A tale of two giants: **Macrophages** and **Megakaryocytes** host persistent viruses

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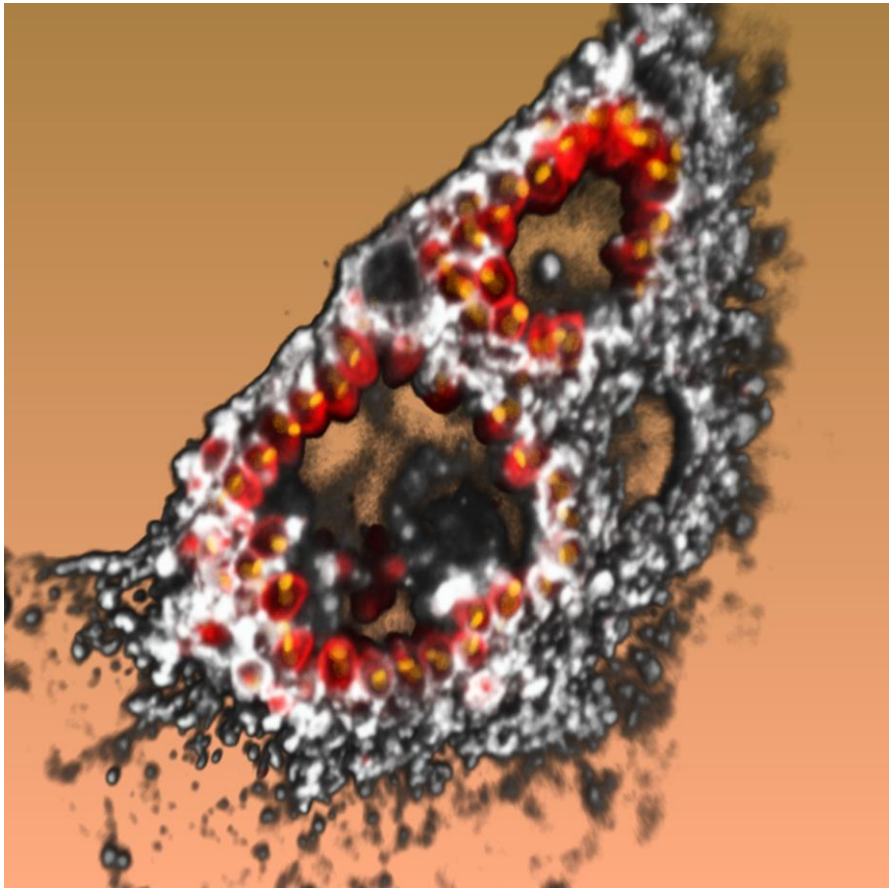
The first giant



Pessoa et al., *PLos Pathog* 2019

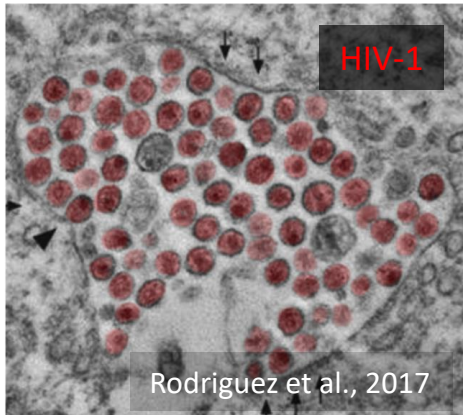
Macrophage

(n.) "type of large white blood cell with the power to devour foreign debris in the body or other cells or organisms". From the greek makros *("large") and* phagein *("eater")*

Macrophages (MΦ) are large, tissue-resident specialized myeloid cells of the immune system that recognize, engulf and destroy infectious microorganisms and infected or damaged cells

Several pathogens multiply and persist **in the very same cells supposed to kill them**

HIV is sheltered by MΦ in **virus-containing compartments (VCC)**



HIV persistence: reservoirs are a major barrier for a definitive cure

1.5 million
people newly infected in 2020

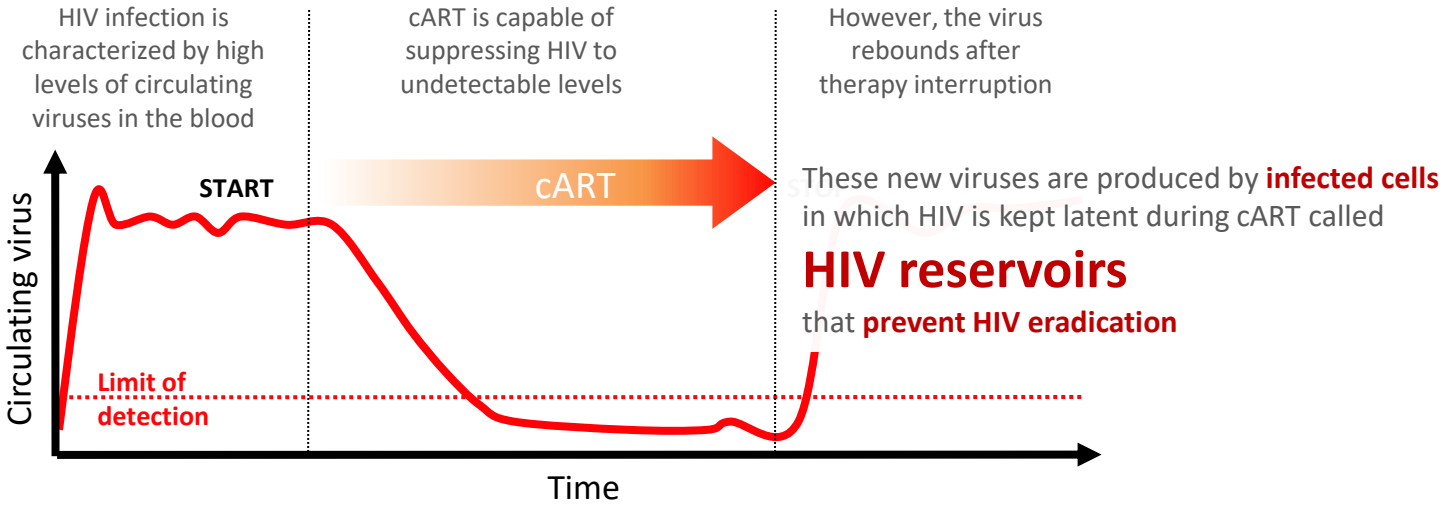
0.7 million
HIV-related deaths in 2020

38 million
people living with HIV

Source: WHO/UNAIDS 2020

73% of them receive **combination antiretroviral treatment (cART)**

cART **cannot eradicate** HIV



These new viruses are produced by **infected cells** in which HIV is kept latent during cART called

HIV reservoirs

that **prevent HIV eradication**

HIV hides in these reservoirs that are **not sensitive** to current therapies

What and where are HIV reservoirs?

- **Reservoir:** Infected cell population that allows persistence of replication-competent HIV-1 in patients virally suppressed by cART regimens on the order of years (Eisele & Siliciano, 2012)
- Several studies in the late 1990s identified a reservoir of HIV in long-lived cells in the body that persists despite ART

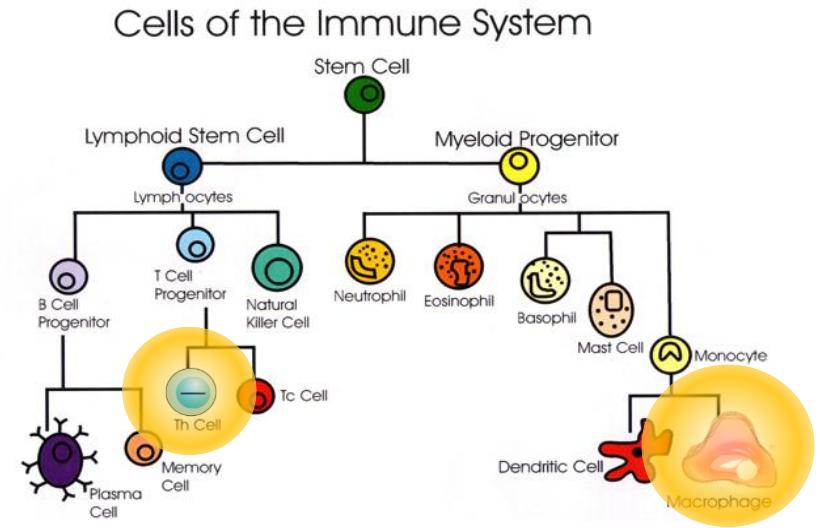
Recovery of Replication-Competent HIV Despite Prolonged Suppression of Plasma Viremia

Joseph K. Wong,* Marjan Hezareh, Huldrych F. Günthard, Diane V. Havlir, Caroline C. Ignacio, Celsa A. Spina, Douglas D. Richman

Identification of a Reservoir for HIV-1 in Patients on Highly Active Antiretroviral Therapy

Diana Finzi, Monika Hermankova, Theodore Pierson, Lucy M. Carruth, Christopher Buck, Richard E. Chaisson, Thomas C. Quinn, Karen Chadwick, Joseph Margolick, Ronald Brookmeyer, Joel Gallant, Martin Markowitz, David D. Ho, Douglas D. Richman, Robert F. Siliciano*

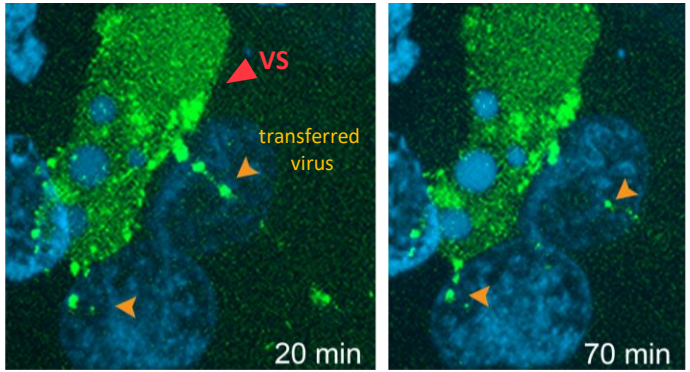
14th November 1997 Science issue

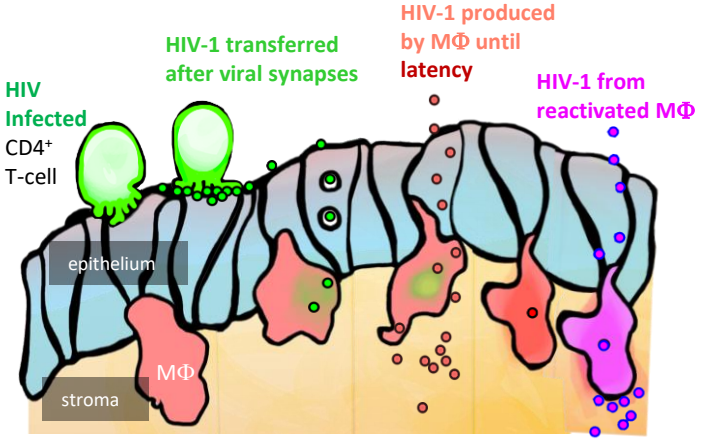


- The best described HIV reservoir are formed in **circulating CD4⁺ T cells**
- **not the only reservoir**, as revealed by HIV genetic analyses (Chun et al, 2000; Cohen et al, 2018)
- **MΦ** are gaining importance as **tissular HIV reservoirs**

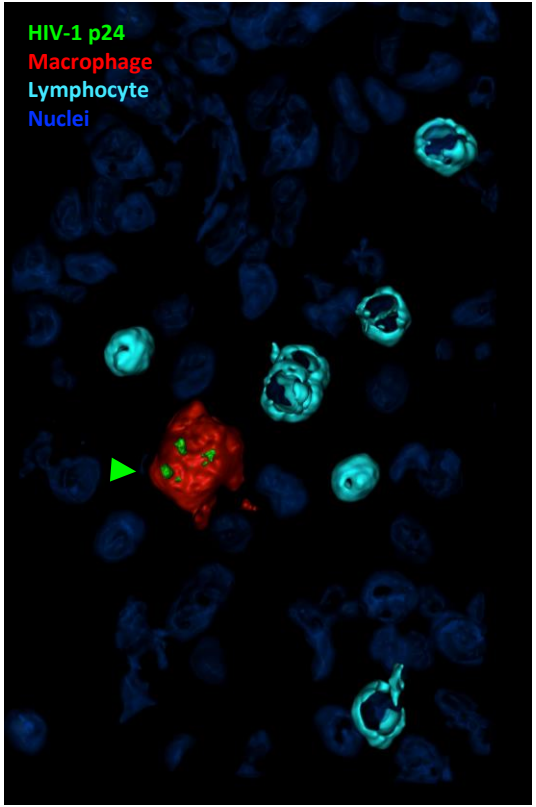
Mucosal MΦ are HIV reservoirs *in vivo*

Viral synapses at epithelium favors MΦ infection

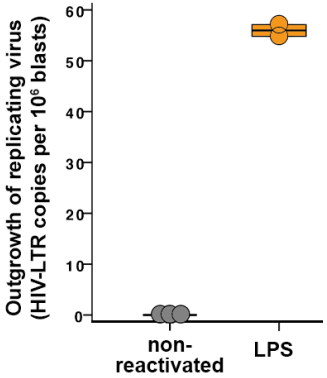


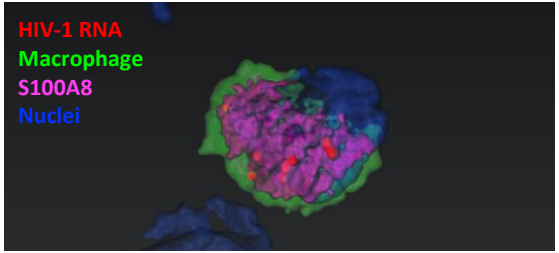


Mucosall MΦ are **HIV reservoirs with VCC's**



Maintained via **immunometabolism**

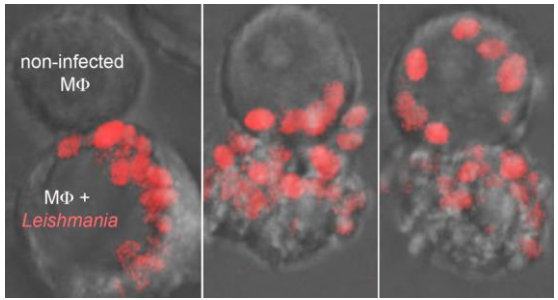




Sennepin, Real et al., *Front Immunol* 2017
Real et al., *Cell Reports* 2018
Real & Bomsel, *Med Sci (Paris)* 2019
Ganor, Real et al, *Nat Microbiol* 2019
Real et al, *Methods in Mol Biol* 2022
Real et al, *Nat Comm* 2022

A myeloid pathway of **spreading**

Mechanism of persistence:
pathogens **spread from cell to cell**



Real et al., *Cell Microbiol* 2014

Photo: H. Kratky, shutterstock.com

Original hypothesis:

MΦ viral reservoirs are fueled by
platelets sheltering HIV

Platelets are responsible for hemostasis
but also involved in the **immune response**

Platelets **internalize HIV** *in vitro*

Youssefian et al., *Blood* 2002

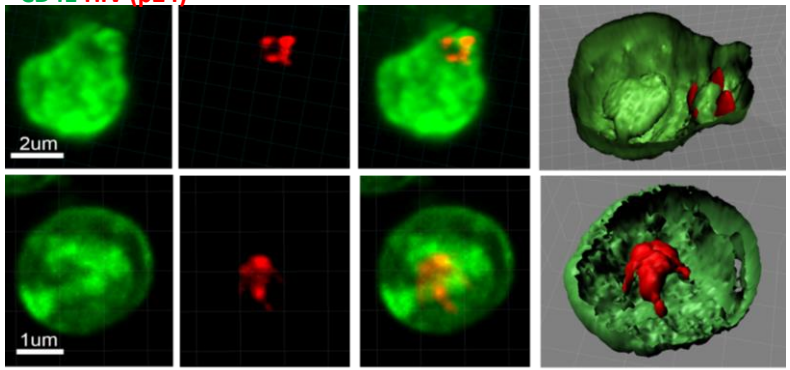
Platelets are short-lived and **scavenged** by MΦ:
if harboring HIV, they could **transfer** infectious HIV to tissue MΦ

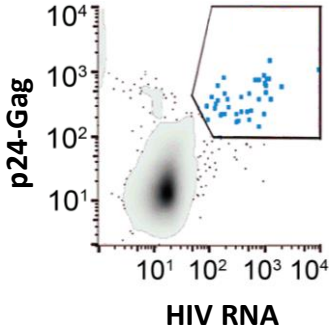
Would this be a mechanism of **HIV persistence**?

Platelets and HIV **persistence**

HIV is sheltered in platelets from cART-treated individuals with **undetectable viral load**

CD41 HIV (p24)

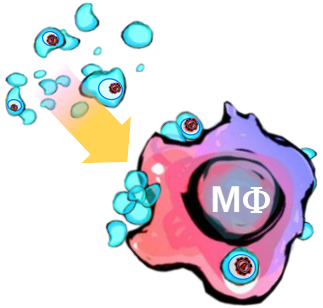


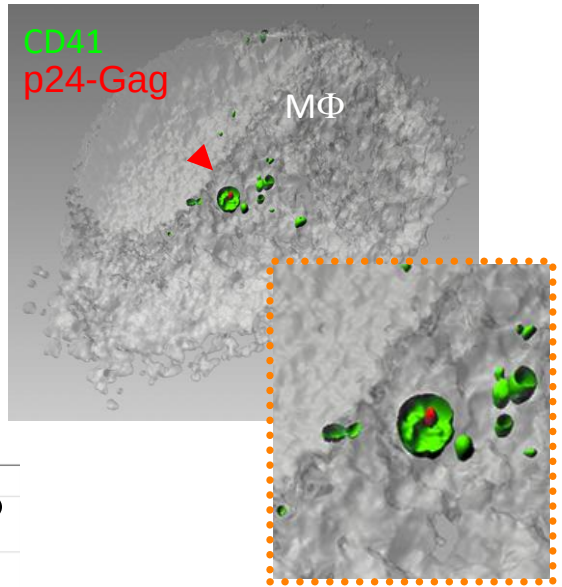


Not negligible!
Platelet production:
20 million per hour
(Lefrançais et al, Nature 2017)

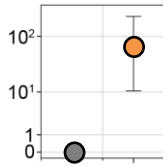
20,000 HIV⁺ platelets produced per hour

HIV sheltered in platelets is **infectious to macrophages**





Integrated HIV DNA
(copies per 10^6 MΦ)



MΦ uptake of:

healthy donor platelets
HIV⁺ platelets
+anti-αIIβ3

Infection is blocked by an **anti-platelet drug**

Real et al., *Sci Transl Med*, 2020
Real et al., *Médecine/Sciences*, 2021

Our questions

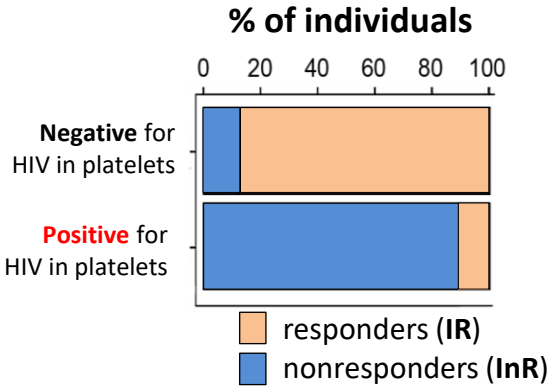
1- **Origin:** How does virus go in there?

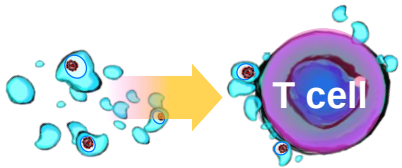
2- **Fate:** What’s the impact to immune system?

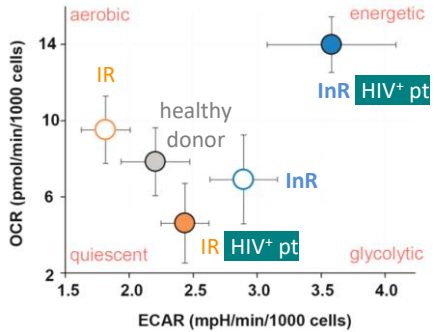
What do individuals with virus-containing platelets have in common?

Immunological non-responders (InRs)

HIV in platelets correlates with **immunological failure**





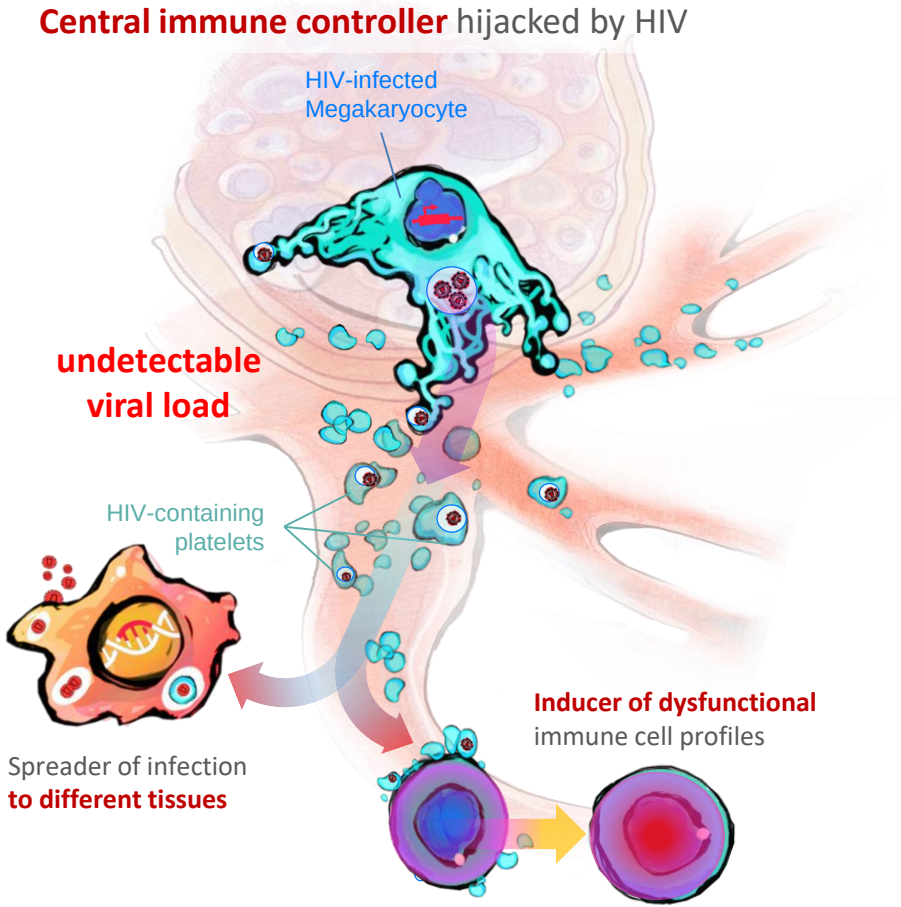


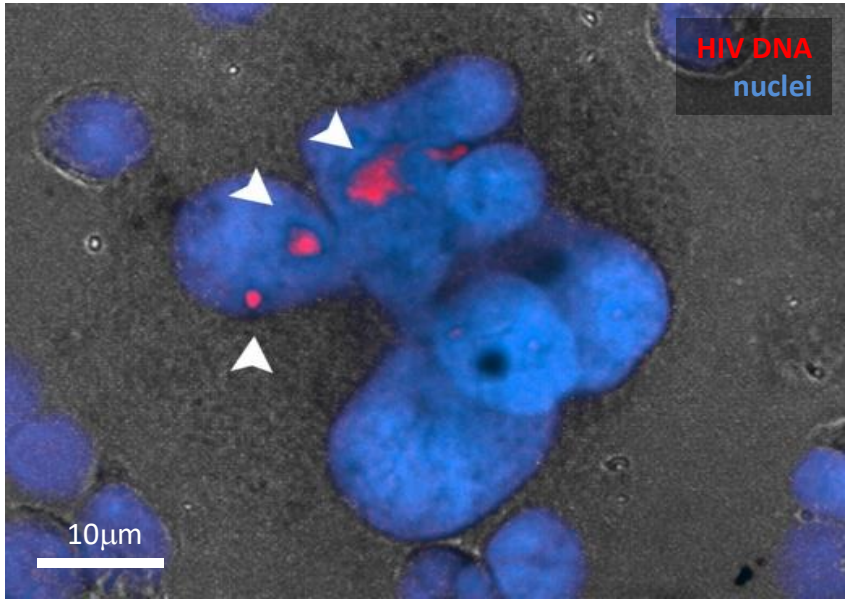
virus-containing platelets indicate poor prognosis

Long-term immunological failure?

Real et al., *Sci Transl Med*, 2020
Real et al., *Médecine/Sciences*, 2021
Zhu et al., *Frontiers in Immunology* 2022

The second giant





Real et al., *Sci Transl Med*, 2020

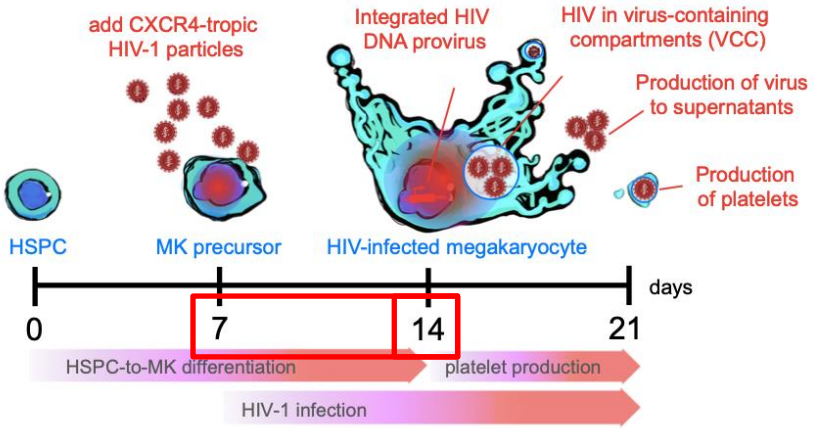
Megakaryocyte

(n.) "a large cell that has a lobulated nucleus, is found especially in the bone marrow, and is the source of blood platelets"

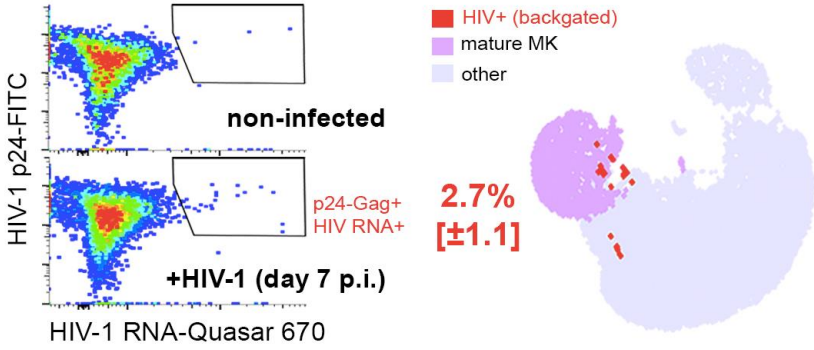
from the greek megas *("great")*, karyon *("nut, kernel")* *and* cyte *("cell")*

Megakaryocytes can be **infected *in vitro*** by HIV-1

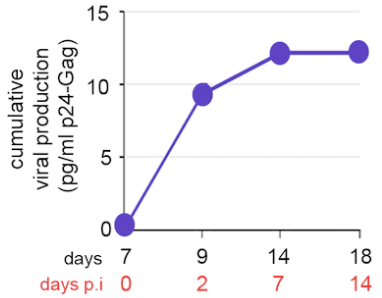
Infection protocol of **HSPC-derived MK**



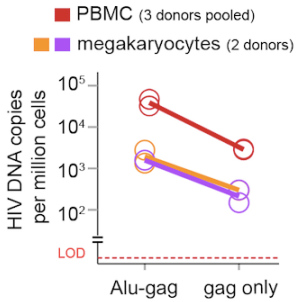
Infected MK display viral **RNA and protein**

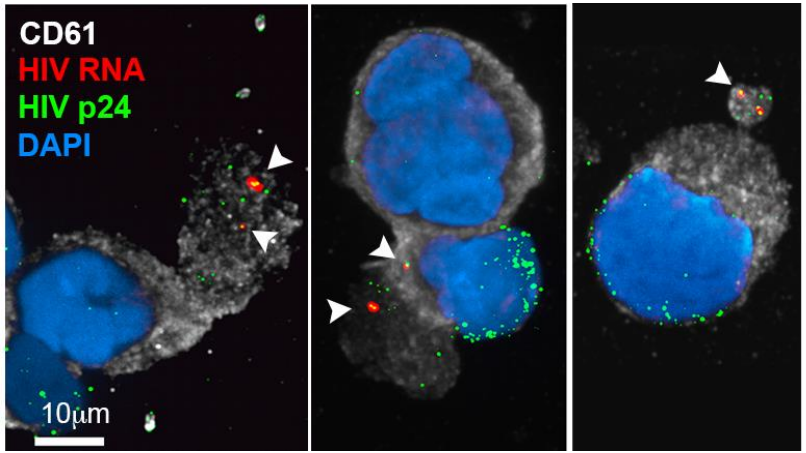


Self-limiting viral production



Integrated HIV DNA

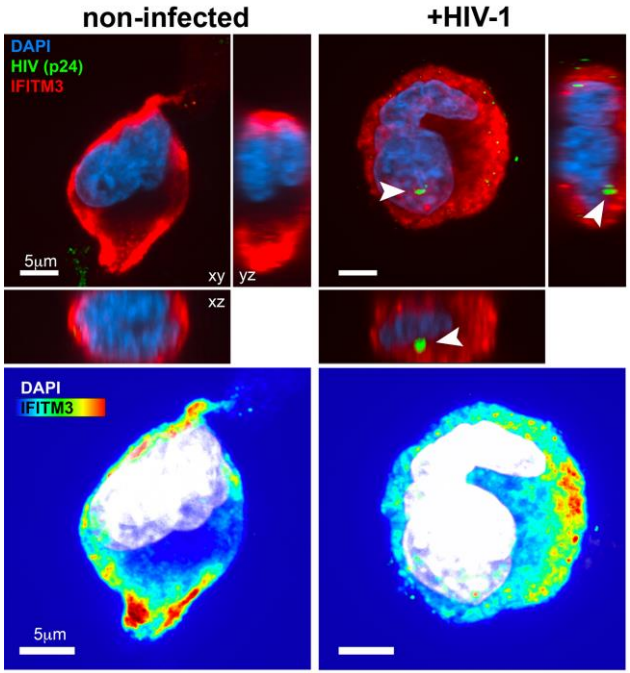


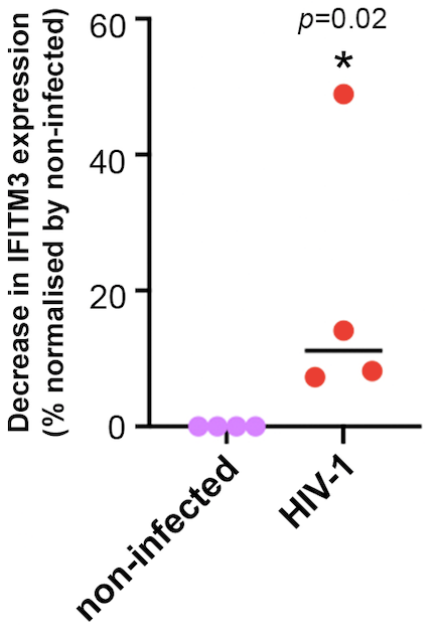


Bentaleb et al., *under review*

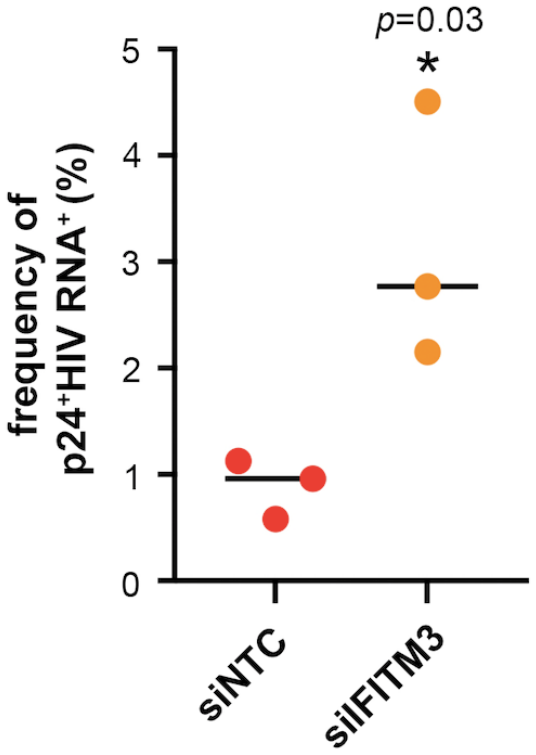
Megakaryocytes host HIV-1 via IFITM3 downregulation

IFITM3 is downregulated in infected MK



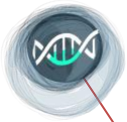


IFITM3 KD favors HIV-1 infection



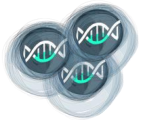
Bentaleb et al., *under review*

HIV persistent infection **in lymphoid *versus* myeloid** compartments

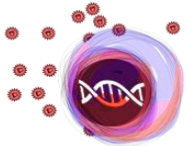


Latently infected memory CD4⁺ T cells are the best described HIV reservoir

1. **HIV DNA integrated** in their genomes but do not express viral proteins

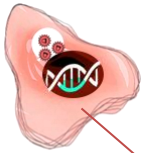


2. **long-lived in circulation**; proliferation under homeostasis and clonal expansion when activated



3. **cART stop replication**. Reactivate viral production upon cART interruption

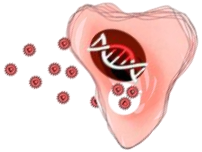
4. susceptible to **HIV cytopathic effects** and **Cytotoxic T lymphocytes (CTLs)-mediated killing**



Latently infected macrophages as HIV reservoirs

1. **HIV DNA integrated** in their genomes that keeps transcriptional activity

2. wide **tissue** distribution, **long life-span** and self-renewal capacity



3. **in contrast to T-cells**, MΦ **store** viral particles in **Virus-containing vacuoles (VCC)** and **stop releasing viruses** in absence of cART

4. susceptible to **HIV infection** but, **in contrast to T cells**, resistant to its cytopathic effects **and CTL**

Infected megakaryocytes are new players in HIV persistence

1. **HIV DNA integrated** in their genomes that keeps transcriptional activity. **High ploidy**

2. **Bone marrow-resident,** but also found in lungs. Short life span prevents reservoir formation, except if **progenitors are infected**

3. **VCCs** potentially present and **inherited** by daughter platelets

4. susceptibility to **HIV cytopathic effects and CTL unknown**

Myeloid viral reservoirs must be targeted for efficient therapeutics in HIV/AIDS

“THINK BIG”

Acknowledgments

Team Chronicity of Viral Infections [CVI]

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