



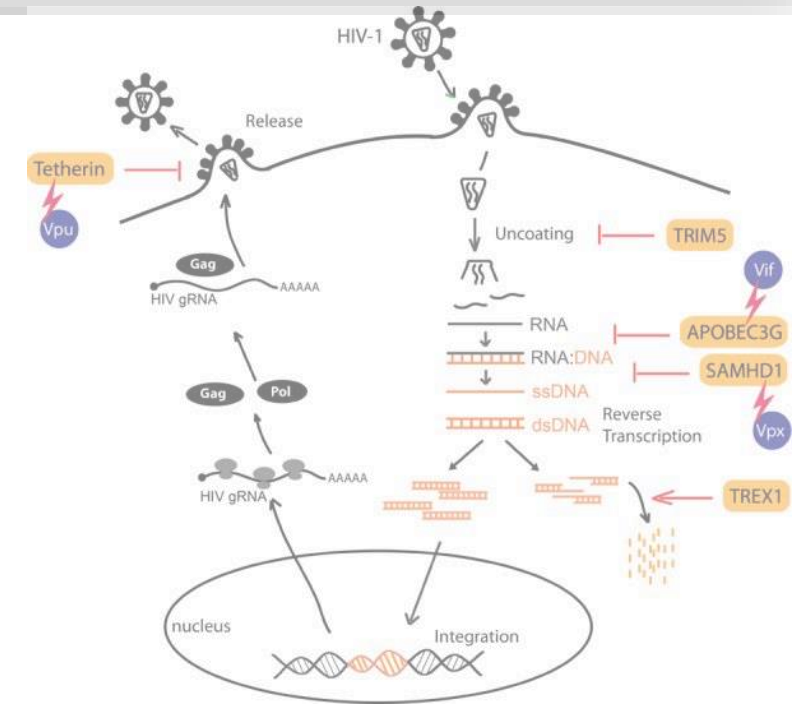
HOT TOPICS IN HIV

Vaccines, immune recovery and eradication

Host **Restriction Factors** Modulating **HIV** Latency & Replication in **Macrophages**

26th October 2023

📍 Palau Macaya, Barcelona



Prof. Guido Poli
San Raffaele University &
Scientific Institute, Milano, Italy

poli.guido@hsr.it



HOT TOPICS IN HIV

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and eradication

1. Introduction. Why macrophages should be taken seriously in HIV infection?
2. Host restriction factors modulating HIV latency & replication in macrophages
3. M1-polarized MDM. An *in vitro* model to study reversible latency in primary human macrophages
4. Conclusions & Perspectives

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HIV Infection of CD4⁺ T cells & Macrophages

Similarities & Differences

CD4 ⁺ T Lymphocytes		Macrophages
CD4 (primary), CCR5 & CXCR4	Entry Receptors	CD4 (primary), CCR5
Yes	Cell proliferation	No
Yes	Cell depletion (<i>in vivo</i> and <i>in vitro</i>)	No

After: I. Pagani et al., *Int. J. Mol. Sci.* 23:3021, 2022

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Well-demonstrated in latently infected “resting memory” cells	Role as viral reservoir in cART-treated individuals	Strong evidence in support of Tissue-Resident Macrophages



The “Classic” View of the Mononuclear Phagocyte System

the MPS “*Monocytes in the circulation constitute a mobile pool of relatively immature cells on their way from the place of origin to the tissues*” (69). Moreover, monocytes should now be fur-



Z.A. Cohn



R. Van Furth



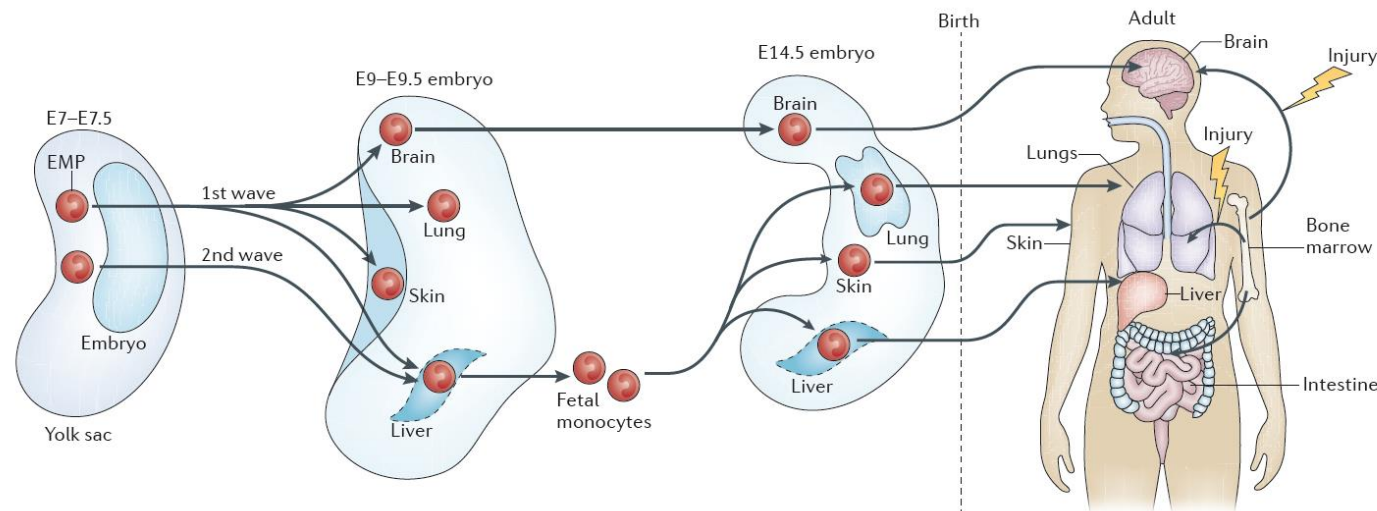
J.G. Hirsch

FIGURE 1 | Selected experimental pathologists and immunologists who coined the term mononuclear phagocyte system.

[Mononuclear phagocytic system: new classification of macrophages, monocytes and of their cell line]. *Bull World Health Organ* (1972) 47:651–8.

Bone Marrow Independent Origin of Most Tissue Resident Macrophages (TRM)

- ❖ In adult life, TRM undergo a slow, are responsible for tissue homeostasis without requiring supply of blood monocytes.
- ❖ Due to their **intrinsic resistance to cell death** and to **homeostatic proliferation**, infected TRM qualify for upgrade to “first class” reservoirs of latent, replication-competent HIV



Yonit Lavin, Arthur Mortha, Adeb Rahman and Miriam Merad

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Profound immunodeficiency, opportunistic infections, cancers	Main pathogenic correlates	Tissue pathology, brain infection (HIV Encephalitis)

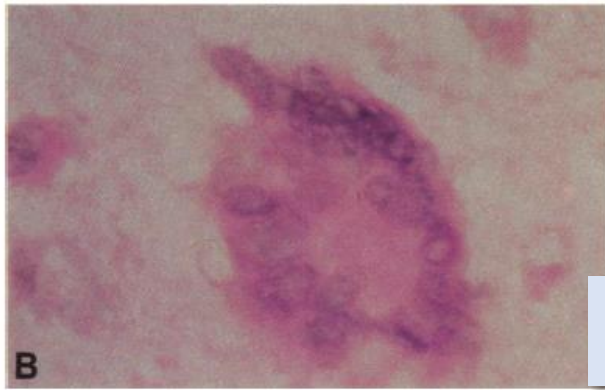
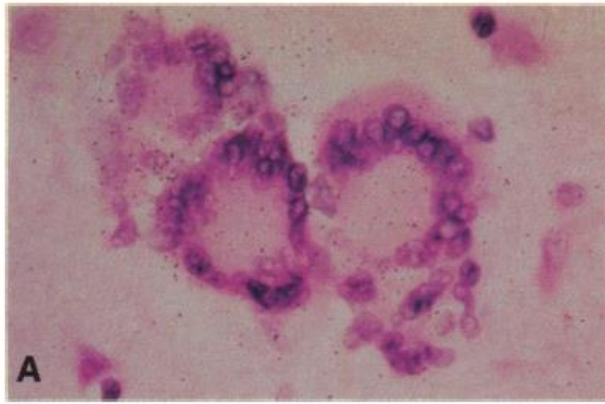


Detection of AIDS Virus in Macrophages in Brain Tissue from AIDS Patients with Encephalopathy

SCOTT KOENIG, HOWARD E. GENDELMAN, JAN M. ORENSTEIN, MAURO C. DAL CANTO, GHOLAM H. PEZESHKPOUR, MARGARET YUNGBLUTH, FRANK JANOTTA, ALLEN AKSAMIT, MALCOLM A. MARTIN, ANTHONY S. FAUCI*

Virus Isolation From and Identification of HTLV-III/LAV-Producing Cells in Brain Tissue From a Patient With AIDS

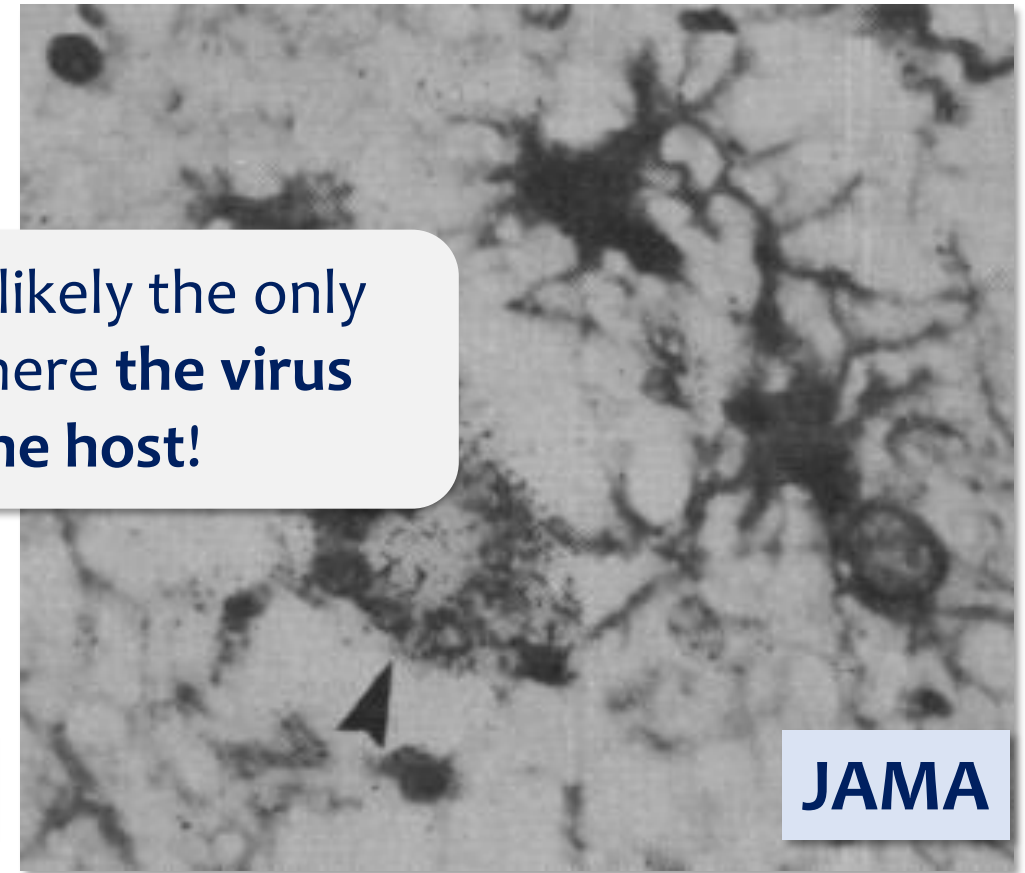
Suzanne Gartner, PhD; Paul Markovits, DVM; David M. Markovitz, MD; Robert F. Betts, MD; Mikulas Popovic, MD, PhD



HIV-1 Encephalitis is likely the only clinical condition where **the virus directly kills the host!**

Science

1986



JAMA

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Plasma membrane only	Virion budding and release	Plasma membrane and VCC (Virus-Containing Compartments)

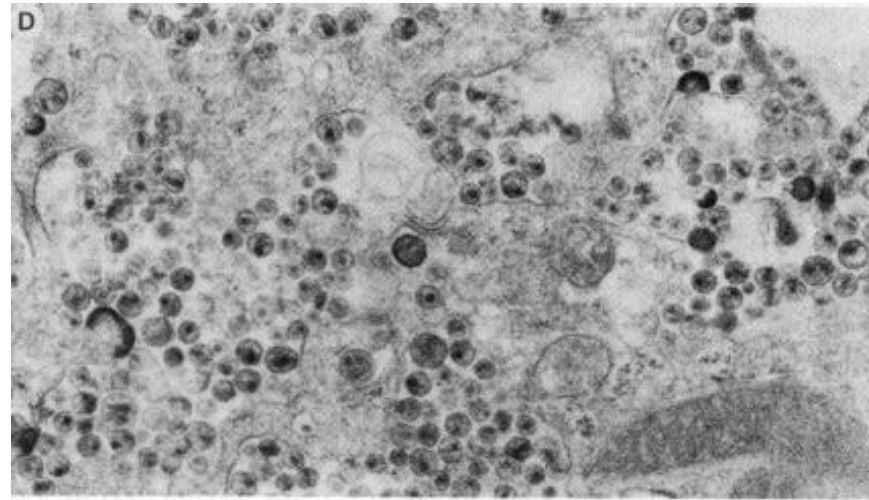
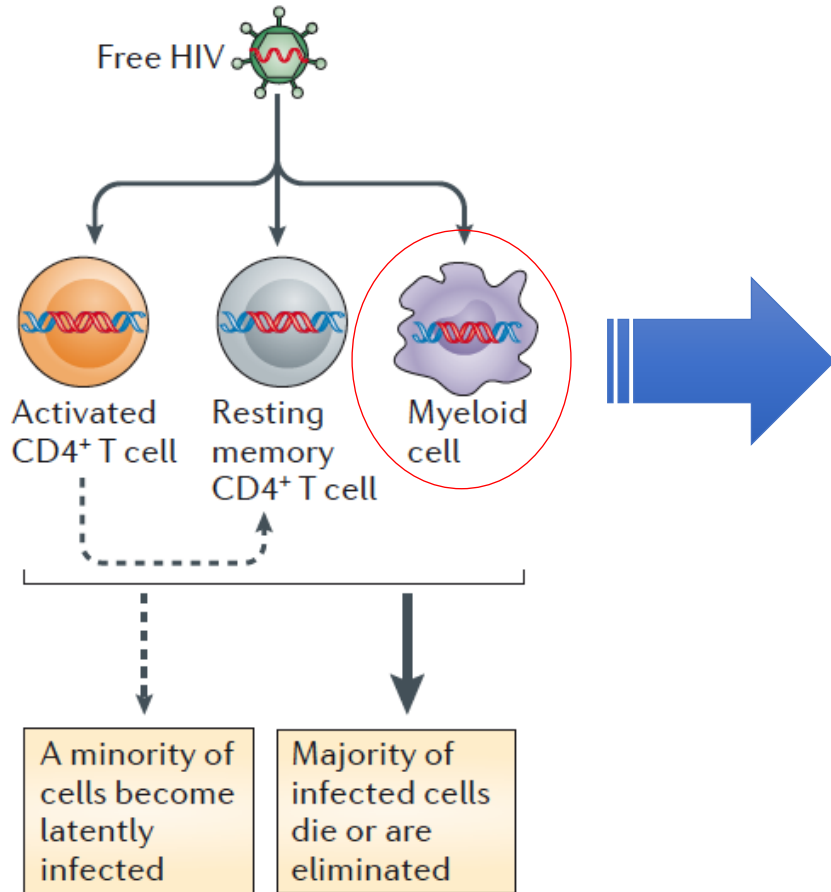


After: I. Pagani et al., *Int. J. Mol. Sci.* 23:3021, 2022

Towards an HIV cure: a global scientific strategy

The International AIDS Society Scientific Working Group on HIV Cure

Establishment of latency

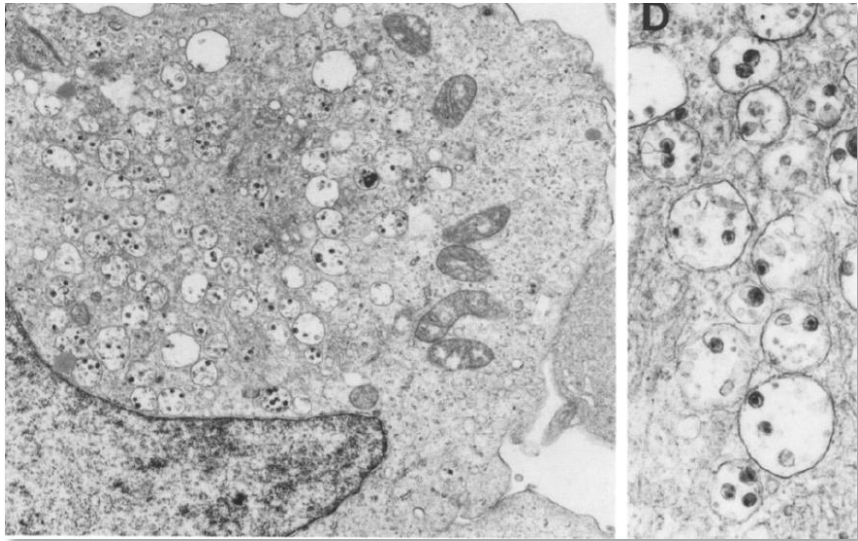


Accumulation of HIV
in **Virus Containing
Compartments (VCC)**

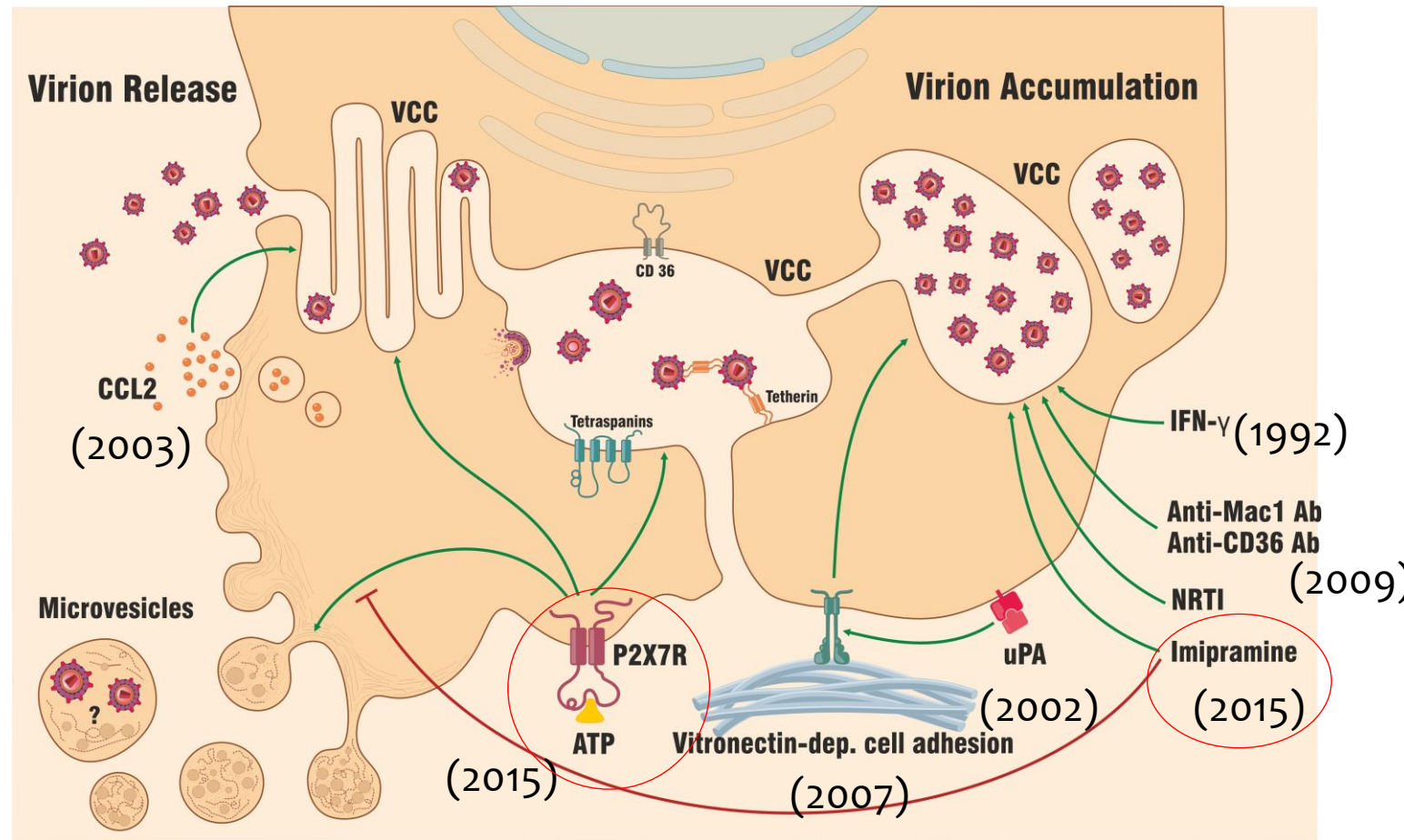


**“Trojan Horse” Model
of HIV Persistency**

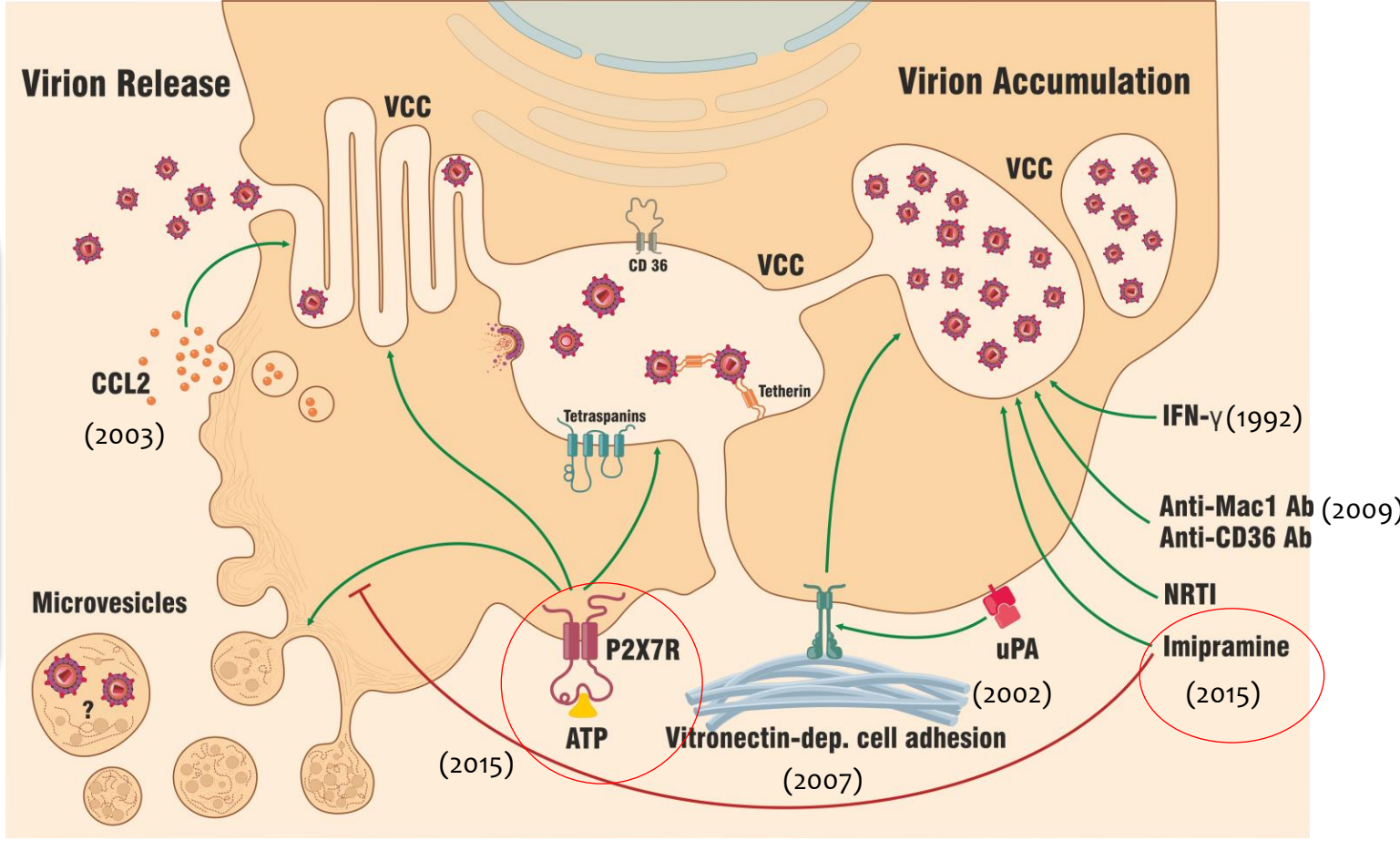
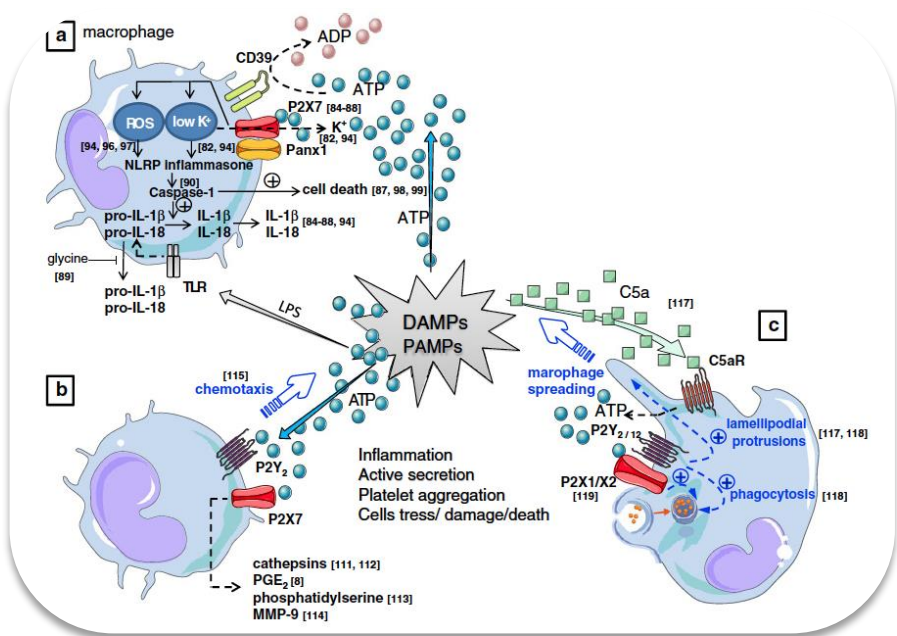
Multiple Signals Favor the Accumulation of Virions in Mø Virus-Containing Compartments (VCC)



P. Biswas et al., *J. Exp. Med.* 1992





Extracellular ATP induces the rapid release of HIV-1 from virus containing compartments of human macrophages



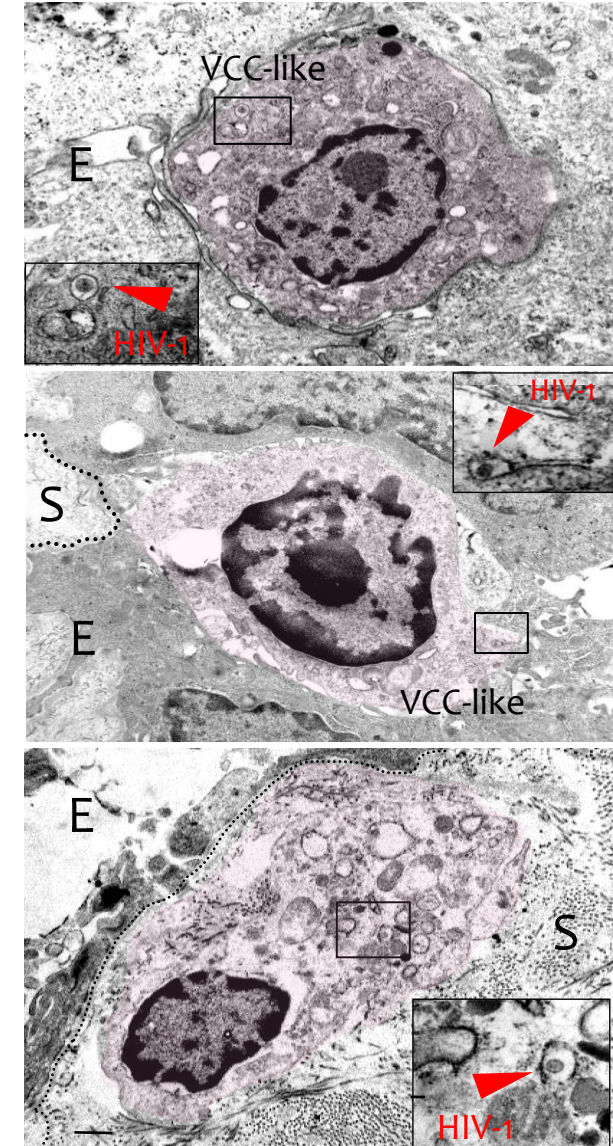
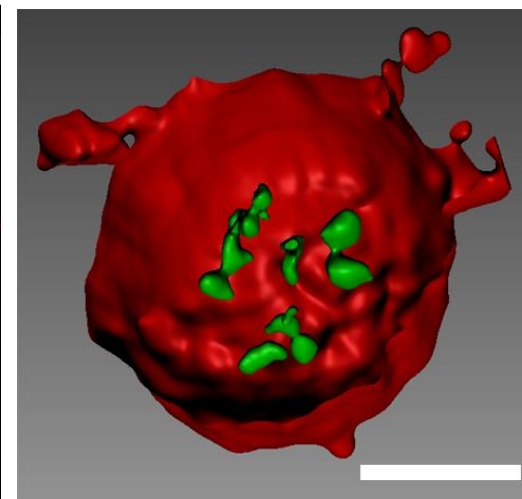
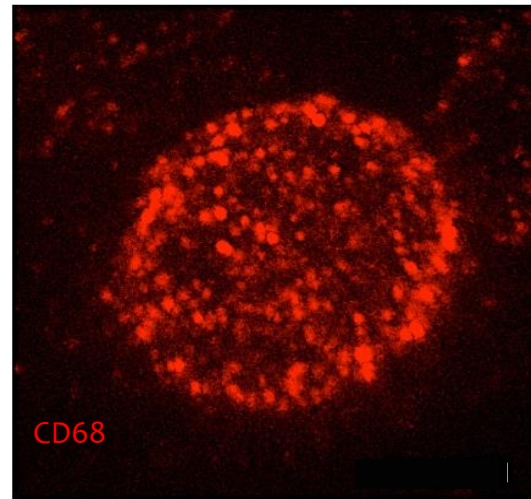
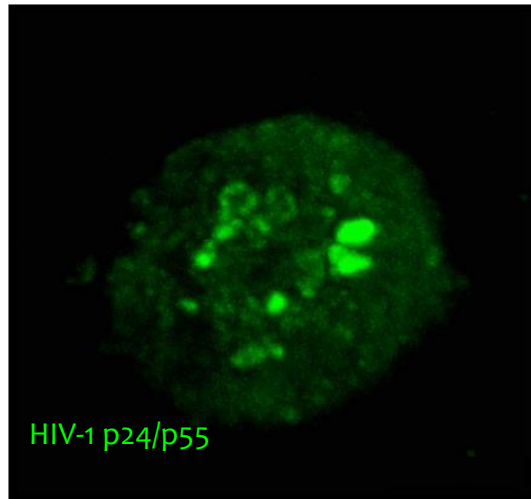
F. Graziano et al., PNAS Plus, 2015

HIV-1 reservoirs in urethral macrophages of patients under suppressive antiretroviral therapy

2019

Yonatan Ganor ^{1,2,3*}, Fernando Real ^{1,2,3,12}, Alexis Sennepin^{1,2,3,12}, Charles-Antoine Dutertre^{2,3,4,11}, ... Morgane Bomsel

- ❖ M ϕ -associated HIV was contained in **Virus Containing Compartments (VCC)**
- ❖ Urethral CD3⁺ T cells did not contain HIV-1 DNA/RNA





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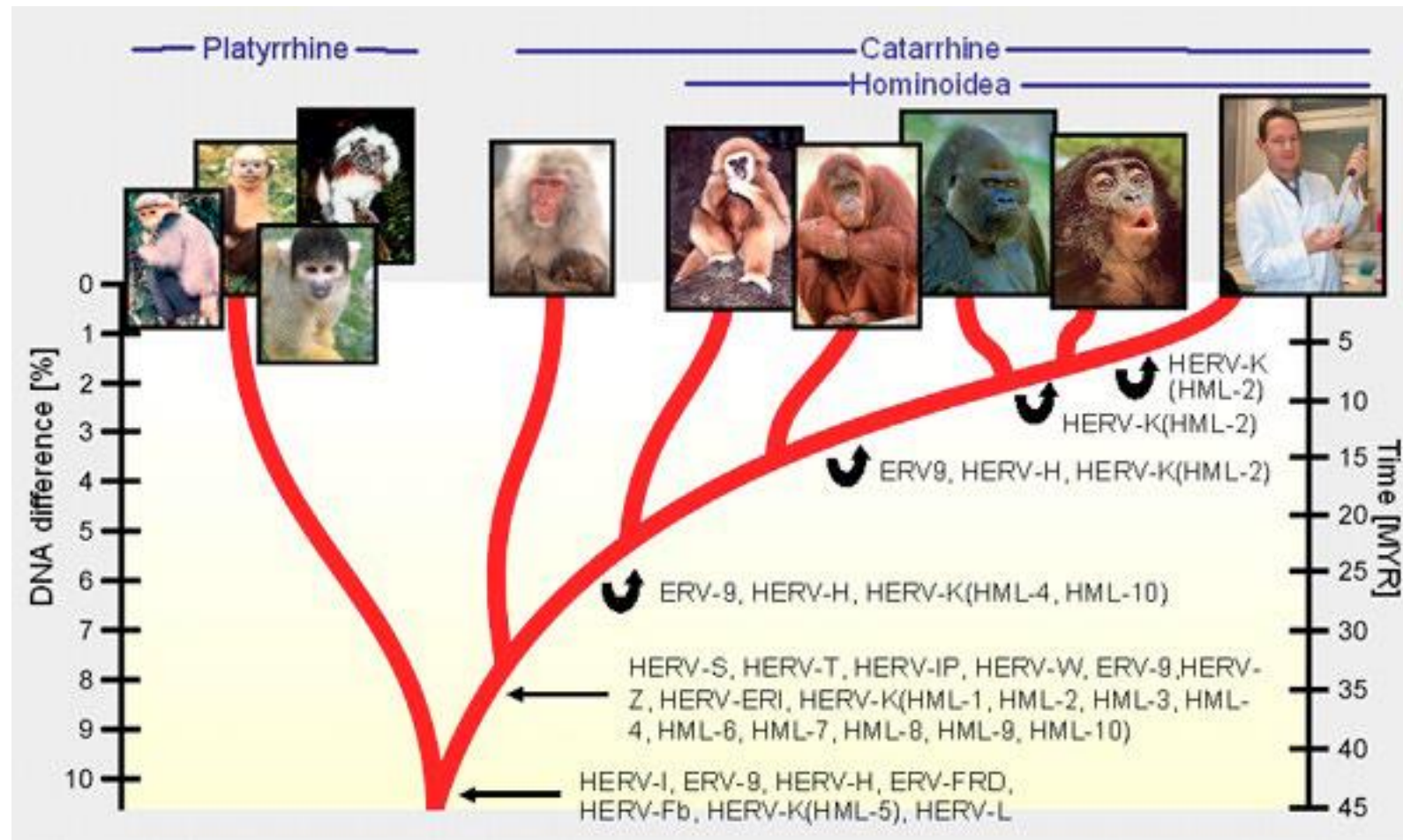
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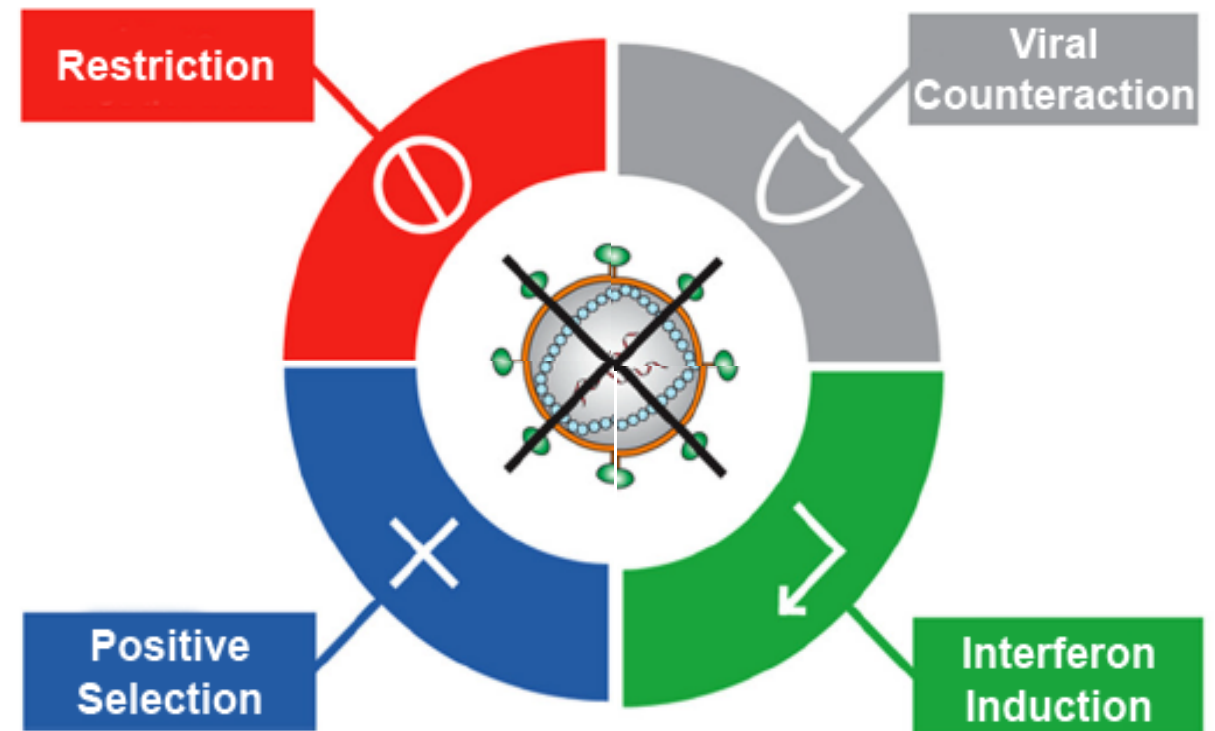
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Retroviruses and Reverse-Transcribing Transposable Elements Have Been Invading their Host's Genomes for Millions of Years...

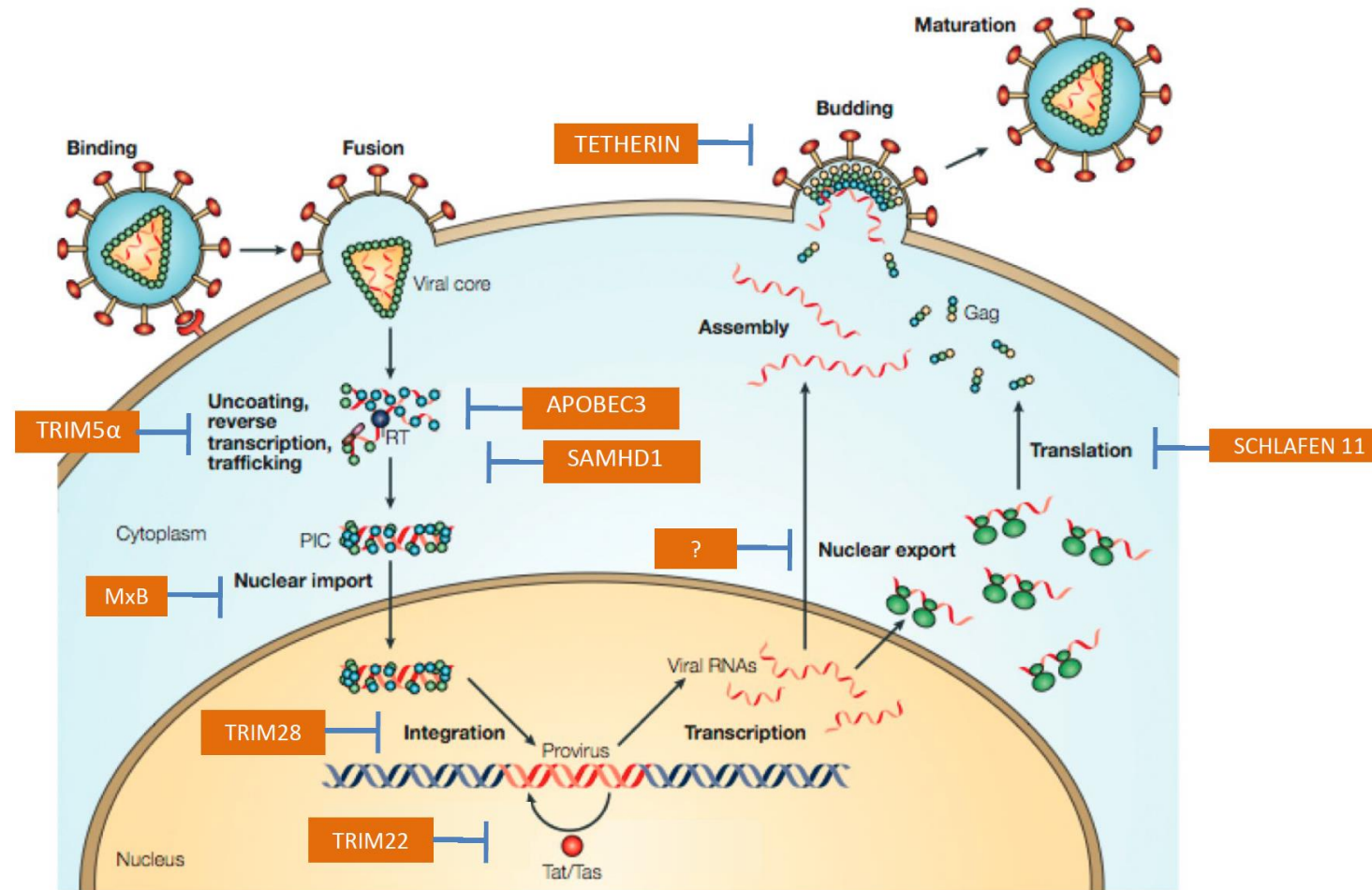


...Conversely, Host Organisms Have Evolved Protective Strategies Including the Expression of **Intracellular Restriction Factors (RFs)**

- ❖ Direct cause of a significant **decrease in viral infectivity**
- ❖ **Positive selection signature** due to a direct evolutionary competition
- ❖ Often strongly **induced by interferon**
- ❖ Targeted by potent **viral counter-restriction factors**



...Conversely, Host Organisms Have Evolved Protective Strategies Including the Expression of **Intracellular Restriction Factors (RFs)**



Adapted from Nisole et al., Nat. Rev Microbiol, 2005

...But **HIV** Has Evolved Countermeasures To Efficiently Evade Host Restriction Factors. **The Red Queen Hypothesis**

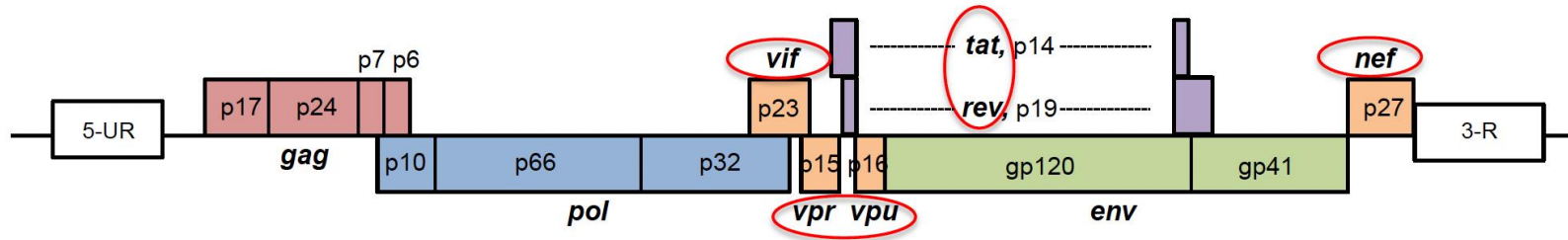


The **Red Queen** lecturing Alice,
by John Tenniel

The Red Queen hypothesis, named after Lewis Carroll's *Through the Looking Glass* in which the queen tells Alice, '*It takes all the running you can do to keep in the same place,*' says that **parasites and their hosts are in a constant evolutionary arms race**. Each has to evolve ever-better ways of out-witting the other to avoid losing out.

Paterson S. et al., *Nature* **464**, 275-278, 2010

...But **HIV** Has Evolved Countermeasures To Efficiently Evade Host Restriction Factors. The Role of Its “**Accessory Genes**”



Gene	Protein	Function
<i>tat</i>	Tat (p14)	Transactivation during viral transcription
<i>rev</i>	Rev (p19)	Regulation of viral splicing (4.5 and 9 Kb)

Regulatory Genes

<i>vif</i>	Vif (p23)	Counteraction of APOBEC3G, 3F
<i>vpr</i>	Vpr (p15)	Cell cycle arrest in G2, nuclear import
<i>vpu</i>	Vpu (p16)	Downregulation of CD4, Tetherin
<i>nef</i>	Nef (p27)	Immune evasion (MHC-I, CD4)

Accessory Genes

A Subset of Restriction Factors is Particularly Effective Against HIV Infection of Macrophages

“Early RF”

HIV Entry

Serinc3 and 5

IFITM1,2,3

REAF

CH25H

Post-Entry & RT

APOBEC3(A)

Tet2

SAMHD1*

TREX1

Mx2/MxB

A Subset of Restriction Factors is Particularly Effective Against HIV Infection of Macrophages

“Early RF”

”Intermediate RF”

HIV Entry

Post-Entry & RT

Transcriptional repressors

Serinc3 and 5

APOBEC3(A)

NF-kB1 (p50)

IFITM1,2,3

Tet2

Homodimers

REAF

SAMHD1*

TRIM22

CH25H

TREX1

CIITA

Mx2/MxB

p21/Waf1

HUSH Complex

A Subset of Restriction Factors is Particularly Effective Against HIV Infection of Macrophages

“Early RF”

HIV Entry

Serinc3 and 5
IFITM1,2,3
REAF
CH25H

Post-Entry & RT

APOBEC3(A)
Tet2
SAMHD1*
TREX1
Mx2/MxB

”Intermediate RF”

Transcriptional repressors

NF-kB1 (p50)
Homodimers
TRIM22
CIITA
p21/Waf1
HUSH Complex

”Late RF”

Virion Budding & Release

BST-2/Tetherin
GBP-5
Mannose R
MARCH1,2, 8

A Subset of Restriction Factors is Particularly Effective Against HIV Infection of Macrophages

“Early RF”

HIV Entry

Serinc3 and 5
IFITM1,2,3
REAF

CH25H

Post-Entry & RT

APOBEC3(A)
Tet2
SAMHD1*

TREX1

Mx2/MxB

“Intermediate RF”

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“Late RF”

Virion Budding & Release

BST-2/Tetherin
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MARCH1,2, 8

Targeted by HIV-1/-2* Accessory Proteins

Adapted from: I. Pagani et al., Int. J. Mol. Sci. 2022

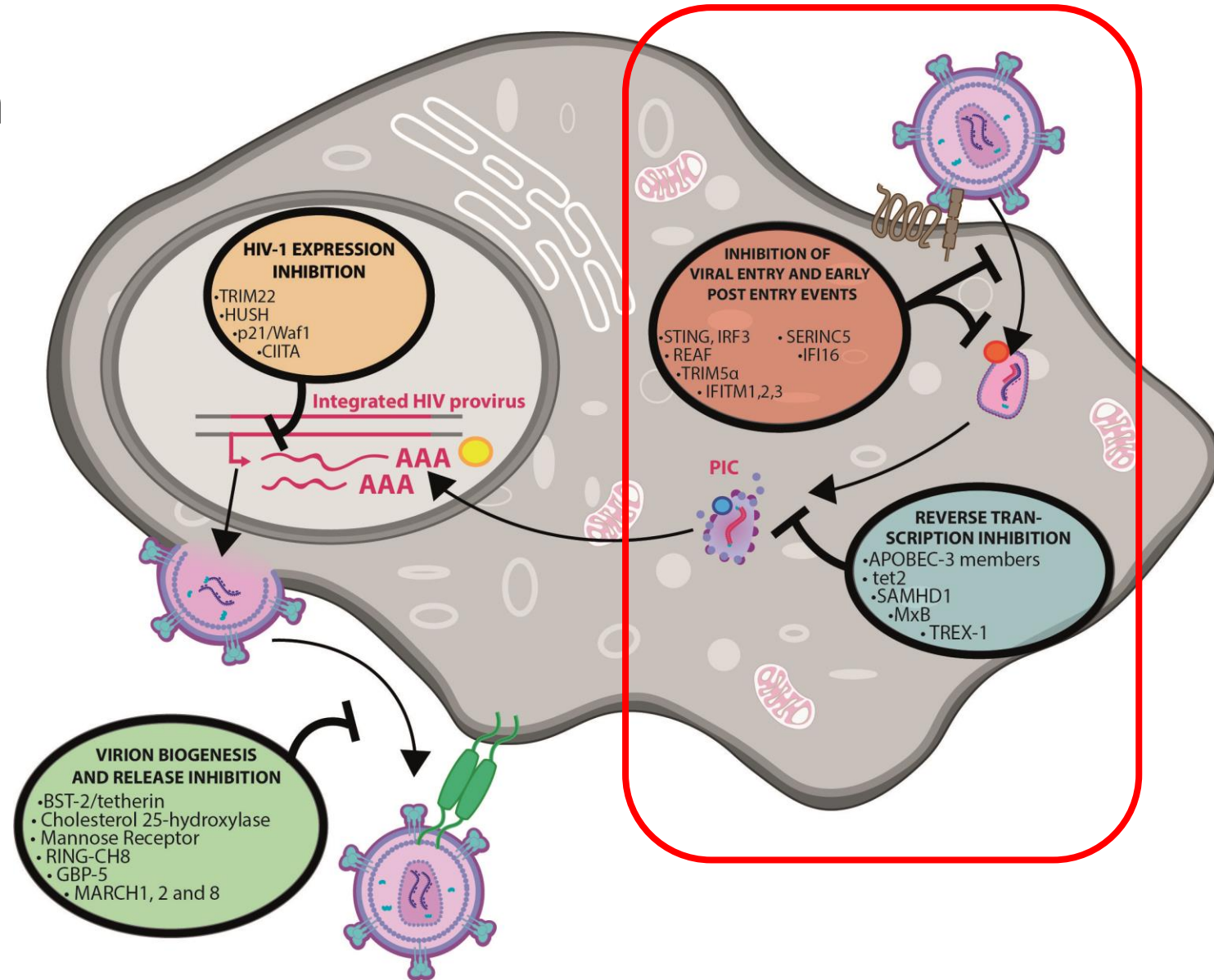
Restriction Factors

Antagonizing HIV Infection and/or Replication

- ❖ “Early RF” prevent or curtail viral entry, reverse transcription or integration of proviral DNA



Optimal restriction!



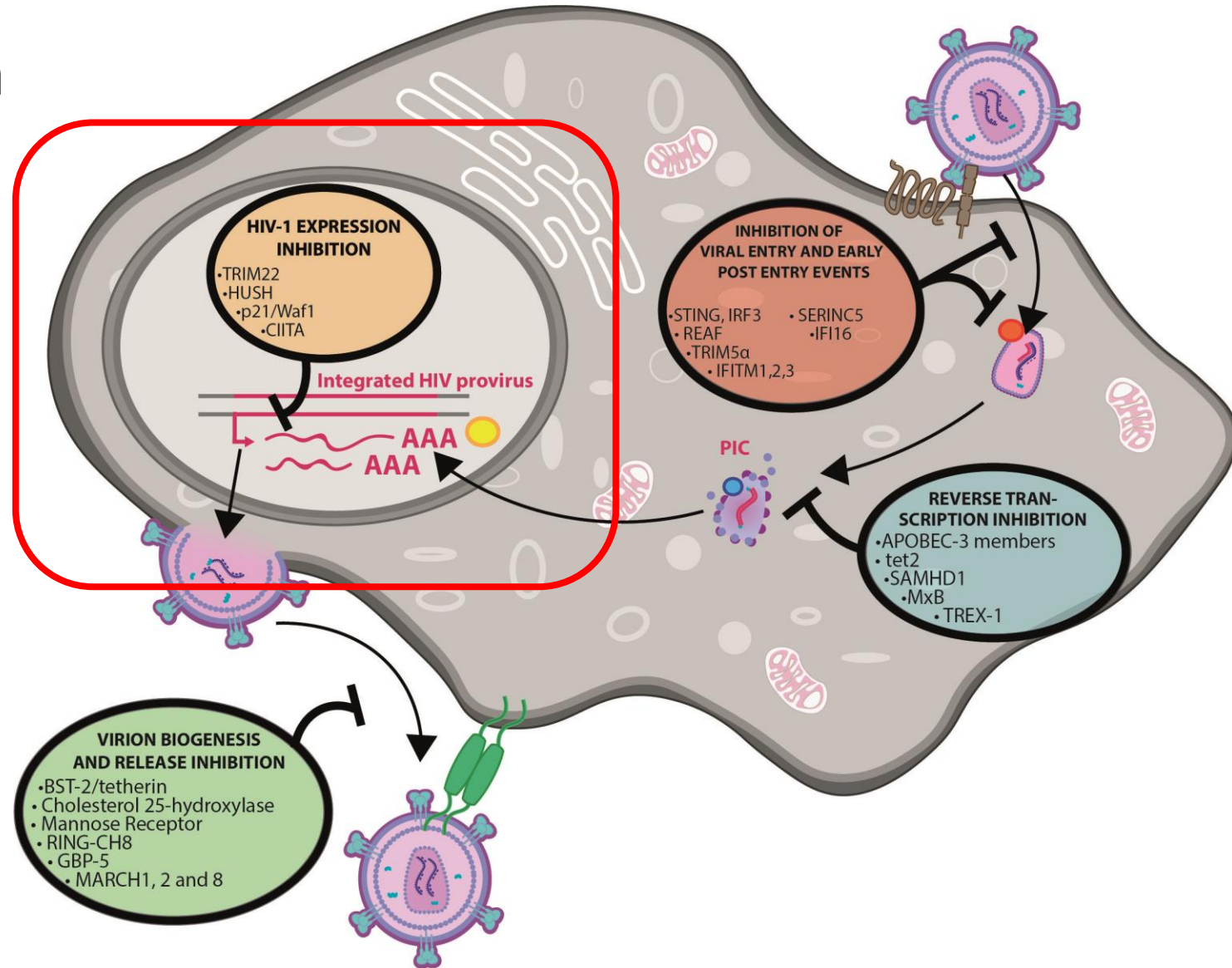
Restriction Factors

Antagonizing HIV Infection and/or Replication

- ❖ “Intermediate RF” repress proviral transcription



Relevance for “Cure”-related studies



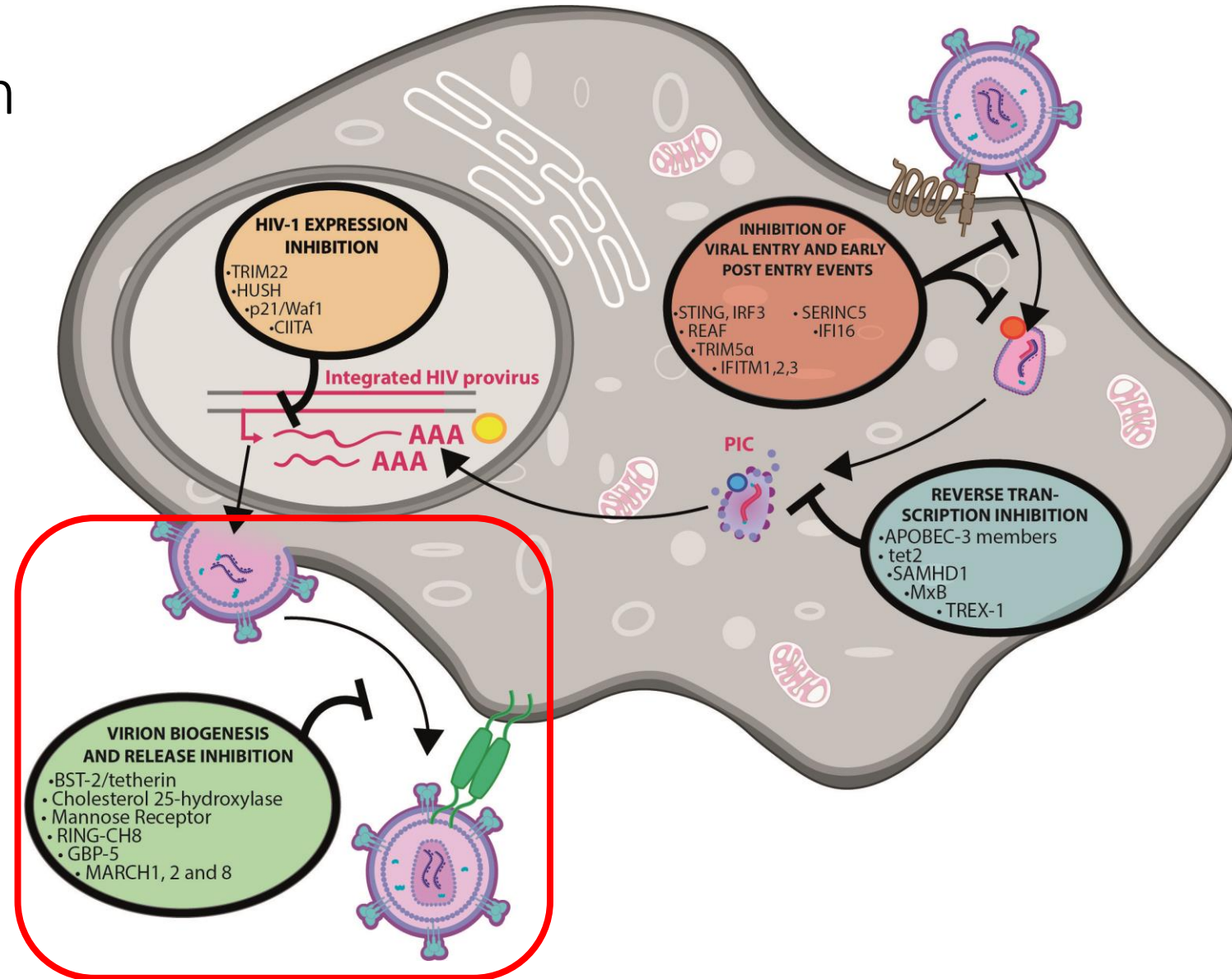
Restriction Factors

Antagonizing HIV Infection and/or Replication

- ❖ “Late RF” target the final steps of the HIV life cycle impeding the release of mature infectious virions



Relevance for drug discovery





Review

Host Restriction Factors Modulating HIV Latency and Replication in Macrophages

Isabel Pagani ¹, Pietro Demela ², Silvia Ghezzi ¹, Elisa Vicenzi ¹, Massimo Pizzato ³  and Guido Poli ^{2,4,*} 

Int. J. Mol. Sci. **2022**, *23*, 3021. <https://doi.org/10.3390/ijms23063021>



HOT TOPICS IN HIV

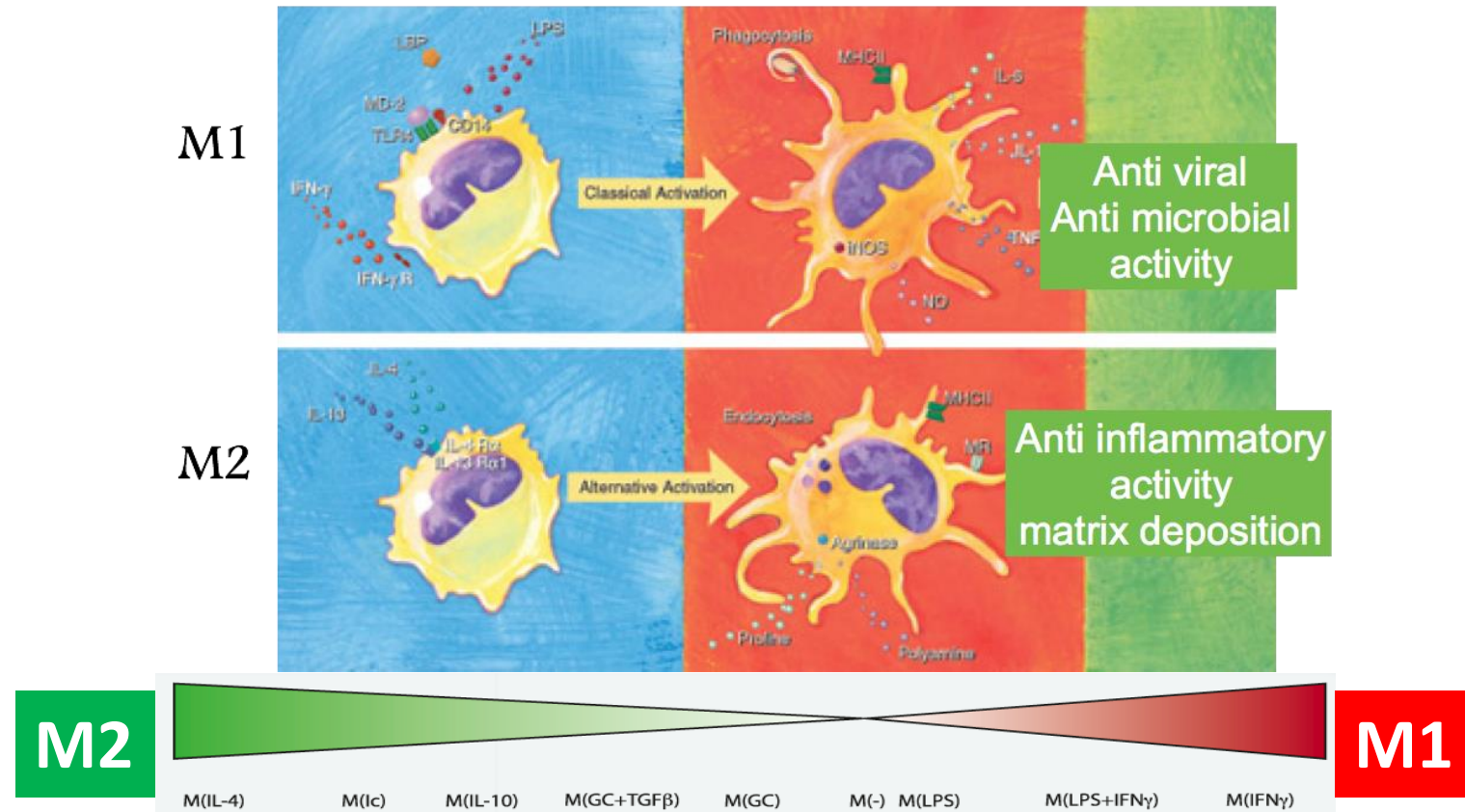
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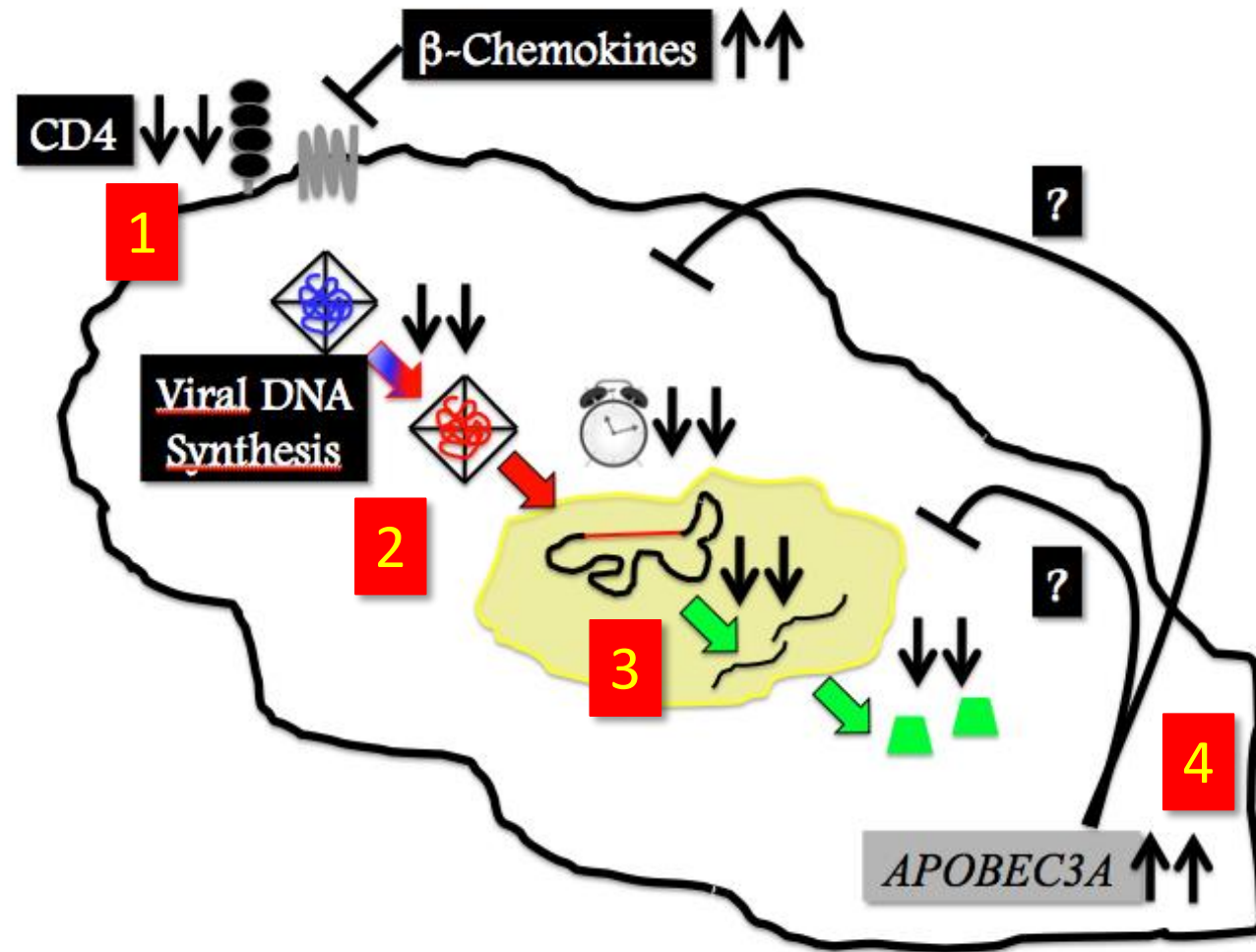
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M1 (Pro-Inflammatory) M ϕ Polarization Contains/Eliminates Microbial Infections and Activates T Cell Adaptive Immunity



After: A. Mantovani, Eur J Immunol 2007; P.J. Murray et al., Immunity, 2014

M1 Polarization Generates a Hostile Environment for HIV-1 Replication in MDM



A Model of Reversible HIV Latency in Primary M1-Polarized MDM

SCIENTIFIC REPORTS

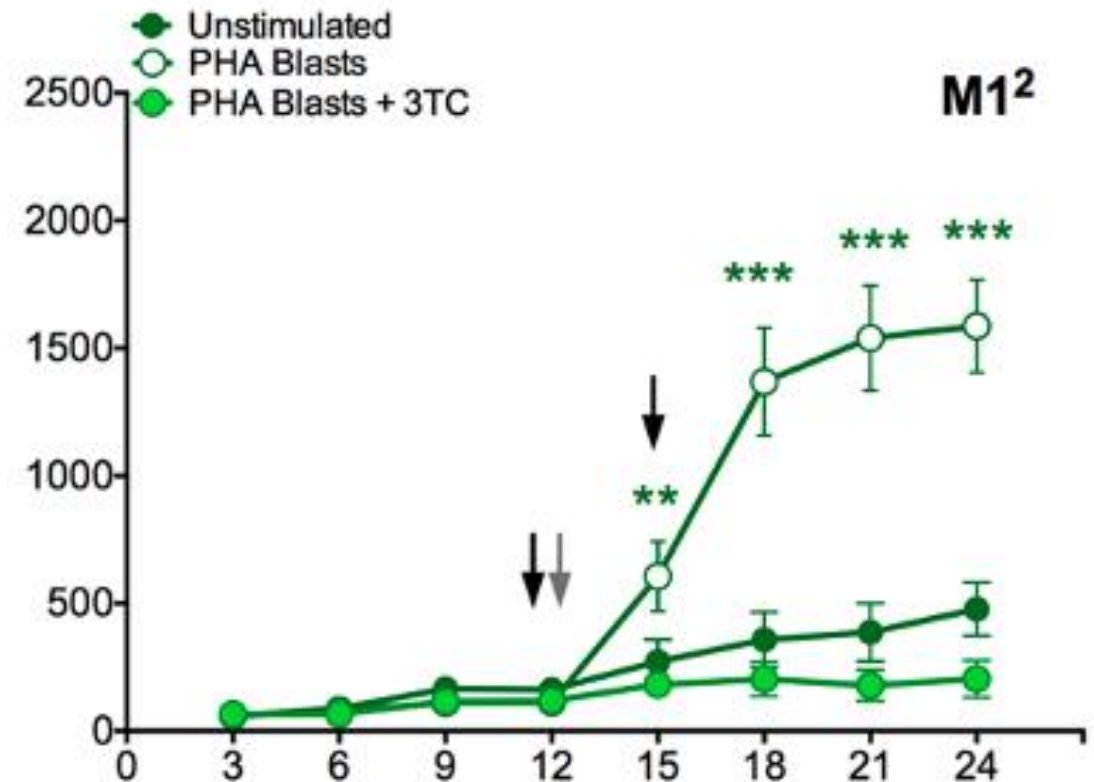
OPEN

Reversible Human Immunodeficiency Virus Type-1 Latency in Primary Human Monocyte-Derived Macrophages Induced by Sustained M1 Polarization

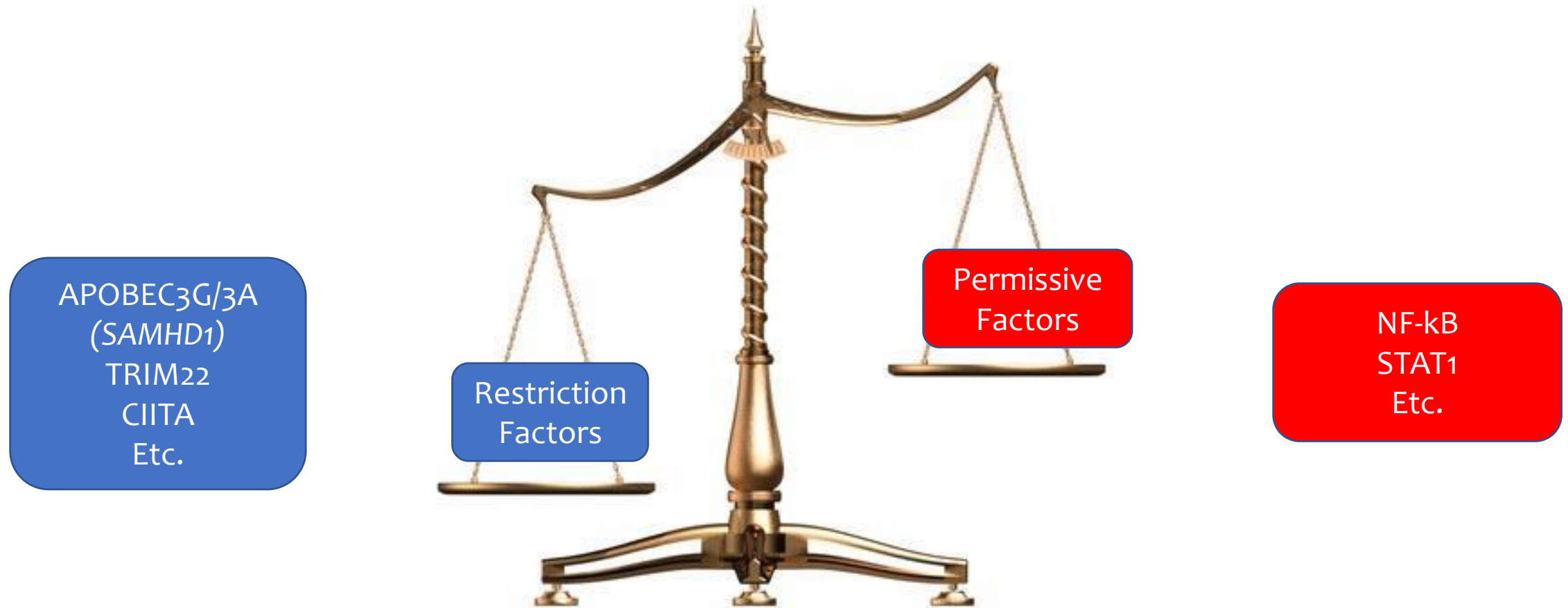
ed: 11 January 2018
ted: 13 August 2018
ed online: 24 September 2018

2018

Francesca Graziano^{1,4}, Giulia Aimola¹, Greta Forlani², Filippo Turrini¹, Roberto S. Accolla², Elisa Vicenzi¹ & Guido Poli^{1,3}

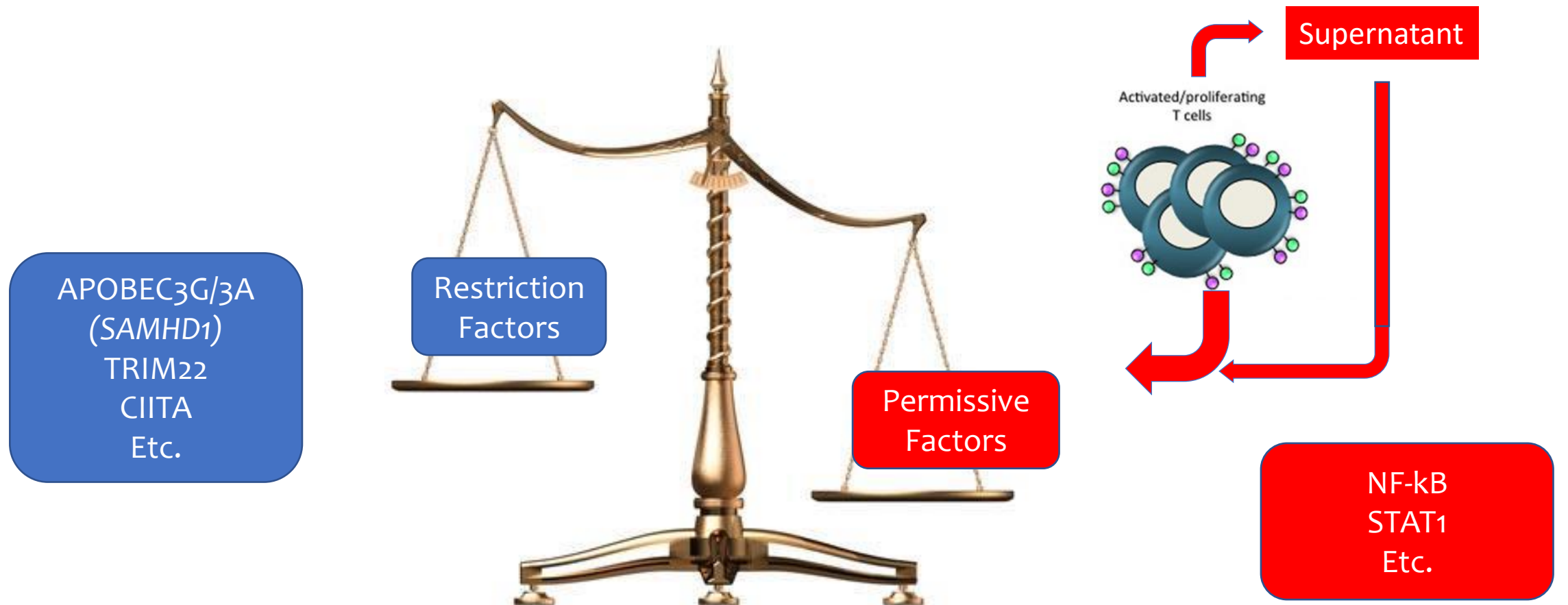


M1² MDM Model of Reversible HIV-1 Latency. Constitutive Dominance of Restrictive vs. Permissive Factors



M1² MDM Model of Reversible HIV-1 Latency.

PHA Blasts Tilt the Balance in Favor of Permissive Factors





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HOT TOPICS IN HIV

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Thank You!

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- ❖ Several features of macrophage infection support the concept that macrophages, and particularly TRM, should be considered primary reservoirs of replication-competent HIV together with CD4+ T lymphocytes
- ❖ Restriction factors (RF) are “natural drugs” encoded by our cells to defend them from invading pathogens, including HIV
- ❖ Some RF are particularly expressed in macrophages vs. T cells suggesting their peculiar role in counteracting infection of these cells
- ❖ Some of these RF are “druggable” targets that should be considered for developing new pharmacological strategies against HIV infection

A Model of Reversible HIV Latency in Primary M1-Polarized MDM

SCIENTIFIC REPORTS

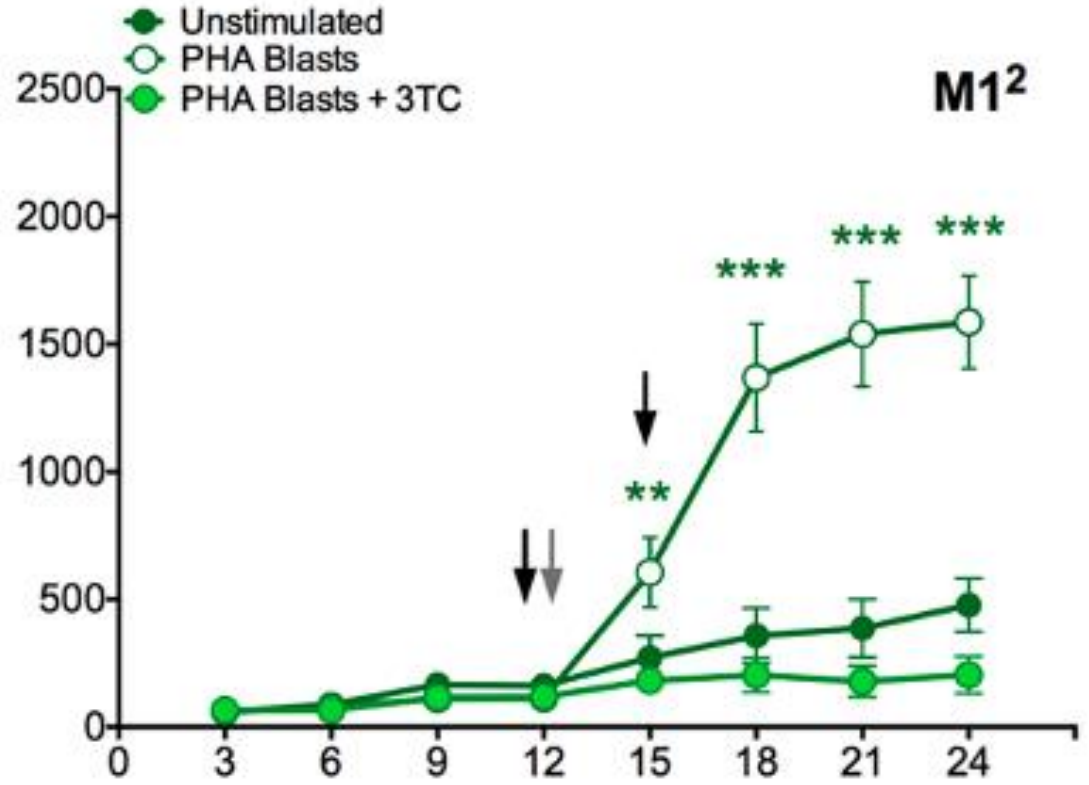
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Reversible Human Immunodeficiency Virus Type-1 Latency in Primary Human Monocyte-Derived Macrophages Induced by Sustained M1 Polarization

Francesca Graziano^{1,4}, Giulia Aimola¹, Greta Forlani², Filippo Turrini¹, Roberto S. Accolla², Elisa Vicenzi¹ & Guido Poli^{1,3}

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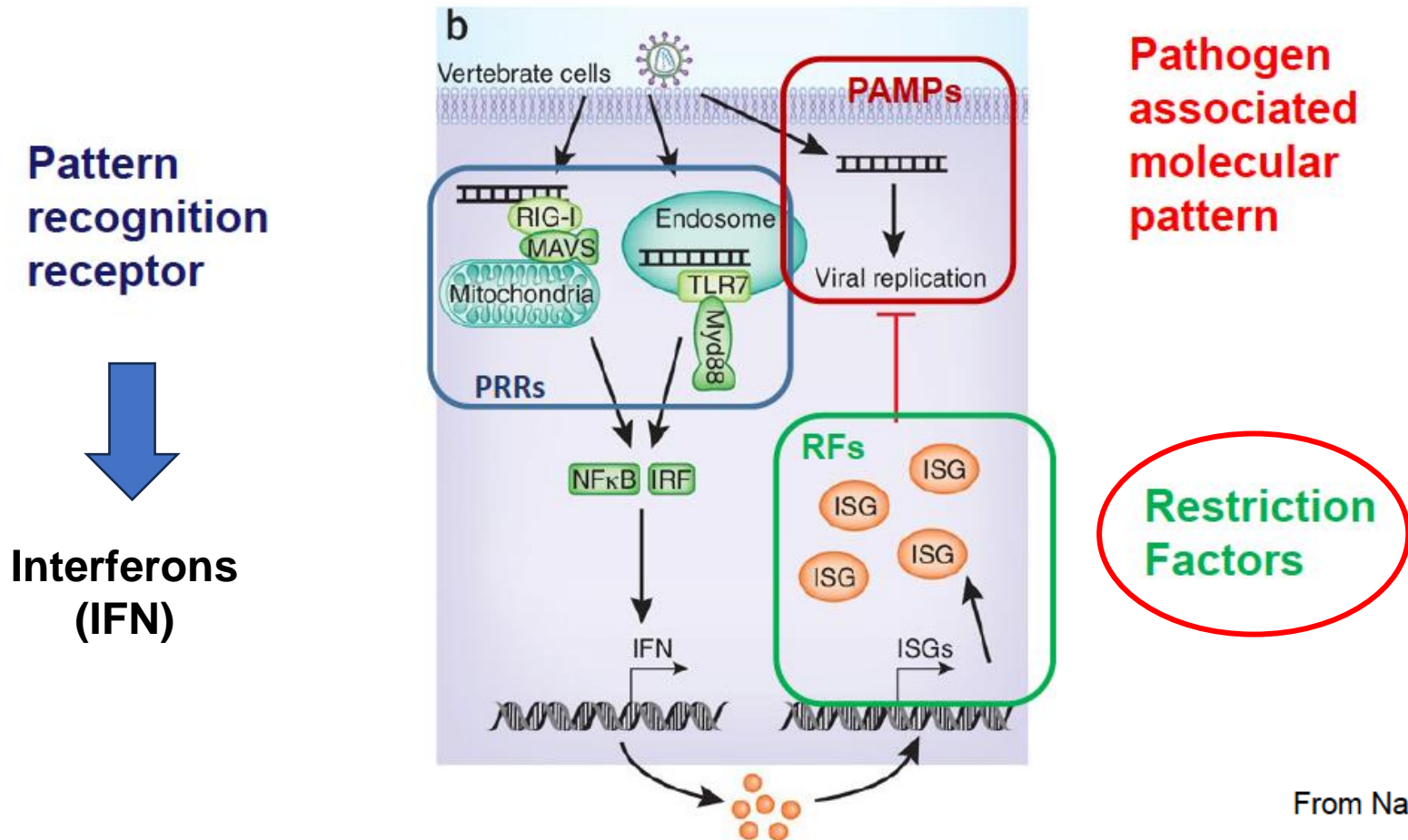




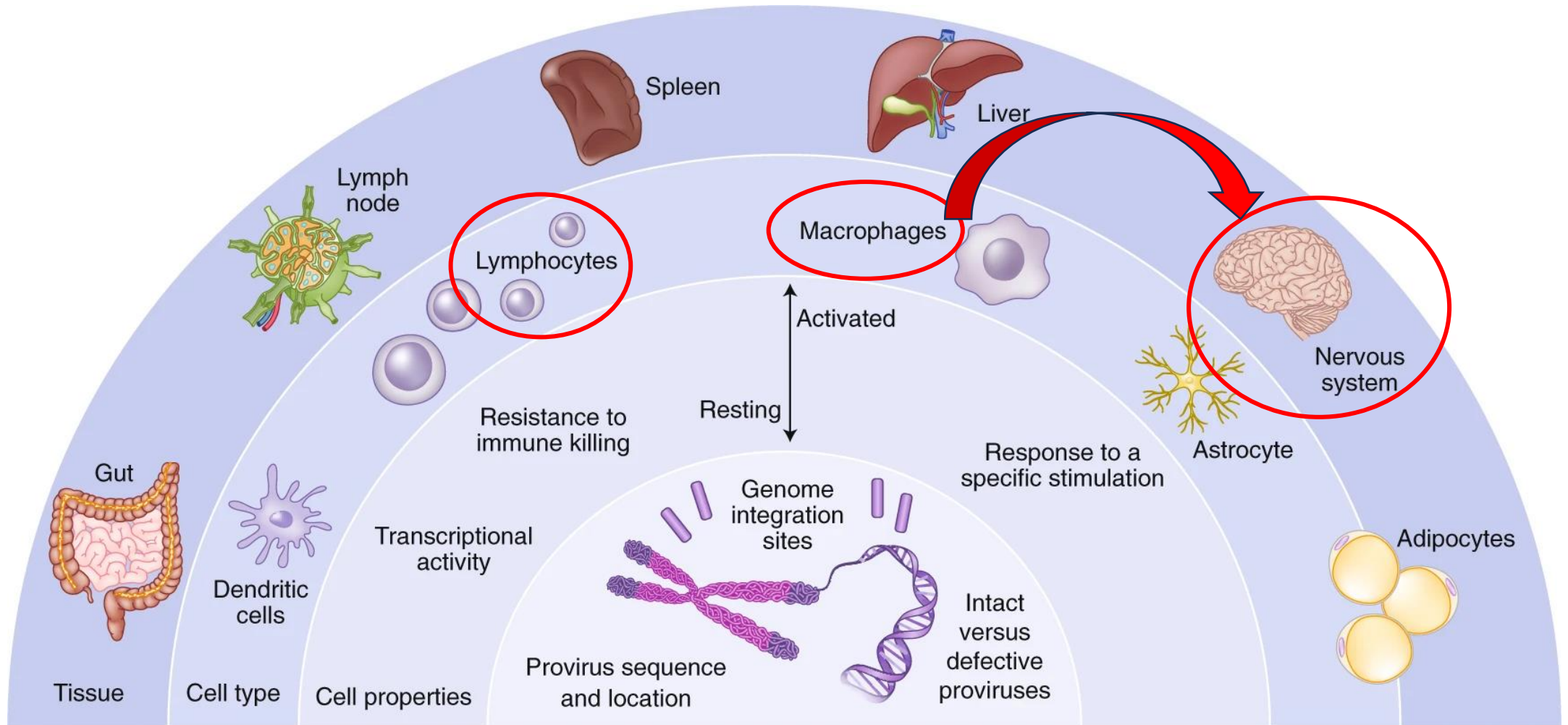
Innate Immunity Prevents
Inter-Species Transmission
of Pathogens



Innate Immunity Is Our First Line of Defense from Infections



Landscape of HIV Reservoir(s)



Major Skepticisms on Whether Macrophages Contribute To “The HIV Reservoir” in Pts. Under cART. **Points & Counterpoints**

- ❖ Infected “Resting Memory” CD4 T cells are long-lived due to homeostatic proliferation

- ❖ Mø can phagocytose infected CD4+ T cells → “false positive” signal

- ❖ “Contaminant” T cells may explain the positive HIV signal attributed to tissue Mø.

- ❖ Different robust *ex vivo/in vitro* models of reversible HIV latency in primary CD4 T cells.

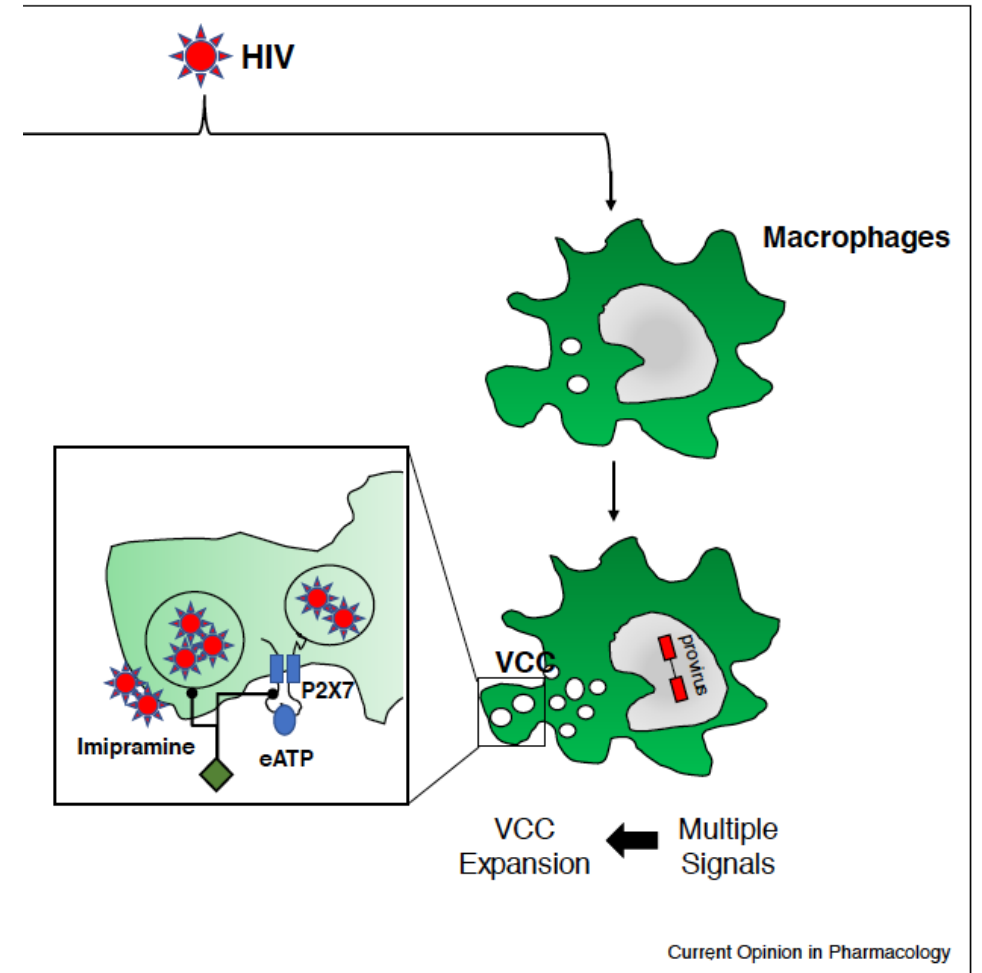
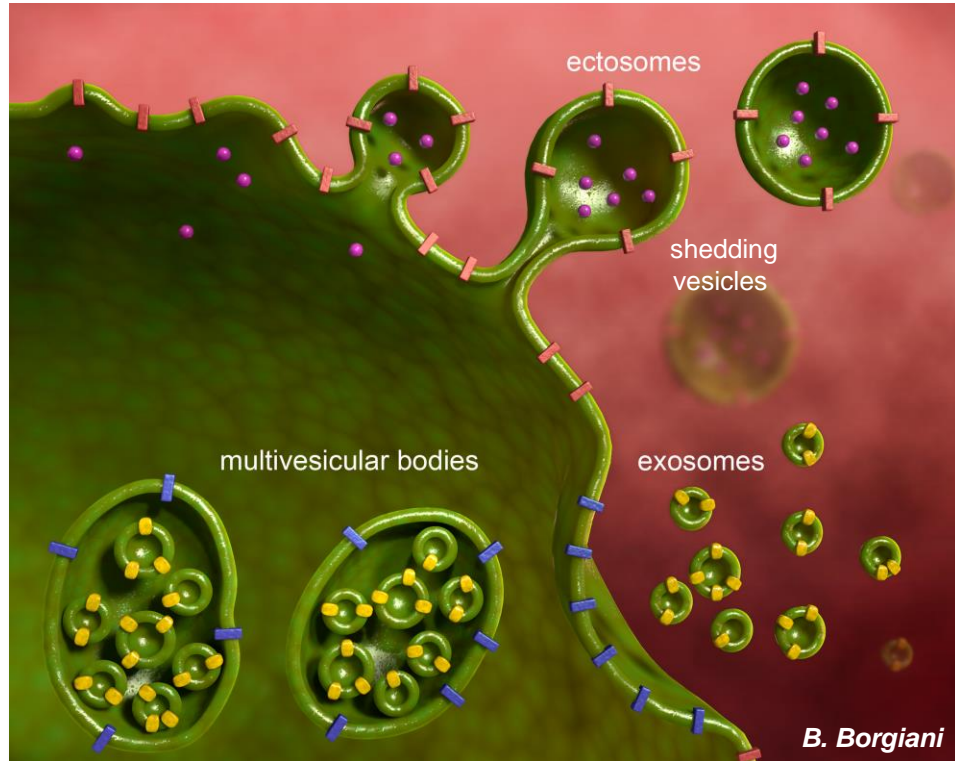
- ❖ BM-independent tissue-resident Mø are long-lived due to homeostatic proliferation.

- ❖ The fate of virus derived from phagocytosed T cells is undefined (*degradation? infection?*).

- ❖ Studies in NHP and humanized mice have demonstrated infection of tissue Mø in the absence of “contaminant” T cells.

- ❖ Lack of robust *ex vivo/in vitro* models of reversible HIV latency in primary Mø.

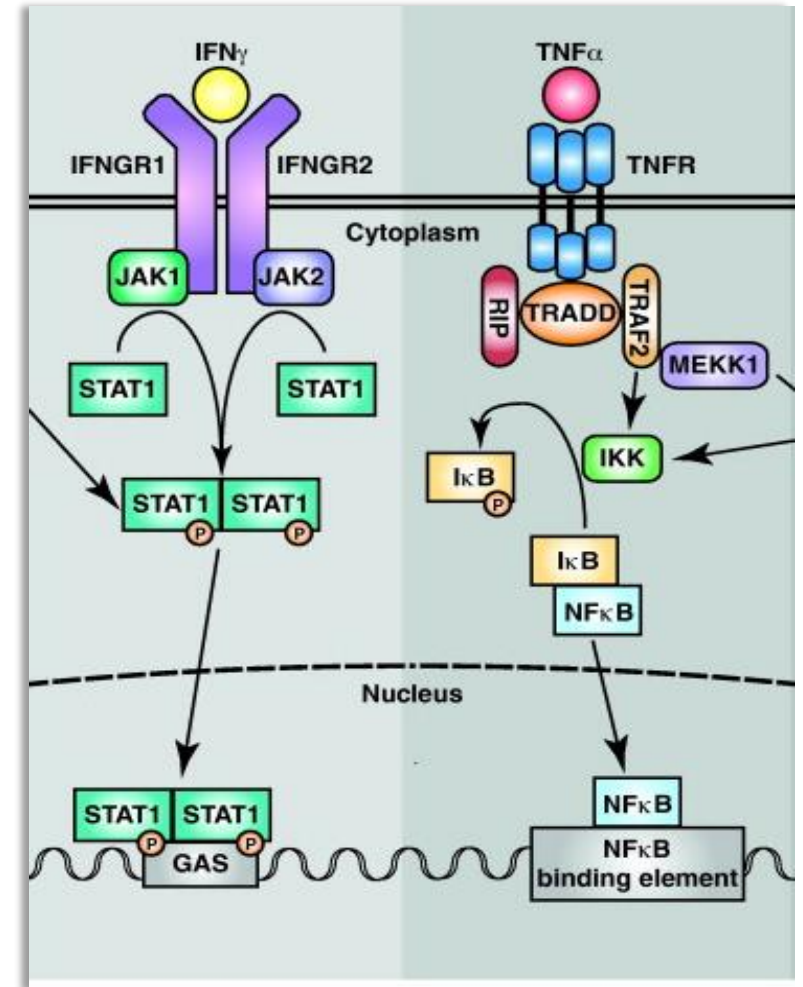
Extracellular ATP Induces the Release of VCC-Associated HIV Virions



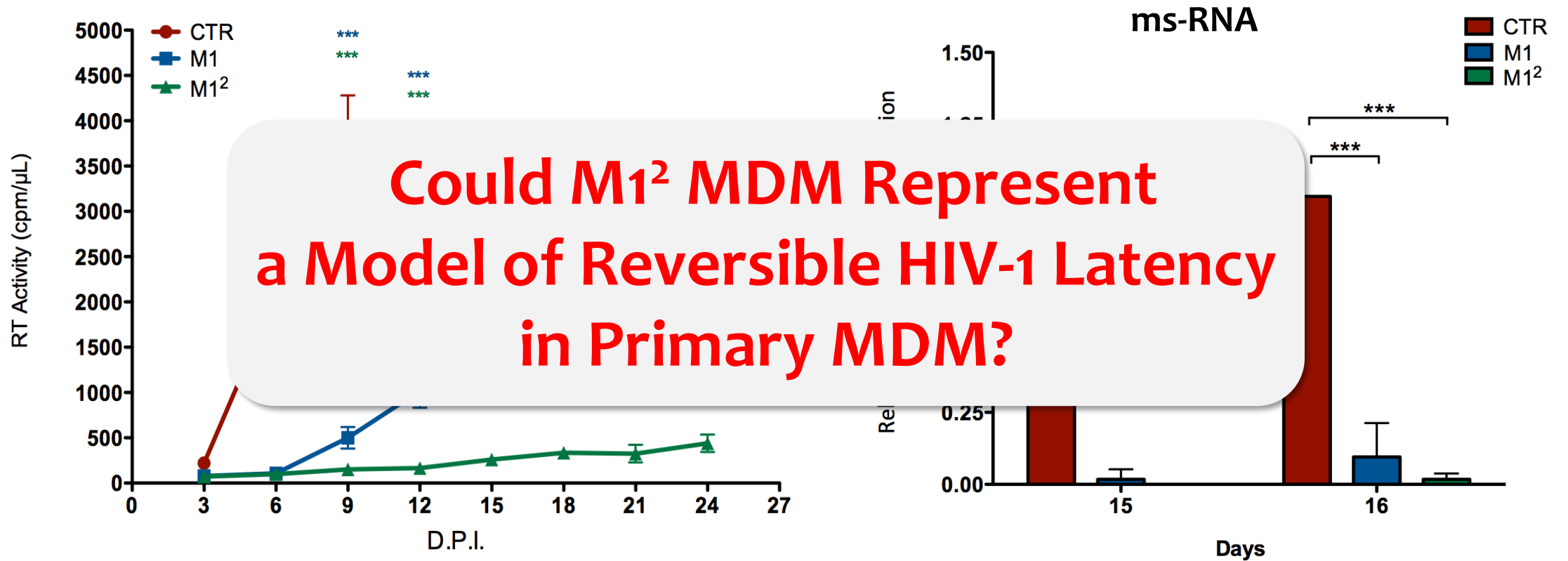
After: F. Graziano et al., PNAS, 112:E3265-73, 2015

A Paradox in **M1**-Restriction of HIV-1 Replication in Primary Human MDM

- ❖ M1 restriction of HIV replication in MDM is induced by **IFN- γ** +**TNF- α** , two pro-inflammatory cytokines known to trigger or potentiate proviral transcription via activation of **STAT1** and **NF- κ B**, respectively.
- ❖ Thus, would *restimulation* of already infected M1-MDM with **IFN- γ** +**TNF- α** promote HIV-1 expression?



M1² MDM Are Characterized by a Silent HIV-1 Transcriptional Profile



Selective HIV Reactivation in **M1²** MDM by PHA Blast Coculture

- Co-culture with allogenic PHA blasts induces a robust reactivation of virus production *exclusively* in M1²-MDM

