End of life research

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Cure over life cycle
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HIV persistence in deep tissues

- **Why is this important?** Studying deep tissue reservoirs in humans (i.e. HIV reservoir in organs) is difficult for obvious safety and ethical reasons. Deep tissues are accessible when people living with HIV give their body to science.

- **What is the objective?** To define how much HIV persists in different parts of the human body during long-term antiretroviral therapies.

- **What did we find?** Both intact and defective HIV persist in many deep tissues, and identical HIV can be found in different organs.

- **What are the implications for a cure?** To specifically target the tissues in which most of the intact (i.e. replication-competent) virus is found.
Why studying anatomical HIV reservoirs?

- Antiretroviral therapies (ART) do not eradicate HIV.
- HIV reservoirs studies are, for the vast majority, performed on circulating CD4+ T cells.

Understanding where and how HIV persists in deep tissues is a prerequisite for the development of curative strategies.
We report here a case study of 2 people living with HIV (PLWH) and under long-term suppressive ART who gave their bodies for HIV research to the Canadian Collaboratory CanCURE in 2018.

We obtained a high number of near-full length proviral sequences to identify potential anatomical sites of viral rebound caused by persistent intact HIV genomes.
Methodology

- Brain
- Spinal cord
- Lungs
- Liver
- Duodenum
- Jejunum
- Ileum
- Colon
- Rectum
- Mediastinal LN
- Axillary LN
- Hilar LN
- Spleen
- Mesenteric LN
- Inguinal LN
- Testes
To evaluate and quantify the presence of persistent latently infected cells in the different tissues collected post-mortem from 2 ART-treated participants

To assess the integrity, the clonality and the distribution of the proviral populations in multiple tissues
Participant #1:
From Ottawa

- 67-year-old HIV+ male diagnosed with AIDS-related symptoms in May 1987
- Undergoing palliative care, Medical Assistant at Death (March 16th, 2018)
- Records of opportunistic infections
- Multiple ART regimens (mono or combination)
- Reported taking his medication until one day before his death
- Had neither AIDS related diseases at the time of death nor other illness
Participant #2:
From Edmonton

- 68-year-old HIV+ male, **diagnosed HIV in 2003**
- Died of non-Hodgkin large B cell lymphoma (June 12th, 2018)
- **Two ART regimen** during his life
- Undetectable viral load at the time of death.
- Diabetes, hypertension, HCV infection, HIV-associated peripheral neuropathy and minor neurocognitive disorder
Total HIV DNA was detected in all tissues analyzed (n = 15 and 14, respectively).

Highest HIV DNA levels are found in the different lymph nodes (Ottawa) or liver and spleen (Edmonton).

Each dot represents an independent measure from a different piece. Empty dots represent samples with undetectable values and are plotted at the limit of detection (calculated from cell input).
Characterizing the persistent tissue reservoir

Limiting dilution of extracted DNA of a small piece of tissue, based on total HIV DNA quantification.

Near-full length amplification of HIV genome

Type of analysis:
- Methodology:
  - Is there any defect such as?
    - Inversion
    - Hypermutation(s)
    - Large internal deletion(s)
    - Stop codon(s)/Frameshift(s)
    - Packaging signal or major donor site defect(s)
    - Small internal deletion(s)
  - if not
    - Intact

- Integrity
- Clonality
  - >2 proviruses that are 100% identical

Softwares:
- MAFIT
- Geneious (ref. HXB2)
- HIV Database "QC Tool"
- HIV Database "GeneCutter"

Circular consensus sequencing (CCS) reads >30X

PCR1
646
9686

PCR2
651
9676
Characterizing the persistent tissue reservoir

Ottawa participant

- 300 proviral genomes, ranging from 150 to 9064 bp (mean of 5797 bp);
Characterizing the persistent tissue reservoir

Ottawa participant

- **300 proviral genomes**, ranging from 150 to 9064 bp (mean of 5797 bp);
- From **14 deep-tissues**, between 1 and 67 HIV sequences per tissue.
• 300 proviral genomes, ranging from 150 to 9064 bp (mean of 5797 bp);

• From 14 deep-tissues, between 1 and 67 HIV sequences per tissue.

• 2% of the 300 proviral sequences are intact. They were found in the spleen (2), in the mediastinal (2) and mesenteric (1) lymph nodes.
Characterizing the persistent tissue reservoir

Edmonton participant

- 141 proviral genomes, ranging from 490 to 9051 bp (mean of 4848 bp);
Characterizing the persistent tissue reservoir

Edmonton participant

- **141 proviral genomes**, ranging from 490 to 9051 bp (mean of 4848 bp);
- From **8 deep-tissues**, between 6 and 33 sequences per tissue.
Characteizing the persistent tissue reservoir

- 141 proviral genomes, ranging from 490 to 9051 bp (mean of 4848 bp);
- From 8 deep tissues, between 6 and 33 sequences per tissue.
- 26% of proviral sequences (36) are intact. They were found in all analyzed tissues except for inguinal LN and duodenum.

Intact sequences, although rare, are mainly found in lymphoid organs, but can also be retrieved in other deep tissues (lungs, liver, gut).
- Approximately 50% of the reservoir in deep tissues is composed of clonal expansions (i.e., 100% identical).
- Clonally expanded HIV genomes were observed in every deep tissue where more than 1 provirus was sequenced.
In both participants, **each tissue shares identical proviruses with other anatomical sites**

Many of these shared clones have **also expanded locally**.
A given tissue shares clonal proviral sequences with:

- 3 to 11 of the 13 other tissues (Ottawa)
- 2 to 5 of the 7 other tissues (Edmonton)
Two given organs share from 1 to 11 clones (Ottawa) and 1 to 2 clones (Edmonton).

Lack of compartmentalization of the anatomical reservoir.
There is a significant correlation between the size of a clone (# of copies) and the number of different tissues where these copies are located.
To sum-up

- During long-term antiretroviral therapies, latently infected cells persist in all deep tissues analyzed in this study in both participants, with different frequencies.

- The majority of HIV proviral sequences in deep tissues harbor defects preventing them to be replication-competent.

- The anatomical reservoir harbors intact proviruses, mainly but not exclusively in lymphoid tissues (LNs and spleen).

- Half of the persistent reservoir during long-term ART encompasses clonally expanded proviruses that are frequently found in multiple locations.
In conclusion

In this study, we performed near-full length proviral sequencing of various human deep tissues of 2 PLWH, allowing for a precise genotypic characterization of integrity, clonality and viral distribution of HIV during long-term ART.

This project, which supports the “Last Gift Study” results, highlights the presence of persistent HIV in all collected tissues.

The two participants have different infection and antiretroviral treatment histories, a factor that explains the distinct proviral integrity proportions.

Our results suggest that clonal expansion is an important mechanism of persistence of the HIV reservoir, and that infected cells circulate throughout different anatomical sites.
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