008

IFN 48 Wk
- 7%-11%1,2
- 14%3

IFN 24 Wk
- 2%1

PEG-IFN 48 Wk
- 28-31%1,2

PEG-IFN/Riba 48 Wk
- 42%4

PEG-IFN/Riba Overall
- 63%5

PEG-IFN/Riba with WBD
- 48%4

PEG/R/BCV 48 Wk
- 75%6

Adherent

HCV SVR Rates: Overall

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