Module C: 

*Charting the HIV reservoir in tissues*

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CD4+ T cells – the major reservoir of HIV - “traffic” through blood, but live in tissues

• Only 1-2% of the body’s CD4+ T cells are in the blood at any time.
• Vast majority are in lymph nodes, spleen, and lining of the gut.
• Lymphoid tissues are like cities, where most people live.
• Blood is like the highways that connect cities.
People in cities vote differently than those who live outside of cities

2016 Presidential Election Results by County
(circle size = size of lead)
www.nytimes.com/elections/results/president

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HIV-infected T cells in tissues may be very different than those in blood

• Proportion of CD4+ T cells infected by HIV is 10x higher in gut than in blood.

• Some types of infected CD4+ T cells in lymph nodes and gut don’t even circulate through the blood.

• These cells may also respond differently to therapies to “shock” HIV out of its hiding places and “kill” infected cells.

• For these reasons, we need to be able to measure the virus in these specialized CD4+ T cells in the tissues.
Cell types that live in tissues and may represent unique barriers to HIV cure:

T follicular helper cells (Tfh)
T follicular helper cells (Tfh) are a major reservoir of HIV in lymph nodes

- Tfh cells live in B cell follicles of lymph nodes and "help" B cells make antibodies.
- Tfh cells usually stay in follicles and don’t circulate in the blood.
- These Tfh cells are primary targets of HIV in lymph nodes.
- Produce most of the virus in lymph nodes in untreated infection.

Fukazawa, Nat Med, 2015
HIV-infected Tfh cells may be protected from killing by the immune system

- The B cell follicle (where Tfh cells live) is a “sanctuary site.”

- Cells that normally would kill HIV-infected cells are prevented from entering the follicle.
  - Cytotoxic CD8+ T cells
  - “Natural Killer” cells

- For this reason, infected Tfh cells may be more difficult to clear with immune-based therapies (like TLR stimulators).
Cell types that live in tissues and may represent unique barriers to cure:

Tissue Resident Memory cells (TRMs)
Tissue-Resident Memory (TRM) T Cells

• Most CD4+ T cells circulate freely between blood and lymph nodes.

• Tissue-resident memory T cells remain in the gut (and other tissues in the body) and do not circulate in the blood.
  • We can’t measure these cells by getting a blood sample.

• TRMs may also preferentially support latent HIV infection.
  • This allows the virus to “hide” from the immune system and avoid being killed.
The very mechanism that retains TRMs in tissues may suppress HIV transcription, promoting latency. May make TRMs more resistant to “shock” therapies and less “visible” to the immune system, escaping killing.
Tfh and TRM cells: Progress and Next Steps

• We are now able to measure the amount of HIV in these cells isolated from gut biopsies and/or lymph node biopsies.

• Plan to quantify HIV in Tfh and TRM reservoirs in the context of an analytic treatment interruption study
  – Does the burden of HIV in these cells predict time it takes for HIV to rebound once HIV medications are stopped?

• Assess the impact of “shock” and “kill” strategies like TLR stimulators on these cells.
Characterizing the location and phenotype of cells infected with HIV in tissues
Multicolor staining with HIV-RNA, DAPI, CD20, and CD3

Lymph Node from an untreated HIV-infected individual
Lattice-like staining is in same location as CD20 (B cell follicle) and infected cell is CD20-
Multicolor staining with HIV-RNA, DAPI, CD20, and CD3

HIV infected cell stains with CD3 (T cell receptor)
Multicolor staining with HIV-RNA, DAPI, CD20, and CD3

HIV-producing T cell

Lattice-like HIV-RNA in FRC Network of B cell Follicle

Showing all stains together
Developing methods to more accurately identify signatures of latently infected cells in tissues

If we can identify “signatures” of cells that are “silently” infected with HIV, we might be able to use total-body imaging techniques to track these cells without doing biopsies (Dr. Pillai’s talk).
Currently, we only have a few markers that identify CD4+ T cells enriched for HIV

- Exhaustion markers: PD-1, LAG-3, TIGIT (Fromentin, PLoS Path, 2016)
- Not very sensitive
  - Many HIV-infected cells don’t express these markers
- Not very specific
  - Most CD4 cells that express these markers are not HIV-infected
- Like trying to describe a face by presence of a nose, eyes, and mouth.
We need “Facial recognition software” for the HIV reservoir

- Mass cytometry (CyToF) allows for multi-dimensional phenotyping of T cells.
- Developed a 38-parameter CyToF panel to provide high-resolution phenotyping of T cells.
- Linking this panel to probes for detecting HIV expression
  - Before and after stimulation
If HIV reactivation alters cell phenotype, “Nearest Neighbor” analysis can identify original phenotype.