Zabrina Brumme, PhD
Professor, Simon Fraser University, Canada
Laboratory Director, British Columbia Centre for Excellence in HIV/AIDS

IAS Pathways to an HIV cure meeting 2022:

Cure Advances Globally

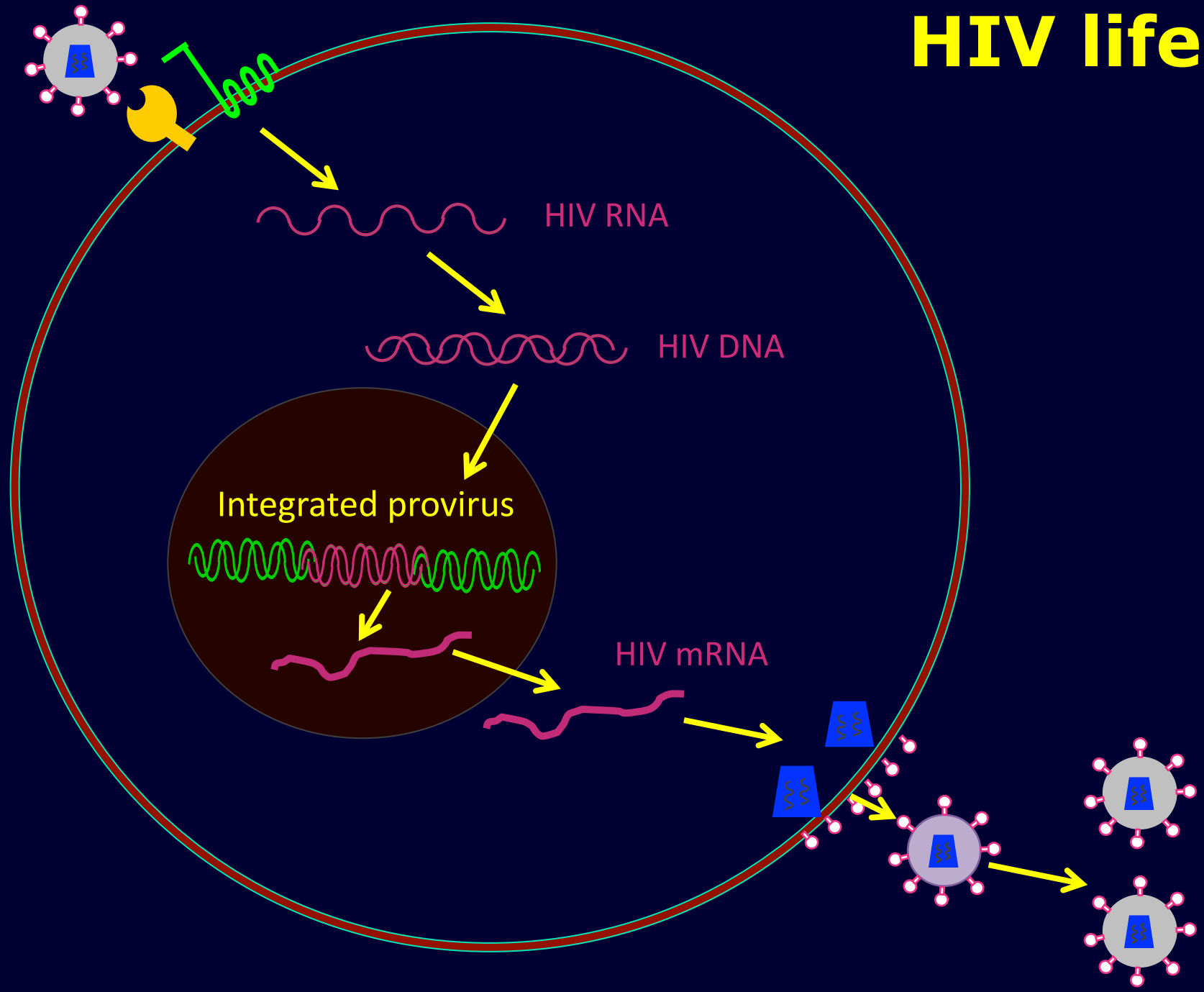
Virology of the reservoir:

a 15 minute summary

AIDS 2022

AIDS 2022

HIV life cycle



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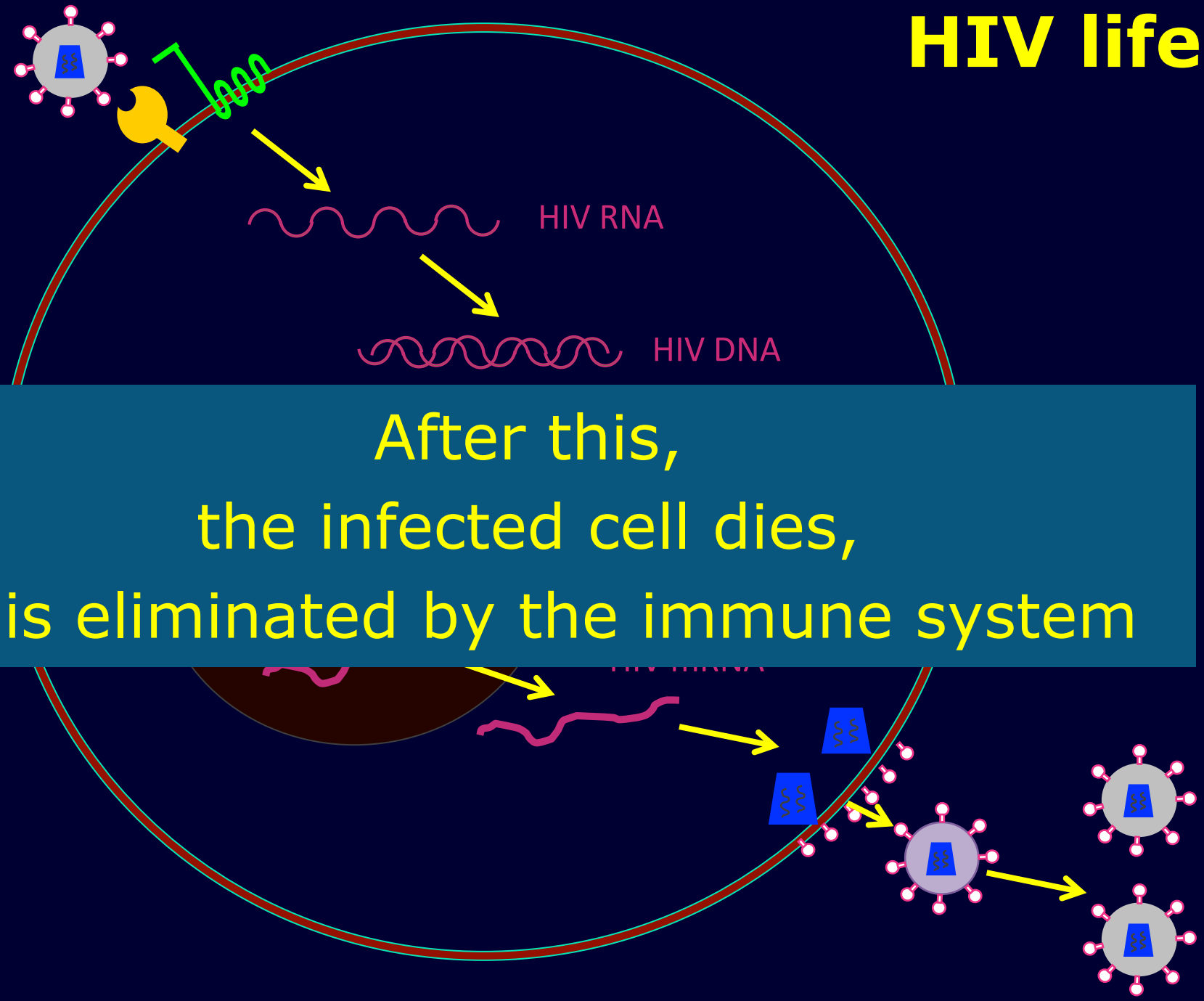
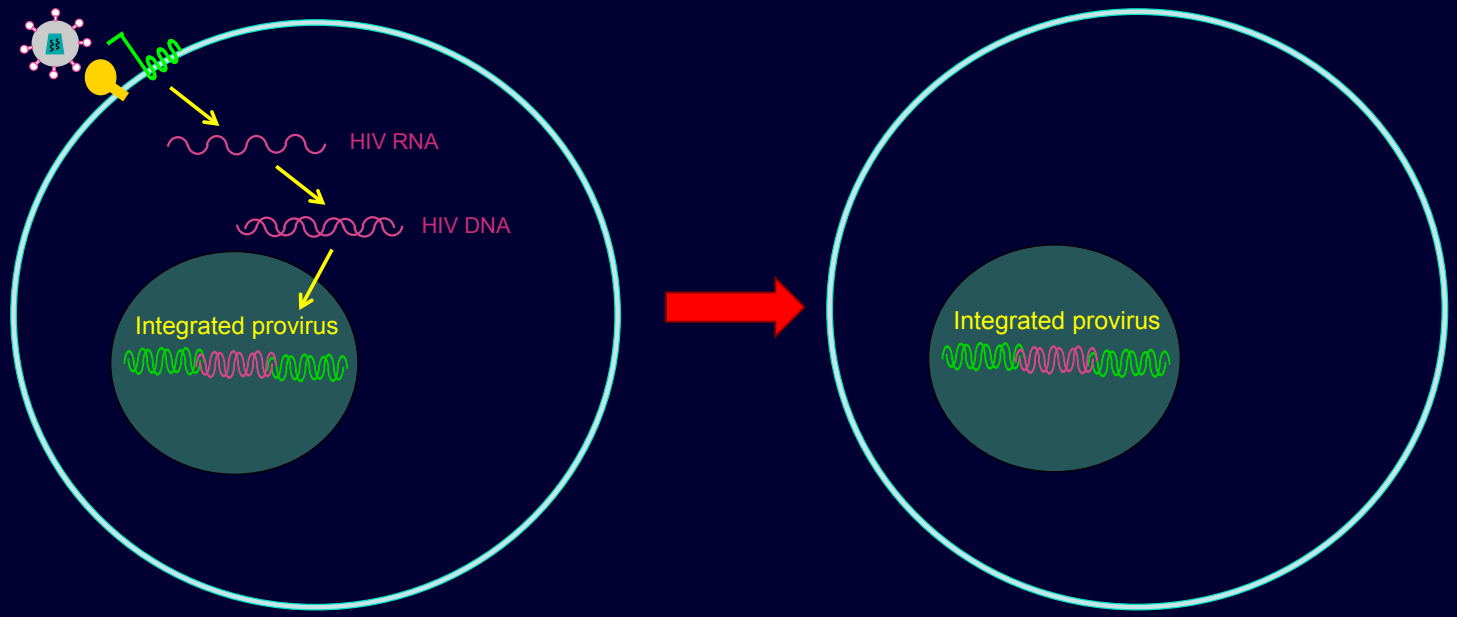


HIV can persist inside cells as an integrated viral genome



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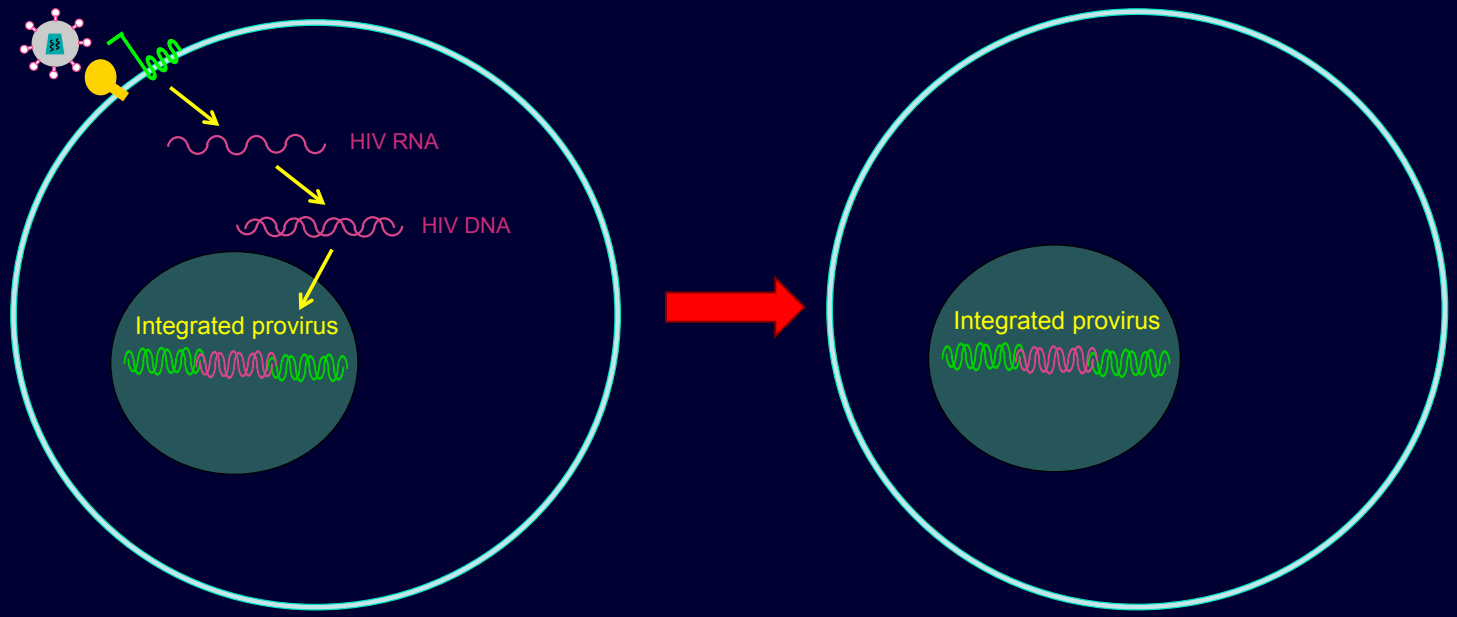

These <u>viral reservoirs</u> are the main barrier to HIV remission and cure:

-> antiretroviral therapies do not eliminate these cells

-> these cells are *largely* invisible to the immune system

-> these cells can persist for years

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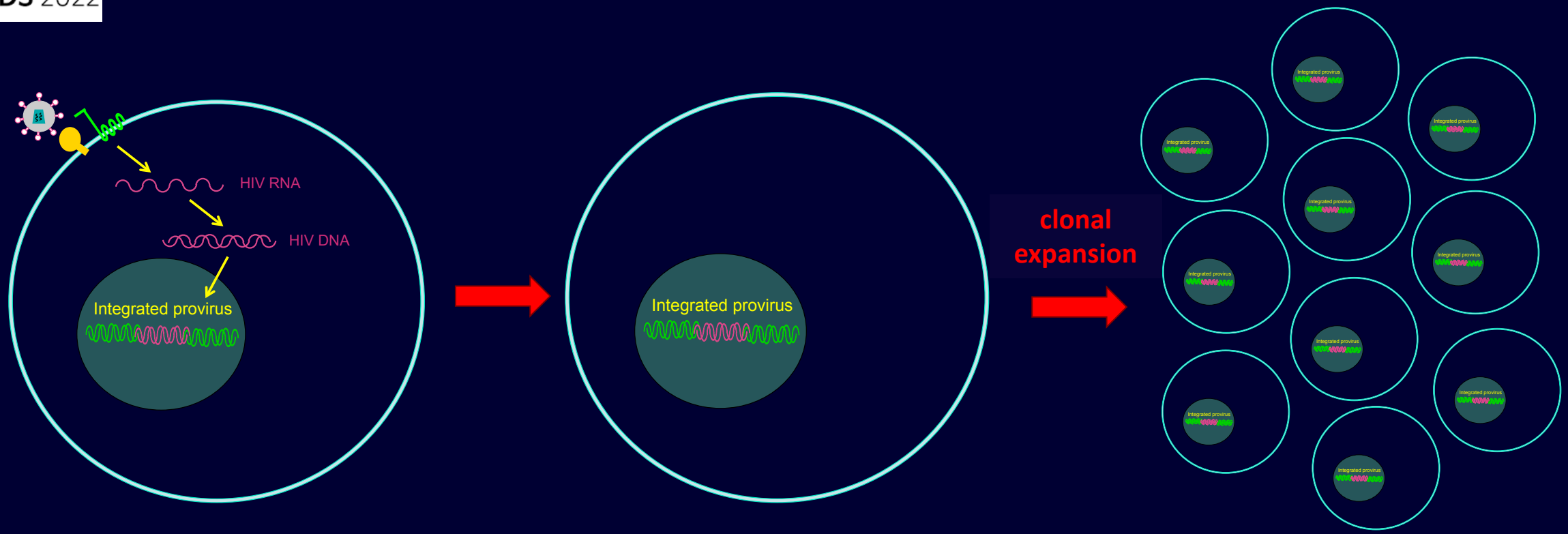


These <u>viral reservoirs</u> are the main barrier to HIV remission and cure:

-> antiretroviral therapies do not eliminate these cells

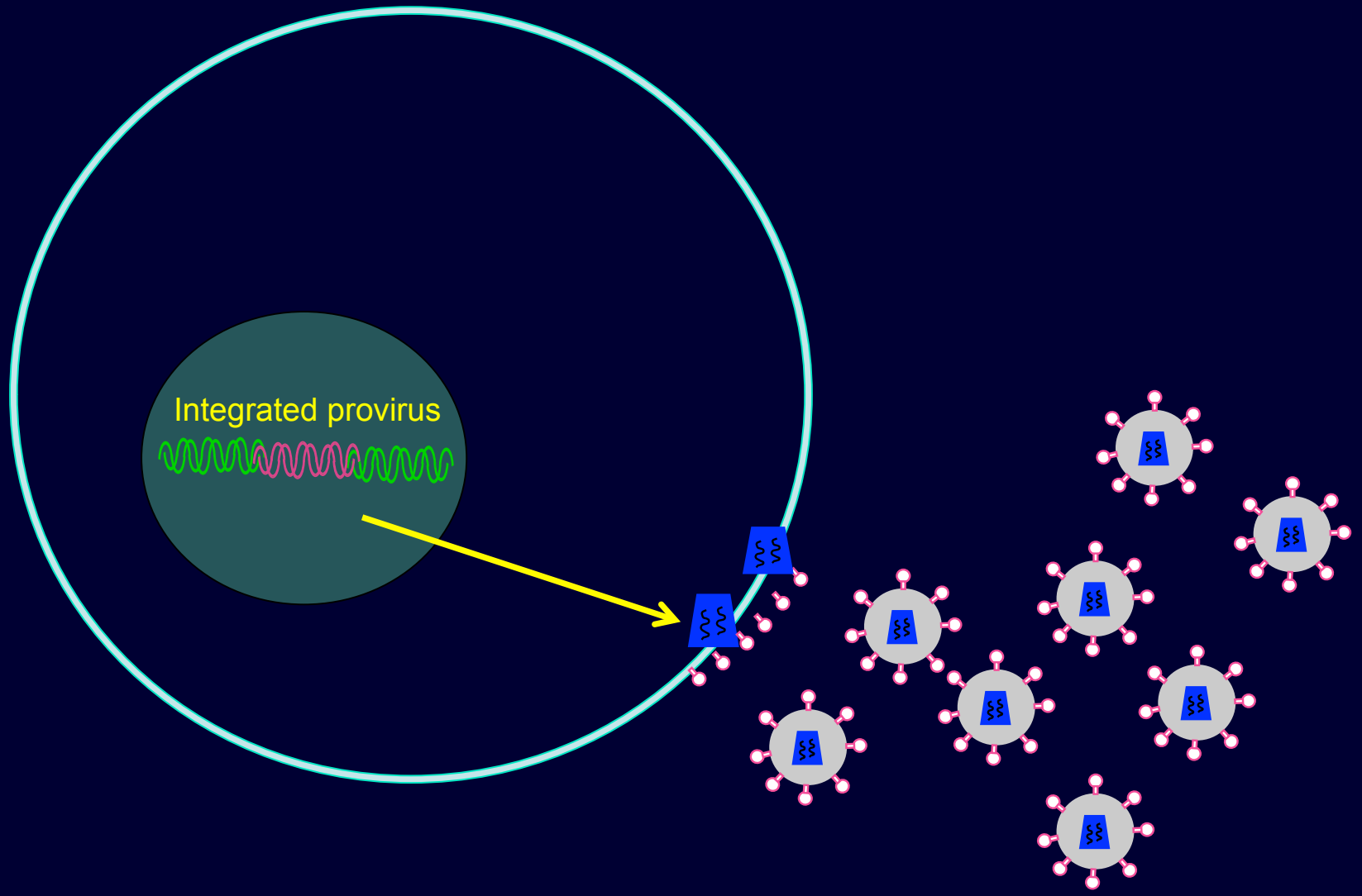
-> these cells are *largely* invisible to the immune system

-> these cells can persist for years

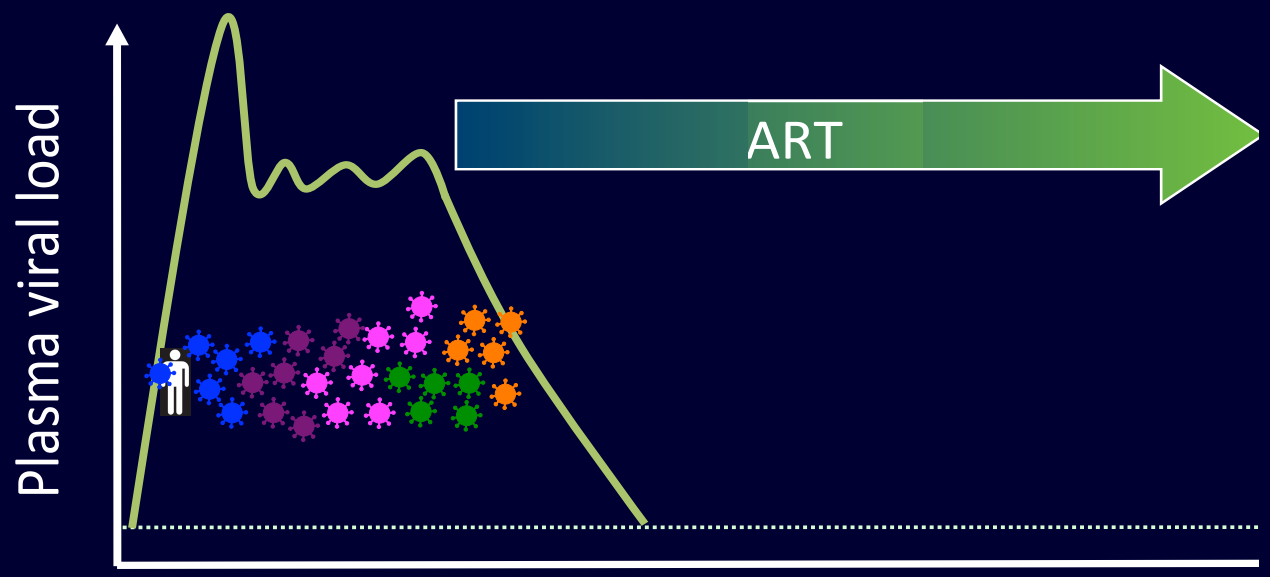
-> these cells can clonally expand, producing daughter cells that also contain integrated HIV within them[1-5]

[1]Maldarelli et al 2014 PMID 24968937; [2]Wagner et al 2014 PMID 25011556; [3]Simonetti et al 2016 PMID 26858442; [4]Hosmane et al 2017 PMID 28341641; [5]Lorenzi et al 2016 PMID 27872306

Viral reservoirs can reactivate at any time to produce infectious HIV



Reservoir dynamics: a two-minute summary



Following transmission, HIV replicates and mutates, producing a genetically diverse viral population

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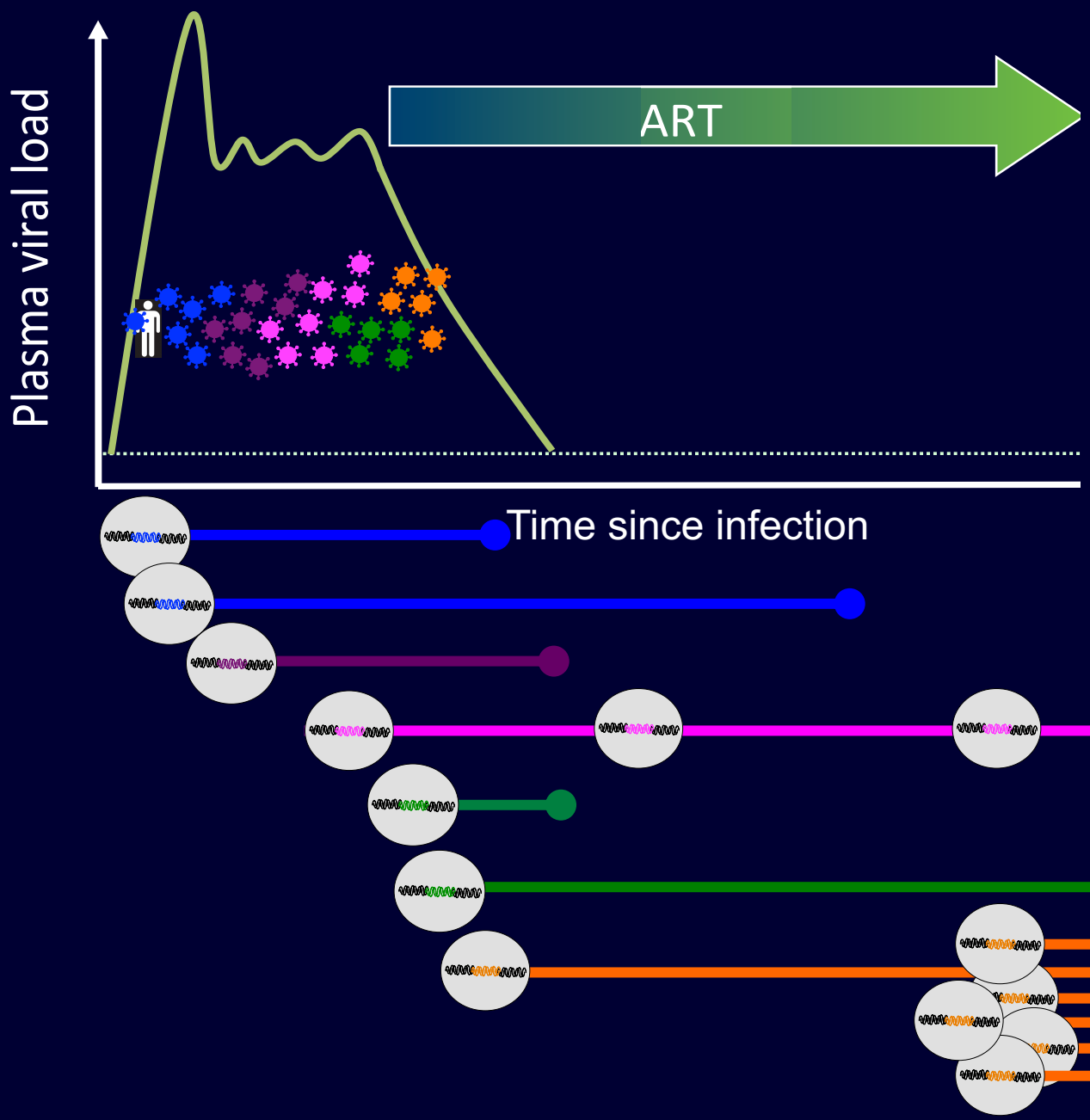

Seeding of the reservoir begins immediately following infection.

Reservoir cells can persist for years.

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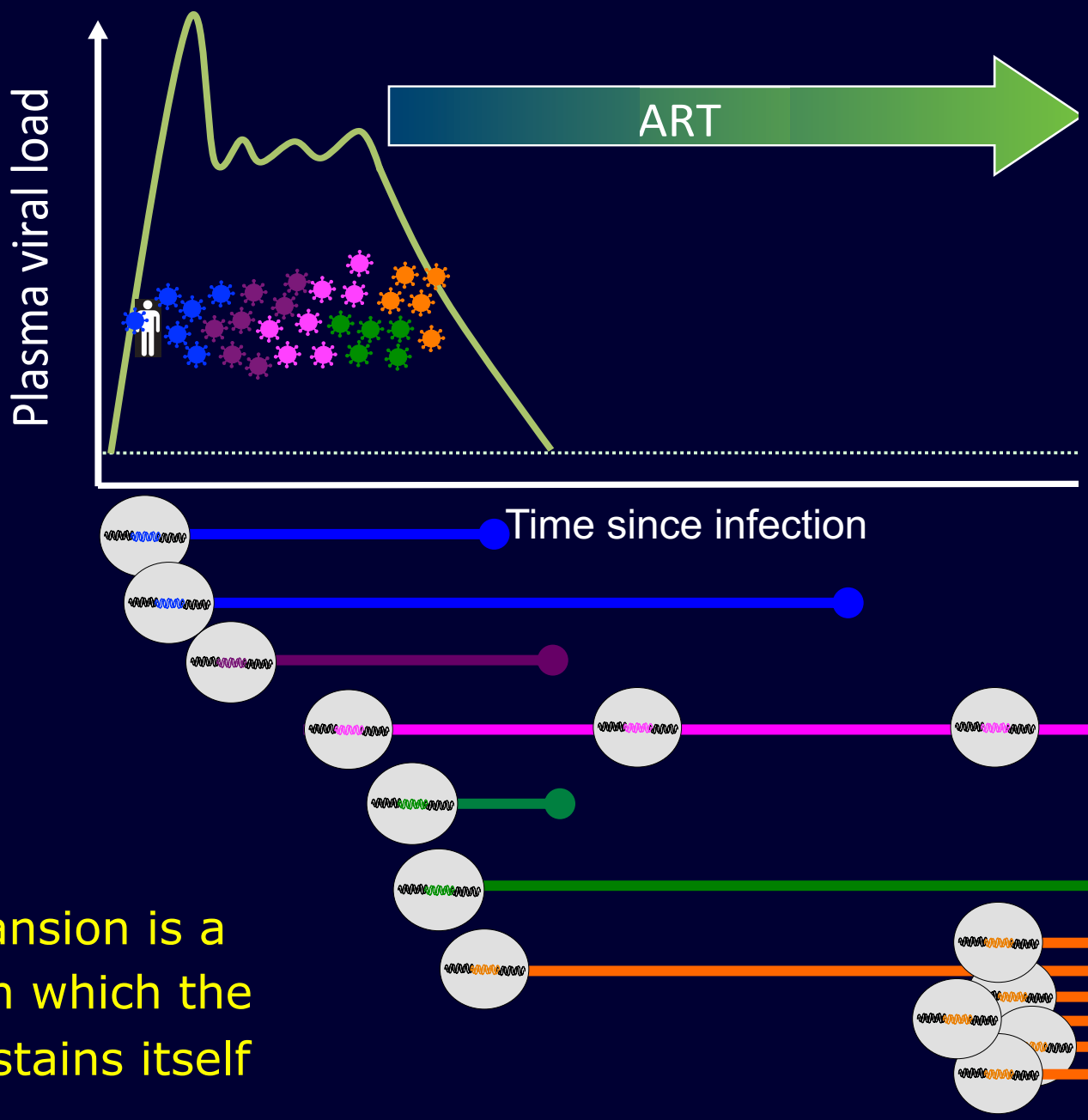

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Clonal expansion is a major way in which the reservoir sustains itself

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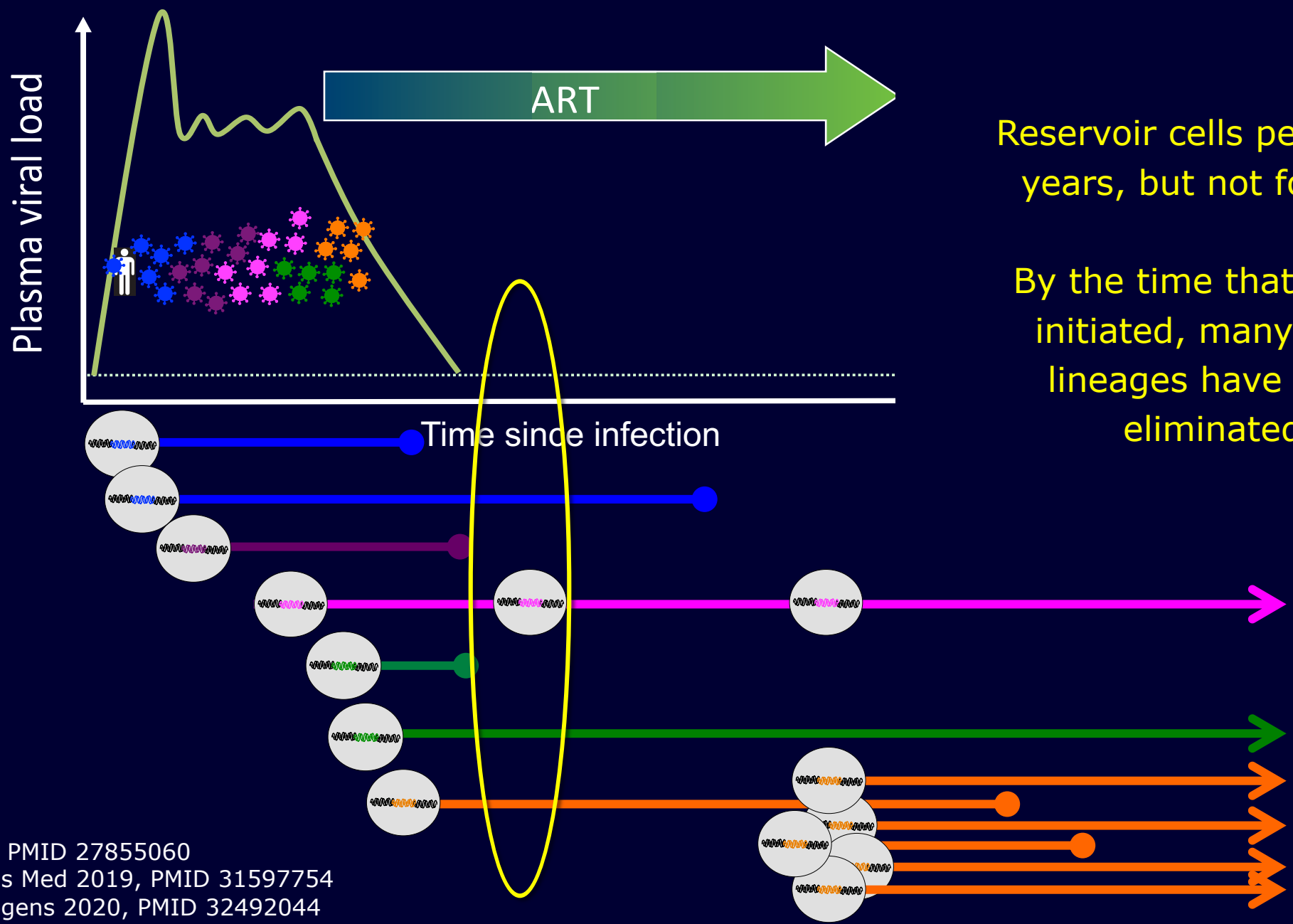
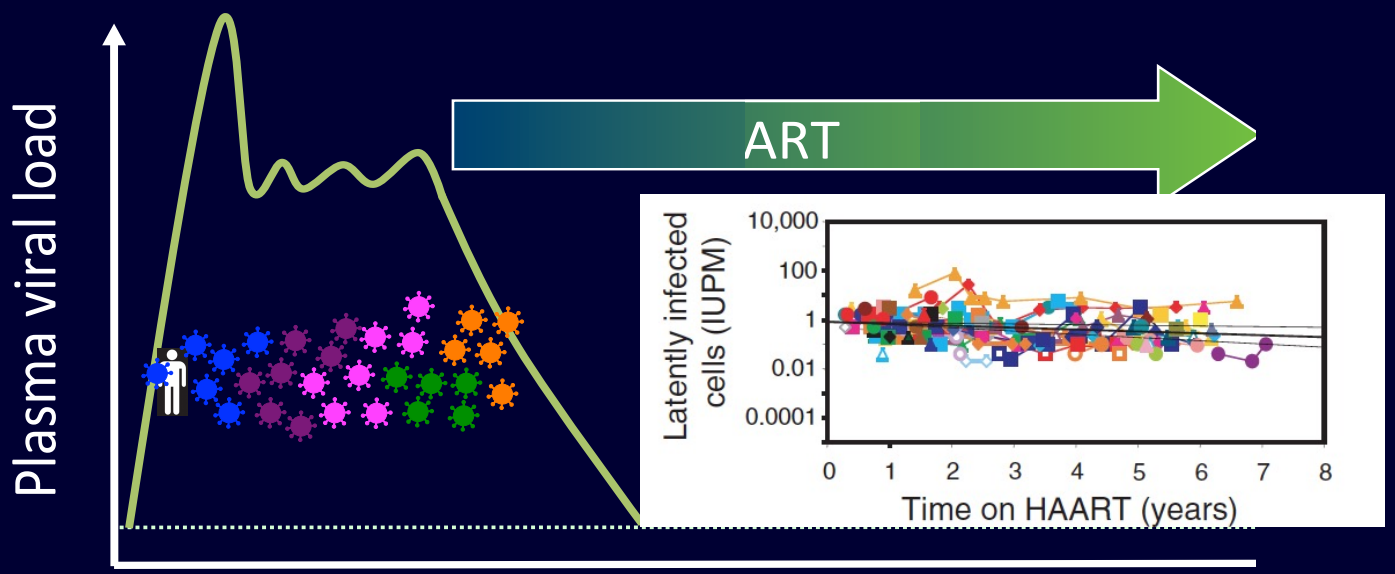


Reservoir cells persist for years, but not forever.

By the time that ART is initiated, many early lineages have been eliminated

Brodin et al, eLife 2016; PMID 27855060
Abrahams et al, Sci Trans Med 2019, PMID 31597754
Brooks et al, PLoS Pathogens 2020, PMID 32492044

Reservoir dynamics: a two-minute summary



On ART, reservoir decay is SLOW

Estimated half-life = 44 months (3.7 years)

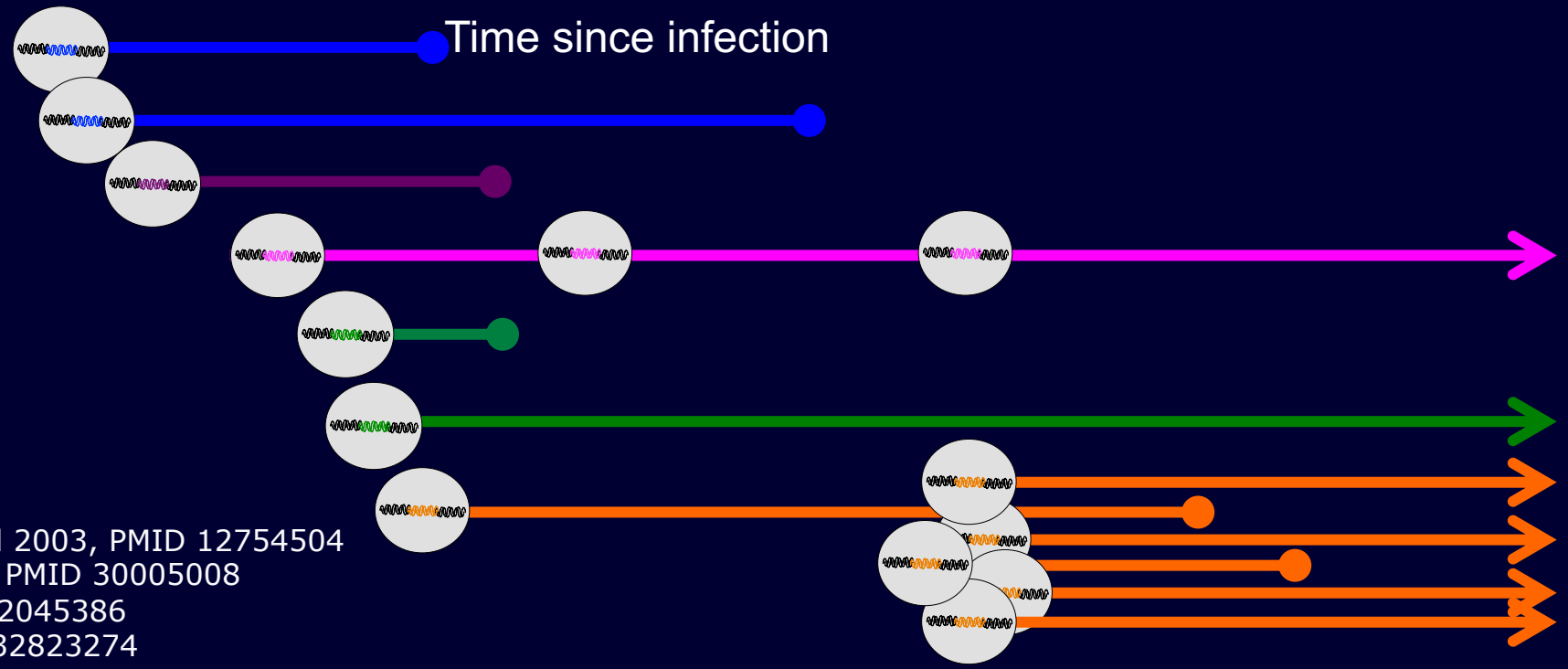


Figure: Siliciano et al, Nat Med 2003, PMID 12754504
Also: Golob et al, AIDS 2018, PMID 30005008
Peluso et al, JCI 2020, PMID 32045386
Gandhi et al, JID 2021, PMID 32823274

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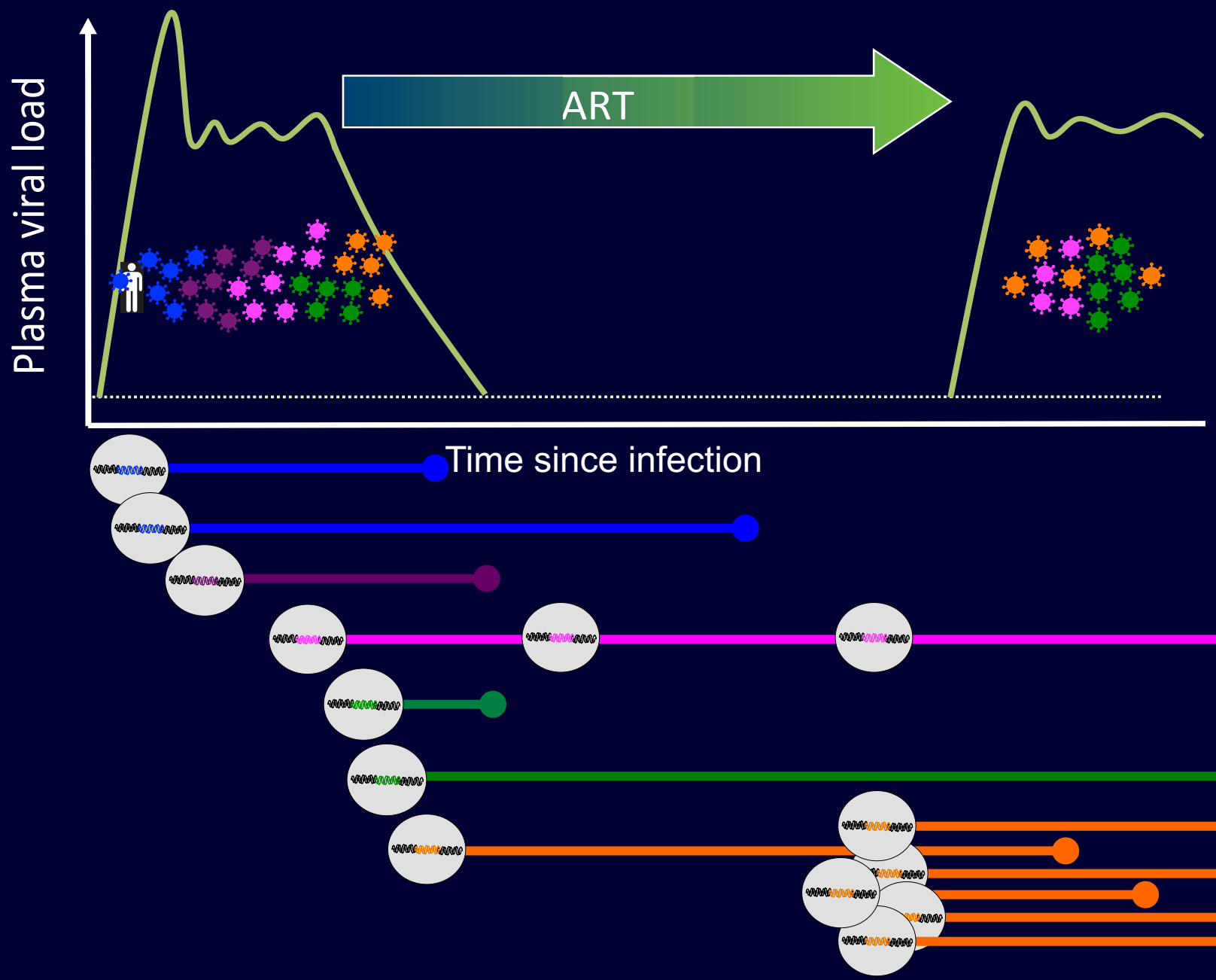
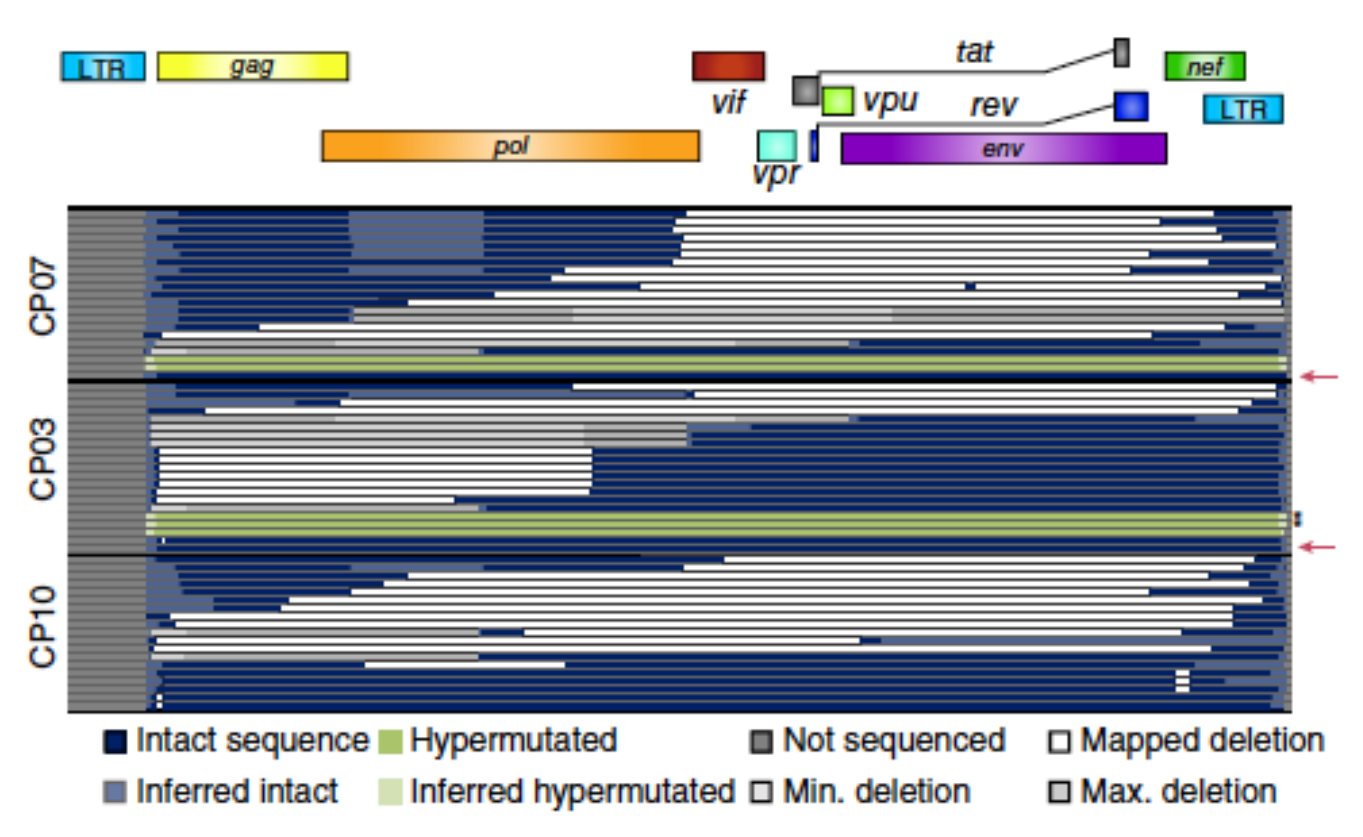
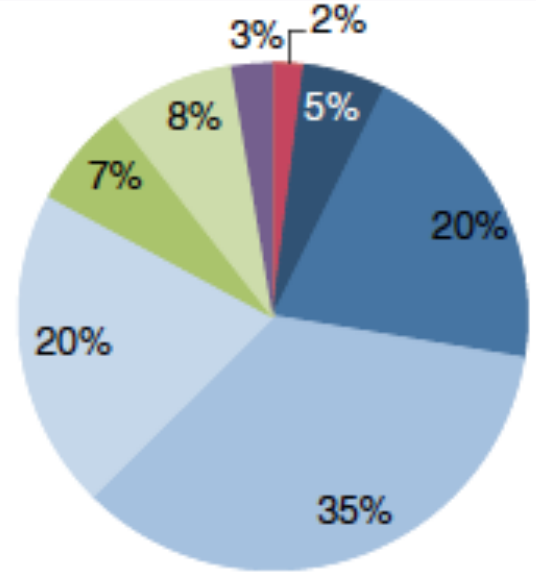


When ART is stopped, HIV re-emerges

Only ~2% proviruses persisting during long-term ART are genetically intact

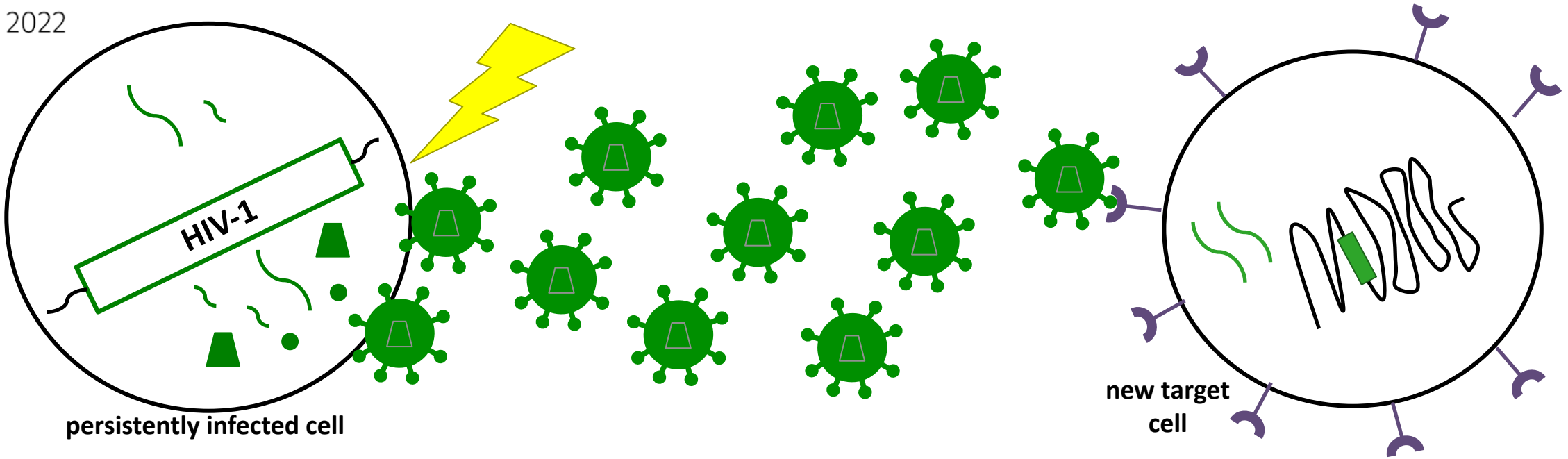




- Intact
- Packaging signal and major splice donor site deletion
- Deletion within 5′ half of the genome
- Deletion within 3′ half of the genome
- Very large internal deletion
- Hypermutated
- Hypermutated and deleted
- Sequence insertions or inversions

Figure from: Bruner et al, Nature Medicine 2016, PMID 27500724.
See also: Ho et al Cell 2013 PMID 24243014

Reservoir = cell harboring intact, replication-competent HIV



Slide by Natalie Kinloch

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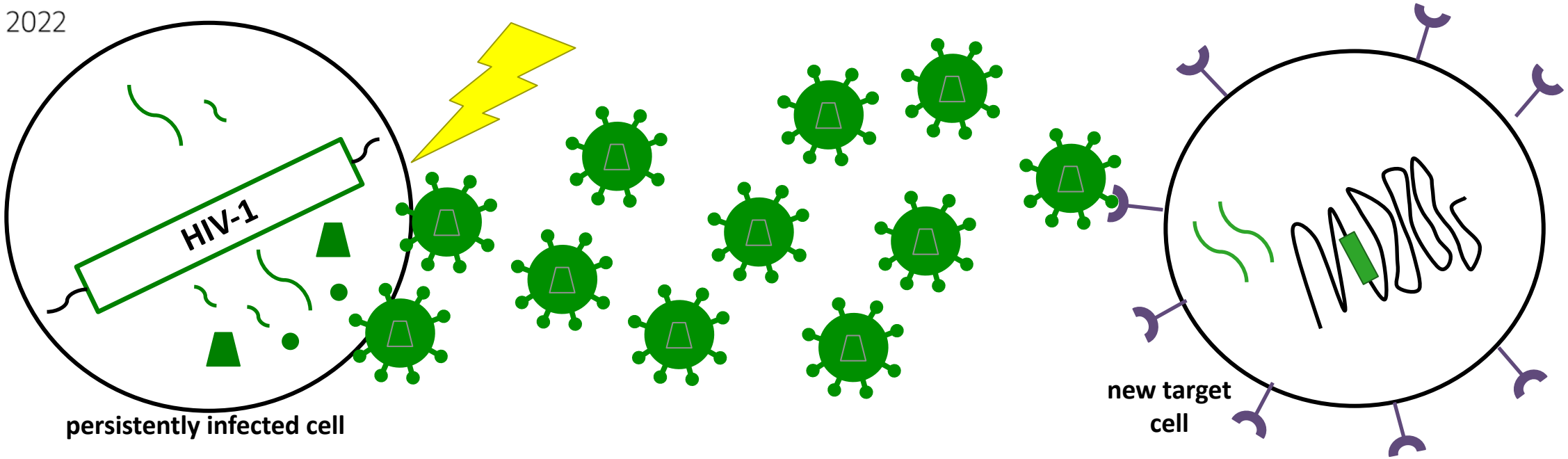

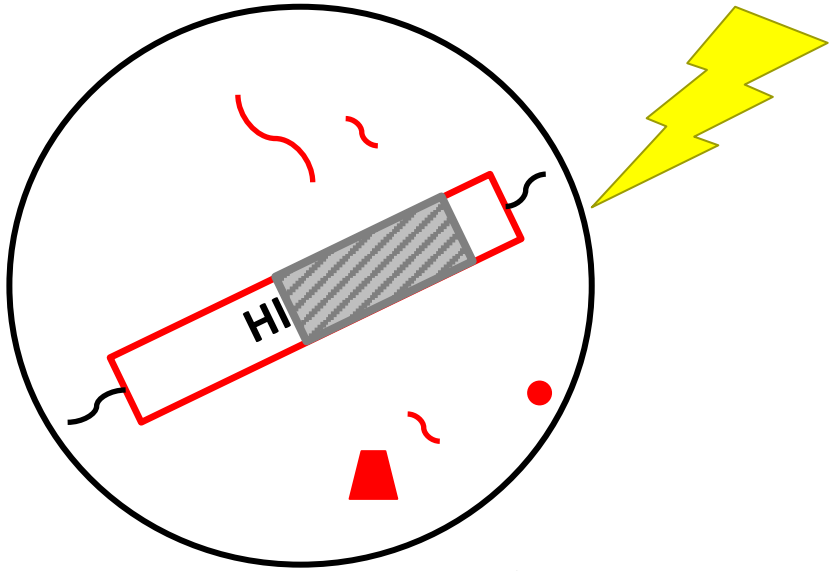

Cells with defective provirus may produce HIV transcripts/proteins[1-3],
but they don't meet the definition of "reservoir"

[1]Imamichi et al 2016 PMID 27432972; [2]Imamichi et al 2020 PMID 32029589; [3]Pollack et al 2018 PMID 29355843

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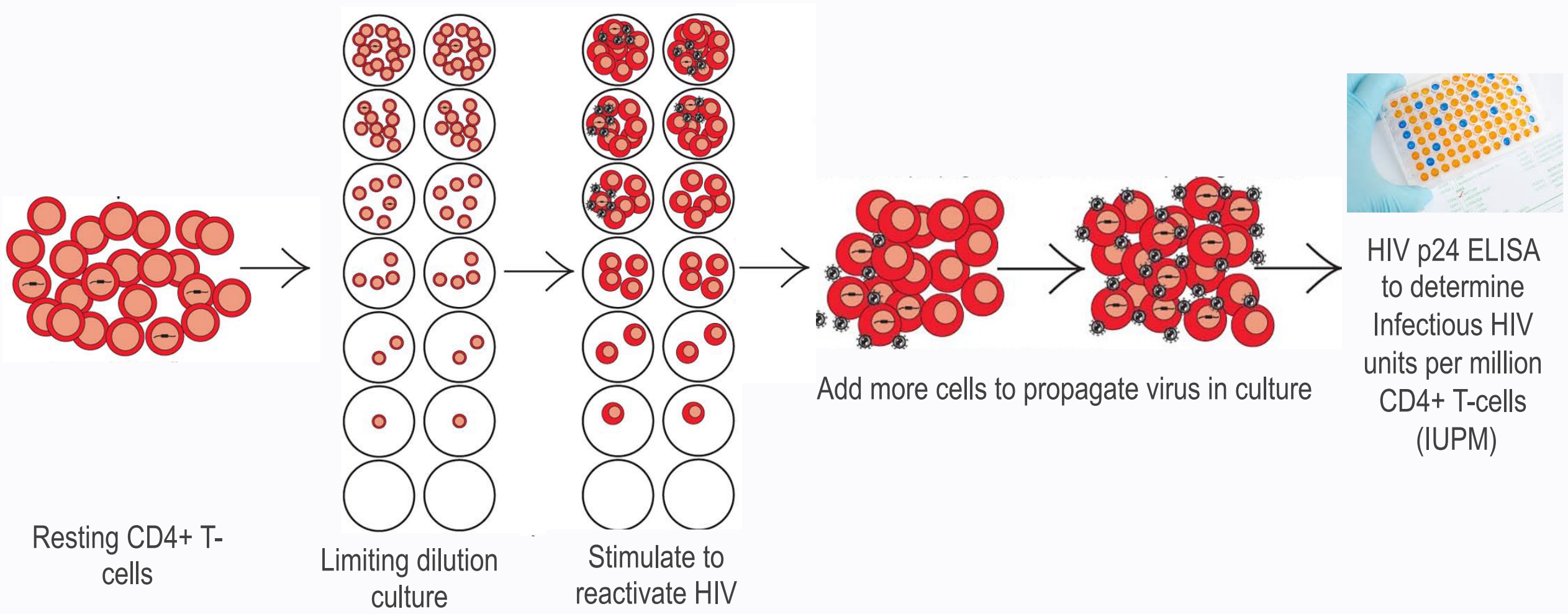
Studying the reservoir: key methods

1. Methods for reservoir ***quantification***

2. Methods for (genetically) ***characterizing reservoir cells***

Reservoir quantification #1: Quantitative Viral Outgrowth Assay (QVOA)



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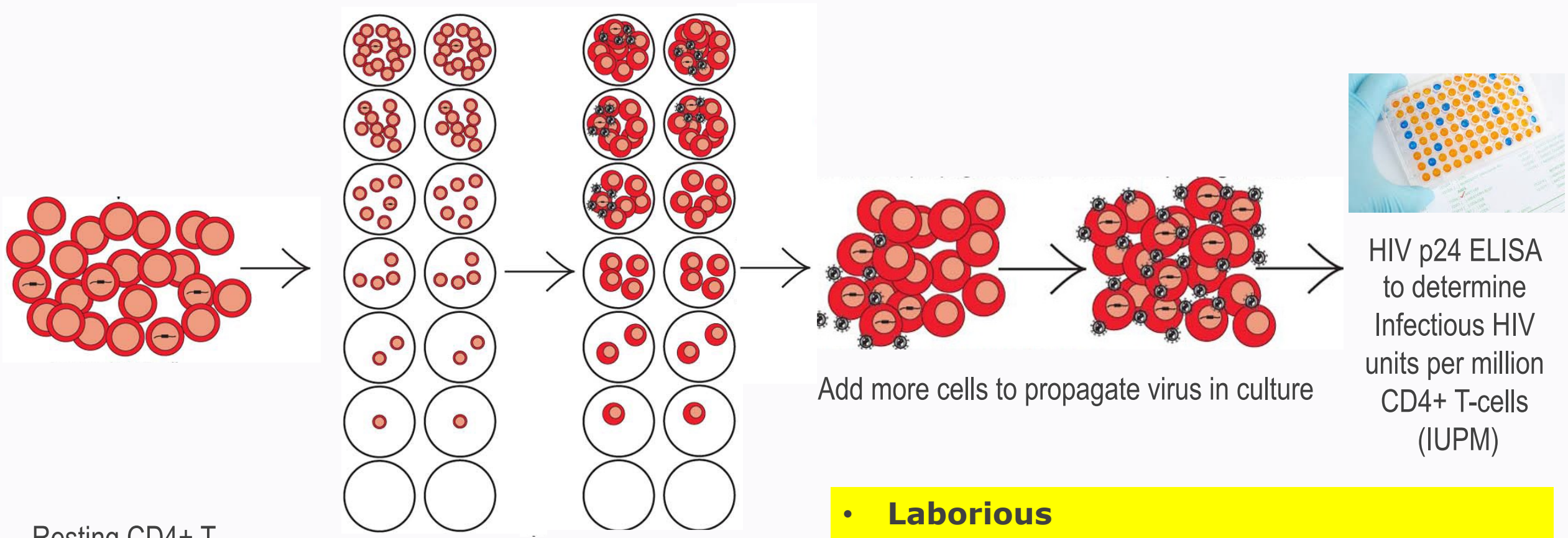


- **Laborious**
- **Requires a lot (~200mL) of blood**
- **Underestimates reservoir, because not all cells reactivate *in vitro***

Adapted from Laird et al PLoS Pathogens 2013; PMID 23737751

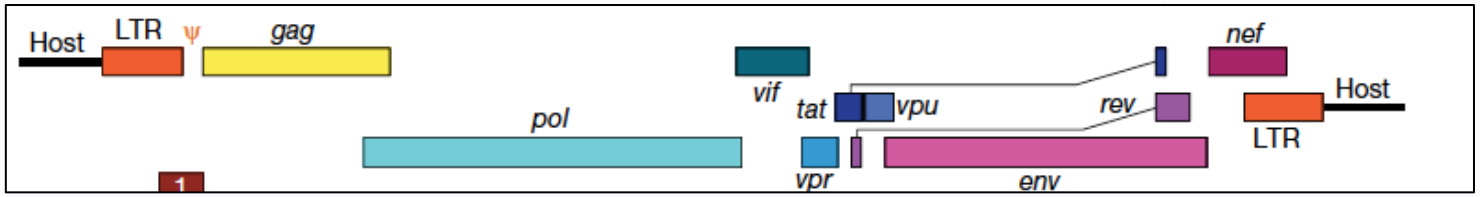
Reservoir quantification #2: The Intact Proviral DNA Assay (IPDA)

Starts with DNA!

Target 1 (ψ)

Detection of both targets jointly discriminates >90% of defective proviruses

Target 2 (env)



Bruner et al, Nature 2019; 566(7):120

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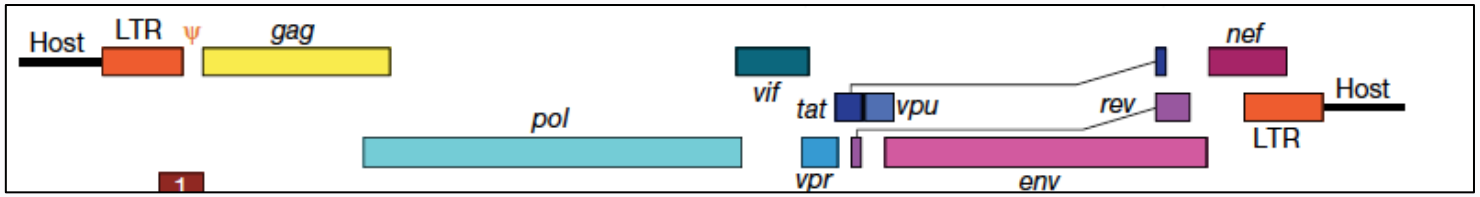

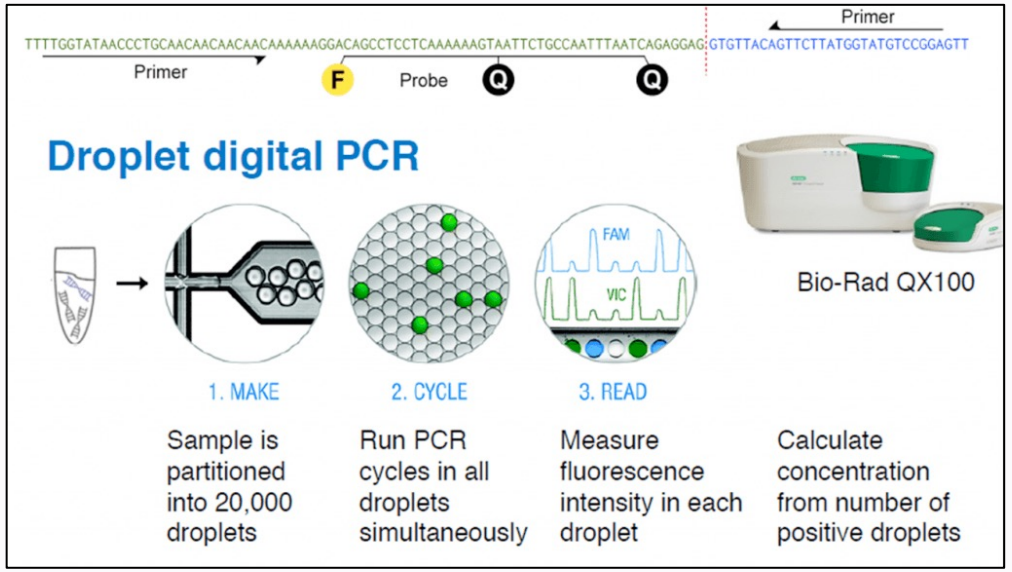

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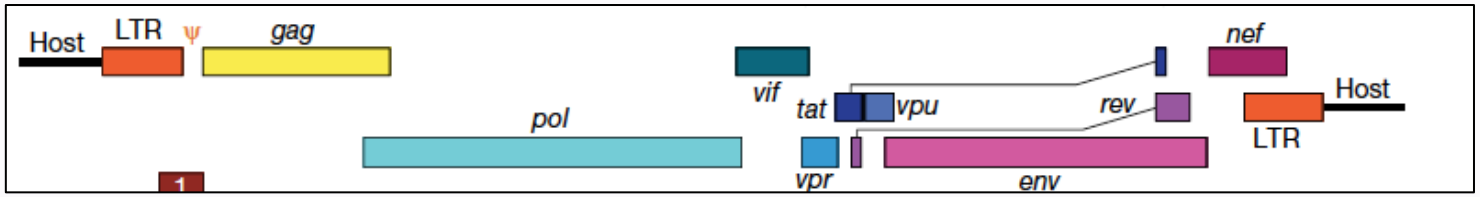

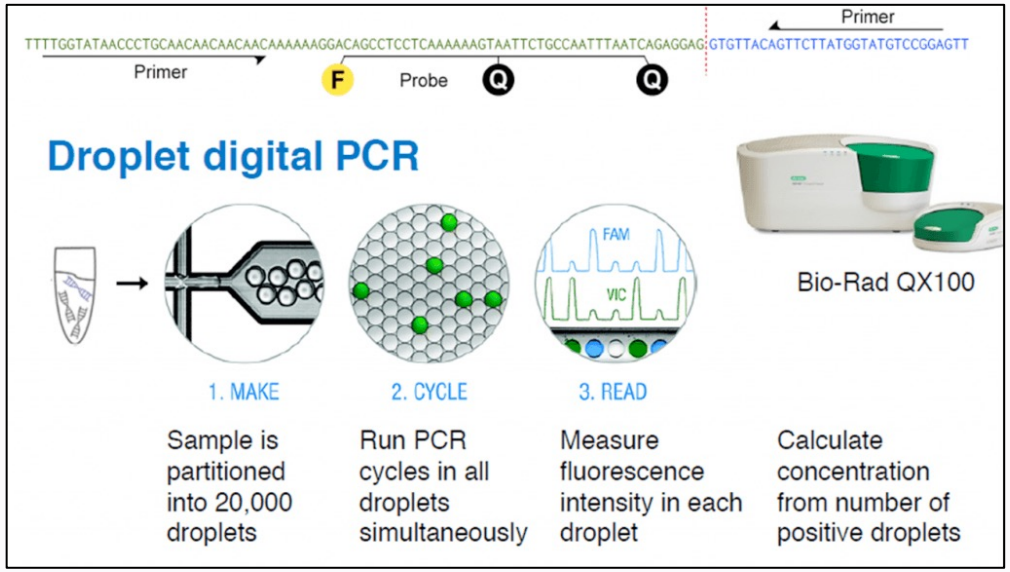

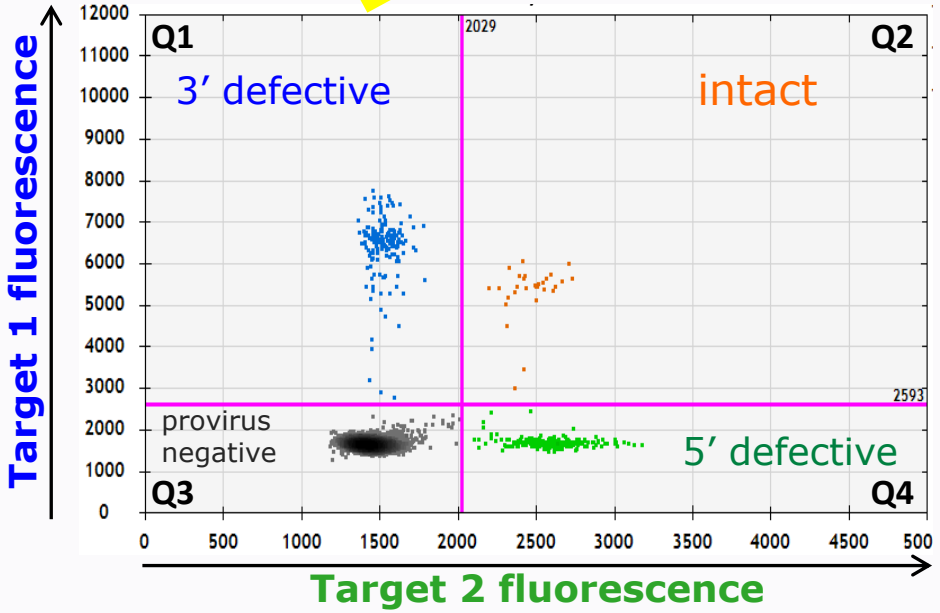

Results expressed as intact proviral genomes per million CD4+ T-cells

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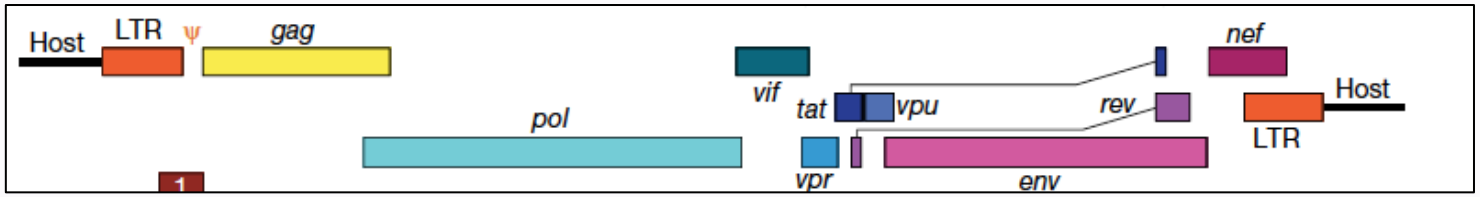

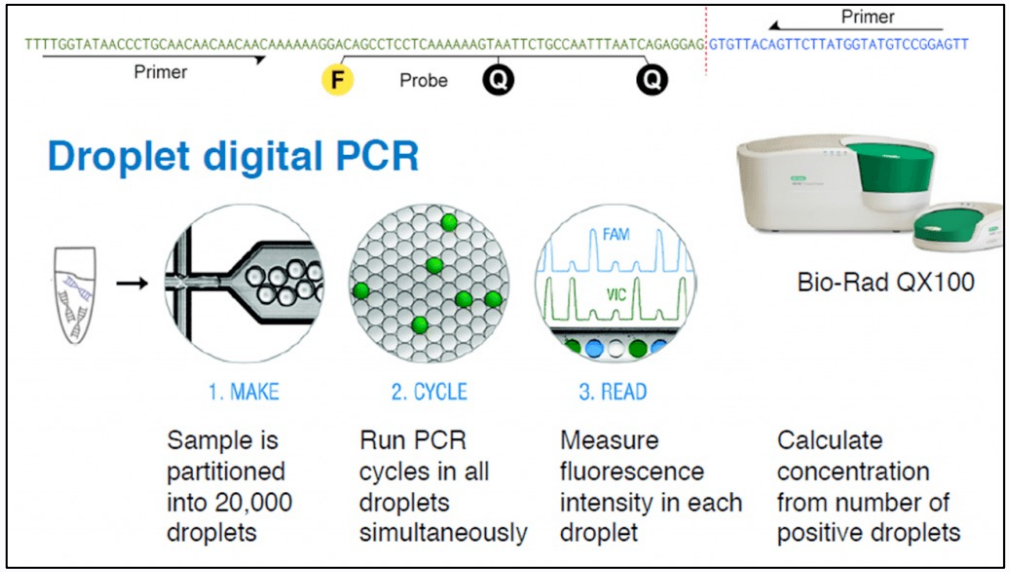

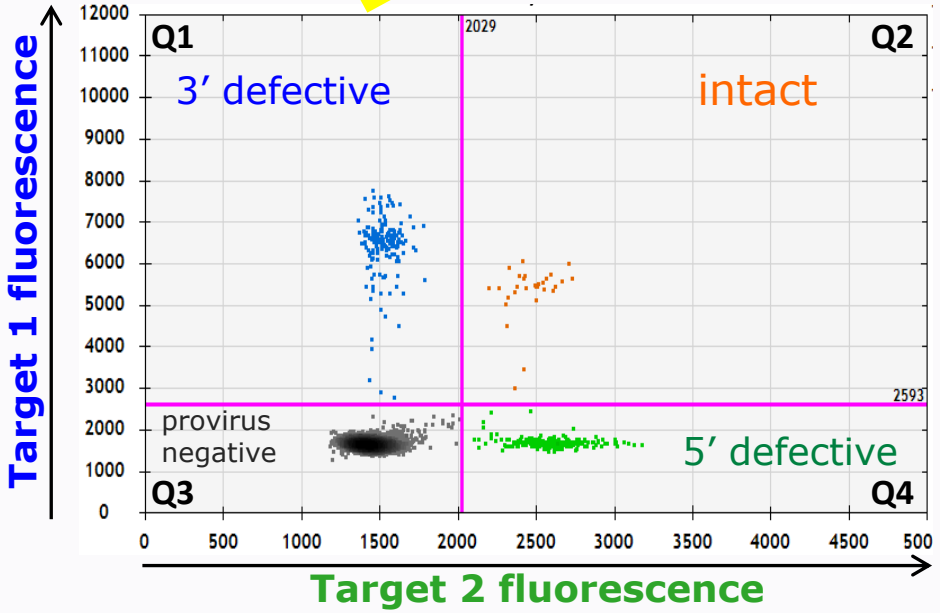

Results expressed as intact proviral genomes per million CD4+ T-cells

- **primers/probes don't capture all HIV variants**

Bruner et al, Nature 2019; 566(7):120

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Reservoir genetic characterization: Full-length individual proviral sequencing

DNA extraction | **96 well plate** | **Visualize amplicons** | **Intactness inference**

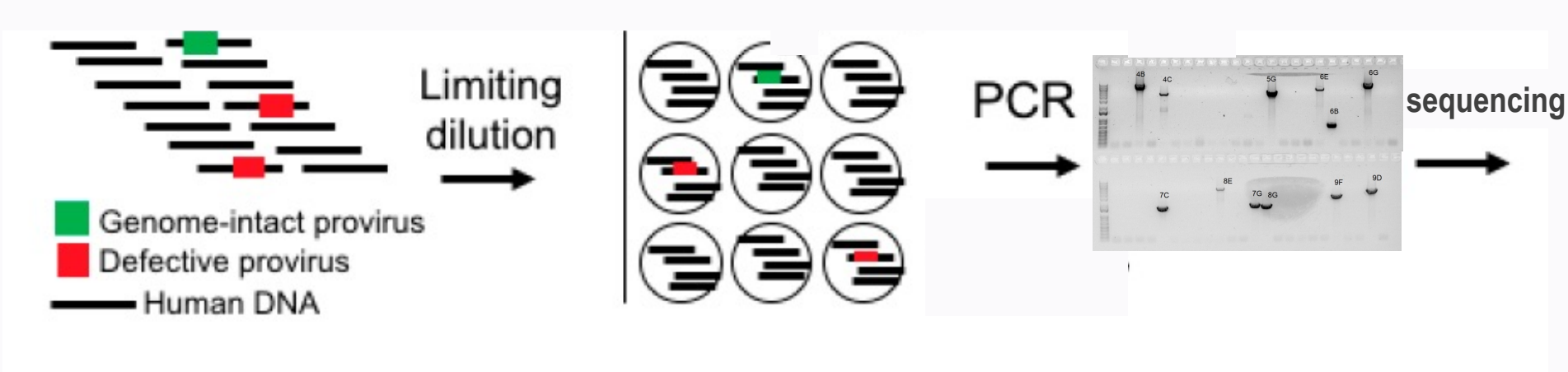

Figure adapted from Lee, Viruses 2021 PMID 34578455 and Patro et al, Viruses 2021 PMID 34960744.
Also see Lee GQ et al, J Clin Investig 2017 PMID 28628034 ; Hiener et al Cell Rep 2017 PMID 29045846
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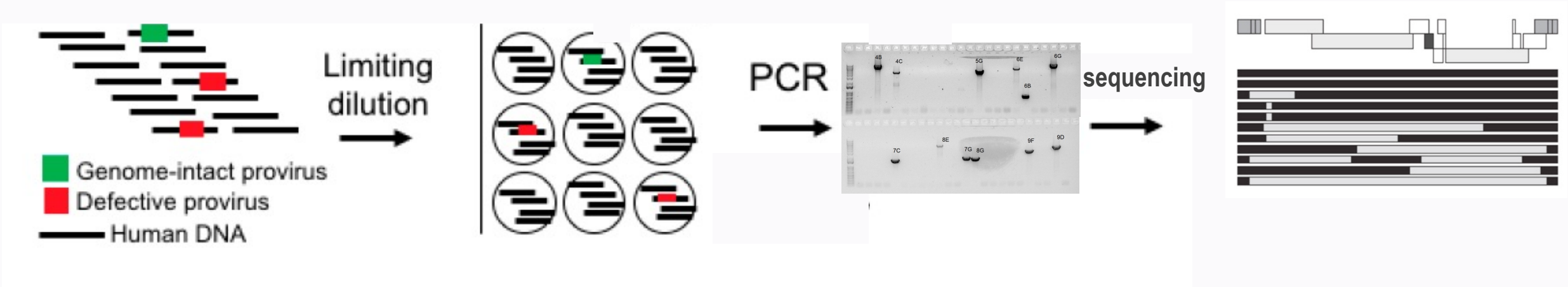

LIMITATION: This approach does not allow simultaneous study of other reservoir attributes (*e.g.* integration site)

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SOLUTION:
"Multiply" your DNA to allow multi-parameter characterization

DNA extraction

96 well plate

Amplify
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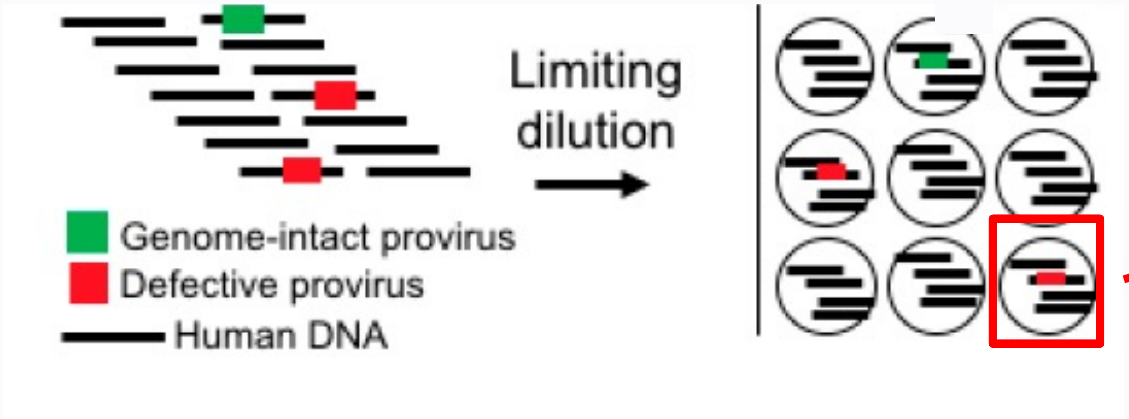

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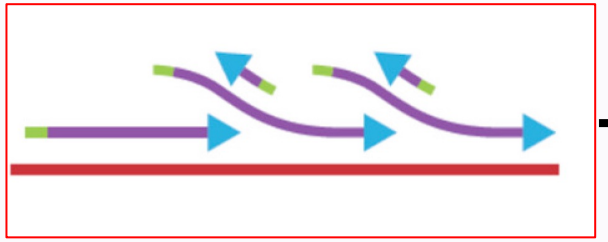
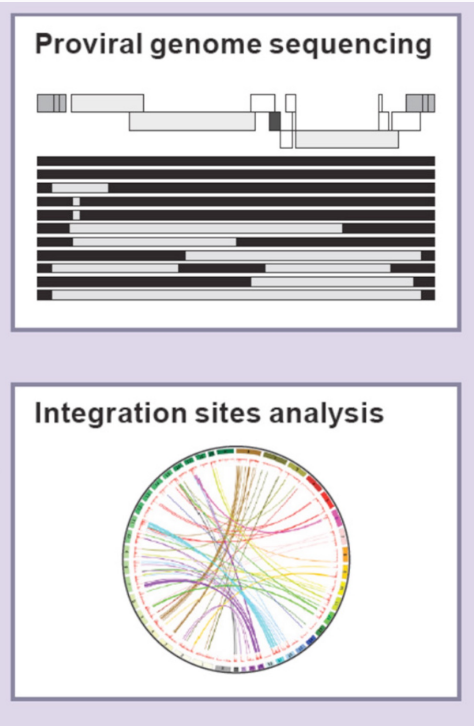


Multiparameter characterization



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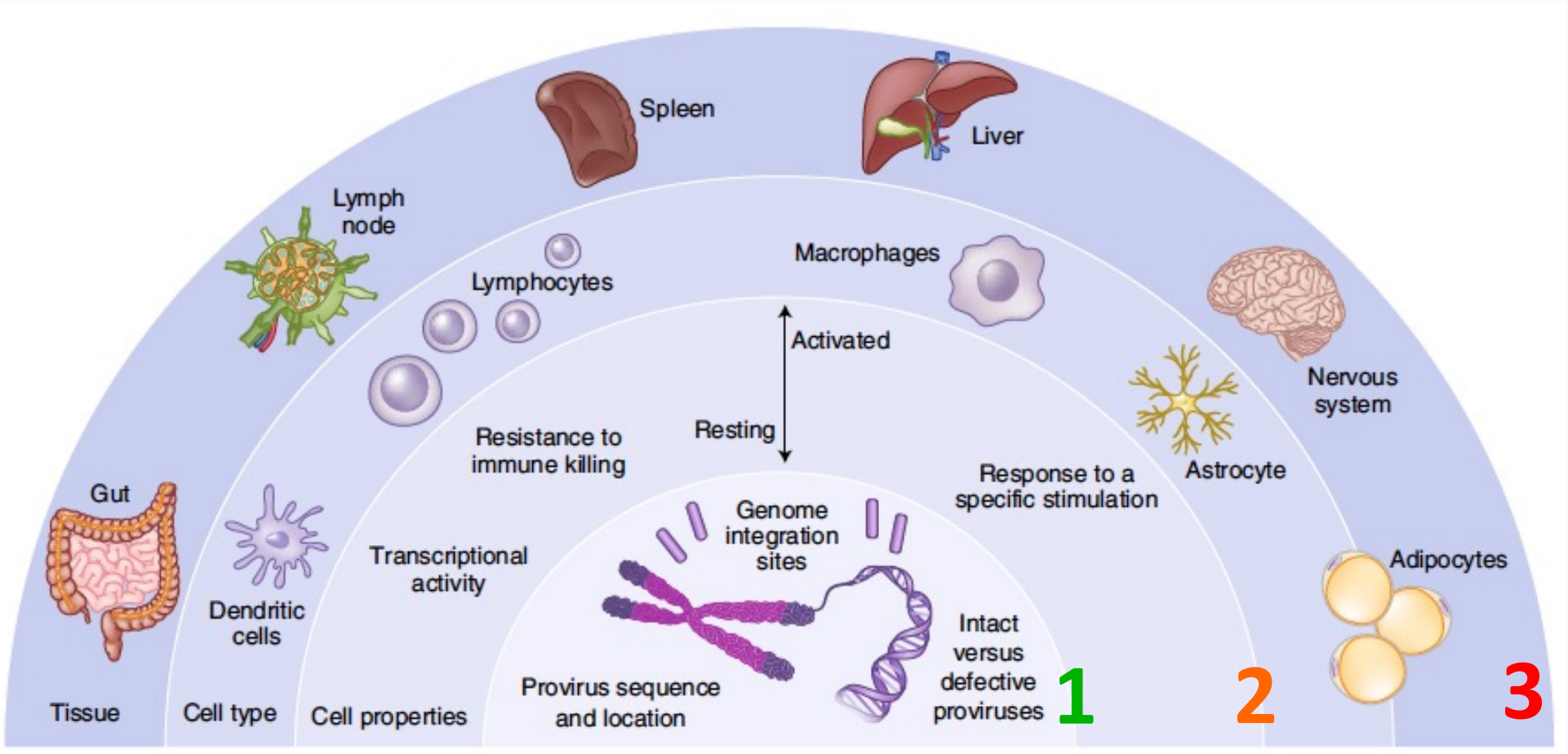
Where is the reservoir? The answer is multidimensional



1. Where in the cell's genome did the provirus integrate?

2. What cell types harbor HIV reservoirs?

3. What tissues in the body harbor HIV reservoirs?

Deeks et al Nat Med 2021; PMID 34848888

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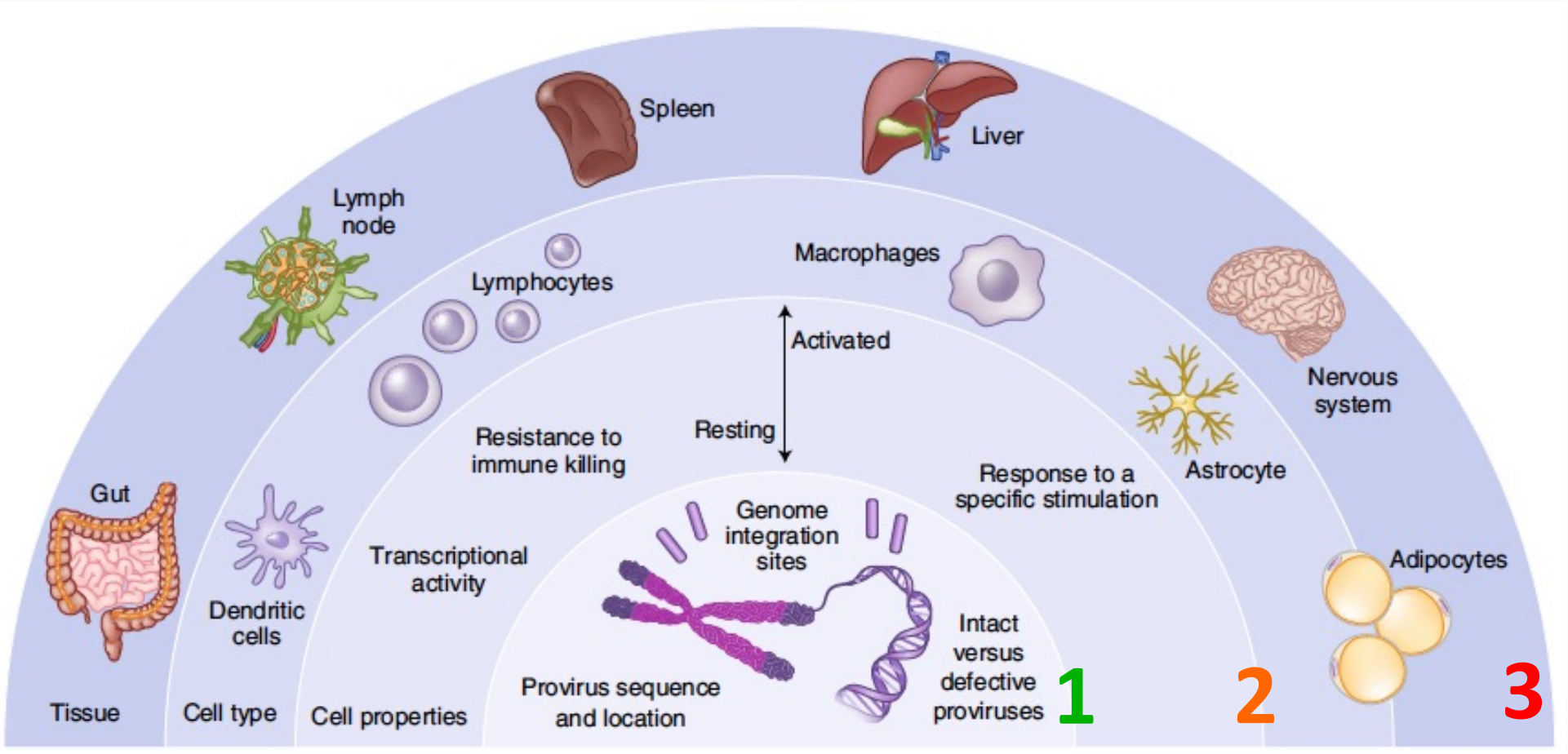

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2. What cell types harbor HIV reservoirs?

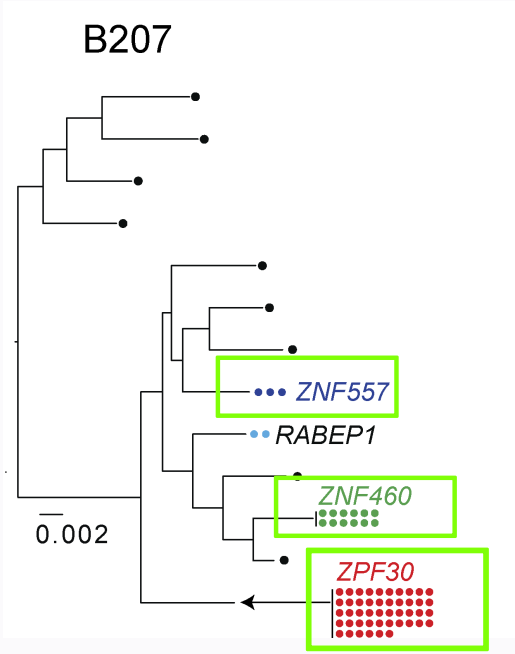
3. What tissues in the body harbor HIV reservoirs?

These locations influence reservoir longevity, reactivation, and genetic composition

Deeks et al Nat Med 2021; PMID 34848888

Integration site influences the likelihood of persistence

Intact proviruses in expanded CD4+ T-cell clones were preferentially integrated within KRAB domain-containing zinc finger (ZNF) genes.



Huang et al JEM 2021 PMID 34636876

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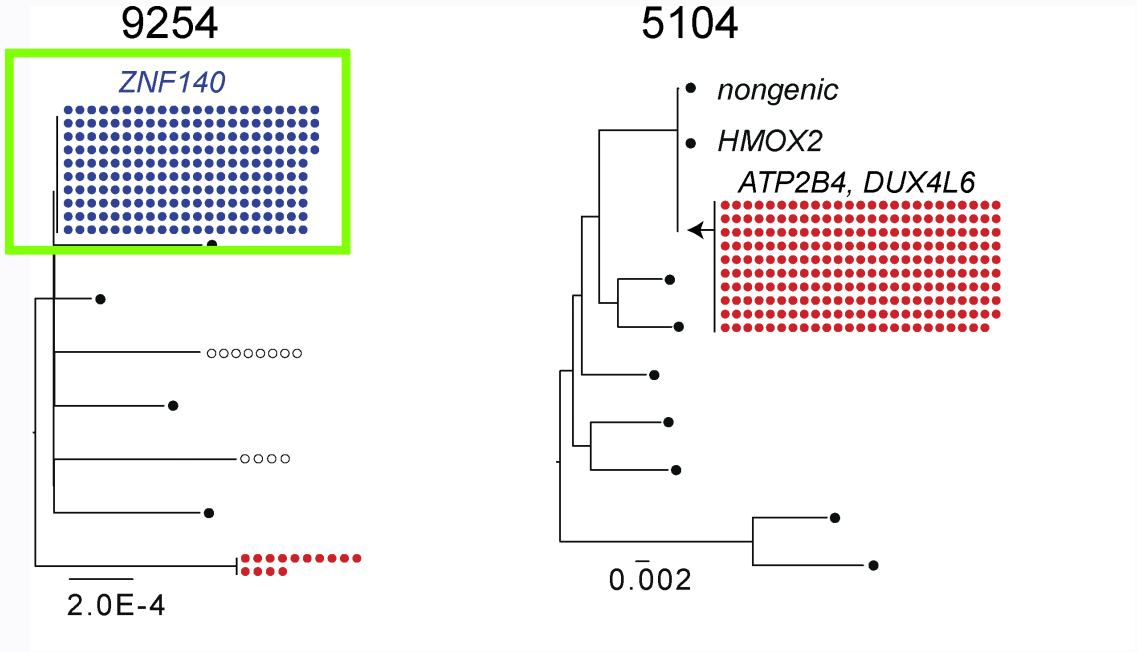
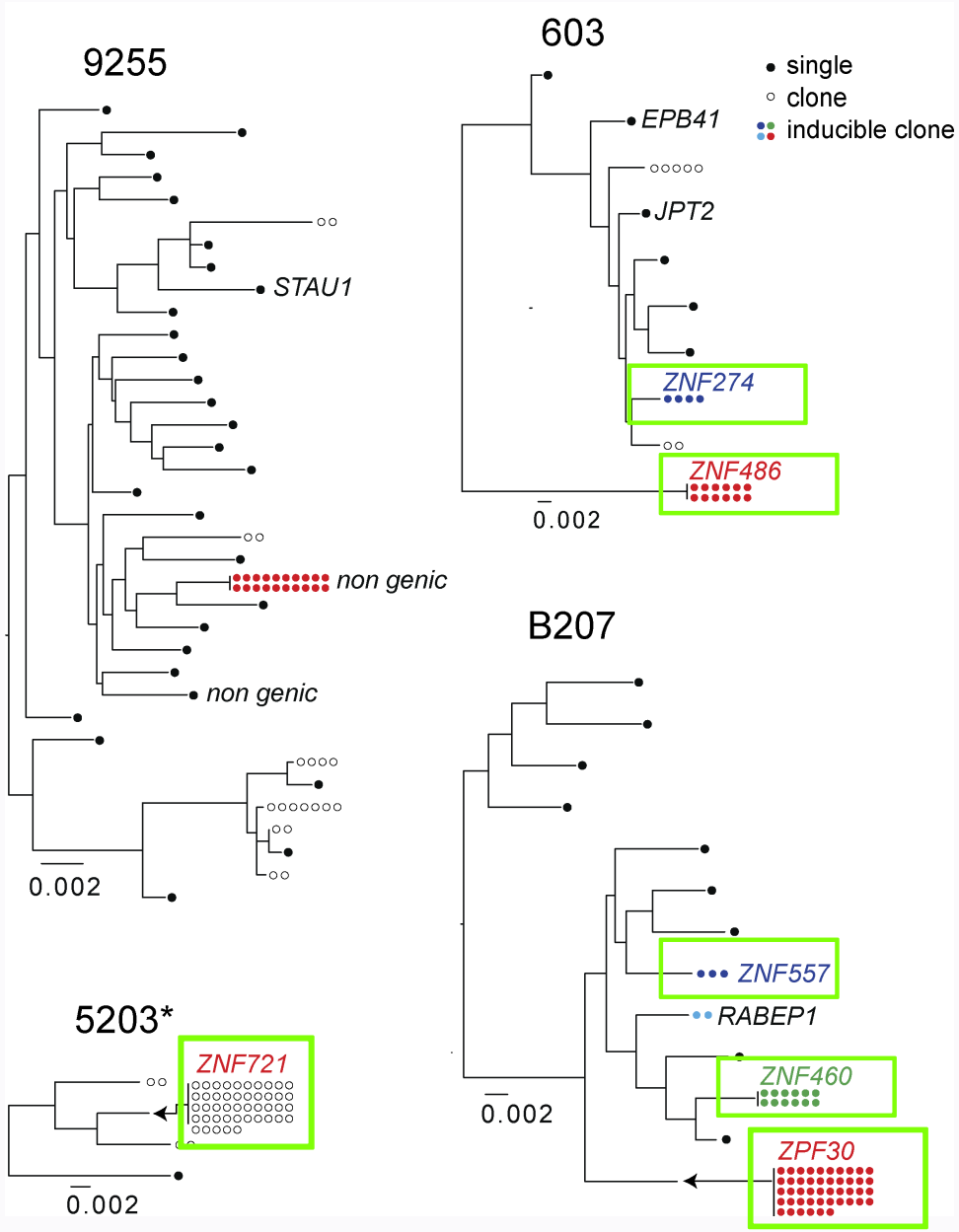
Integration into certain genomic sites may help reservoir cells persist following clonal expansion





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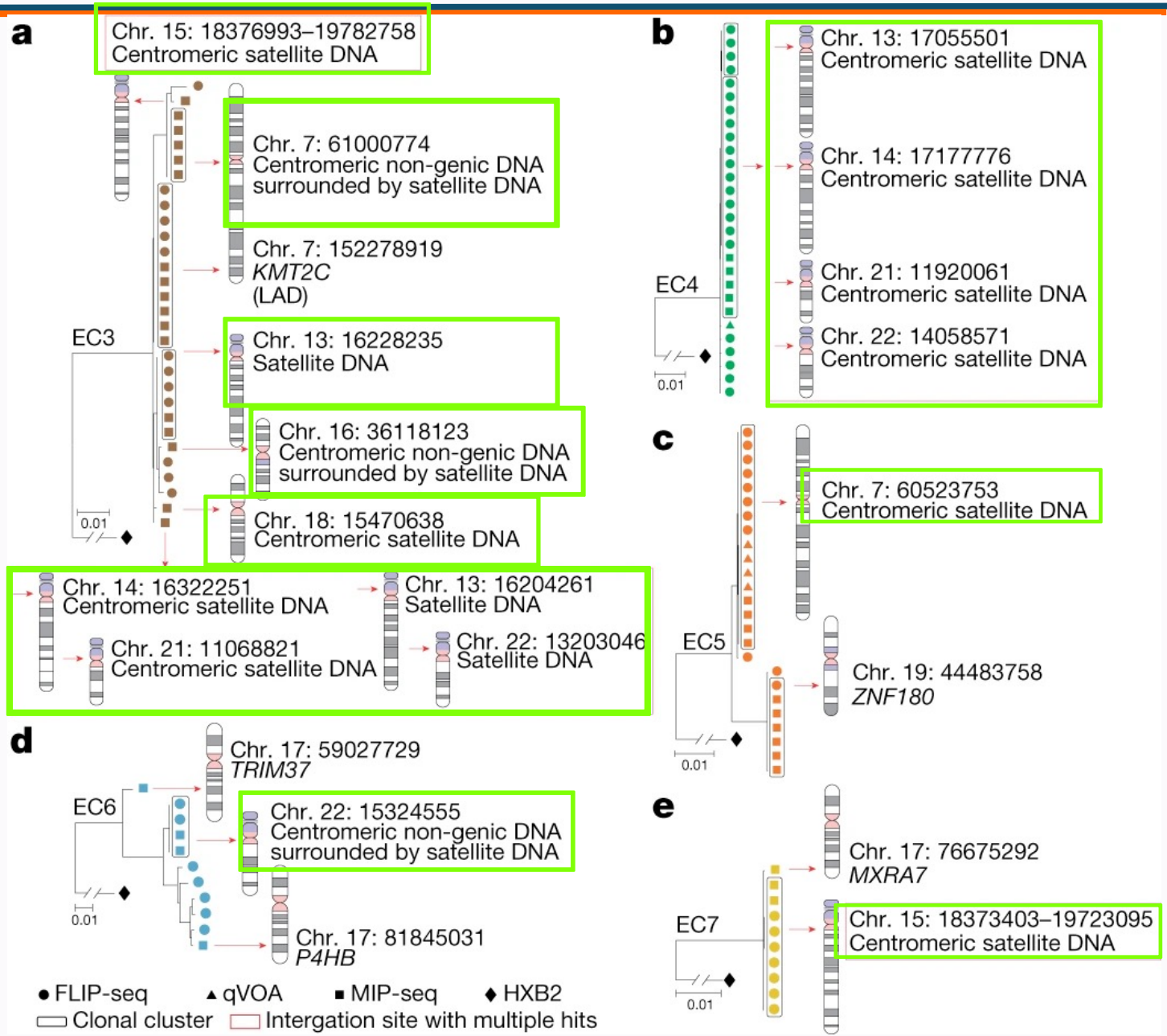
Integration site influences the likelihood of persistence

In Elite Controllers, clonally expanded proviruses tend to be integrated into transcriptionally inactive regions (*e.g.* centromeric satellite DNA)



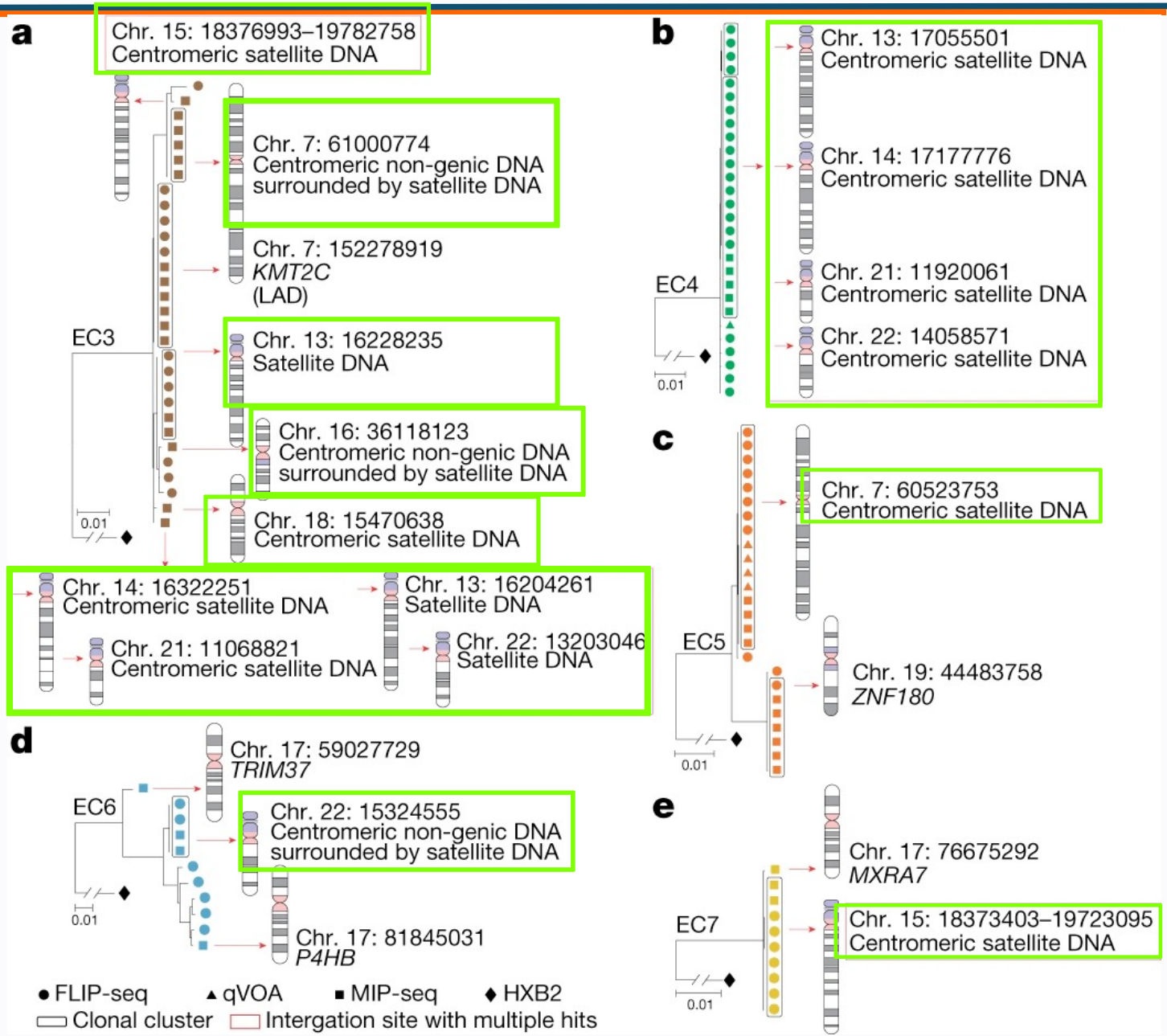
Jiang et al, Nature 2020 PMID 32848246

Integration site influences the likelihood of persistence

In Elite Controllers, clonally expanded proviruses tend to be integrated into transcriptionally inactive regions (*e.g.* centromeric satellite DNA)

This is not because HIV preferentially integrates into these regions.

It is because their immune systems eliminate cells with proviruses integrated into more transcriptionally active regions.



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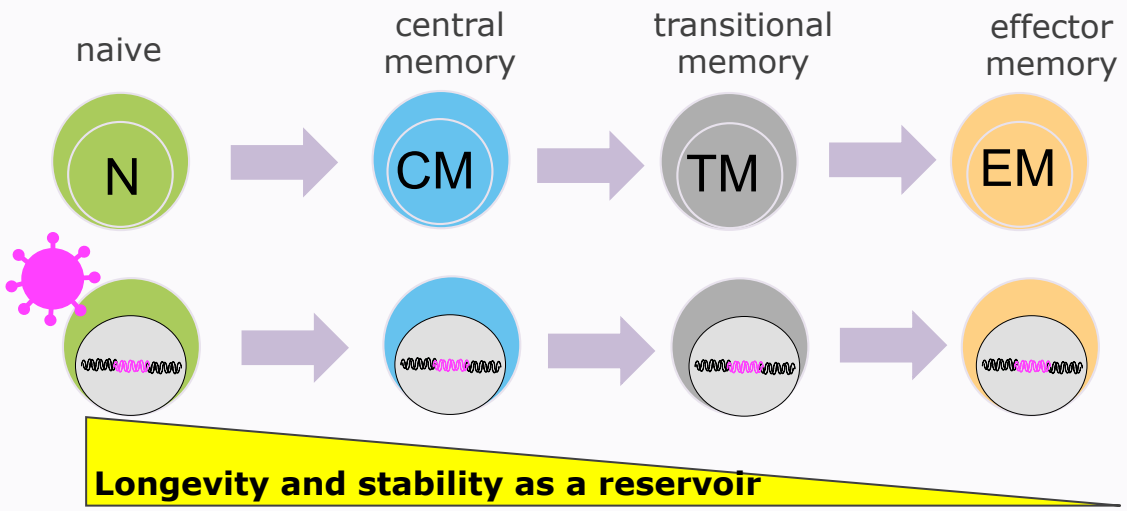
Cell type matters

CD4+ T-cell subsets differ in the degree of clonal expansion

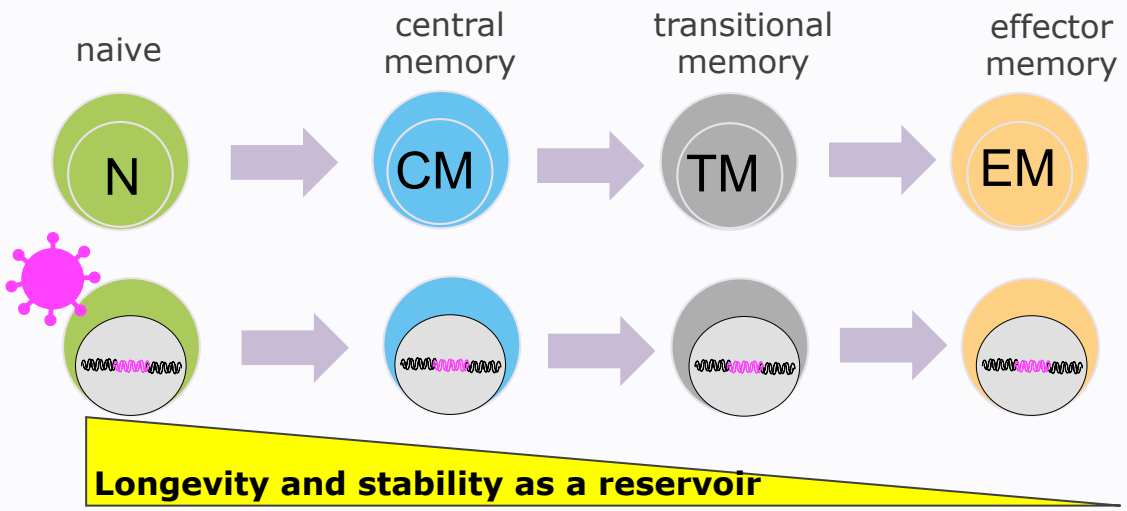


Effector memory cells are enriched in clonally expanded proviral sequences[1,2,3]

[1]Hiener et al 2017 PMID 29045846; [2]De Scheerder el al 2019 PMID 31471273;
[3]Jones et al 2020 PMID 31776273

Cell type matters

CD4+ T-cell subsets differ in the degree of clonal expansion



Effector memory cells are enriched in clonally expanded proviral sequences[1,2,3]

[1]Hiener et al 2017 PMID 29045846; [2]De Scheerder el al 2019 PMID 31471273; [3]Jones et al 2020 PMID 31776273

Macrophages as distinctive reservoirs

Found in all tissues[1-4]

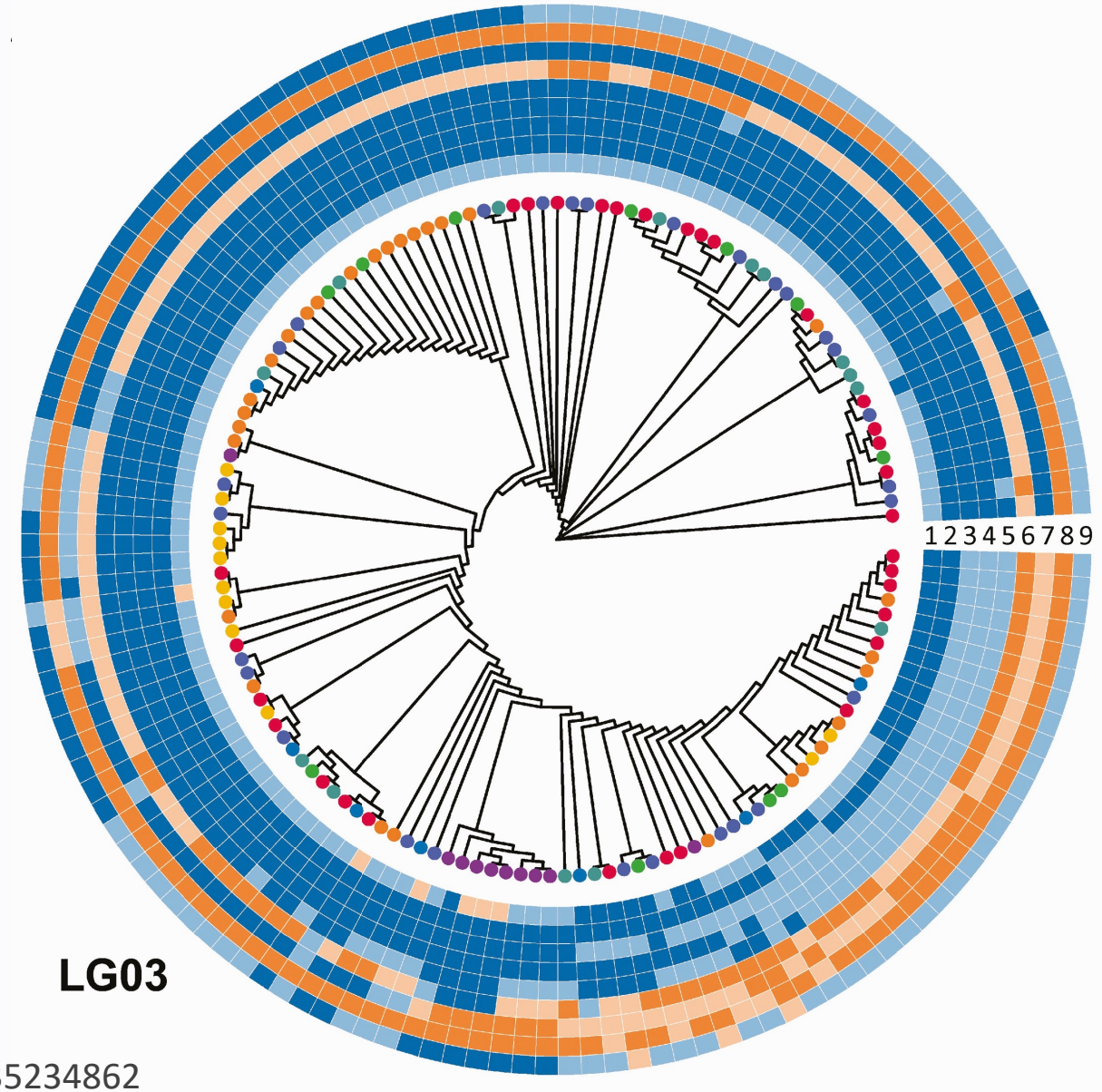
Long-lived; relatively resistant to immune and HIV-mediated killing[5]; reside in sites with reduced ART penetration (*e.g.* CNS).

These properties can yield genetically distinctive proviral populations in certain tissues[6]

[1]Micci et al 2014 PMID 25356757; [2]DiNapoli et al 2017 PMID 28239657; [3]Honeycutt et al 2017 PMID 28414330; [4]Ganor et al 2019 PMID 30718846; [5]Clayton et al 2018 PMID 29670239; [6]Schnell et al 2009 PMID 19390619; [6]Schnell et al 2009 PMID 19390619

How (genetically) distinctive are reservoirs in different tissues?

Proviral diversity in blood is generally representative of that in tissues, and genetic compartmentalization in tissue is generally limited.



Tissue

- Blood/PBMC
- Gastrointestinal tract
- Liver
- Lymphatic tissue
- CNS
- Pancreas
- Heart
- Genito–urinary tract

IC_{50} to various nAbs

- <= 0.1
- <= 1
- <=10
- >10

Last Gift

http://lastgift.ucsd.edu/

Wang et al, Clin Infect Dis 2022 PMID 35234862

How (genetically) distinctive are reservoirs in different tissues?

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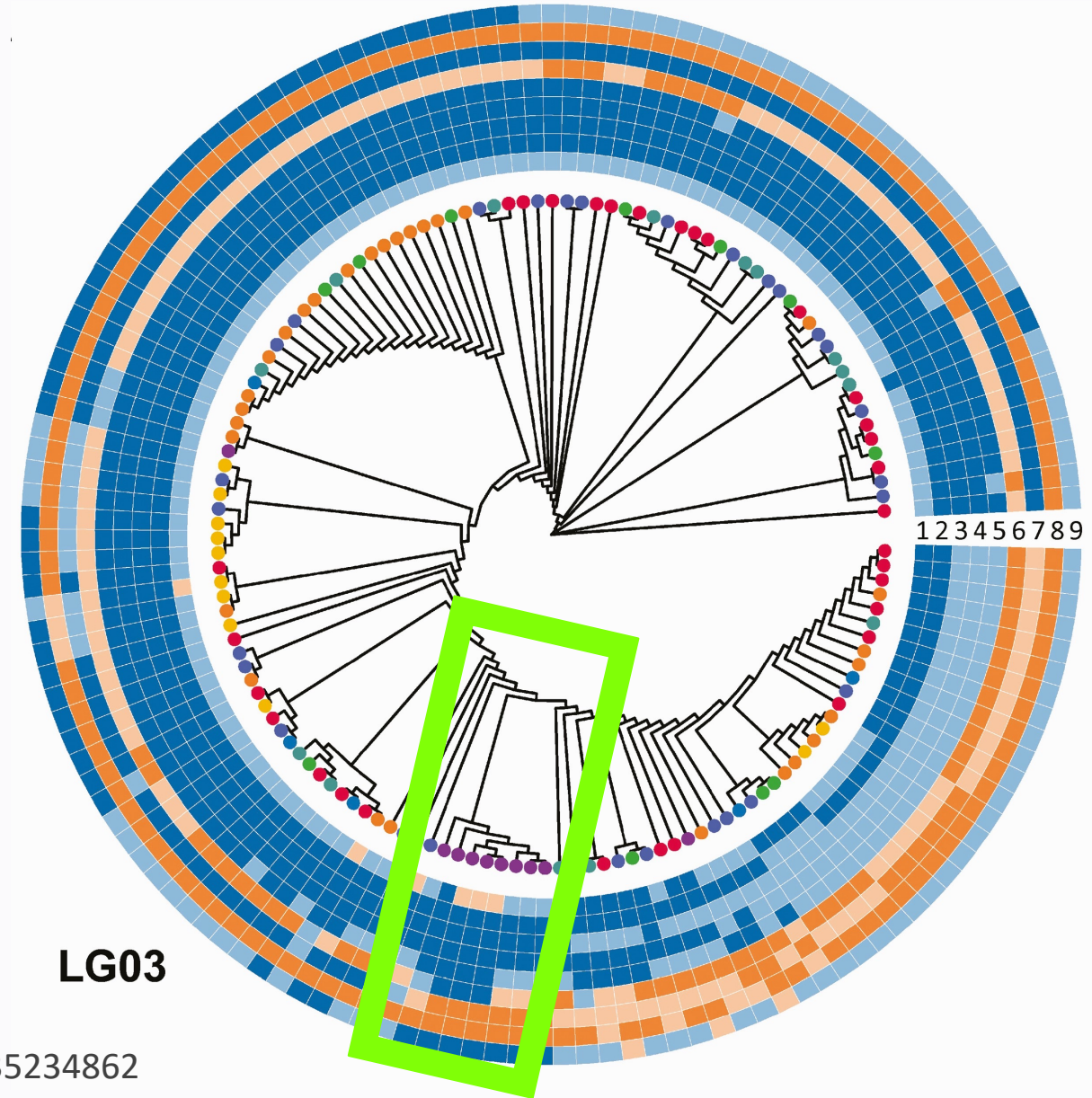
Proviral diversity in blood is generally representative of that in tissues, and genetic compartmentalization in tissue is generally limited.

BUT some tissues may harbor proviral populations with distinctive genetic and functional features (*e.g.* the brain).



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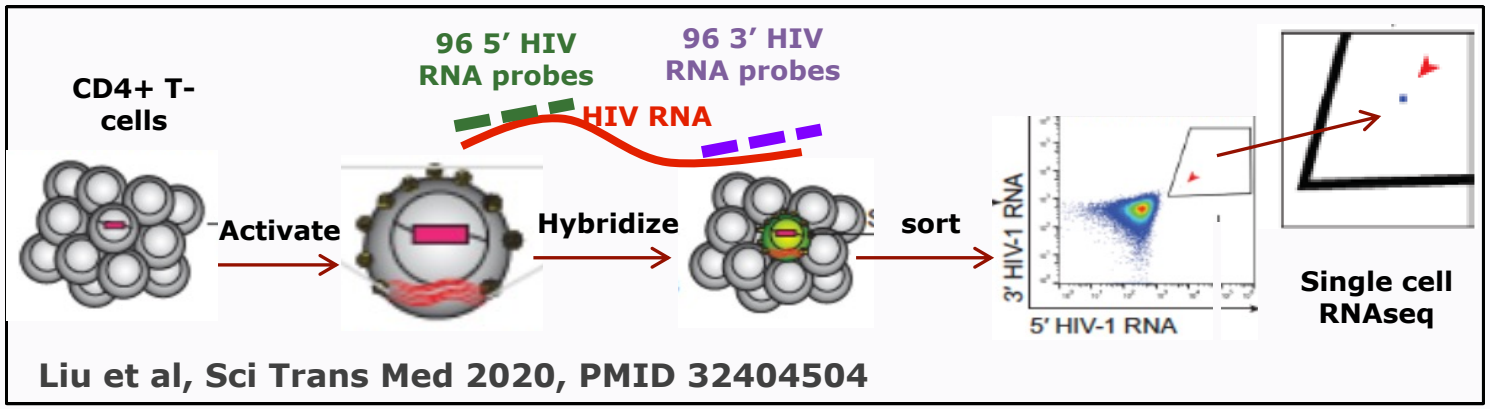
Methods innovation: single-cell reservoir profiling

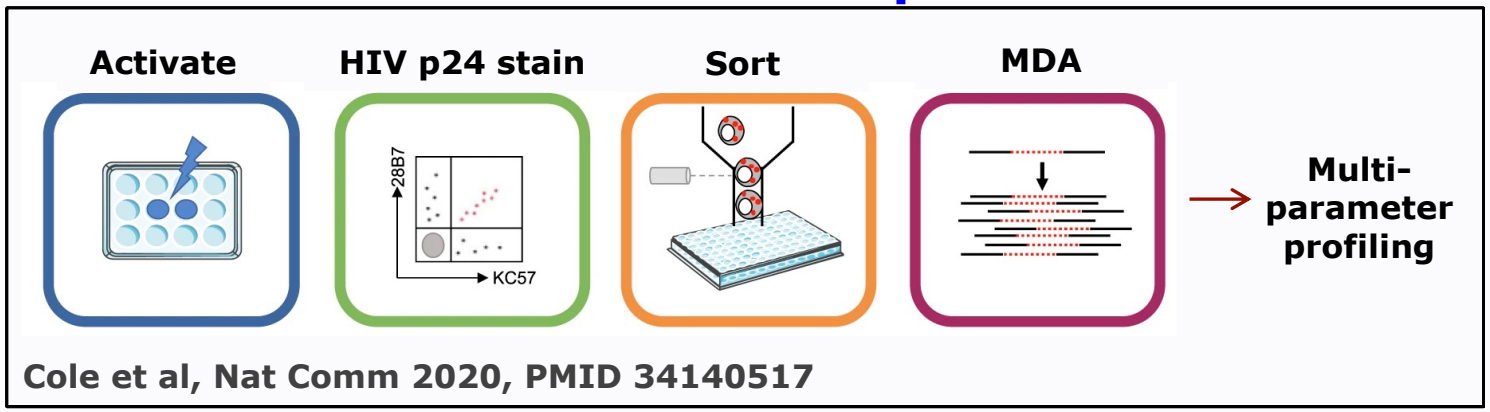
These techniques "fish" out individual reservoir cells for characterization!

HIV SORT-seq



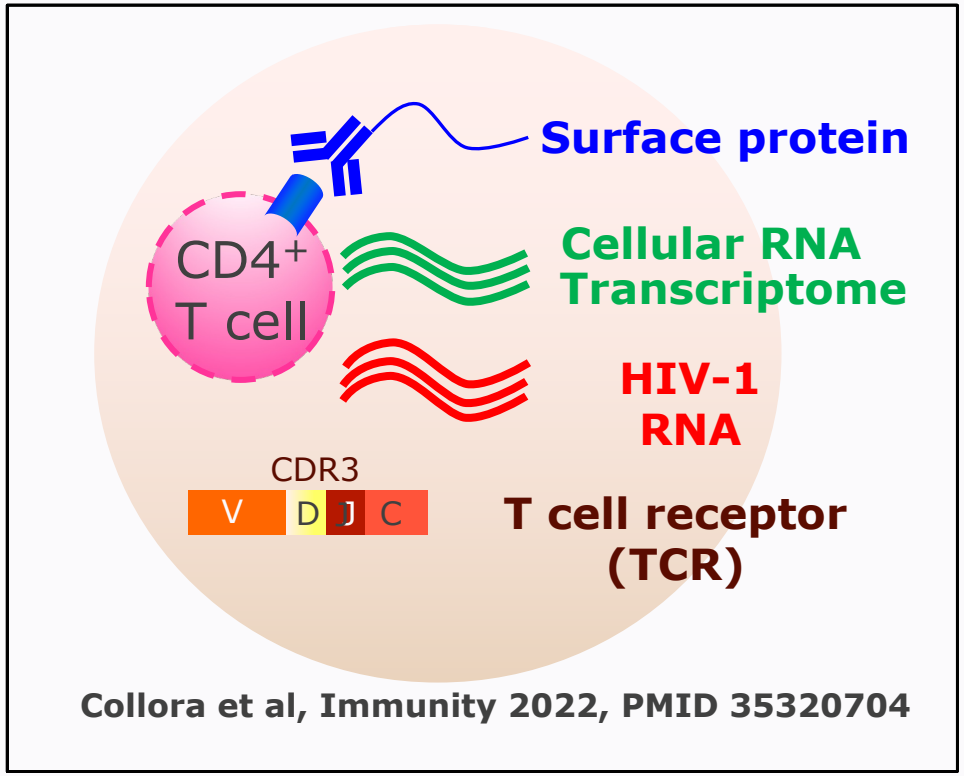
Liu et al, Sci Trans Med 2020, PMID 32404504

HIV STIP-seq



Cole et al, Nat Comm 2020, PMID 34140517

ECCITEseq



Collora et al, Immunity 2022, PMID 35320704

Summary

- Intact, replication-competent HIV proviruses persist throughout the body during ART, and represent the main barrier to cure
- Clonal expansion is a major mechanism that sustains the reservoir
- A provirus' location (genomic, cellular, tissue) can influence its ability to persist
- Methods for reservoir characterization continue to be innovated

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REACH For the Cure Research Enterprise to Advance a Cure for HIV

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