



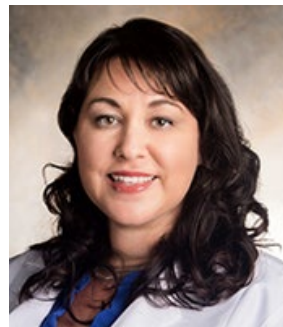
Seminar

Results From CFAR Pilot Awards

Monday, March 20, 2023



Ramon Lorenzo-Redondo, PhD
*Northwestern University
Feinberg School of Medicine*



Kimberly Stanford, MD, MPH
University of Chicago Medicine

New molecular approaches to study the dynamics of HIV-1 tissue reservoirs

Results From CFAR Pilot Awards Seminar

Ramon Lorenzo-Redondo, PhD

Assistant Professor of Medicine (Infectious Diseases)

Bioinformatics Director, Center for Pathogen Genomics and Microbial
Evolution (CPGME)



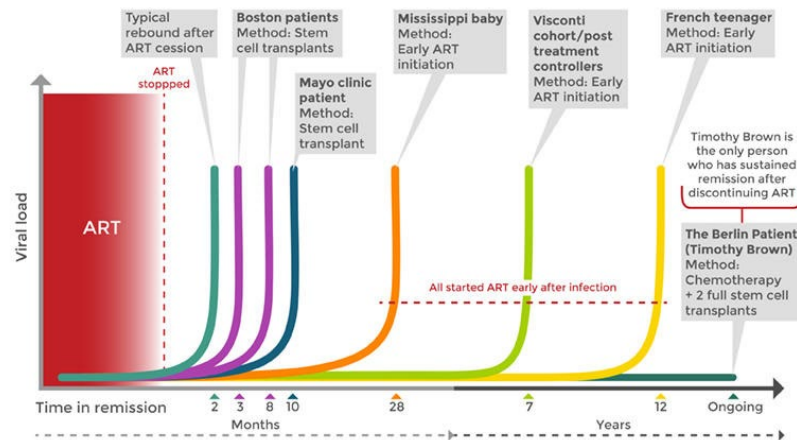
Reservoir main questions

Current ART quickly suppress HIV to levels undetectable in the blood.

However, the virus is not eradicated from the body and in most cases comes roaring back if the drugs are stopped.

- Where the reactivated virus comes from?
- How is the reservoir maintained?
- What are the characteristics of the cells that harbor the reservoirs?
- How is the virus able to retain the variability that it needs to survive?
- How is the virus able to circumvent immunity in tissues to rebound?

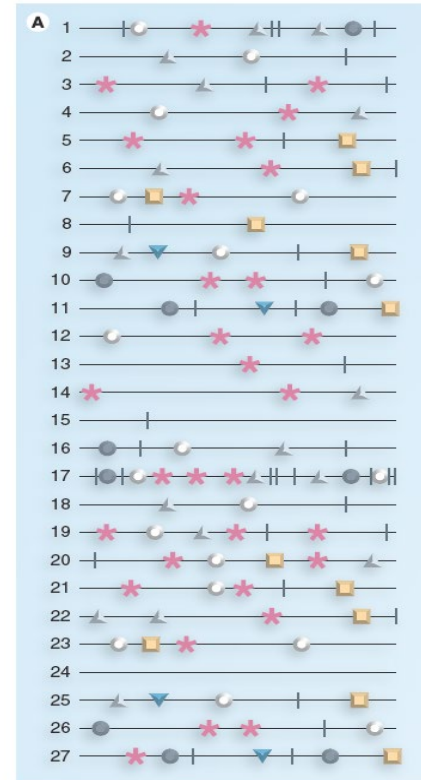
HIV cure trials: Time in remission and viral rebound after ART cessation



AVERT.org Source: IAS Society 'Full recommendations: Towards an HIV cure 2016'

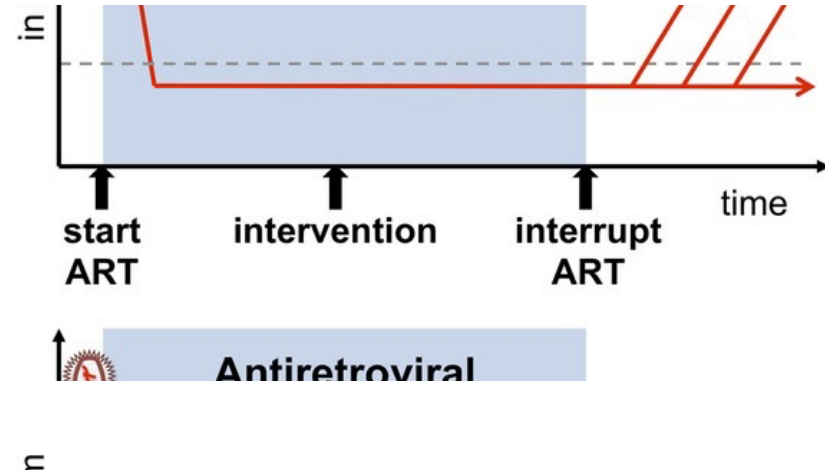
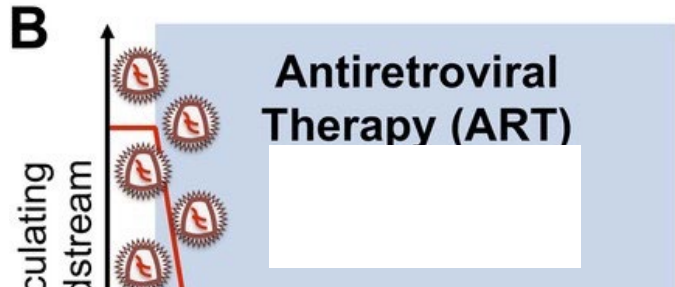
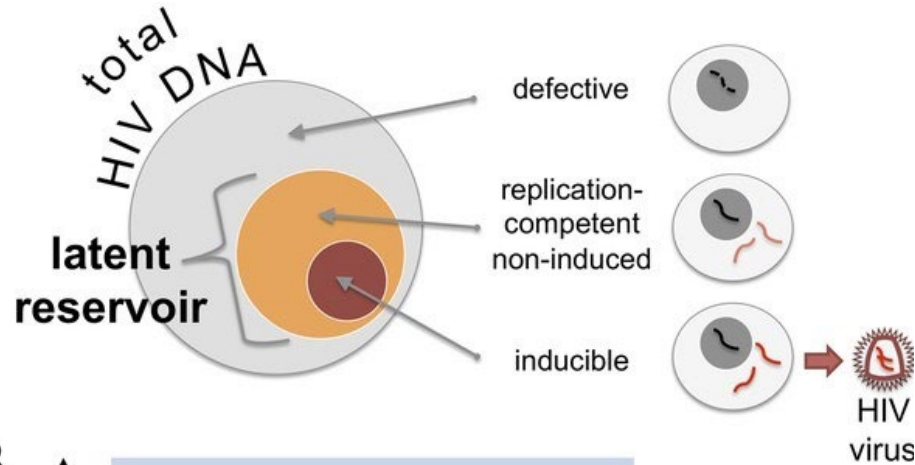
Quasispecies properties

- RNA viruses high mutation rates and progeny production generate a swarm of mutants known as quasispecies.
- Quasispecies, and not single genomes, are the object of the evolution.
- Quasispecies dynamics and properties drive viral infection evolution.
- This population distribution allows the viruses to better adapt to changing environments.



Mutant Spectra in Virus Behavior.
Perales et al. Fut.Virol.2010

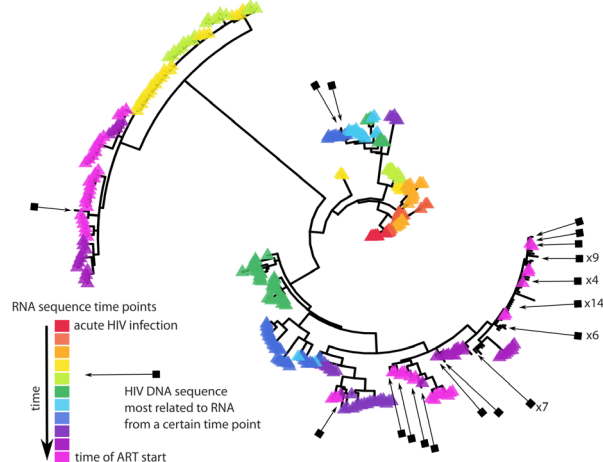
The reservoir “Dogma”



Eshe R Cockerham, Steven G Deeks (2014) eLife

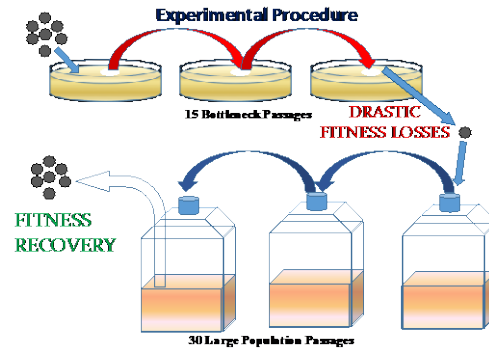
Clonal populations should not produce the rebound

Rebound sequences are related to time of ART start populations and retain variability



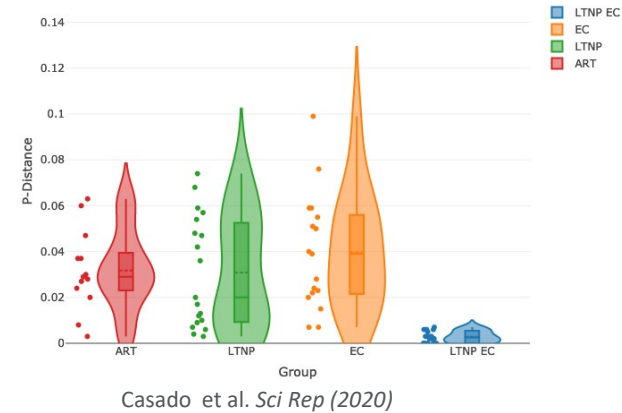
Pankau et al. (2020) PLOS Pathogens

HIV-1 cannot survive extreme losses of diversity

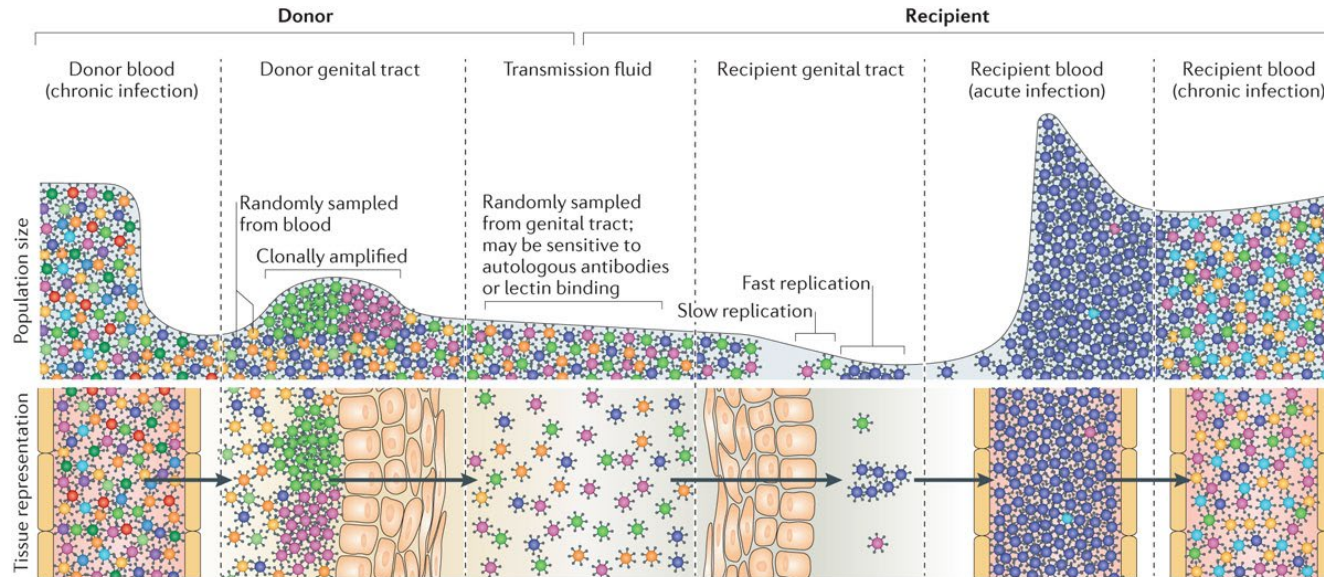


Lorenzo-Redondo R et al.(2011) J Virol.

Extreme low diversity prevents replication

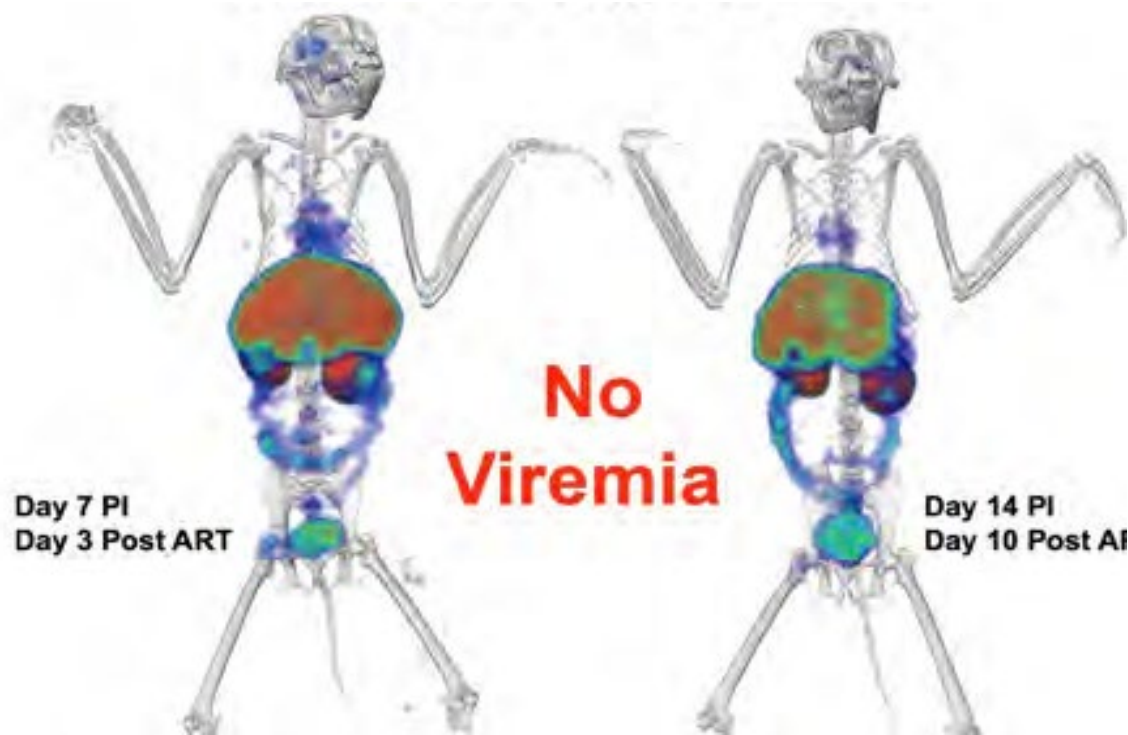


HIV-1 population bottlenecks require pre-existing diversity



Nature Reviews | Microbiology

The Reservoir is everywhere (and now we can find it!)



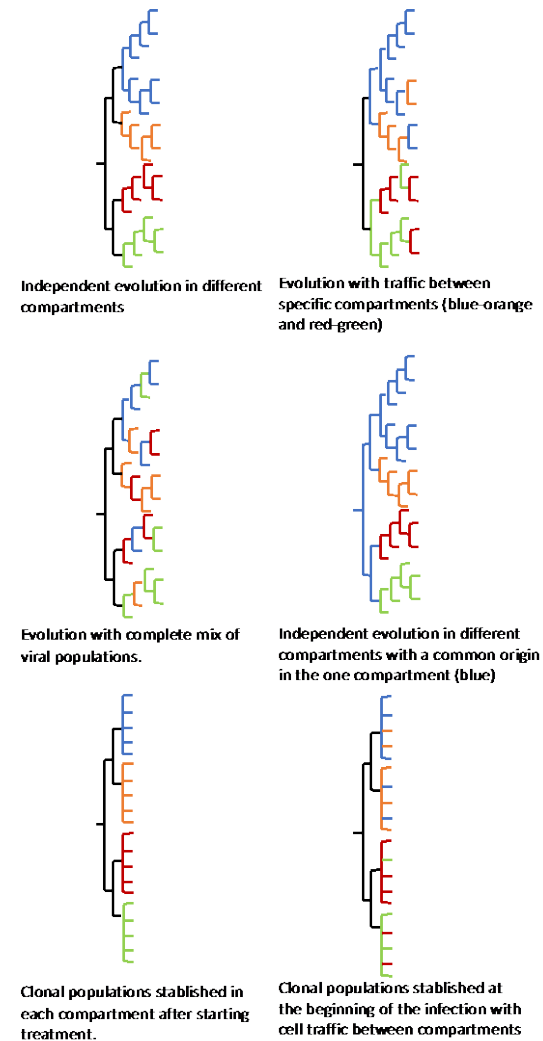
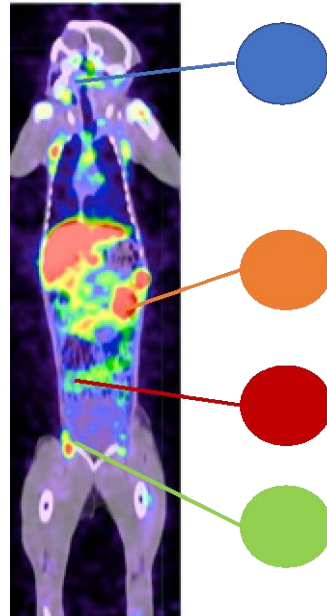
Thomas J Hope



Francois Villinger

CFAR Pilot 1: Assessing the best viral sampling method for an accurate detection of the tissue viral reservoir dynamics

Reservoir Population Dynamics

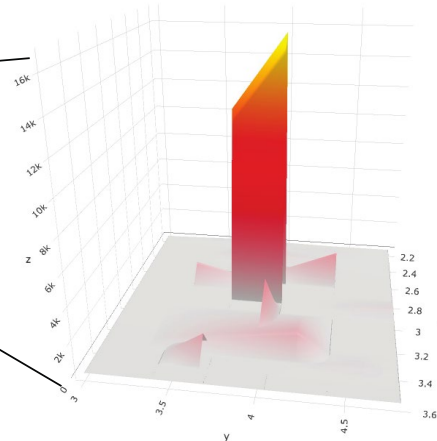
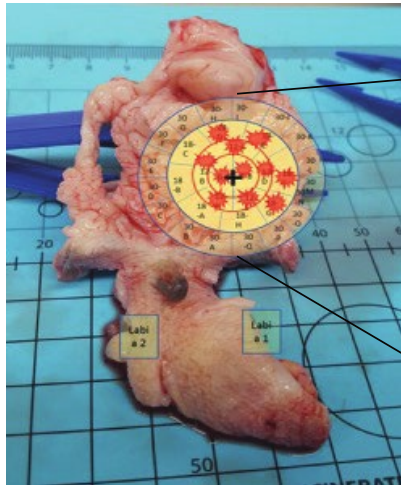


Optimization of SIV sequencing from tissues



Isabelle Clerc, PhD

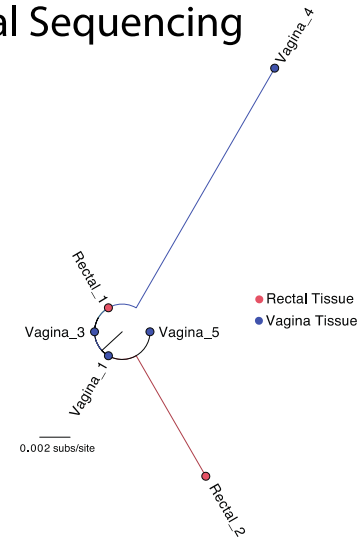
a) Gag qPCR Tissue Levels



SIV copies/10⁶ Cells

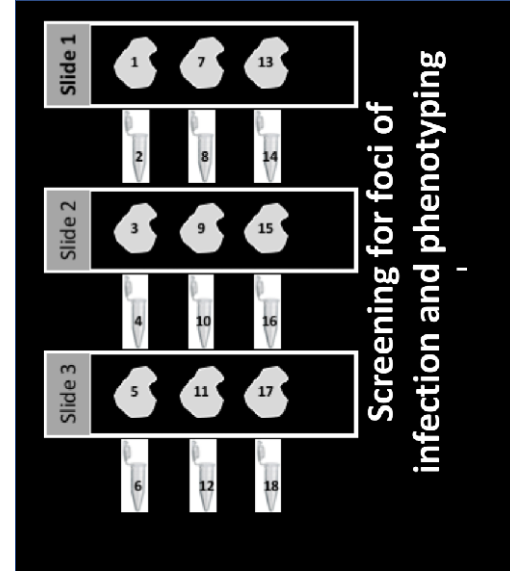
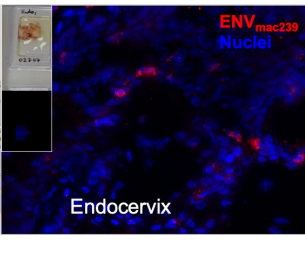
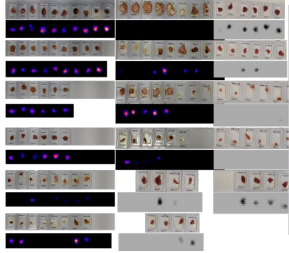
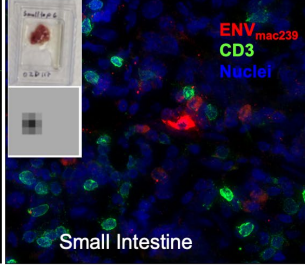
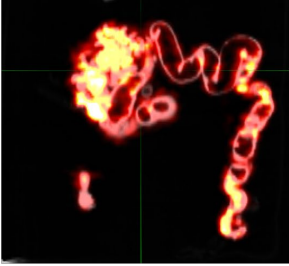
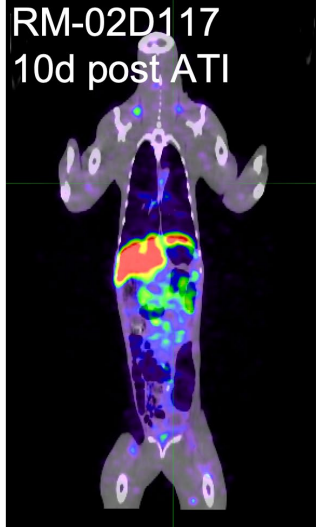


b) Tissue Reservoir Viral Sequencing



PET-CT-Guided SIV reservoir molecular characterization

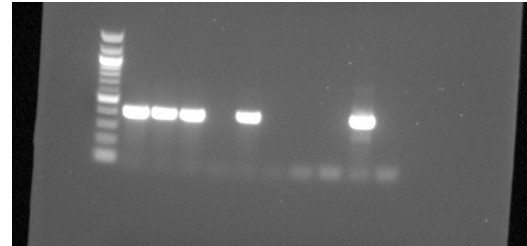
RM-02D117
10d post ATI



Muhammad Shoaib Arif, PhD



Christopher Thuruthiyil



Early rebound intra-host viral populations

TissueClass

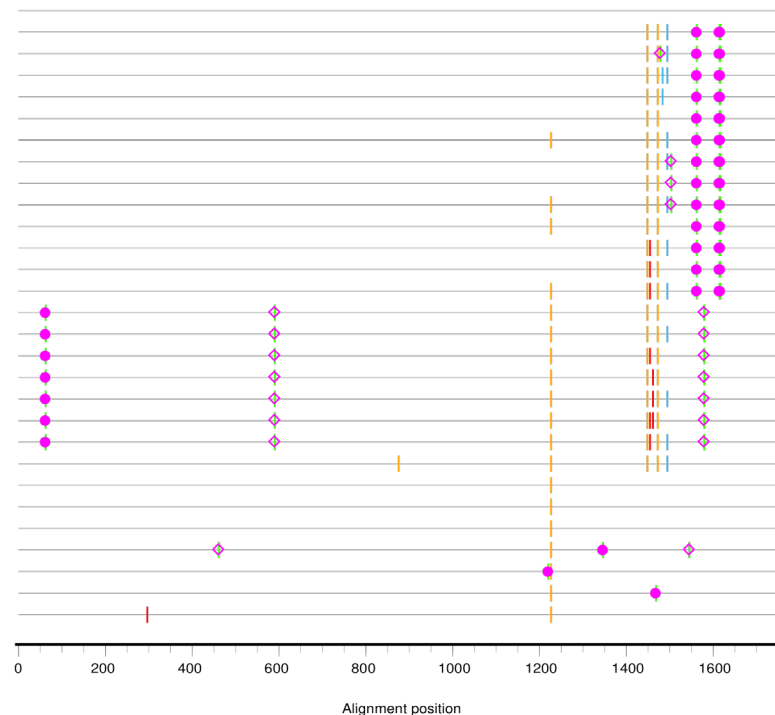
- Ascending Colon
- Descending Colon
- Transverse Colon 1
- Transverse Colon 2

Freq

- 0.2
- 0.4
- 0.6
- 0.8

Legend

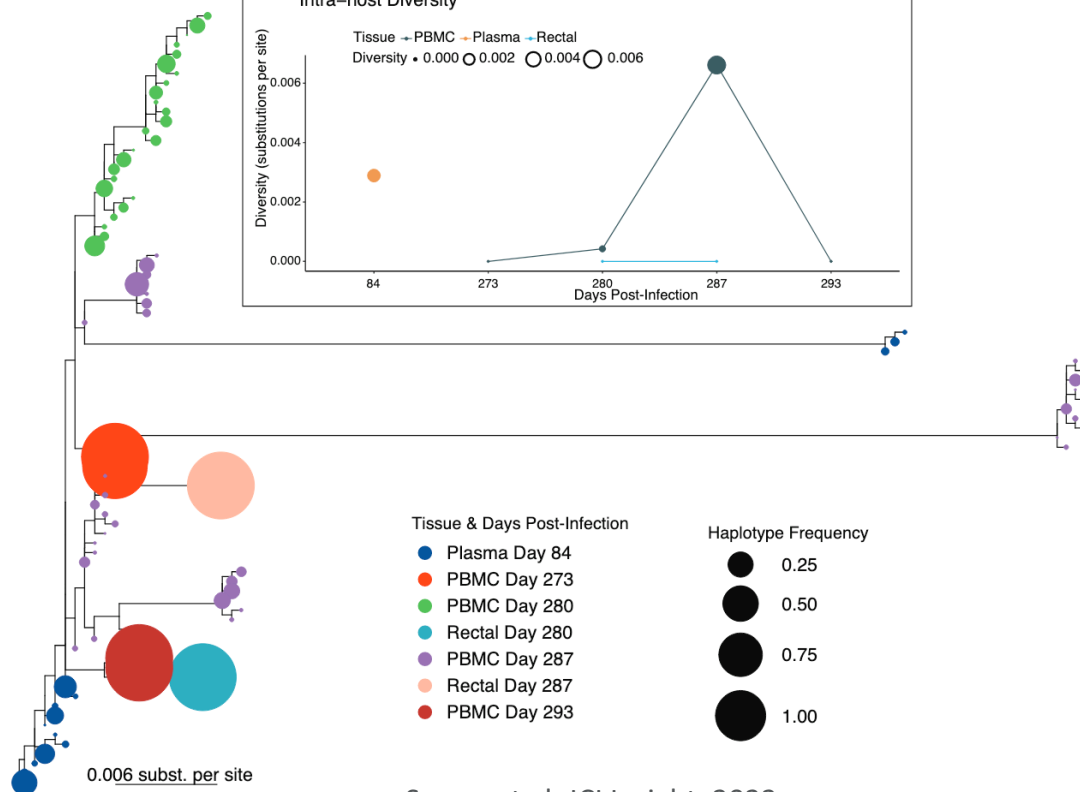
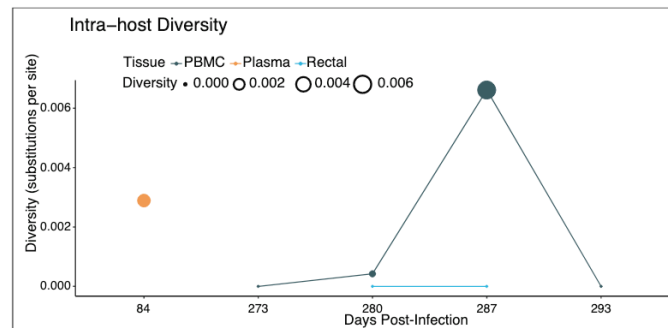
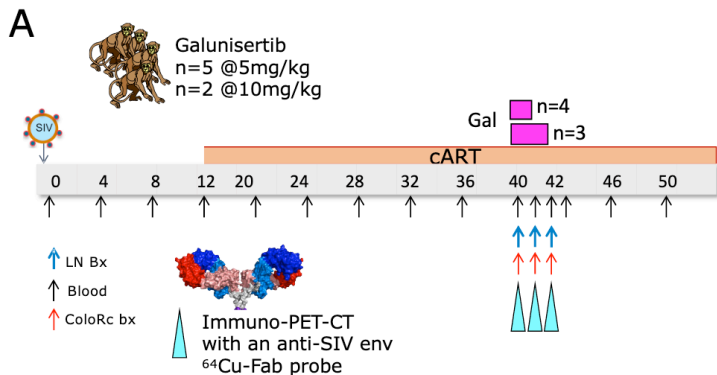
- A C G T GAP
- APOBEC ◇ G→A mutation



Detection of reservoir diversification after reactivation



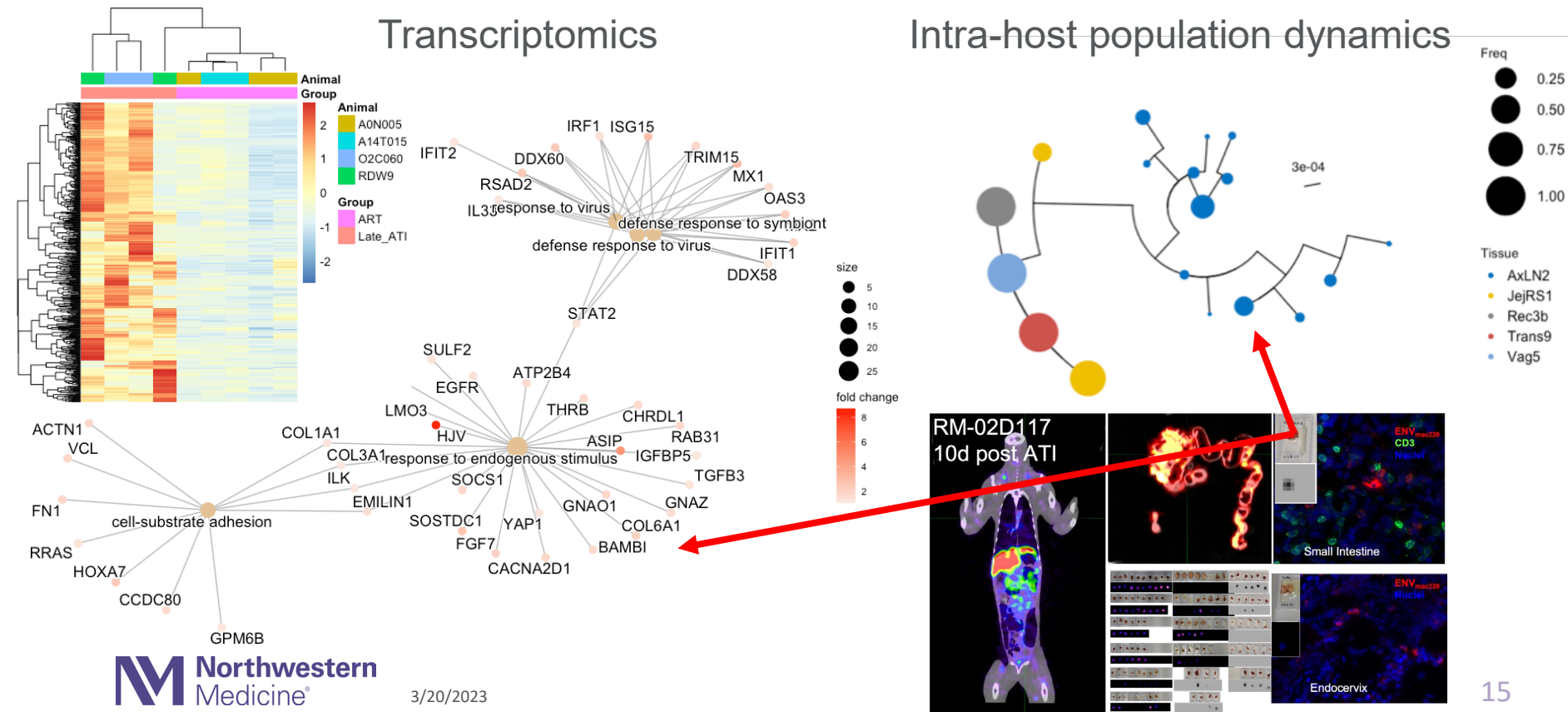
Elena Martinelli



Virus-host system in SIV reservoirs

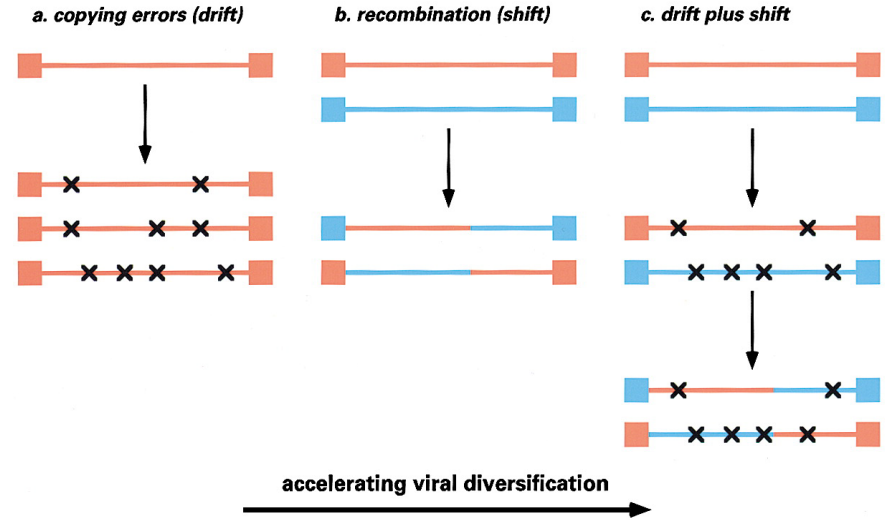
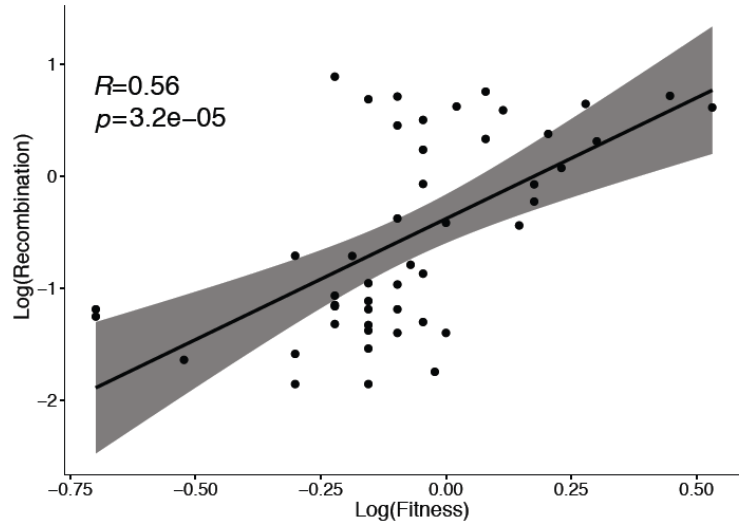
Transcriptomics

Intra-host population dynamics



CFAR Pilot 2: Understanding the role of HIV-1 recombination in the maintenance and rebound of the tissue reservoir

HIV-1 Recombination in the Maintenance and Rebound of the Tissue Reservoir



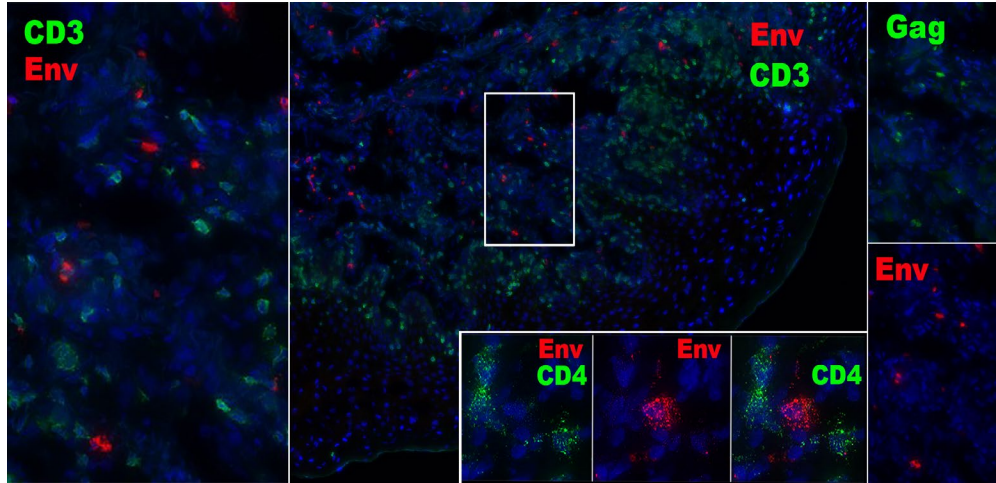
Miguel Arenas, Ramon Lorenzo-Redondo, Cecilio Lopez-Galindez. Influence of mutation and recombination on HIV-1 in vitro fitness recovery. *Molecular Phylogenetics and Evolution*, 2016

Malim et al. *Cell* 2001

Myeloid cells are a key reservoir



Thomas J Hope, PhD

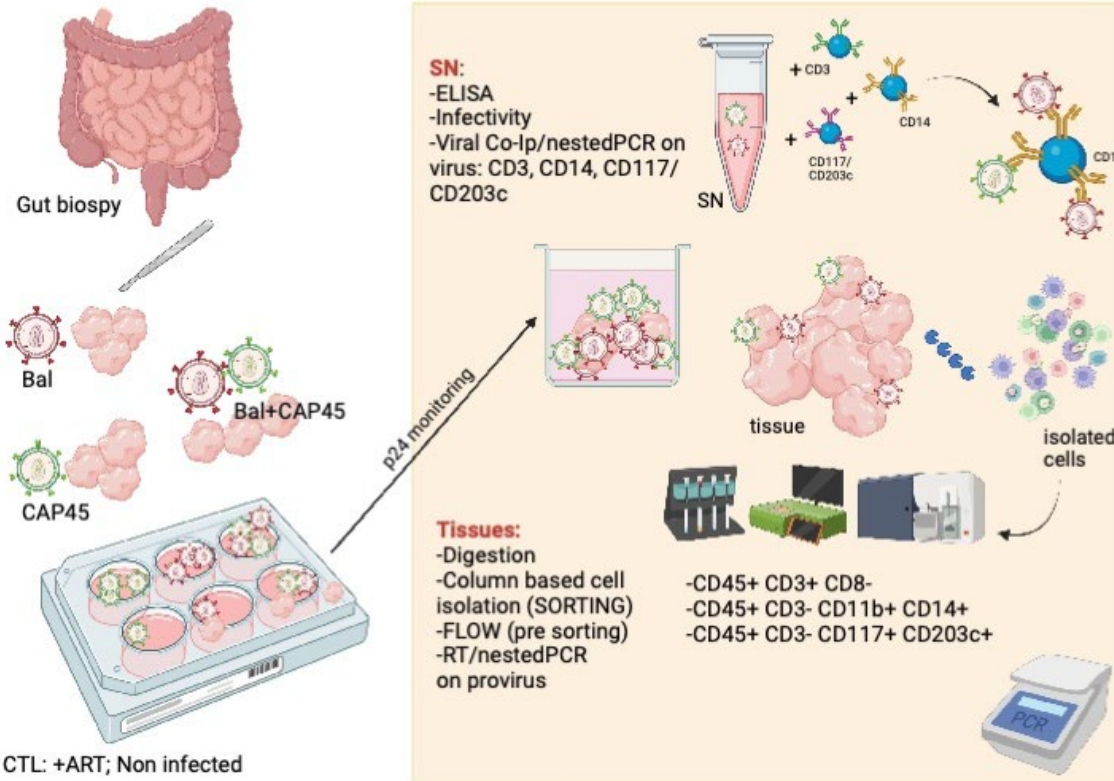


Myeloid cells are exclusively detected in foci of rebounding SIVmac239 5 days after ATI.

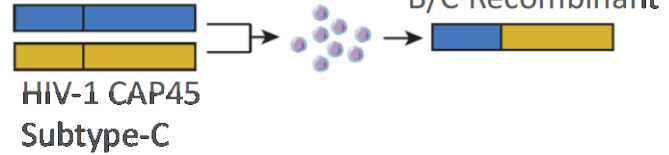
HIV-1 Recombination in different cell types



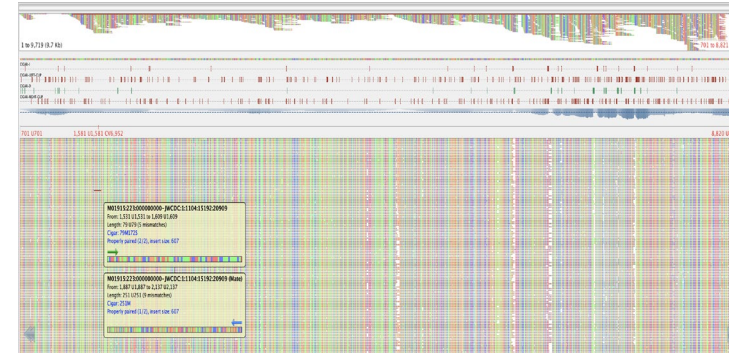
Isabelle Clerc, PhD



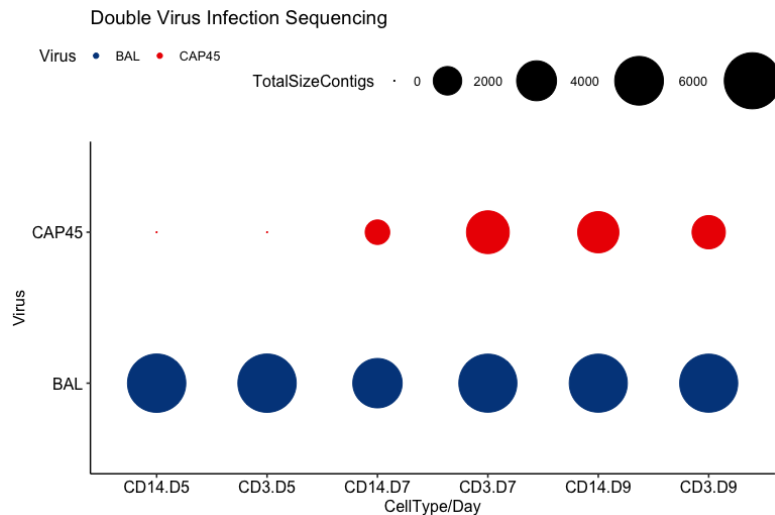
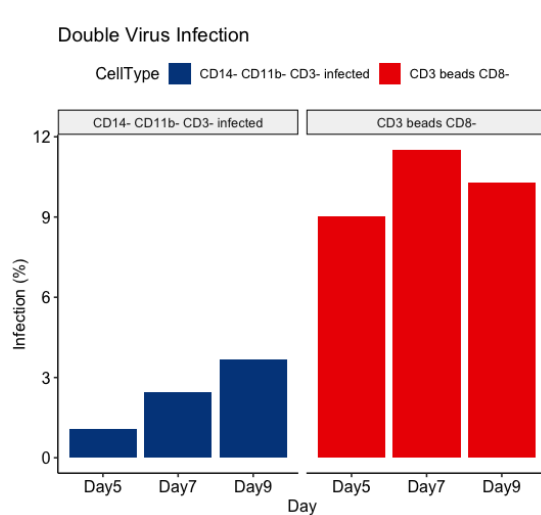
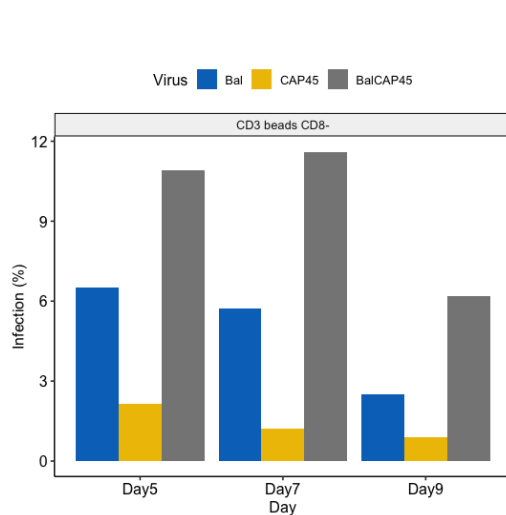
**HIV-1 BAL
Subtype-B**



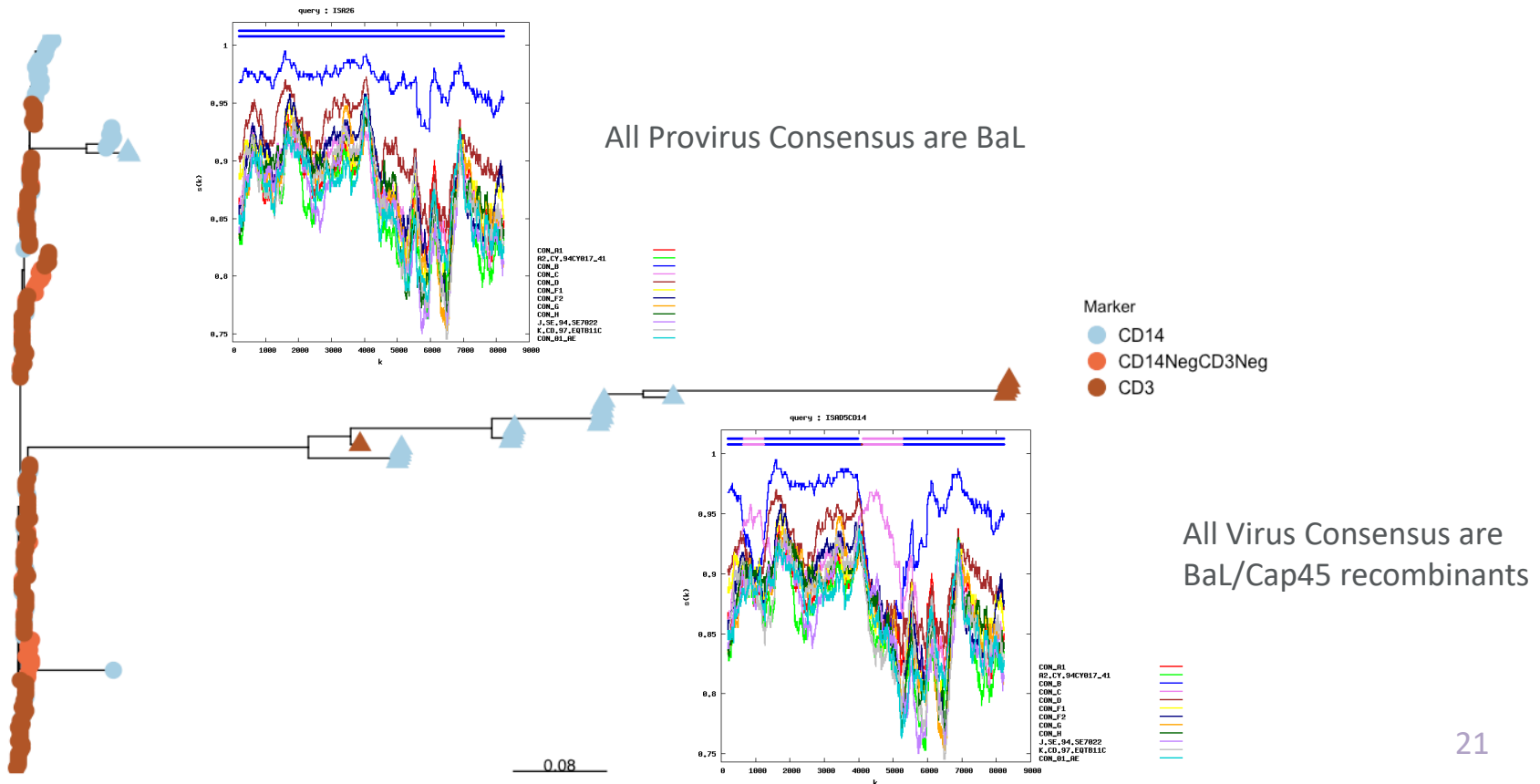
**Full Genome Sequencing
(Sequencing at CPGME)**



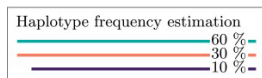
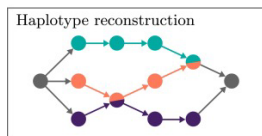
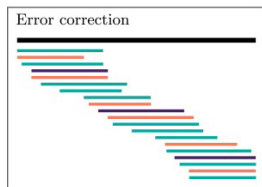
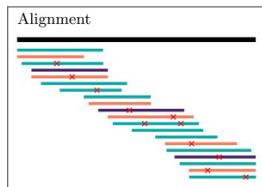
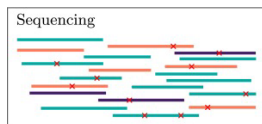
PBMC Infection Test: Cell Type Isolation and NGS



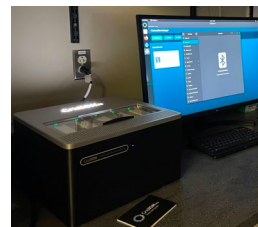
Quasispecies sequences from Cells and Virus



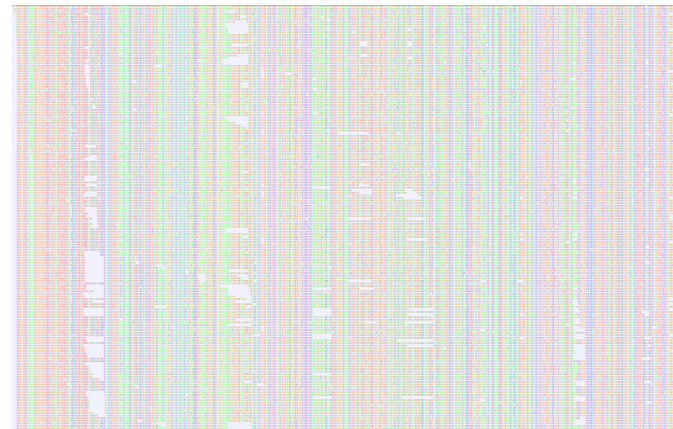
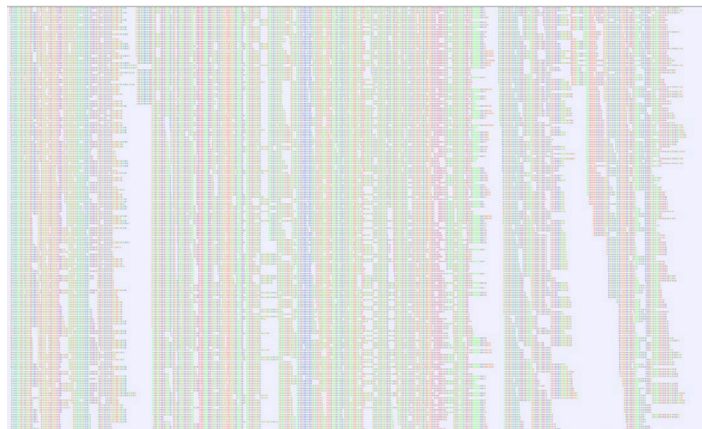
Long-read sequencing now available as a CFAR service



MiSeq read
length: 2 ×
300 bp



GridION read
length: up to
4 MB



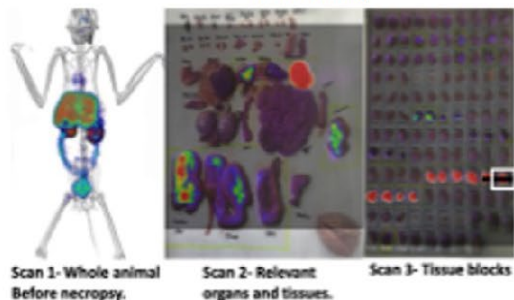
CFAR Supplement: PET-CT-Guided Spatial Transcriptomics of SIV Tissue Reservoirs

PET-CT-Guided Spatial Transcriptomics of SIV Tissue Reservoirs

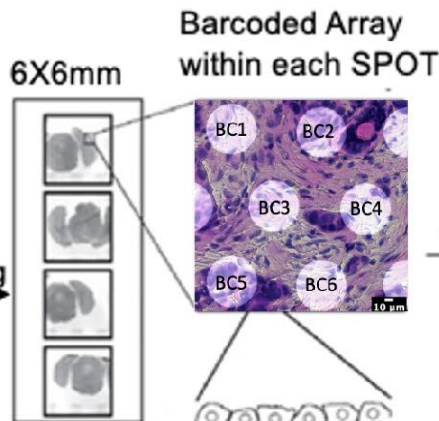
Steps optimized

Screening of tissues

- PET/CT
- Infected vs Non-infected
- RNA Quality
- Tissue Quality

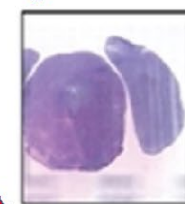


Sectioning



H&E or IF

Permeabilization



Input

10X Visium Slide

- 4x quadrants
- 5000 Spots/quadrant
- Average 1-10 cells/spot

mRNA from Tissue captured on each spot

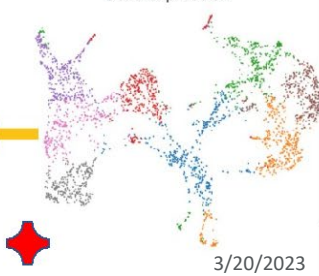
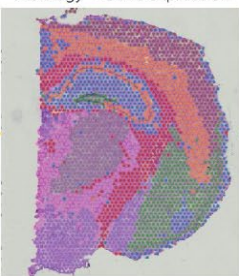


Sequencing
Mapping

	Barcodes			
Gene counts	50	45	11	67
	50	10	00	14
	15	55	98	15
	96	14	15	35

Histology + Gene expression

Gene expression



3/20/2023



Elena Martinelli, PhD

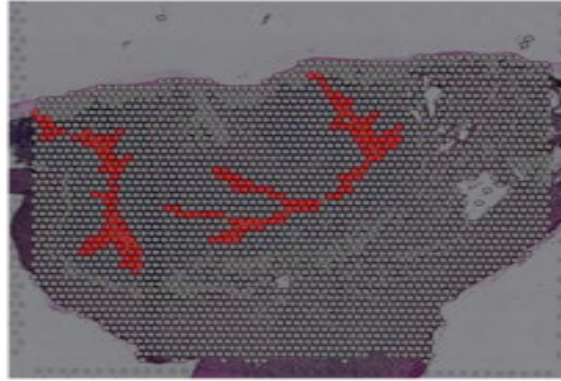
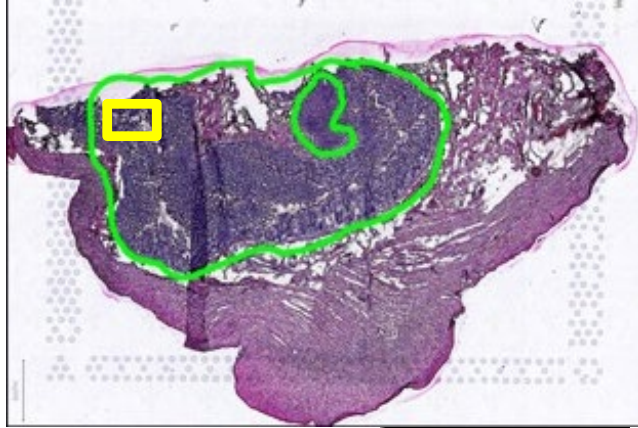


Muhammad Shoaib Arif, PhD

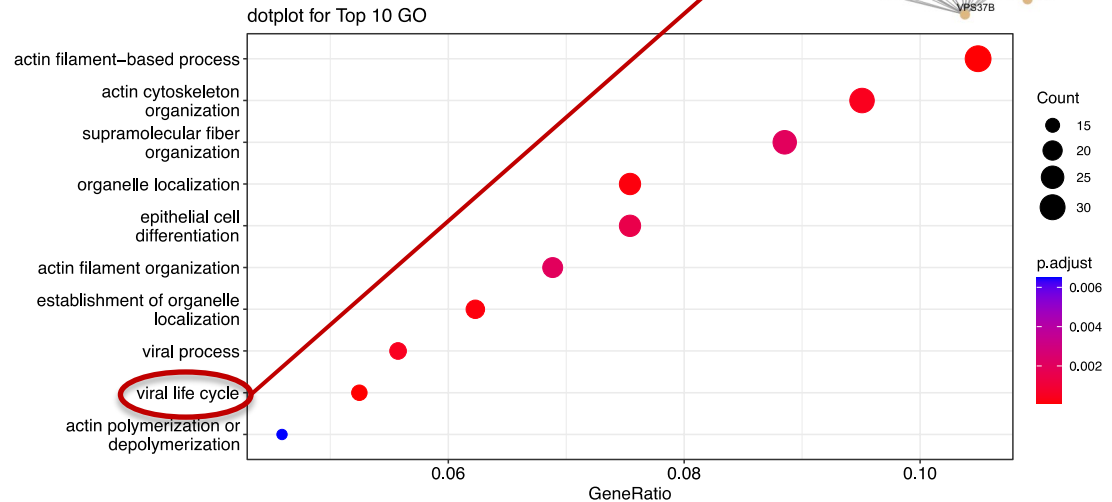
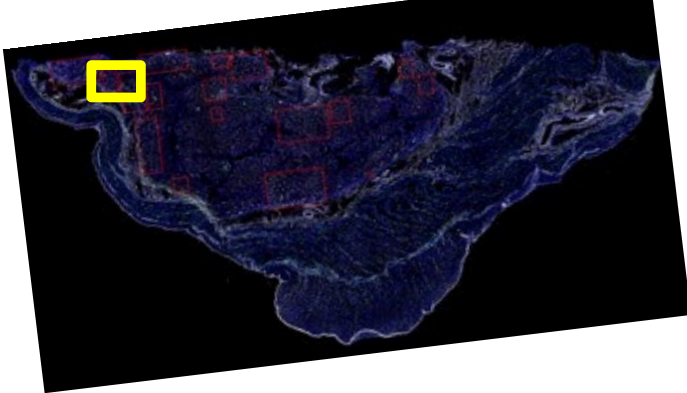
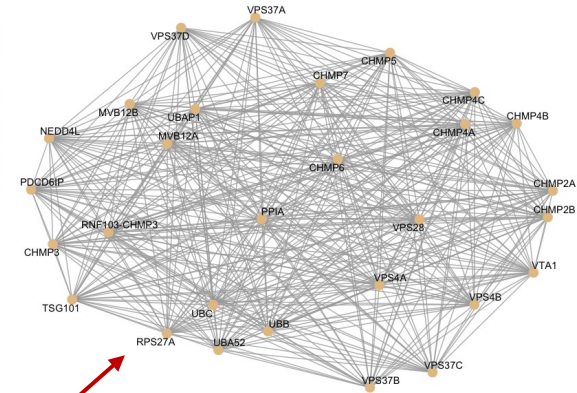


Christopher Thuruthiyil

Cluster associated with areas of infected cells

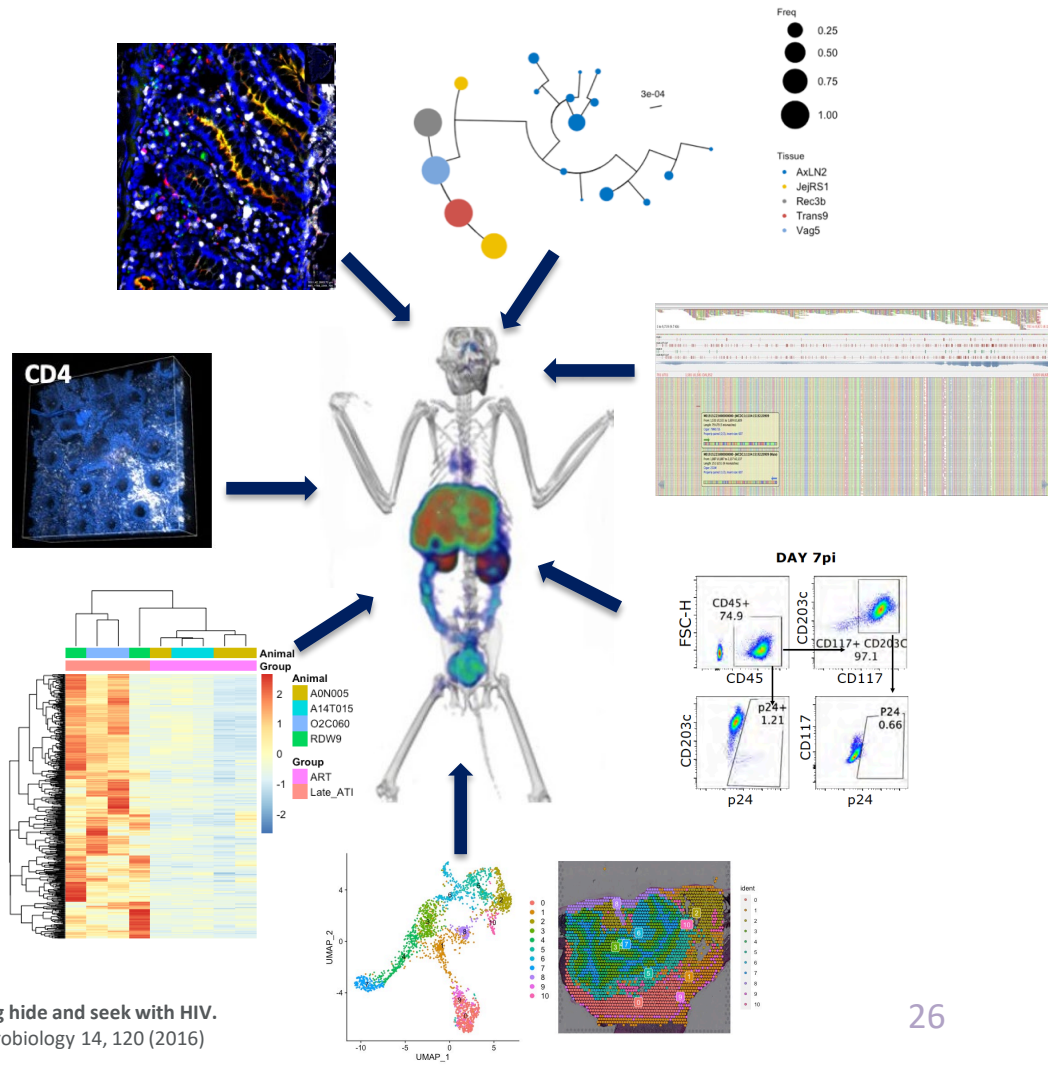
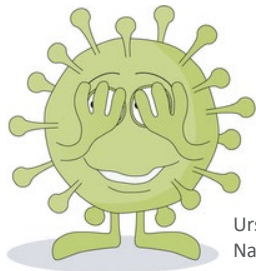


Budding and maturation of HIV virion



Conclusions

- The unique program of CFAR pilot grants has allowed us develop innovative methodology and pipelines to study the HIV-1 reservoirs.
- We have now a set of tools that has the potential to unveil key characteristics of the reservoir virus-host system at an unprecedented level.
- The toolbox is important, but the key is “to have the piece of tissue”. **Now we know where to look to find and characterize the reservoir.**



Value Added by the CFAR

All methods and data generated thanks to CFAR funding have been key for 2 NIH awarded grants

Role of myeloid cells in CNS and systemic reservoirs and rebound (R01)



Thomas J Hope



Elena Martinelli



Francois Villinger

Unraveling the Mechanisms of HIV Persistence and Rebound (P01)



Thomas J Hope



Elena Martinelli



Francois Villinger



Bruce S Bochner

Acknowledgements



Thomas J Hope



Richard T D'Aquila



Muhammad Shoaib Arif



Isabelle Clerc



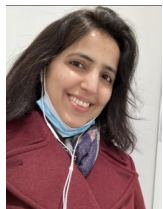
Francois Villingier



Elena Martinelli



Mike McRaven



Sadia Samer



Ed Allen



Christopher Thuruthiyil



Hope Lab

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 - 1R01MH125778
- Third Coast Center for AIDS Research



National Institutes
of Health



NIAID



UNIVERSITY OF LOUISIANA AT LAFAYETTE
NEW IBERIA RESEARCH CENTER
University Research Division





Center for Pathogen Genomics and Microbial Evolution

Thank You!

Questions?