Module R: 

*Recording the HIV Reservoir*

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Fundamentals of HIV persistence

Untreated HIV infection

HIV-infected cell → Virus → Uninfected cells → Newly-infected cells
Fundamentals of HIV persistence

Treated HIV infection

- Long-lived, “latently-infected” cells are invisible to the immune system and may survive for years during ART
When antiretroviral therapy is stopped, the virus returns
We need tools to predict IF and WHEN the virus will rebound.

Diagram:
- Viral load vs. Time
- Antiretroviral therapy
- Question mark indicating uncertainty
HIV reservoir size determines timing of viral rebound

Antiretroviral therapy

Time

Viral load
HIV reservoir size determines timing of viral rebound
We need a *revolution* in reservoir recording
We need more sensitive recording tools

HIV returns in two patients after bone marrow transplant

By Ray Sanchez, Sho Wills and Saundra Young, CNN

Updated 5:31 AM ET, Mon December 9, 2013
Challenge #1: HIV persists at low levels during ART

HIV-infected cells are extremely rare in treated individuals
Fewer than 10 out of a million CD4+ T cells may harbor HIV during antiretroviral therapy

We will find more needles by sampling more of the haystack.

We are using digital droplet technology that enables analysis of 10-fold more cells and DNA than standard approaches.
We can now detect a single copy of HIV DNA in a million CD4+ T cells using digital PCR.
We are using digital PCR to understand how HIV cure strategies affect both virus and host.

Unstimulated CD4+ cells  
- Galectin-9 potently reactivates latent HIV and induces host immunity against HIV

Cells treated with Galectin-9

(Adapted from Abdel-Mohsen et al, *PLoS Pathogens* 2016)
Challenge #2:
Most viruses are defective and cannot grow

HIV has a high mutation rate that enables immune escape and evolution of drug resistance
HIV genomes can be classified into three types

- Infectious, replication competent virus
We are developing a microfluidic chip that will allow us to efficiently measure infectious virus in a clinical sample.

Traditional quantitative viral outgrowth assays ("QVOA") are extremely expensive and laborious.
HIV genomes can be classified into three types

- Benign, defective viral genomes
HIV genomes can be classified into three types:

- Inflammatory, defective viral genomes
Challenge #3: HIV persistence in tissues is likely critical

- The host immune response against HIV may give us critical info about the size of the latent HIV reservoir

Circulating antibodies in blood may inform us about HIV persistence in tissues
Antibodies against HIV may “see” virus in tissues and predict time until viral rebound.

\[ r = 0.7 \]
\[ p = 0.007 \]
We are using *in vivo* imaging to visualize the latent HIV reservoir in infected individuals.
Dr. VanBrooklin and team have labeled VRC01 (broadly neutralizing antibody against HIV) with zirconium-89 and are gathering the data for an FDA Investigational New Drug (IND) submission in Q1 2017.
We have performed FDG-PET/CT imaging of lymph nodes to measure the HIV reservoir in humans. Metabolic activity in lymph nodes is associated with HIV reservoir size. (Tawakol and Hsue, in press 2016)
HIV Imaging Roadmap

Today

18FDG
TSPO

89Zr-VRC-01
PD-1/PD-L1
TLR

18F-Raltegravir
18F-GE-180
18F-AraG

5 years

Direct Disease Marker

Biomarker Development

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FOR HIV CURE RESEARCH
The work we have done to identify a reservoir "biomarker"
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The work we have done to identify a reservoir “biomarker”

- 72 HIV-infected individuals on anti-retroviral therapy
- 2 x 10^7 Cryopreserved cells
- CD4+ T cell isolation
- RNA and DNA extraction
- Immunophenotyping (flow cytometry)
- BLOOD PLASMA
  - Ultrasensitive HIV viral load measurement
  - Anti-HIV antibody profiling
  - Cytokine profiling

- GENOMIC DNA
  - HIV-pol and 2-LTR circle DNA quantitation (droplet digital PCR)
  - Host genotyping
  - Ultra-deep HIV proviral DNA sequencing
  - Exosome analysis
The work we have done to identify a reservoir "biomarker"
The HIV reservoir may be more “active” than we thought
Analytical treatment interruption will help us to build accurate reservoir recording tools.

Collect specimens and record measurements.
Analytical treatment interruption will help us to build accurate reservoir recording tools.

Our recording tells us the reservoir is depleted.