Towards an HIV Cure
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Caroline Dufour
PhD Candidate (CRCHUM/Université de Montréal)

Cure over life cycle

28 July 2022 - Montreal

End of life research

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Community slide

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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.

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Why studying anatomical HIV reservoirs?

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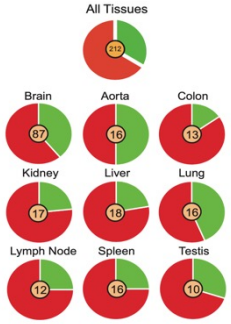
- Antiretroviral therapies (ART) do not eradicate HIV.
- HIV reservoirs studies are, for the vast majority, performed on circulating CD4+ T cells.

American Society for Microbiology | Journal of Virology

HIV DNA Is Frequently Present within Pathologic Tissues Evaluated at Autopsy from Combined Antiretroviral Therapy-Treated Patients with Undetectable Viral Loads

Susanna L. Lamers,[a] Rebecca Rose,[a] Ekaterina Maidji,[b] Melissa Agsalda-Garcia,[c] David J. Nolan,[a,d] Gary B. Fogel,[e] Marco Salemi,[d] Debra L. Garcia,[f,g] Paige Bracci,[f,g] William Yong,[h,k] Deborah Commins,[j] Jonathan Said,[h,k] Negar Khanlou,[h,k] Charles H. Hinkin,[h,l] Miguel Valdes Sueiras,[h,l] Glenn Mathisen,[h] Suzanne Donovan,[h] Bruce Shiramizu,[c] Cheryl A. Stoddart,[b] Michael S. McGrath,[f,g] Elyse J. Singer[h,l]



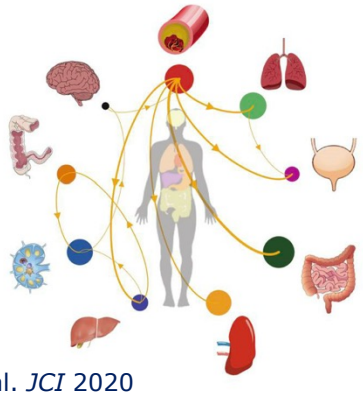
HIV⁺ (red) and HIV⁻ (green) proportions in tissues

S. Lamers et al. *JVI* 2016

JCI The Journal of Clinical Investigation
CLINICAL MEDICINE

HIV persists throughout deep tissues with repopulation from multiple anatomical sources

Antoine Chaillon,[1] Sara Gianella,[1] Simon Dellicour,[2,3] Stephen A. Rawlings,[1] Timothy E. Schlub,[4] Michelli Faria De Oliveira,[1] Caroline Ignacio,[1] Magali Porrachia,[1] Bram Vrancken,[3] and Davey M. Smith[1]

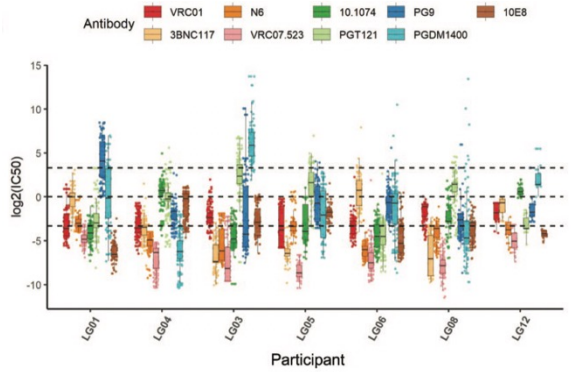


A. Chaillon et al. *JCI* 2020

Clinical Infectious Diseases
MAJOR ARTICLE
IDSA Infectious Diseases Society of America | hivma hiv medicine association

Landscape of Human Immunodeficiency Virus Neutralization Susceptibilities Across Tissue Reservoirs

Chuangqi Wang,[1] Timothy E. Schlub,[2] Wen-Han Yu,[3] C. Sabrina Tan,[4] Karl Stefic,[5] Sara Gianella,[6] Davey M. Smith,[6,7] Douglas A. Lauffenburger,[1] Antoine Chaillon,[6,a] and Boris Julg[8,a,o]



C. Wang et al. *Clin Infect Dis* 2022

SCIENCE SPOTLIGHT: HIV RESERVOIRS IN CELLS AND TISSUES

PROFILING THE PROVIRAL LANDSCAPE IN TISSUES FROM ART-TREATED INDIVIDUALS (ABSTRACT 307)

Weiwei Sun
Ragon Institute, Cambridge, MA, USA

Mathias Lichterfeld's group

virtual CROI2021
Conference on Retroviruses and Opportunistic Infections

Understanding where and how HIV persists in deep tissues is a prerequisite for the development of curative strategies.

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Case study

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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.

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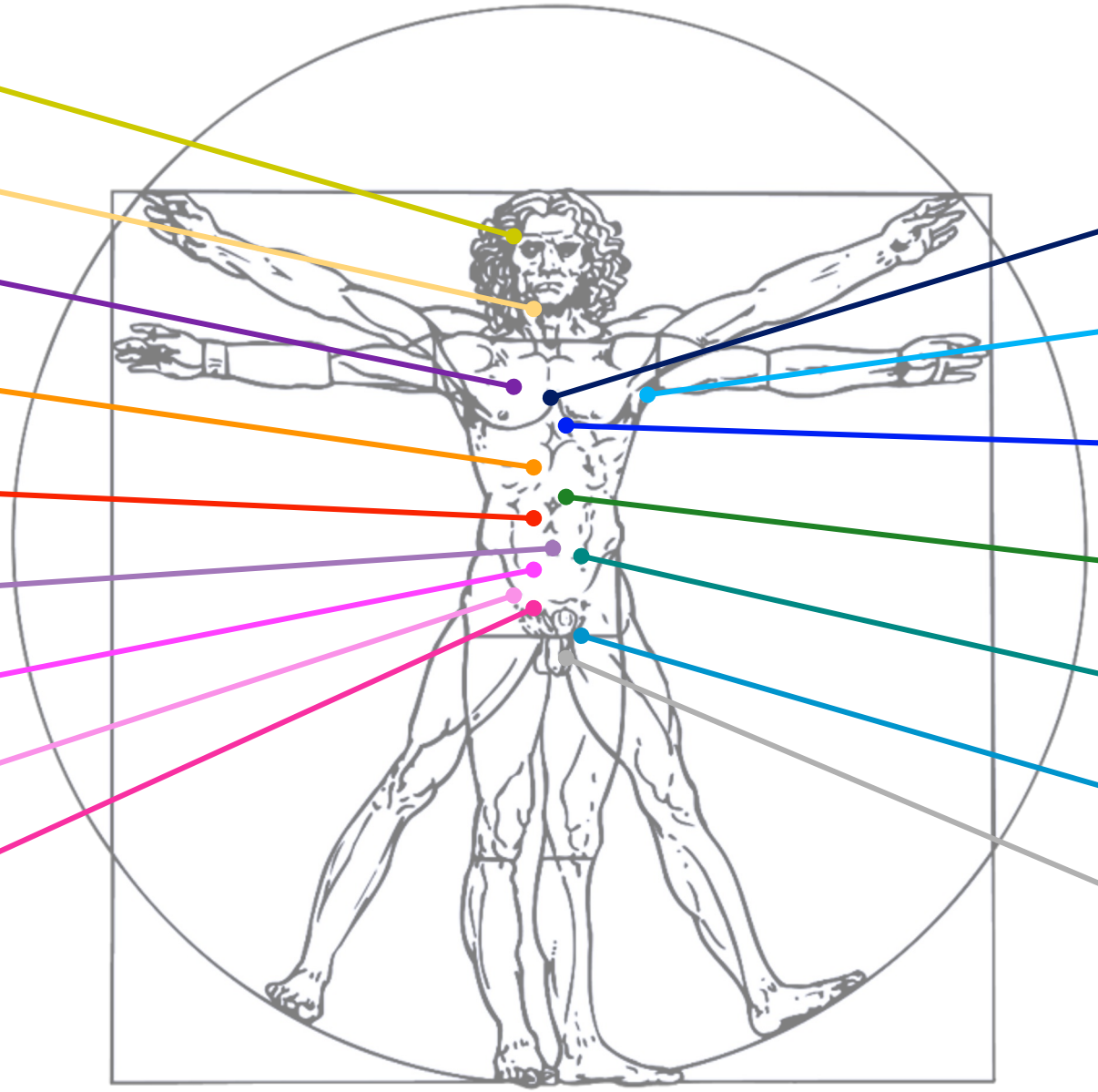
Methodology



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Objectives

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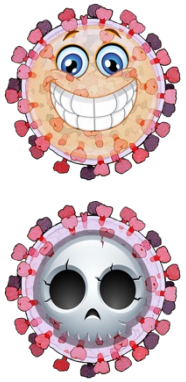
- To evaluate and quantify the **presence** of **persistent latently infected cells** in the **different tissues** collected **post-mortem** from **2 ART-treated participants**

- To assess the **integrity**, the **clonality** and the **distribution** of the **proviral populations** in **multiple tissues**

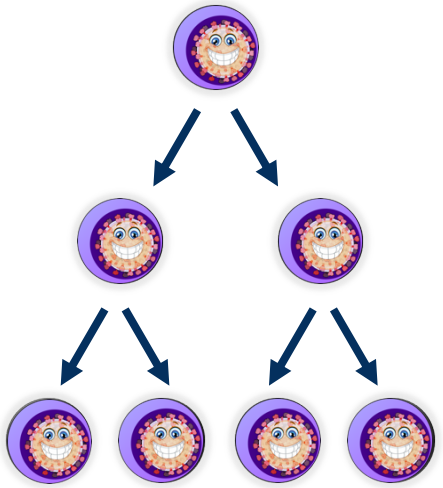
Quantification

Characterization

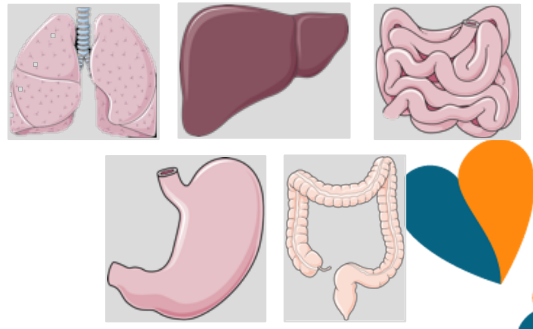
Proviral Integrity



Clonality



Anatomical distribution



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Participants clinical histories

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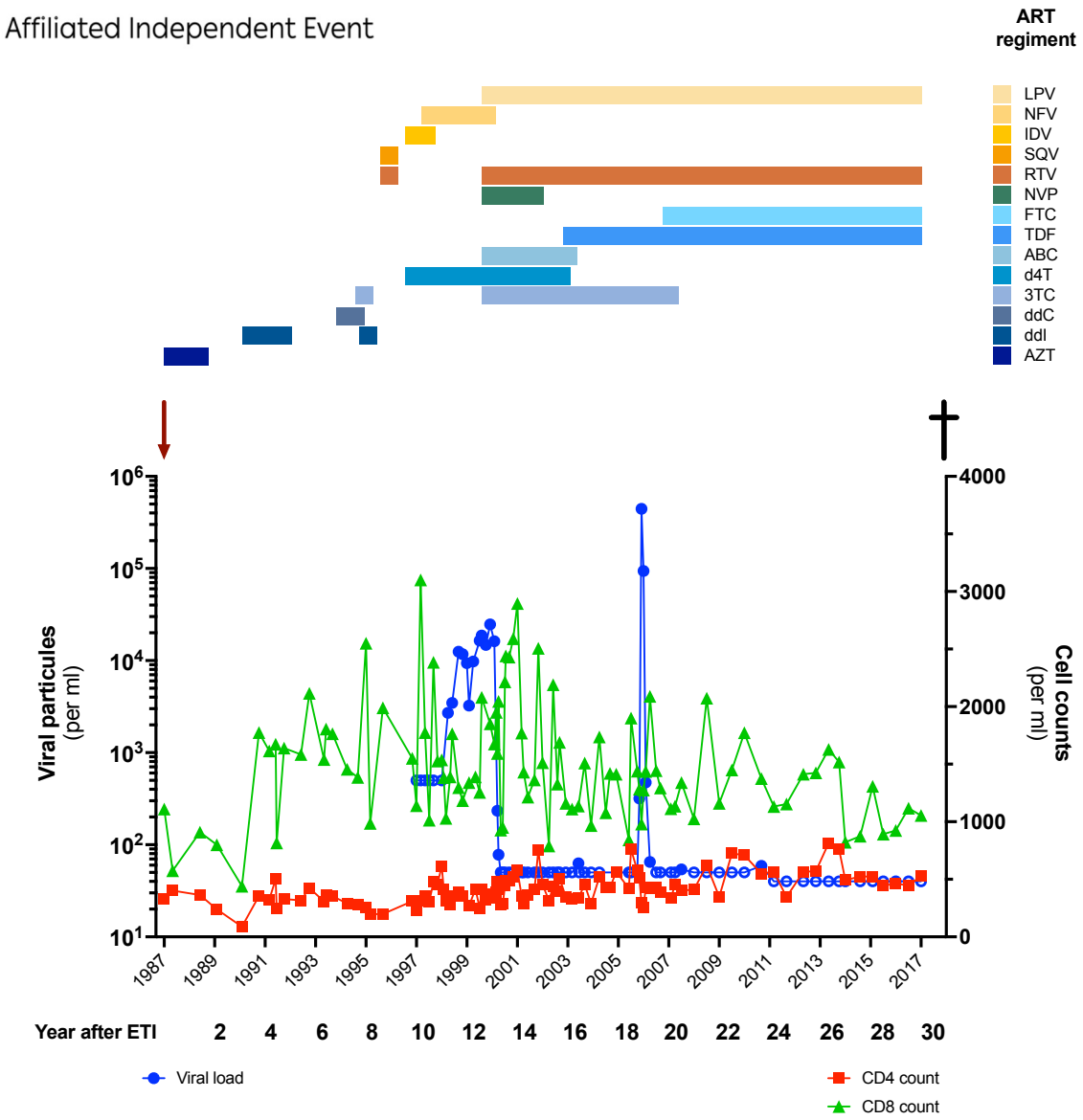

Participant #1 :

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

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Participants clinical histories

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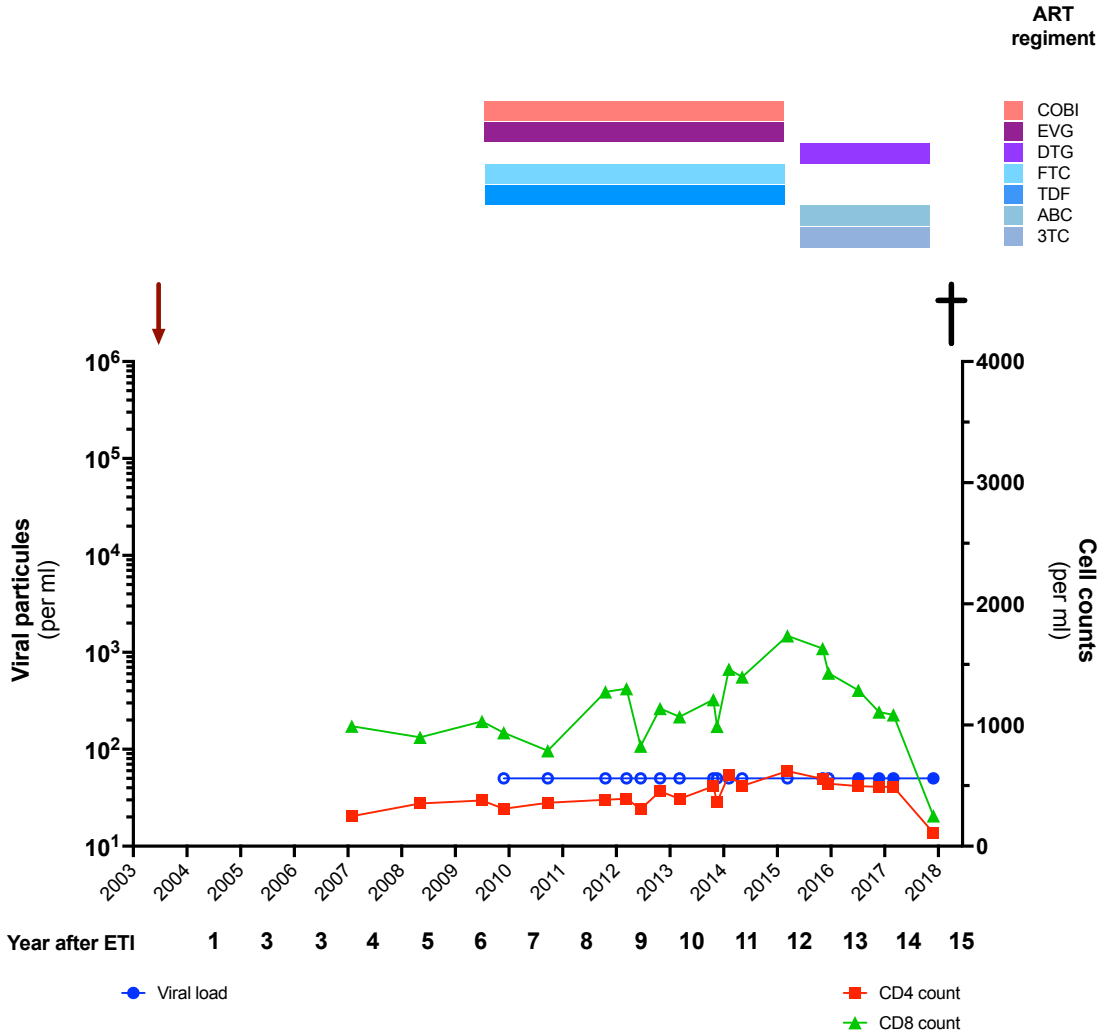


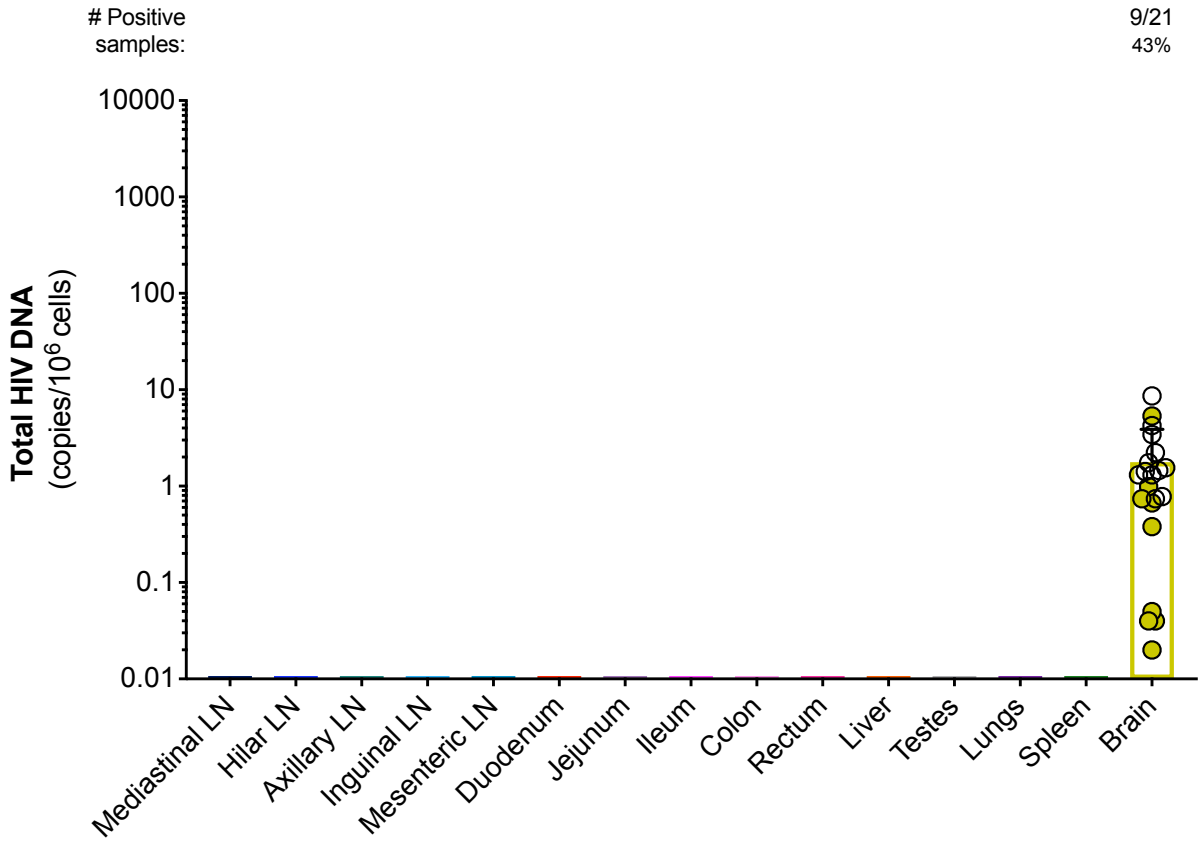
Participant #2 :

From Edmonton

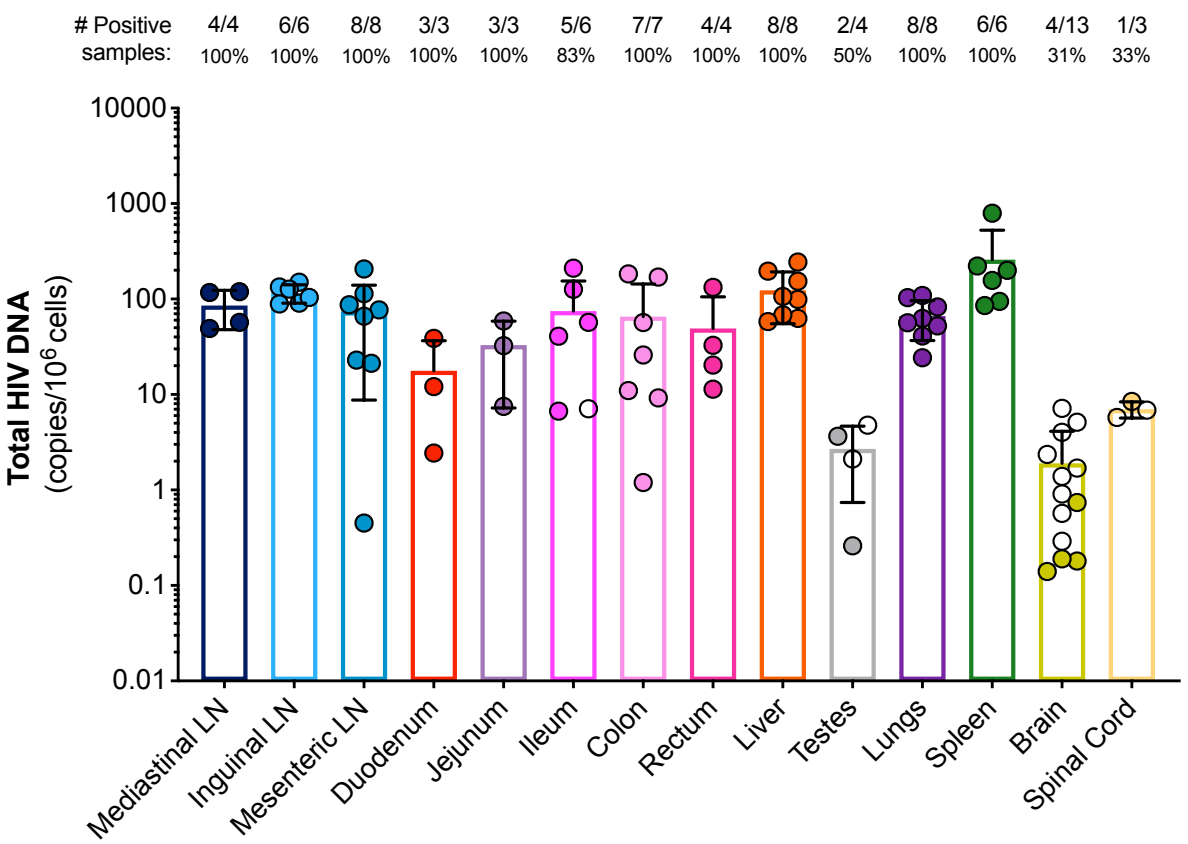
- 68-year-old HIV+ male, **diagnosed HIV in 2003**
- Died of non-Hodgkin large B cell lymphoma (June 12th, 2018)
- **Two ART regimen** during his life
- Undetectable viral load at the time of death.
- Diabetes, hypertension, HCV infection, HIV-associated peripheral neuropathy and minor neurocognitive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

Total HIV DNA quantification in deep tissues

Ottawa participant



Edmonton participant

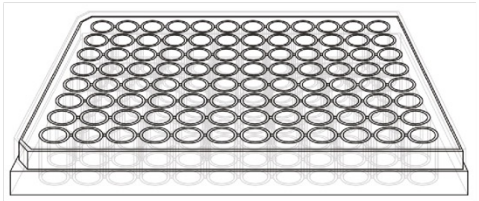


Each dot represents an independent measure from a different piece.
Empty dots represent samples with undetectable values and are plotted at the limit of detection (calculated from cell input).

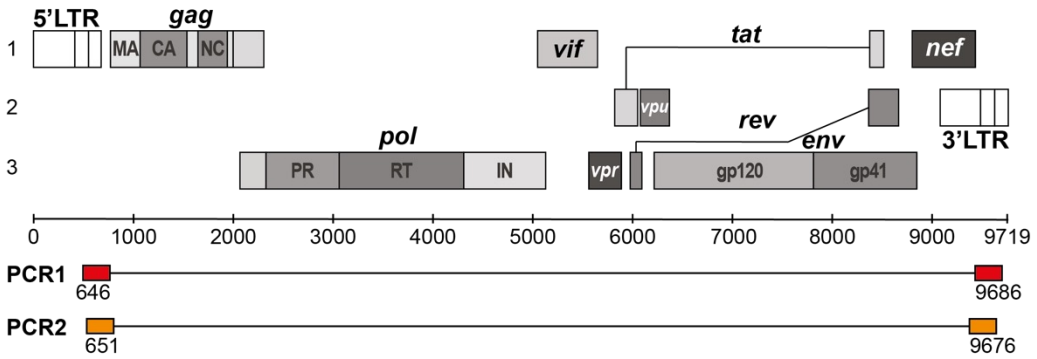
- Total HIV DNA was detected in **all tissues analyzed** (n = 15 and 14, respectively).
- **Highest HIV DNA levels** are found in the different lymph nodes (Ottawa) or liver and spleen (Edmonton).

Characterizing the persistent tissue reservoir

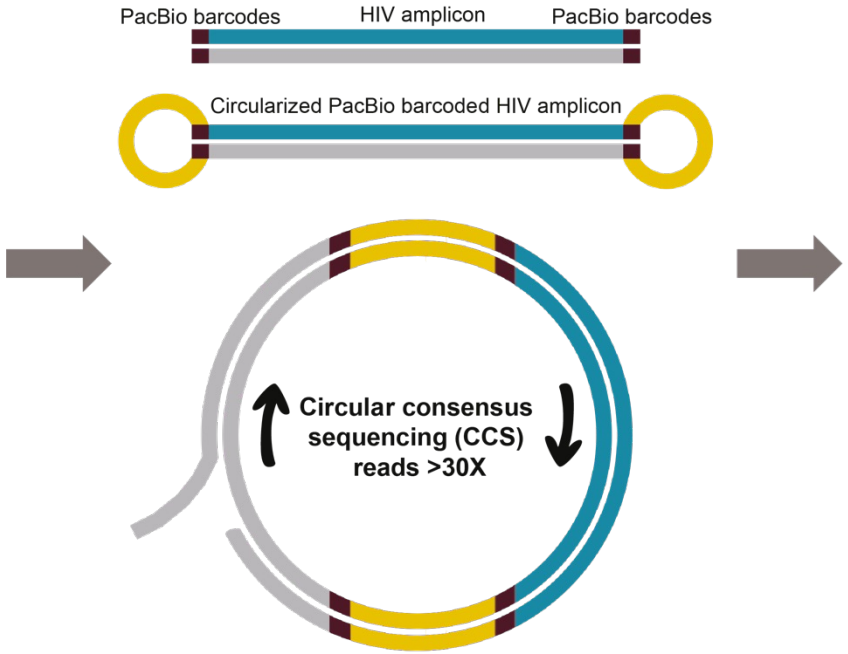
Limiting dilution of extracted DNA of a small piece of tissue, based on total HIV DNA quantification



Near-full length amplification of HIV genome

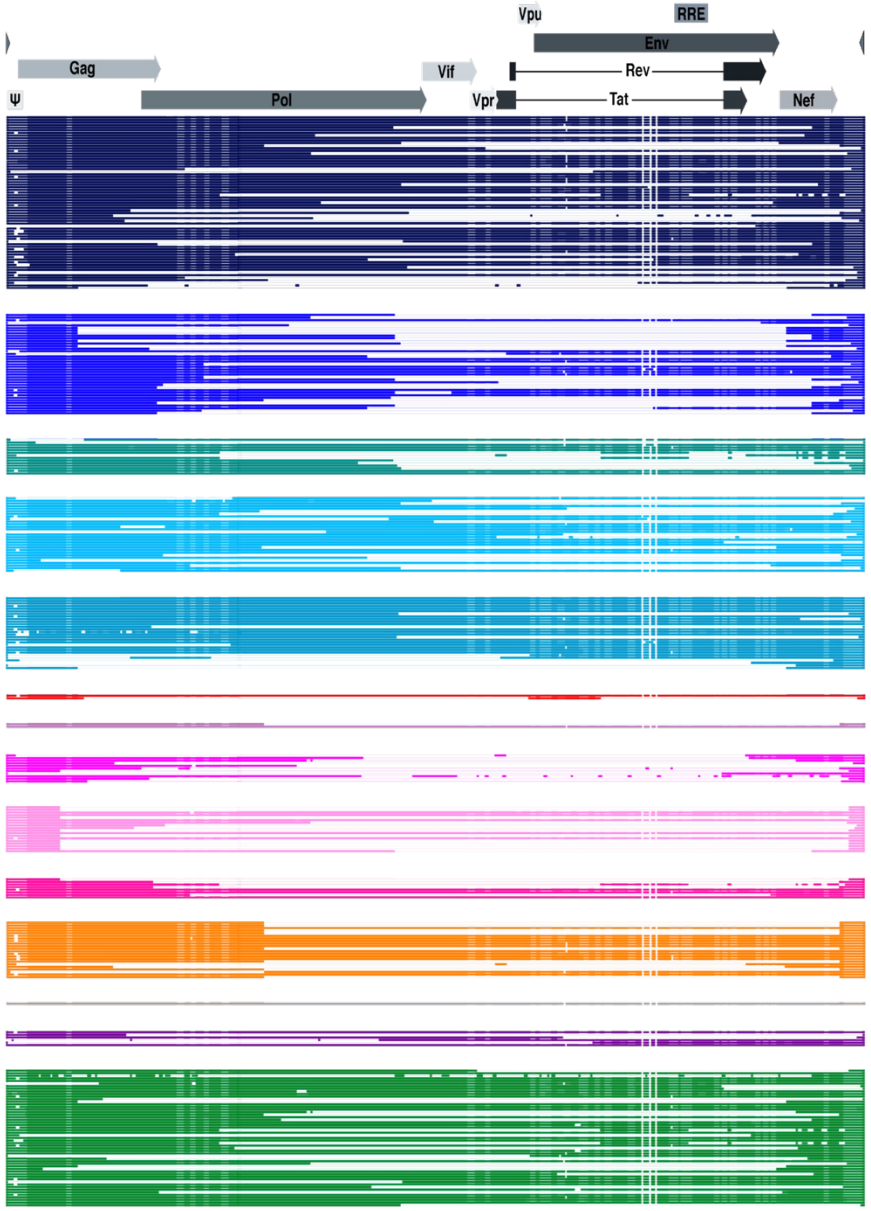


PacBio sequencing



Type of analysis:	Integrity	Clonality
Methodology:	Is there any defect such as? Inversion or Hypermutation(s) or Large internal deletion(s) or Stop codon(s)/Frameshift(s) or Packaging signal or major donor site defect(s) or Small internal deletion(s) if not Intact	≥2 proviruses that are 100% identical
Softwares:	MAFFT Geneious (ref. HXB2) HIV Database "QCTool" HIV Database "GeneCutter"	HIV Database "ElimDupes" Geneious

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

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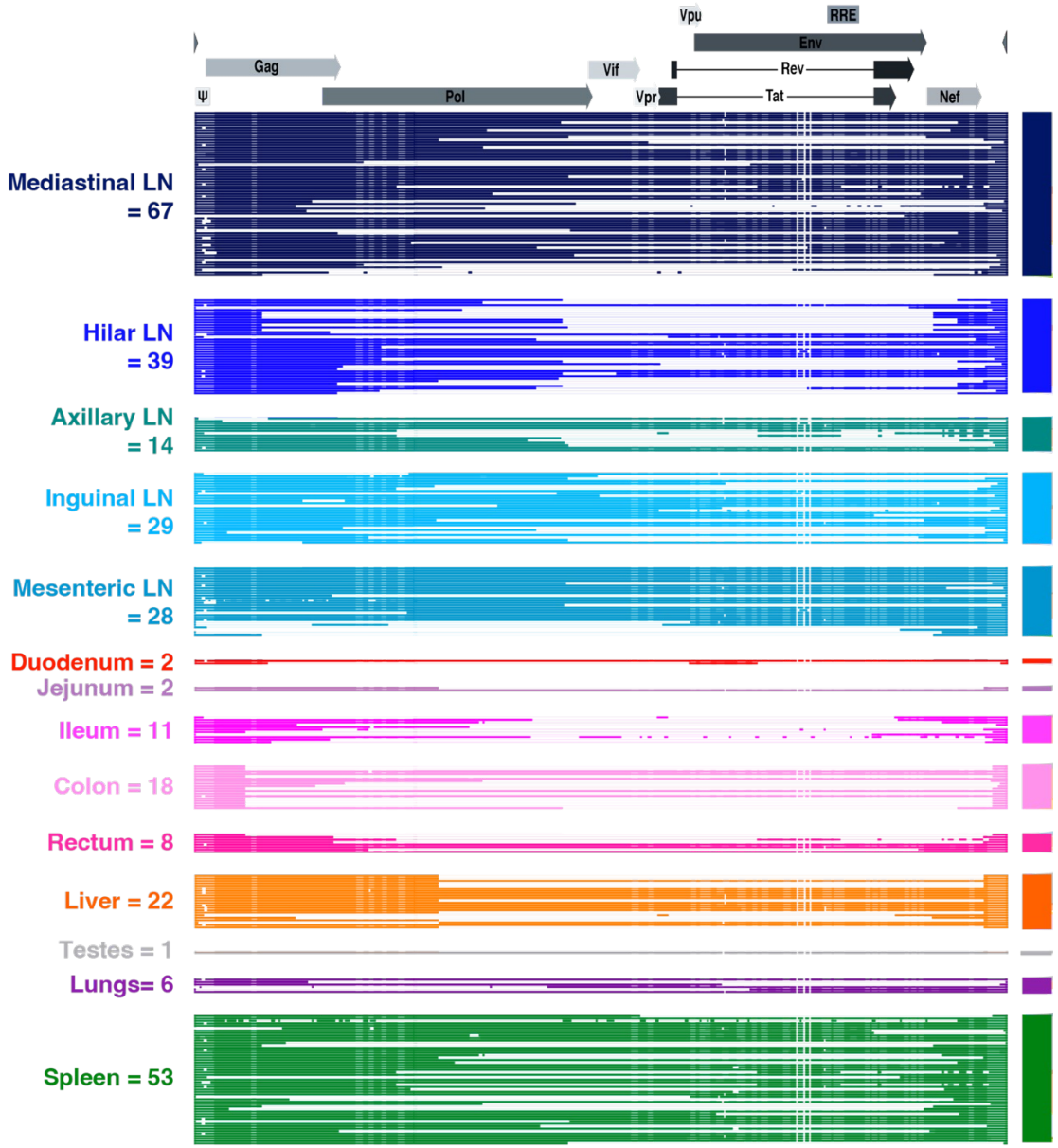

Ottawa participant

- **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.

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Characterizing the persistent tissue reservoir

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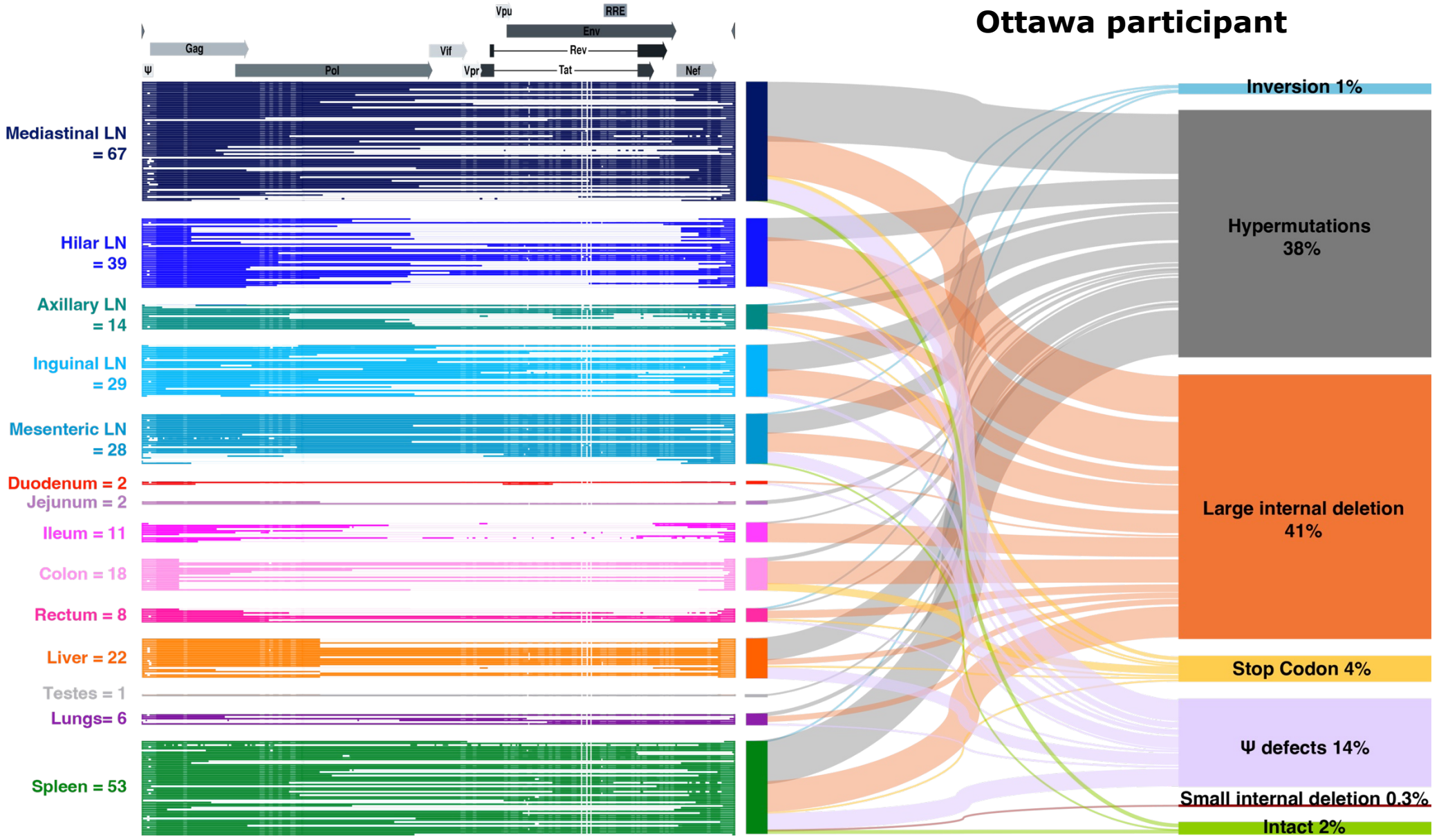
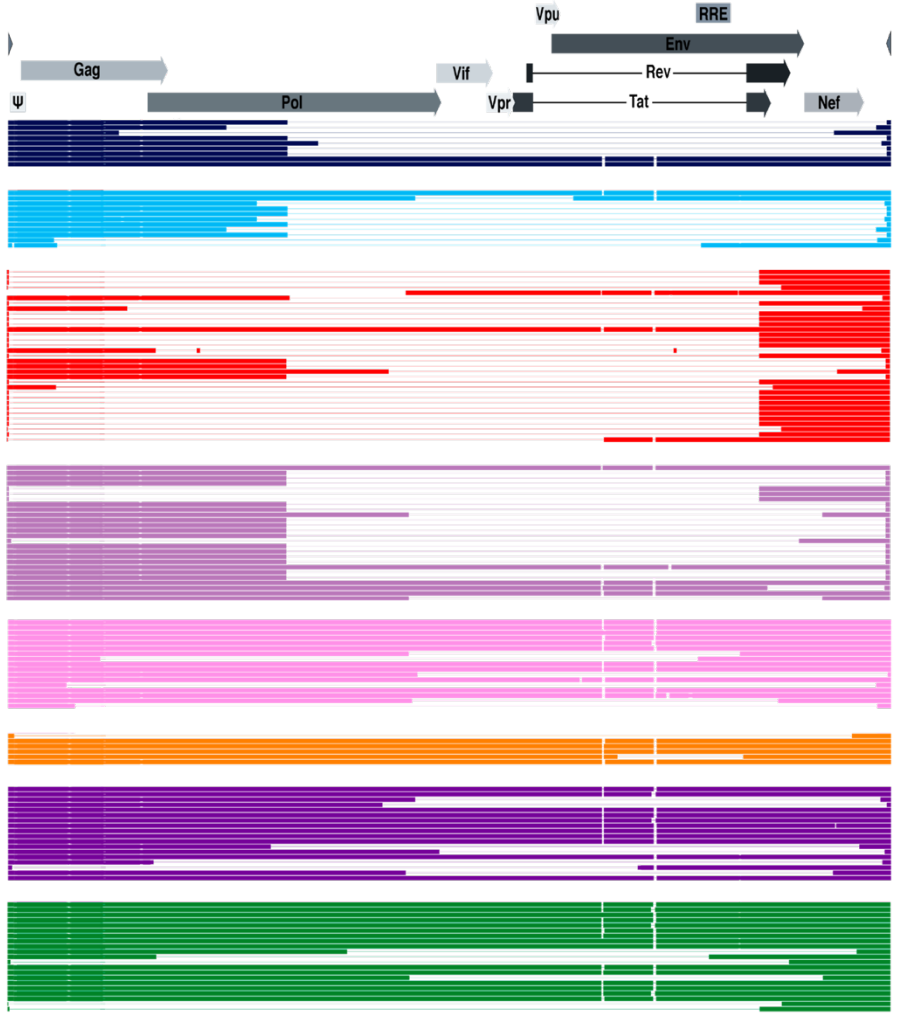


- **300 proviral genomes**, ranging from 150 to 9064 bp (mean of 5797 bp);
- From **14 deep-tissues**, between 1 and 67 HIV sequences per tissue.
- 2% of the 300 proviral sequences are intact. They were found in the **spleen (2)**, in the **mediastinal (2)** and **mesenteric (1) lymph nodes**

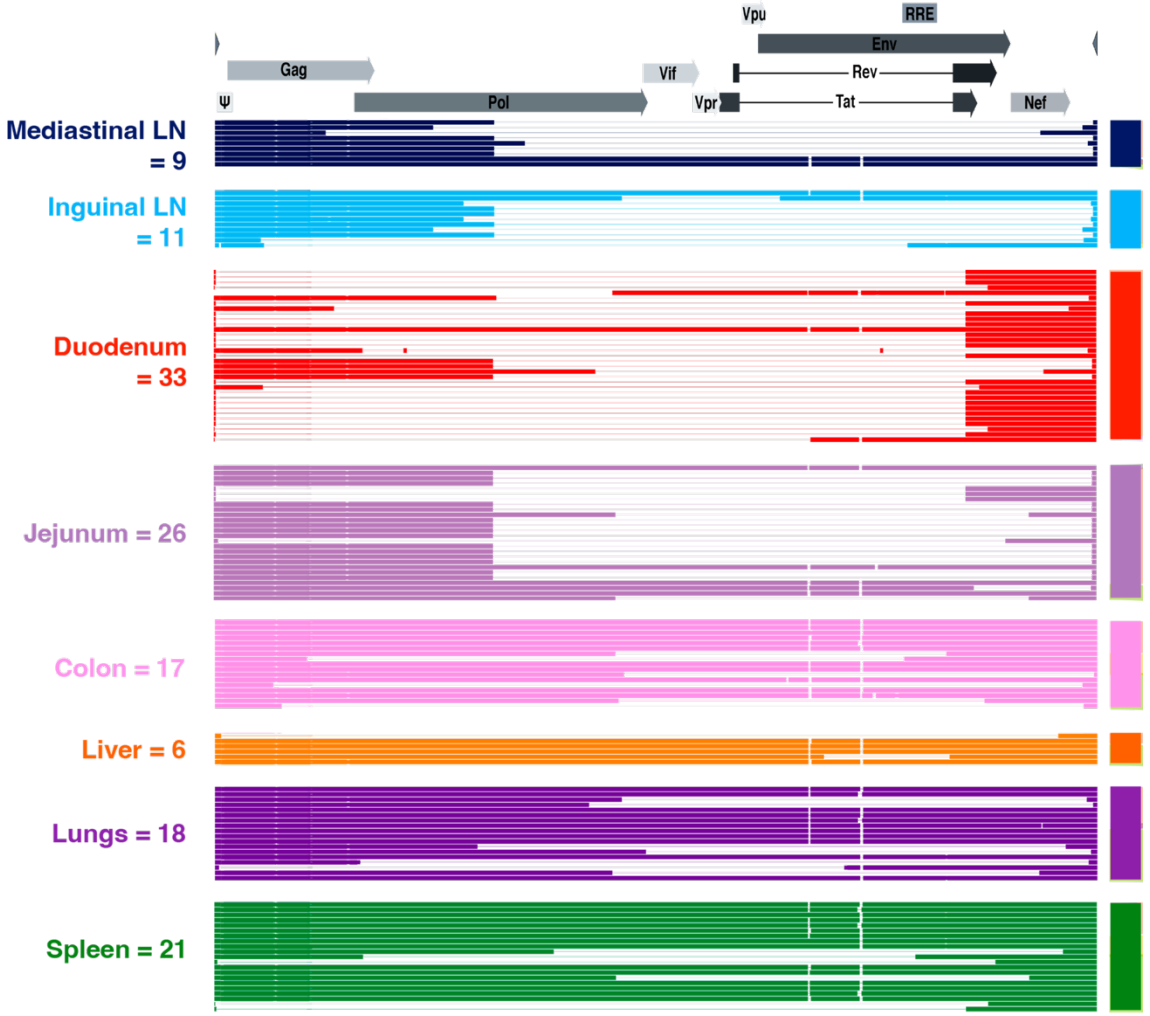
Characterizing the persistent tissue reservoir



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.

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Characterizing the persistent tissue reservoir

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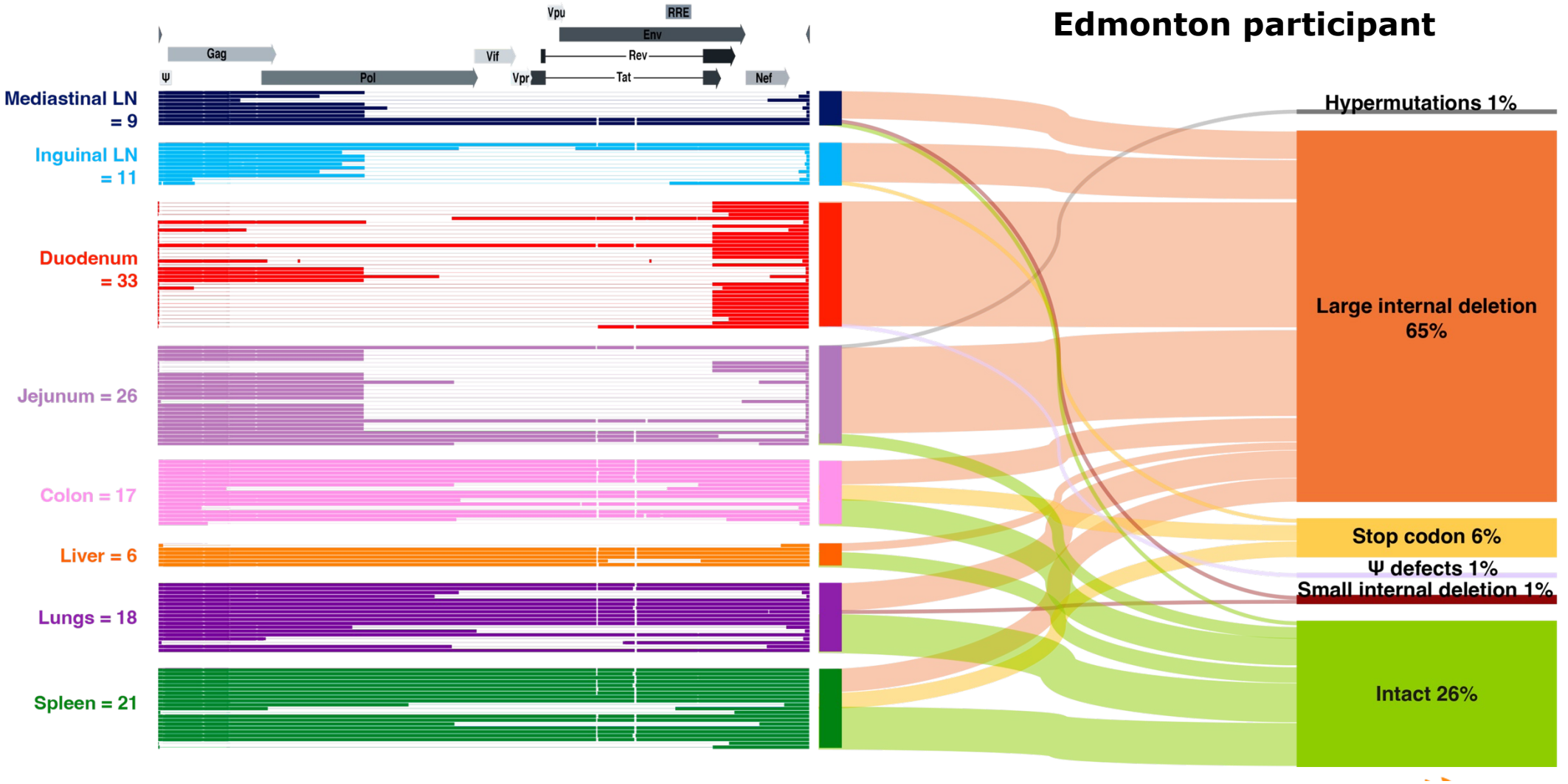

- **141 proviral genomes**, ranging from 490 to 9051 bp (mean of 4848 bp);
- From **8 deep-tissues**, between 6 and 33 sequences per tissue.
- **26%** of proviral sequences **(36)** are **intact**. They were found in **all analyzed tissues except for inguinal LN and duodenum.**

➢ **Intact sequences**, although rare, are **mainly found in lymphoid organs**, but can also be **retrieved in other deep tissues** (lungs, liver, gut).

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Clonality of proviruses in tissue reservoirs

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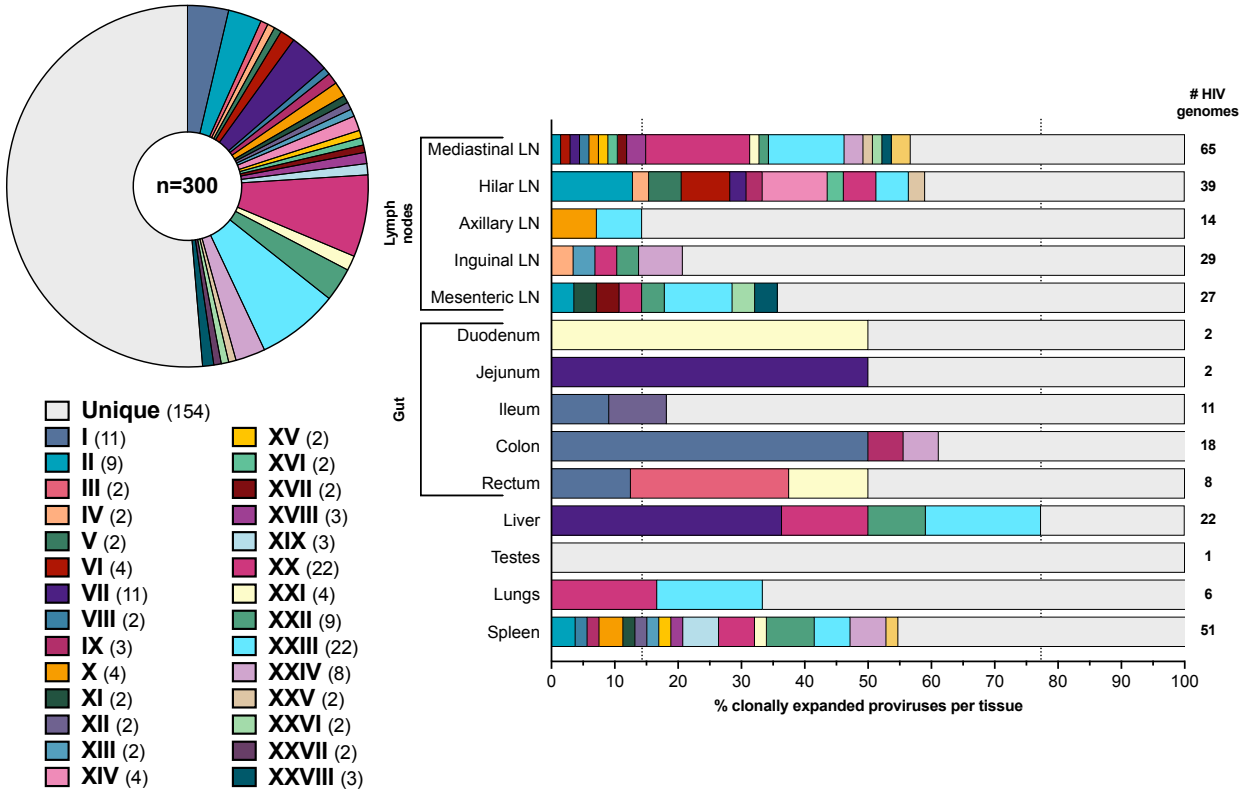
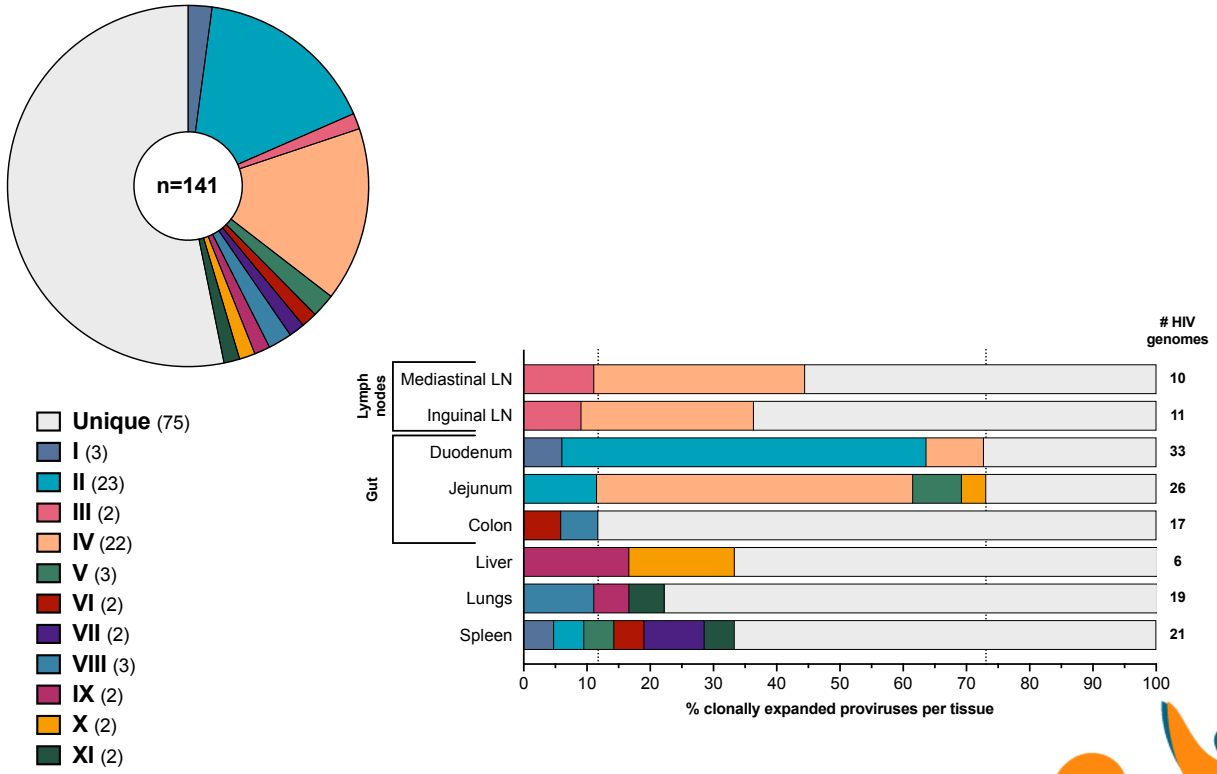


Edmonton participant



- **~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 tissues** where more than 1 provirus was sequenced.

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Distribution of the clonal reservoir in tissues

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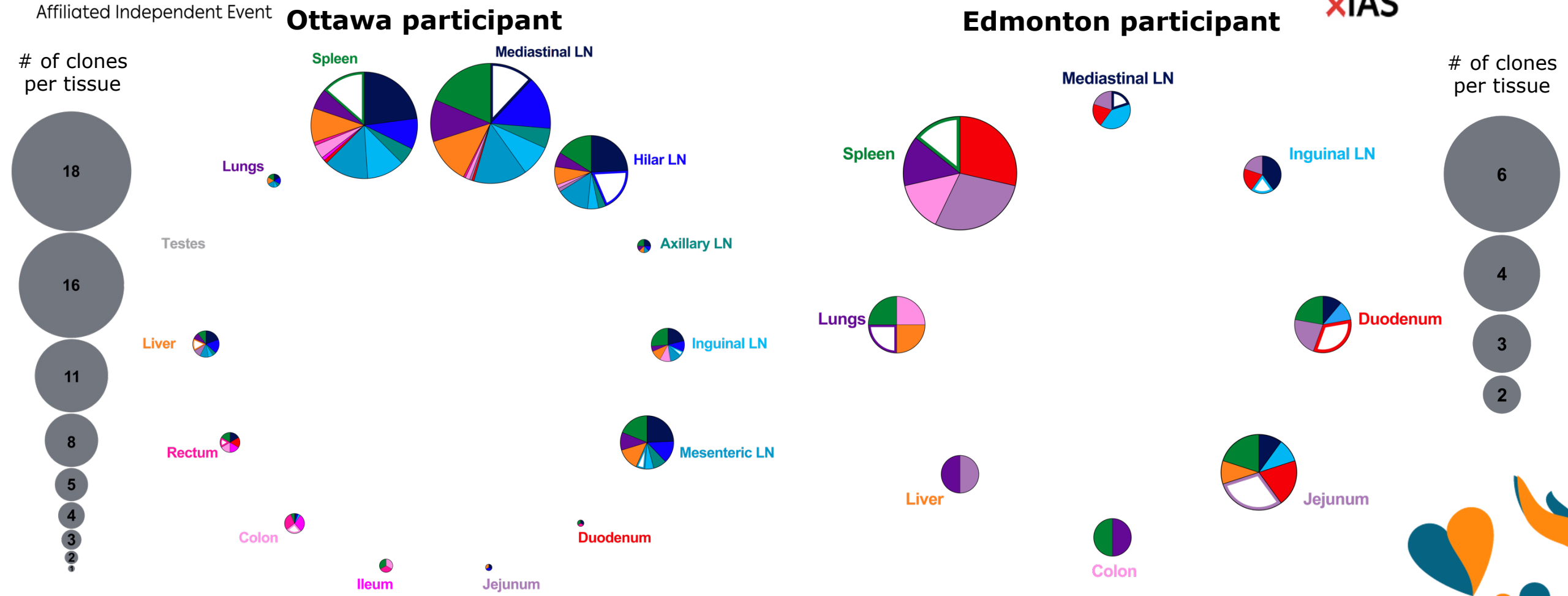

- In both participants, **each tissue shares identical proviruses with other anatomical sites**
- Many of these shared clones have **also expanded locally**.

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Shared clonal proviruses between tissues

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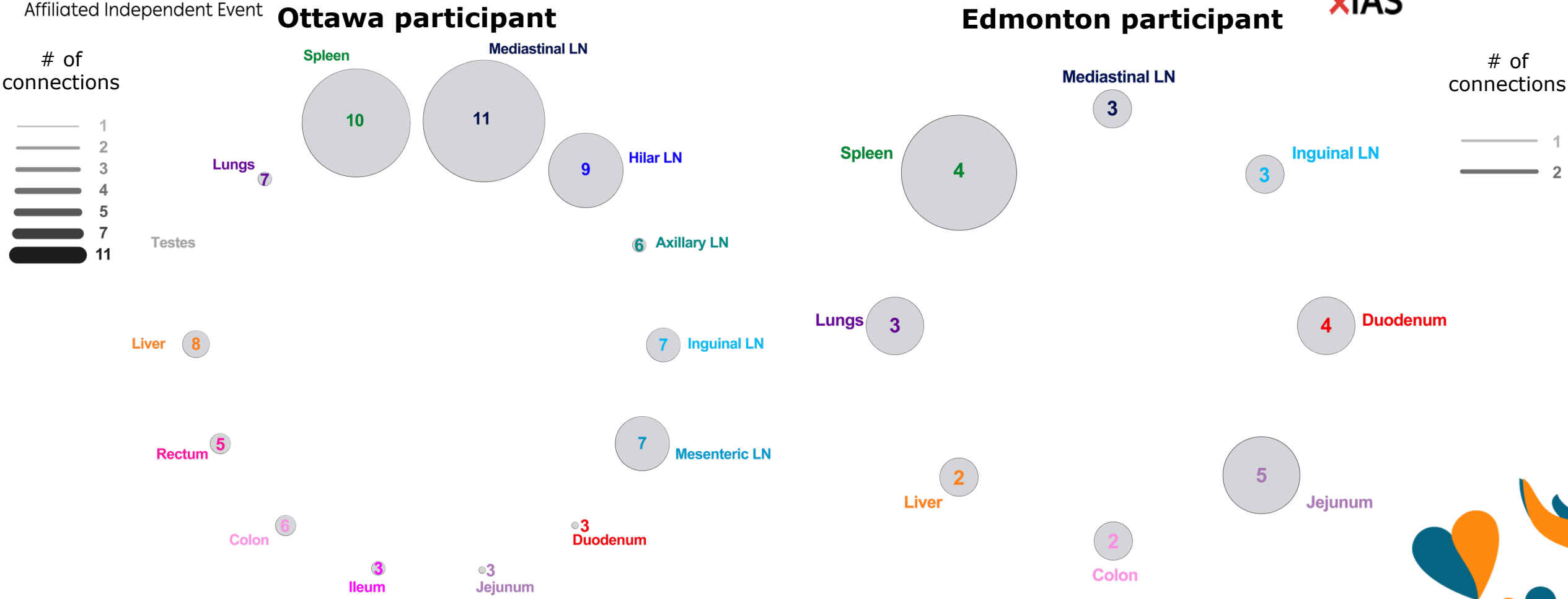

- A given tissue shares clonal proviral sequences with :
  - 3 to 11 of the 13 other tissues (Ottawa)
  - 2 to 5 of the 7 other tissues (Edmonton)

Compartmentalization of the tissue reservoir

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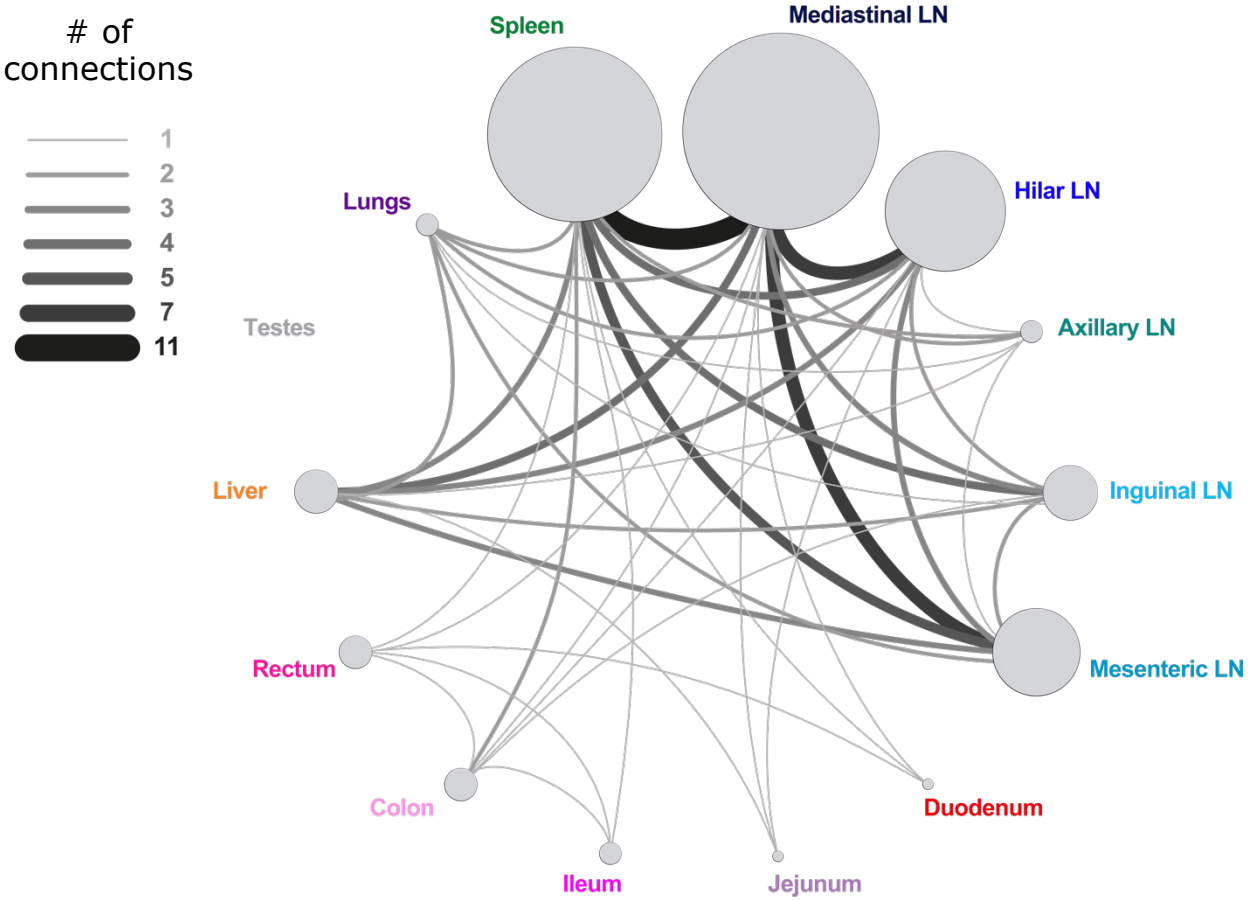
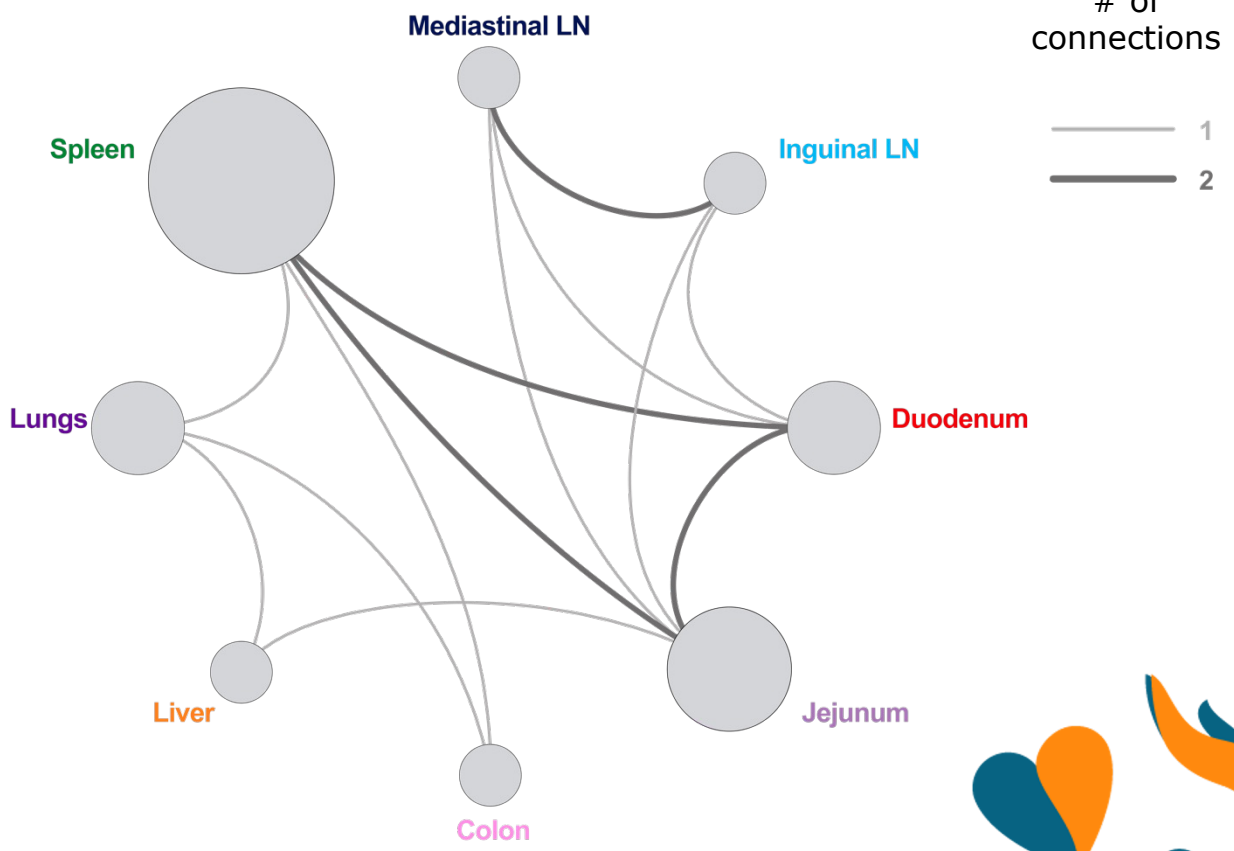
Ottawa participant



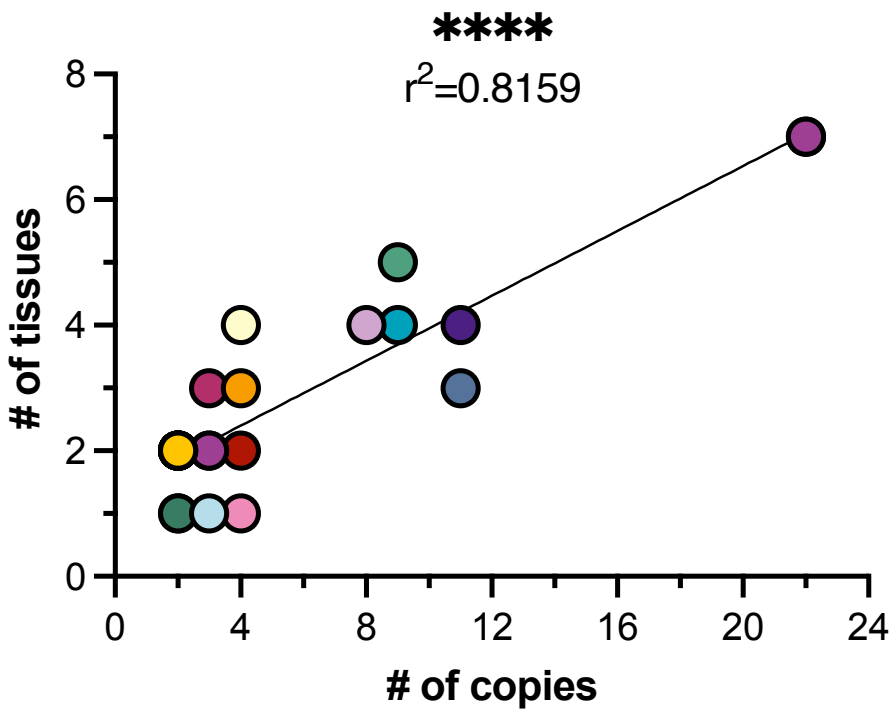
Edmonton participant



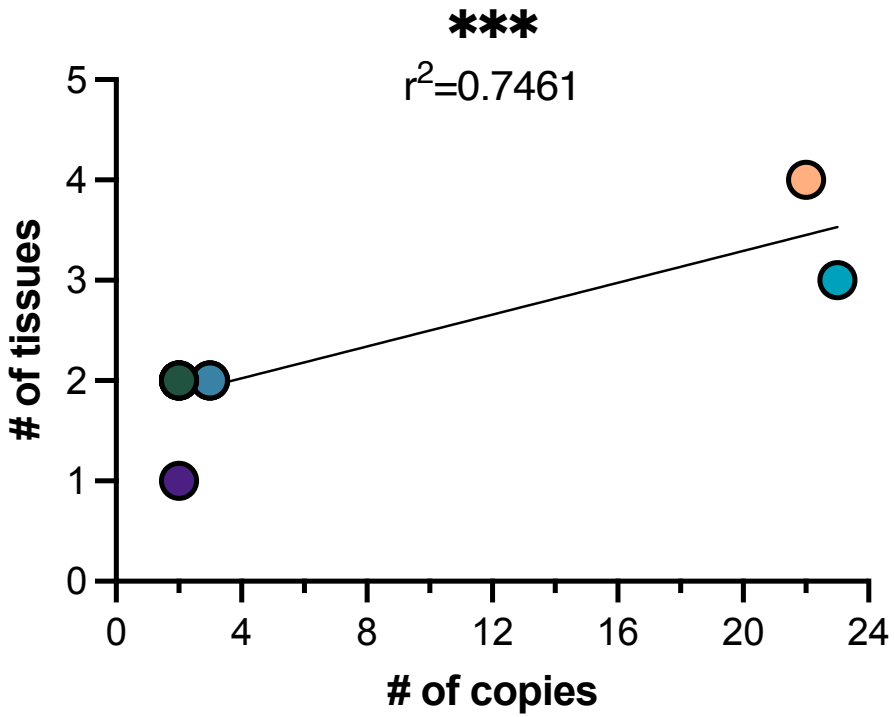
- Two given organs **share from 1 to 11 clones** (Ottawa) and **1 to 2 clones** (Edmonton)
- **Lack of compartmentalization** of the anatomical reservoir

Larger clones are found in more anatomical sites

Ottawa participant



Edmonton participant



- There is a significant **correlation between the size of a clone** (# of copies) and **the number of different tissues** where these copies are located.

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To sum-up

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- 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**.

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In conclusion

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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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Acknowledgments

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U. Montréal

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Ottawa Hospital

Jonathan Angel
Teslin Sandstorm
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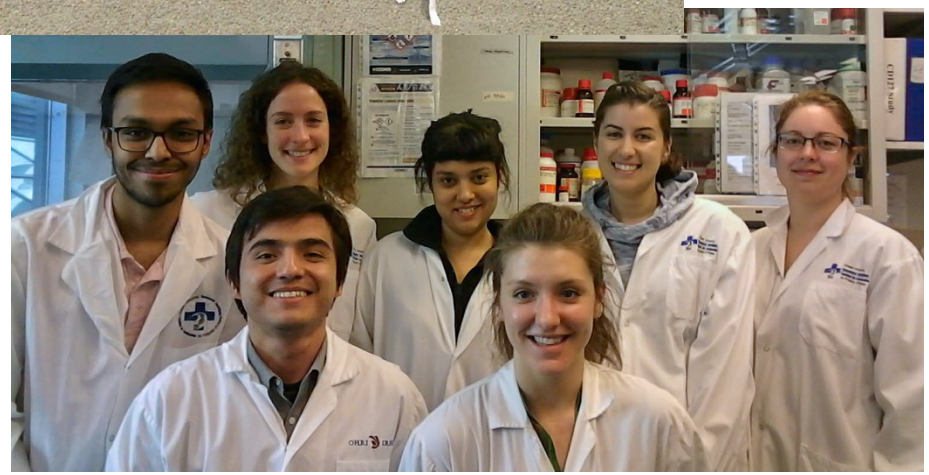
U. Alberta

Christopher Power



The study participants for their generous gift

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Ensemble, nous pouvons GUÉRIR le VIH

Réseau SIDA/MI du FRSQ

IRSC CIHR

Instituts de recherche en santé du Canada | Canadian Institutes of Health Research

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