Silencing the HIV reservoir

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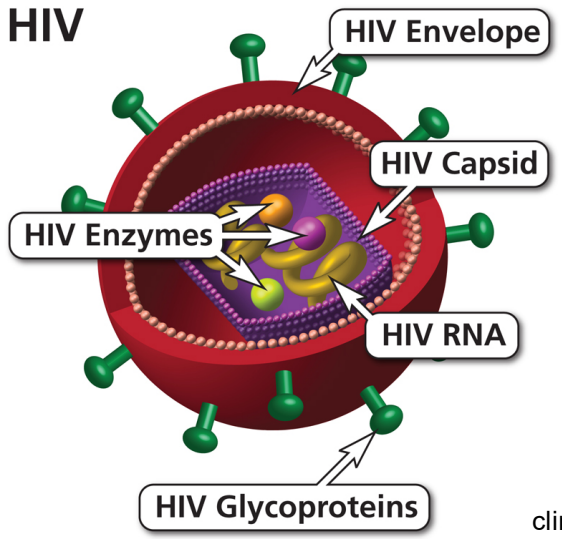
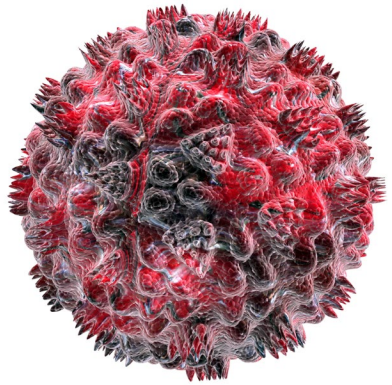
Science Changing Life

Human immunodeficiency virus (HIV)



clinicalinfo.hiv.gov

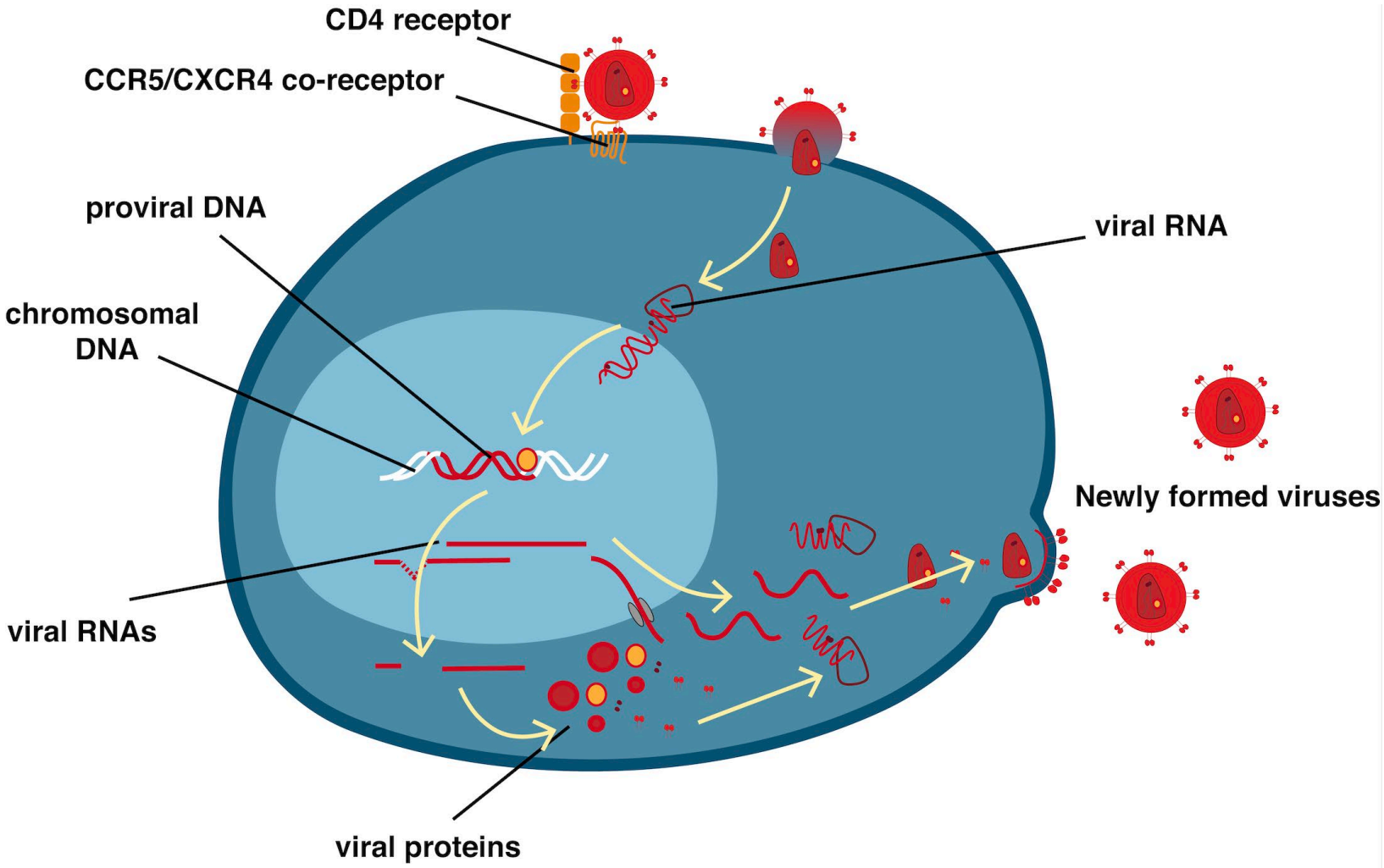


thenativeantigencompany.com

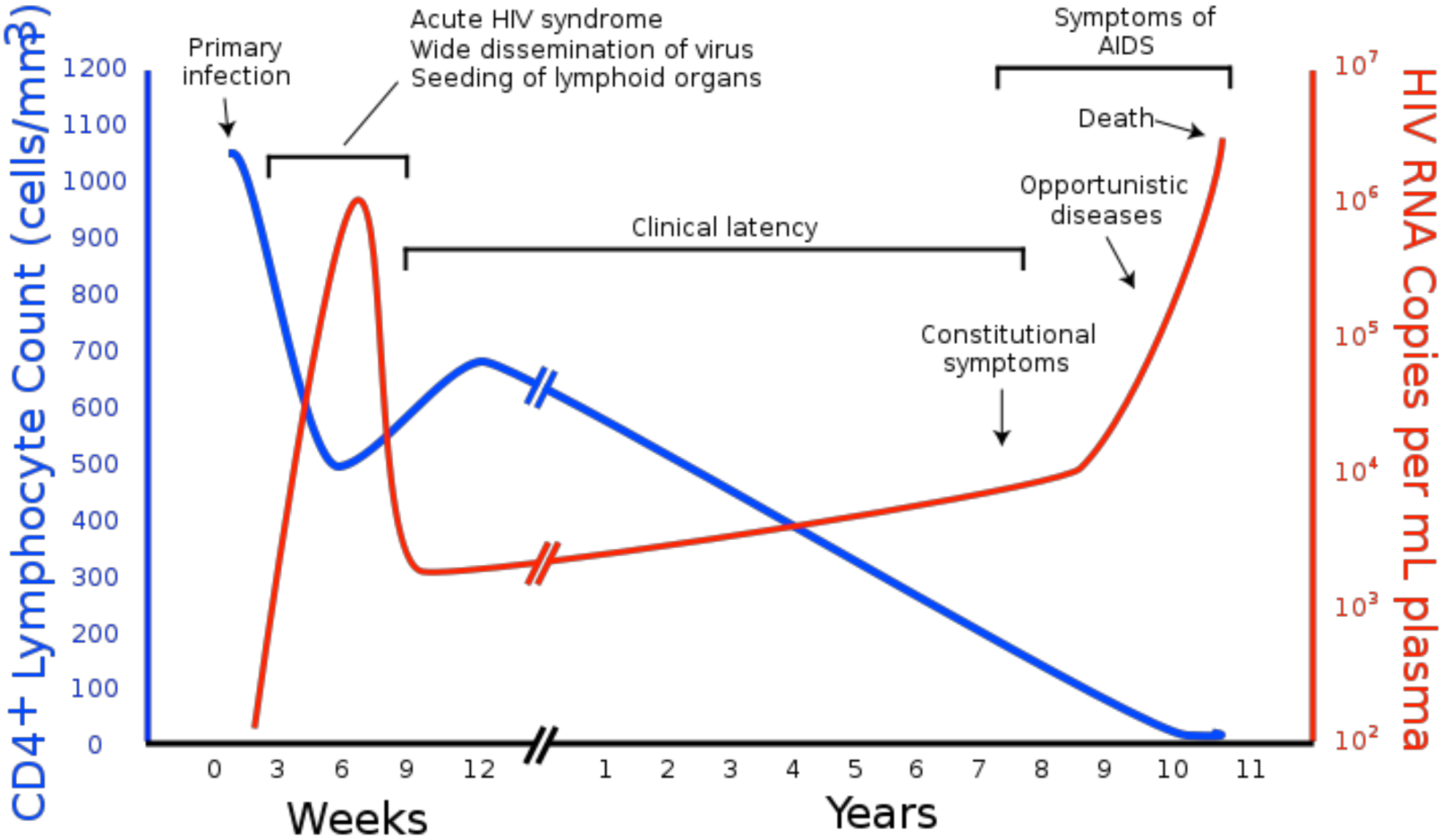
- **HIV is a retrovirus from the genus lentivirus, which literally means “slow virus”, because they take such long time to produce adverse effects in the body.**
- **HIV was estimated to have been introduced in the human population in the late 1940s or early 1950s from the chimpanzee version of HIV called SIV, as a result of African hunters butchering and consuming ape meat**

HIV life cycle

HIV infects immune cell mostly “helper” CD4⁺ T cells and establishes a life-long infection.



Course of untreated HIV infection

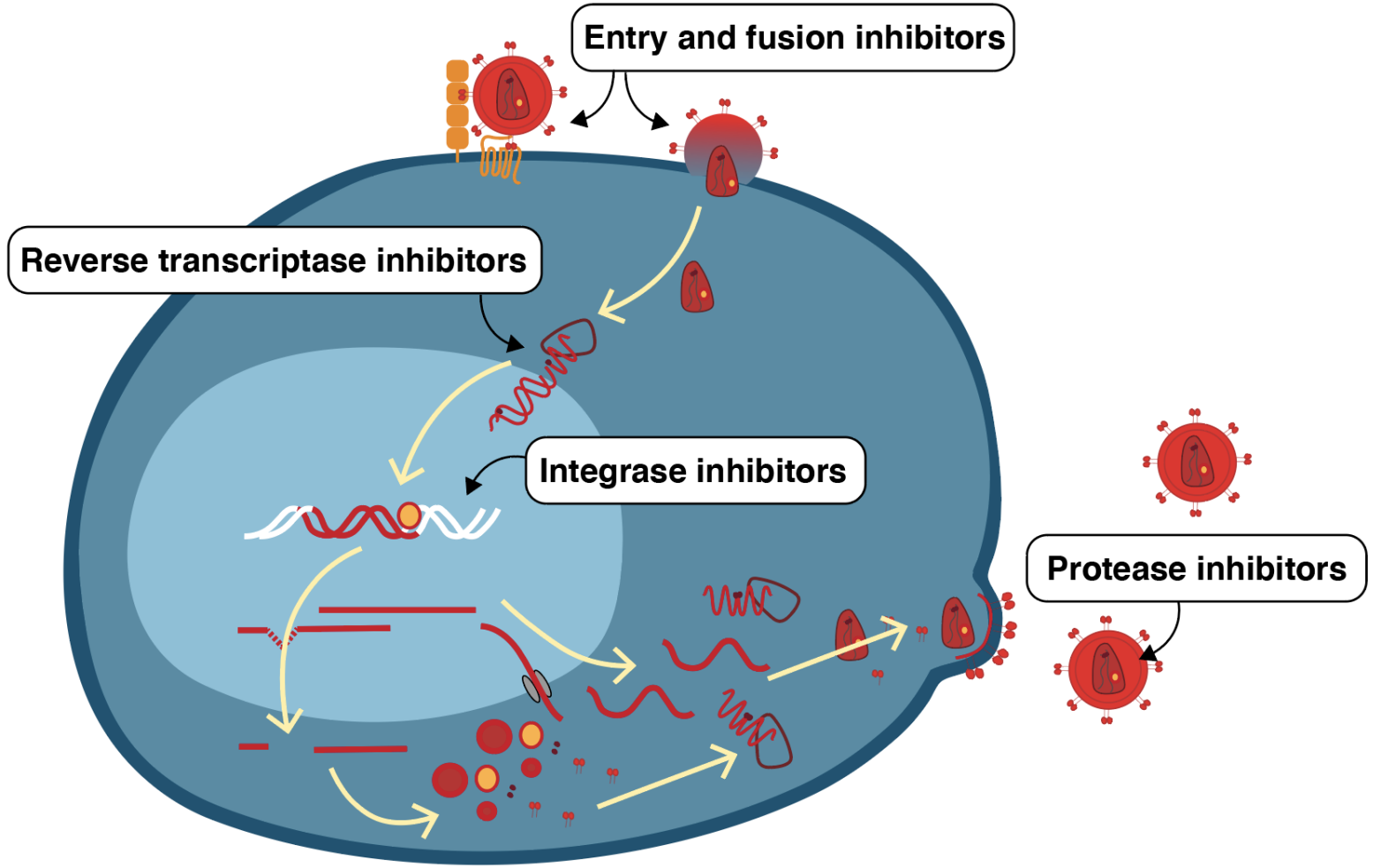


ART - Antiretroviral Therapy

- Introduced in 1996, formalized by FDA in 2001
- Combination of several antiretroviral drugs targeting at least 2 different steps of virus life cycle
- First treatment given to patients, should keep viral load at < 50 copies/ml
- If first ART fails, subsequent treatments are much less likely to succeed (mutants accumulate).

- Without treatment, about 9 out of every 10 people with HIV will progress to AIDS after 10-15 years. Many progress much sooner.
- After HIV has progressed to diagnosable AIDS, the average survival time with antiretroviral therapy is estimated to be > 10 years
- Without antiretroviral therapy, death normally occurs within 2 years.

Targets of current antiretroviral therapy



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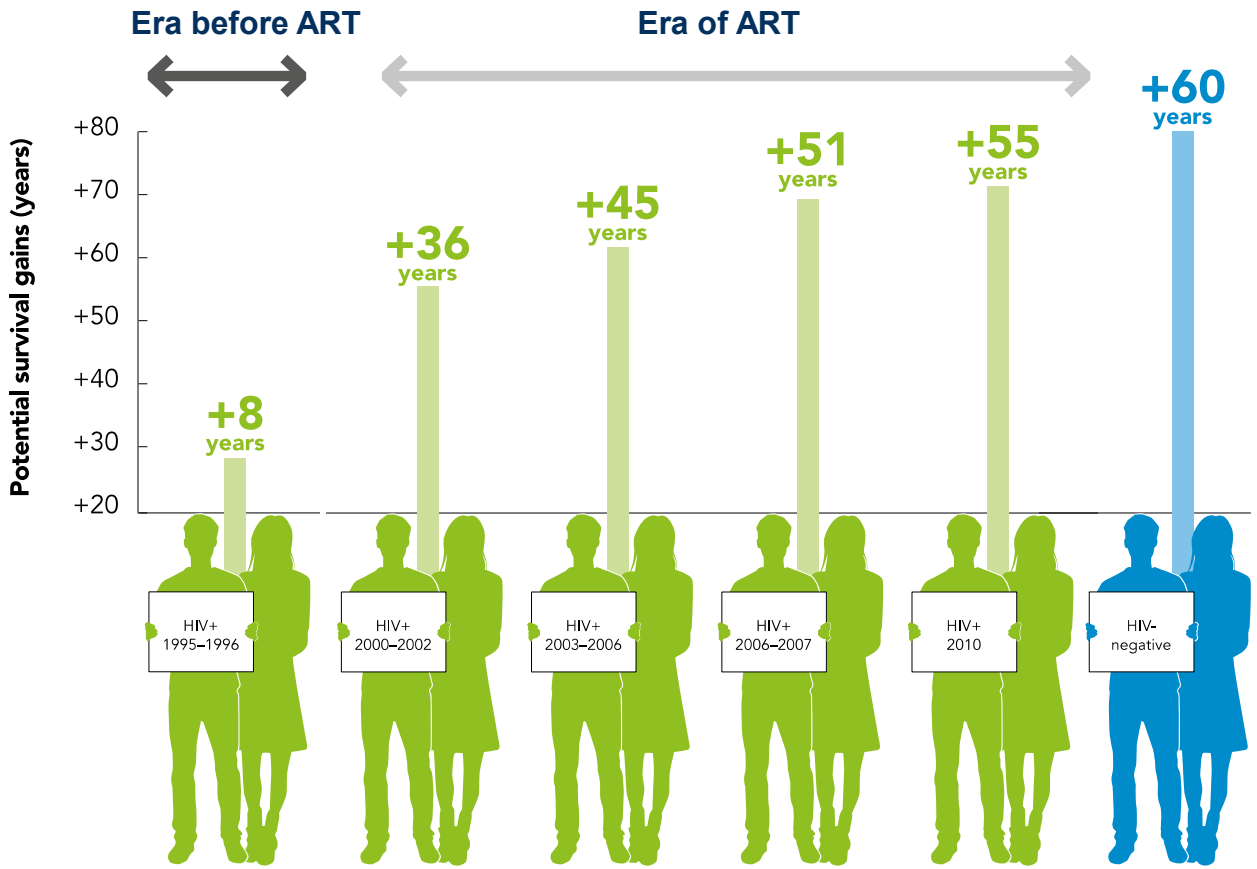
The success of antiretroviral therapy (ART)

Expected survival of a 20-year-old person living with HIV in a high income country



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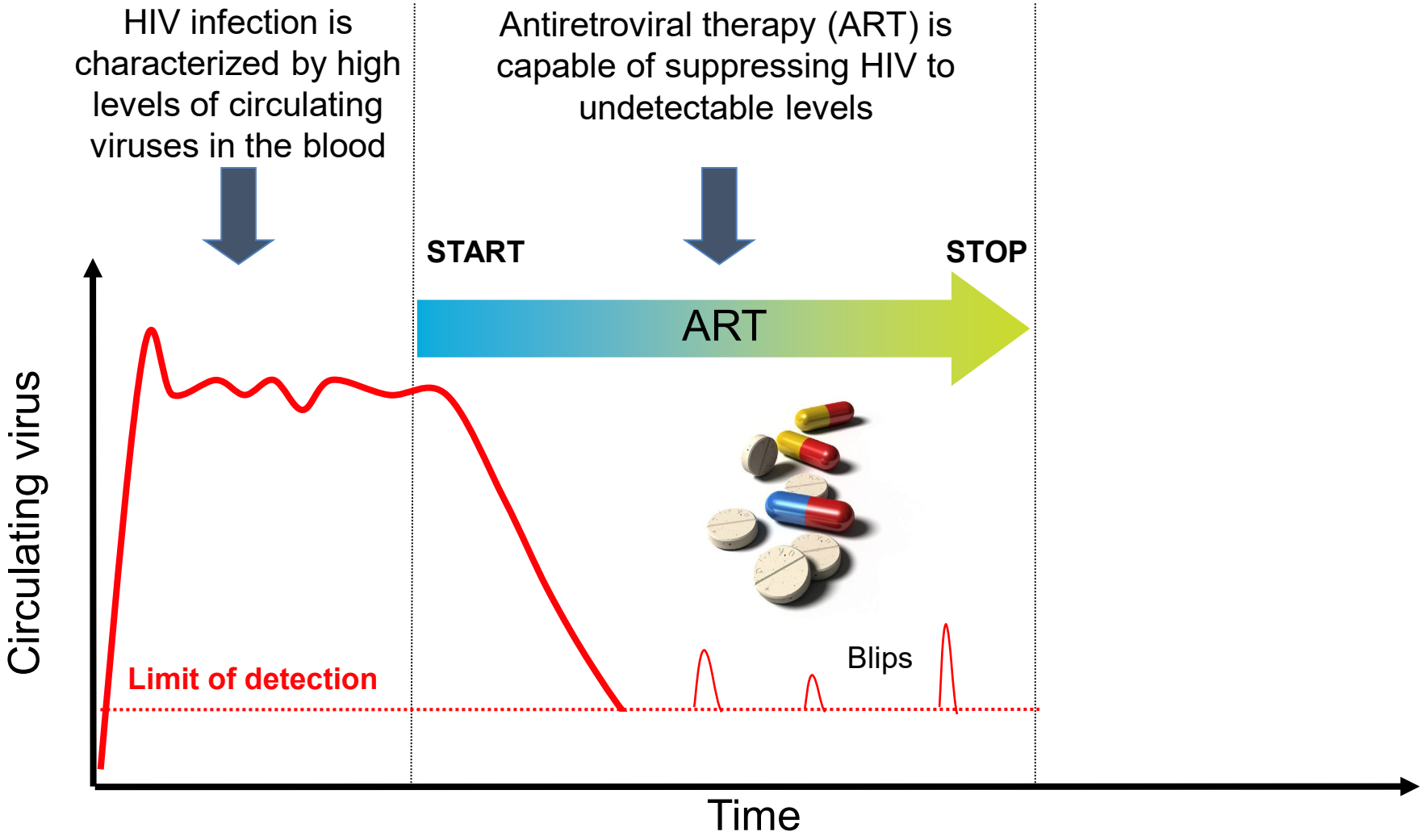
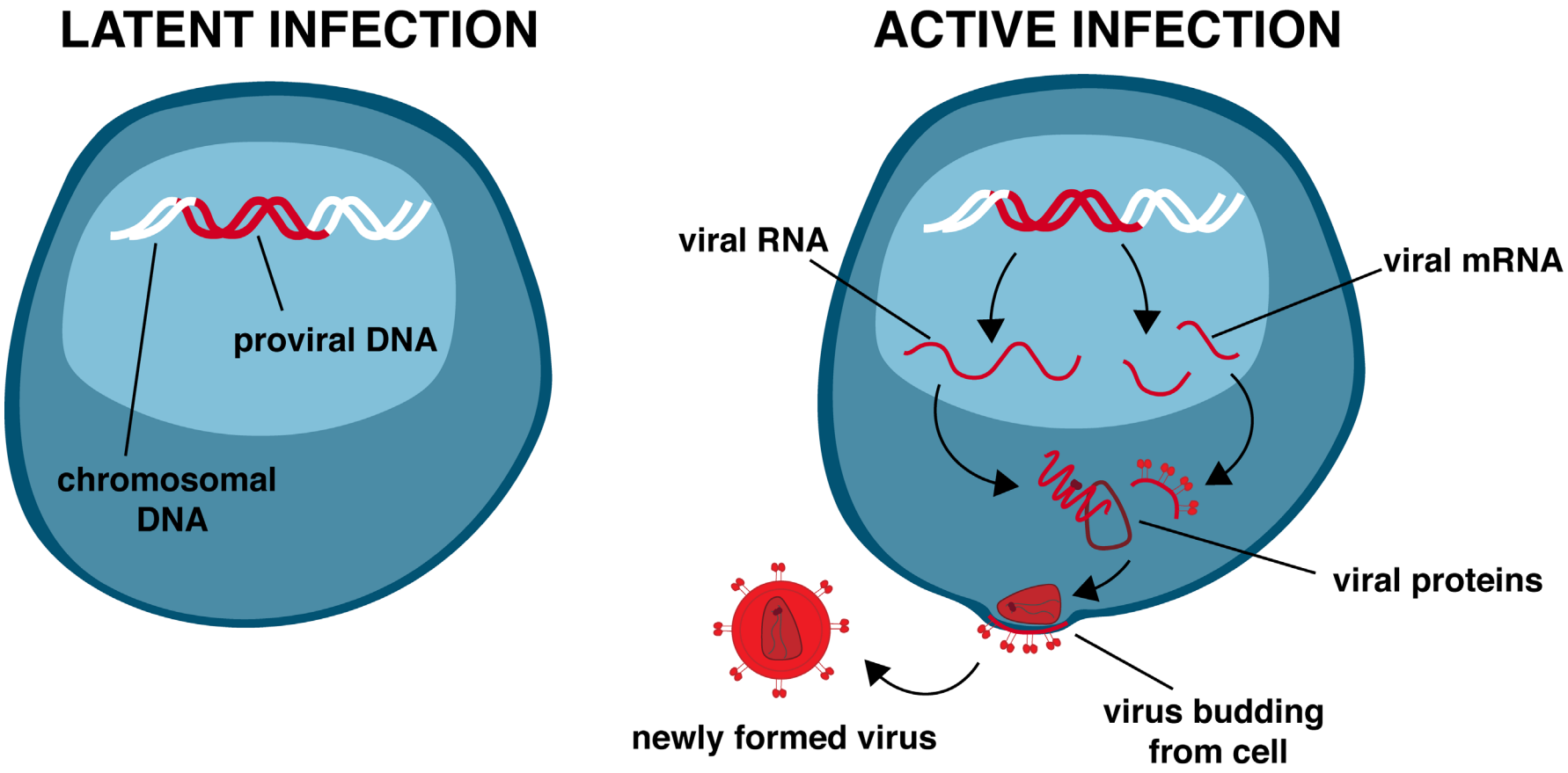
Source: UNAIDS, gap report. Adapted from Lohse et al, 2007; Hoog et al. 2008; May et al, 2011; Hogg et al. 2013

Current anti-HIV drugs do not eradicate HIV



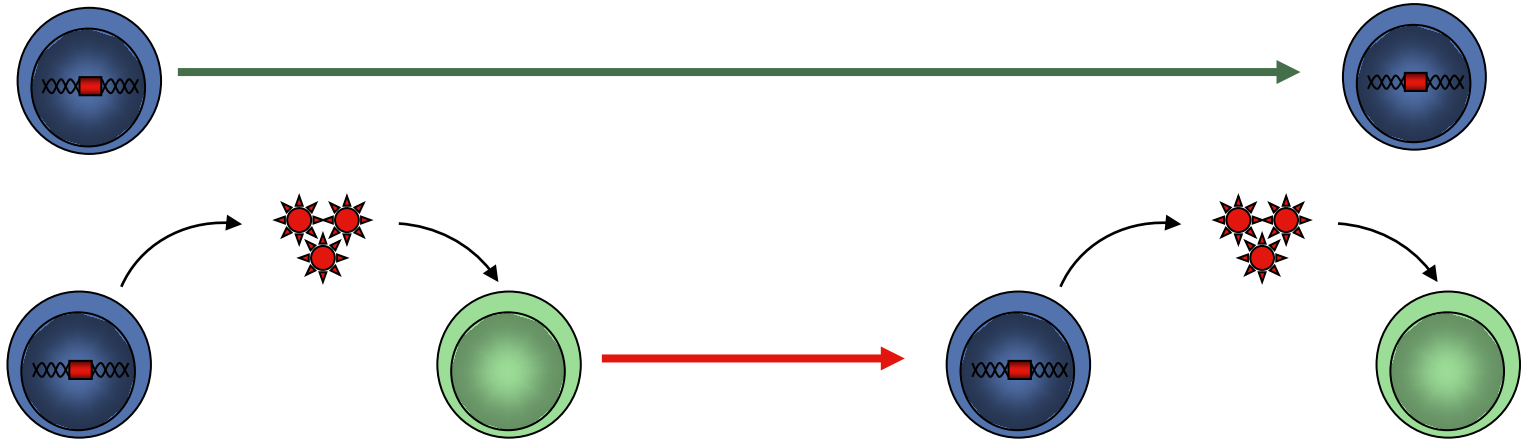
Comparison of latent and active infection

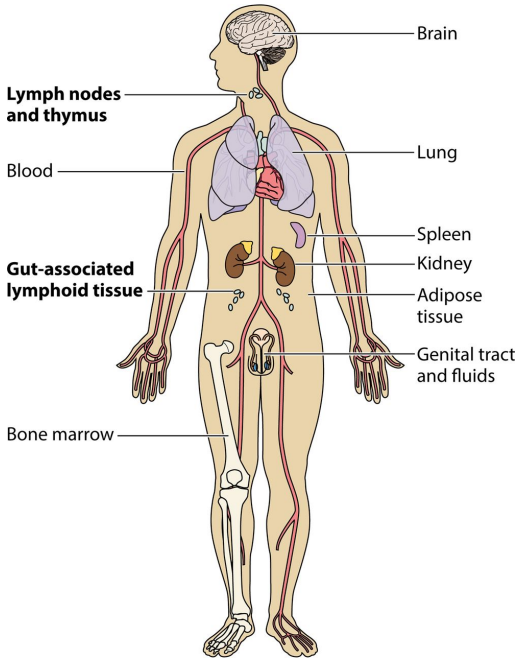


Mechanisms and sites of HIV-1 persistence

Persistence of latently infected cells

Ongoing viral replication at low levels





Sites of HIV persistance:

- Brain
- Lymph nodes
- Peripheral blood
- Gut
- Bone marrow
- Genital tract

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Types of HIV Cures

Functional cure or Remission/control

Virus remains but doesn't rebound after antiviral cocktails are removed

- **Visconti subjects**
- **French teenager**

Sterilizing cure

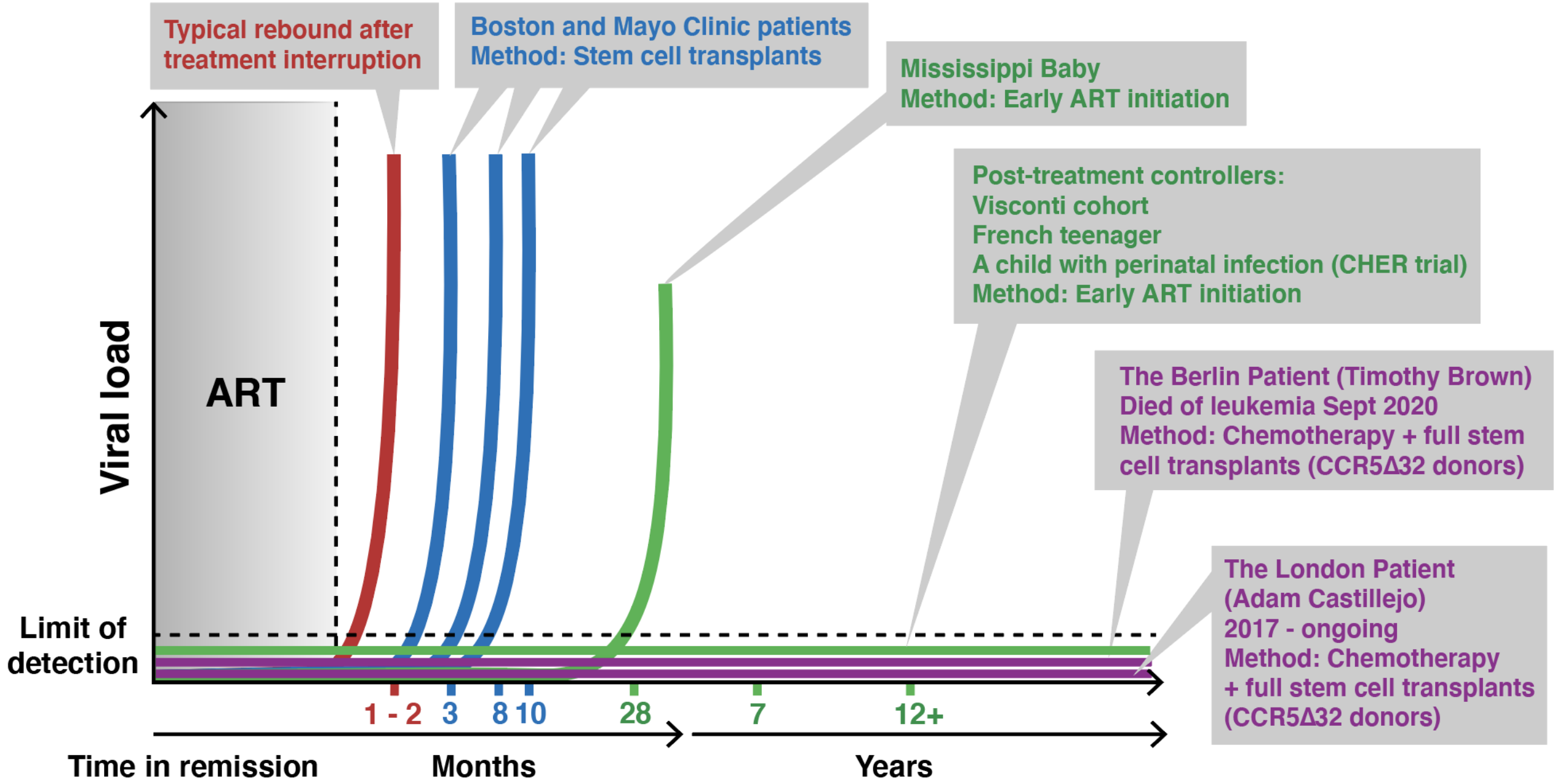
All virus has been eliminated from the body

- **Timothy Brown (2007)**
- **London patient (2017)**

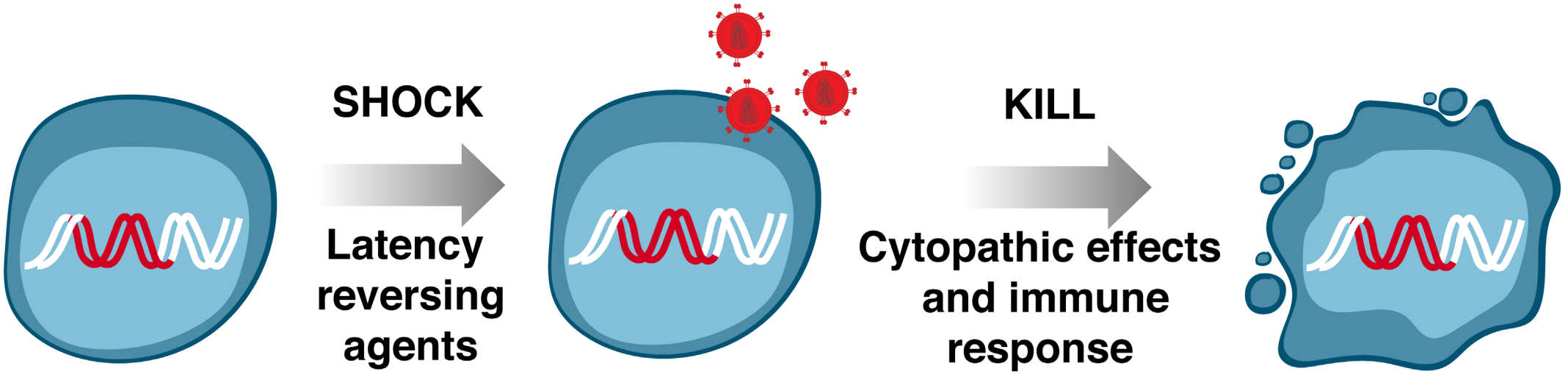
Living with HIV
37.9 million

HIV remission
~ 100+ cases, early treated
(0.0000002%)

HIV eradication
2 cases, the Berlin/London pt
(0.000000002%)


Typical rebound after treatment interruption
Boston and Mayo Clinic patients
Method: Stem cell transplants
Mississippi Baby
Method: Early ART initiation
Post-treatment controllers:
Visconti cohort
French teenager
A child with perinatal infection (CHER trial)
Method: Early ART initiation
The Berlin Patient (Timothy Brown)
Died of leukemia Sept 2020
Method: Chemotherapy + full stem cell transplants (CCR5Δ32 donors)
The London Patient
(Adam Castillejo)
2017 - ongoing
Method: Chemotherapy
+ full stem cell transplants
(CCR5Δ32 donors)
Viral load
ART
Limit of detection
1 - 2
3
8
10
28
7
12+
Time in remission
Months
Years

“Shock-and-Kill” approach to reservoir eradication

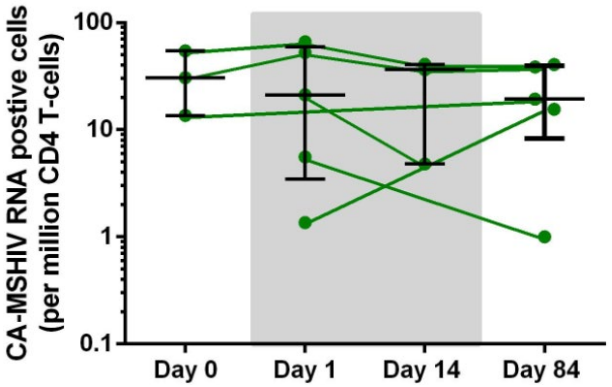


Disrupting latency *in vivo*

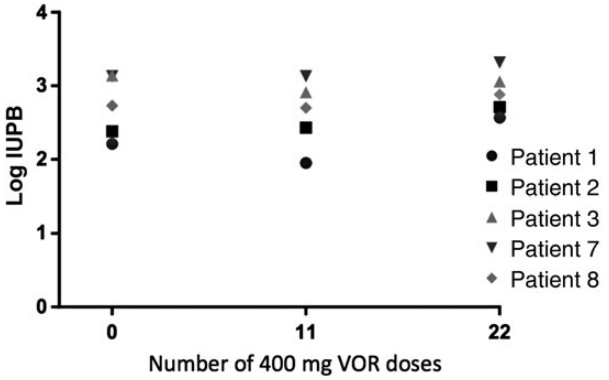
Vorinostat (single dose)

"no […] substantial reduction in the frequency of replication-competent HIV within resting CD4⁺ T cells"

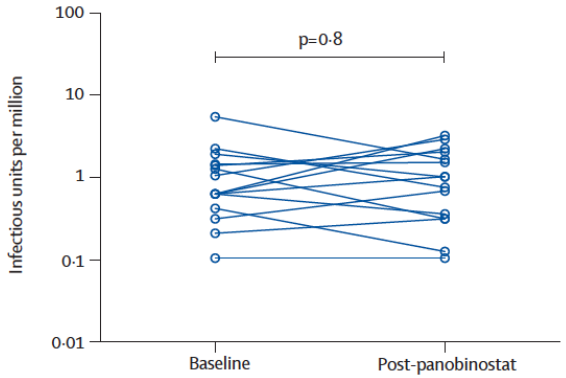
Vorinostat (multiple doses)



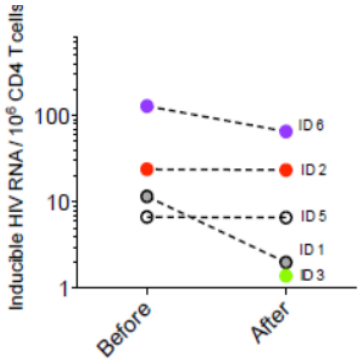
Vorinostat (multiple doses)



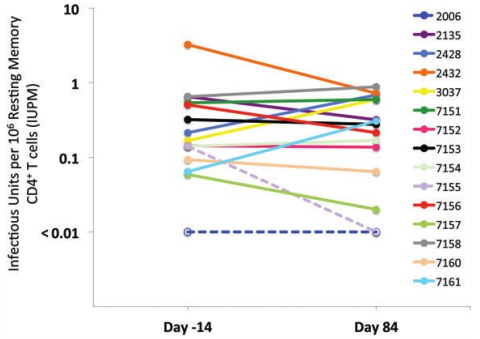
Panobinostat (multiple



Romidepsin (multiple doses)



Disulfiram (multiple doses)



› **… but do not significantly reduce the size of the latent reservoir**

N. Archin et al. Nature 2012; J. Elliott et al. Plos Path 2014; N. Archin et al. JID 2014; T. Rasmussen et al. Lancet HIV 2014; O. Søgaard et al. Plos Pathogens 2014; A. Spivak et al. CID 2014

Hurdles to an effective Shock-and-Kill

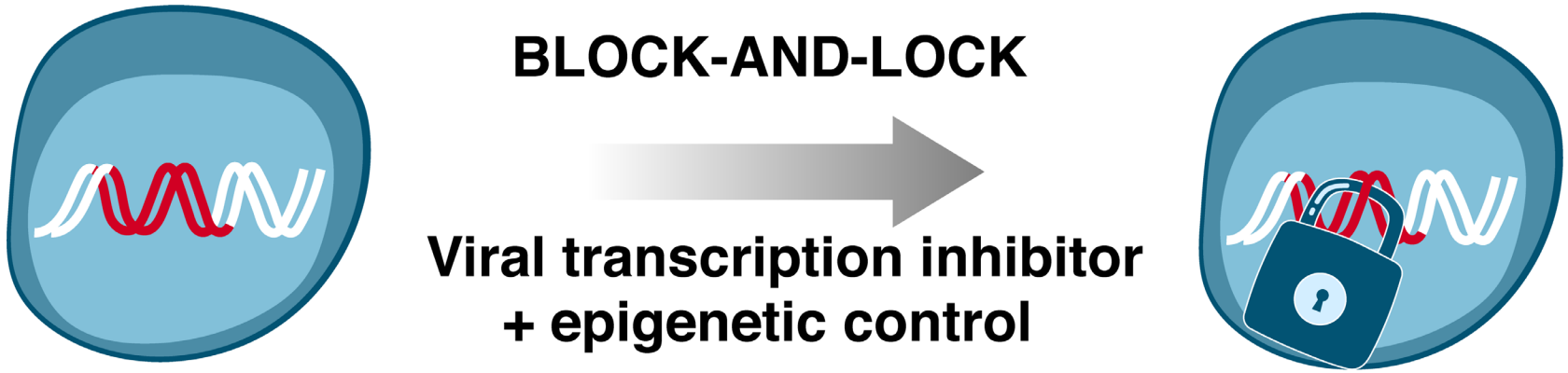
- Levels of reactivation needed to trigger kill not achieved
- Risky! Non-specificity of the Latency reversing agents…cancers?
- HIV-1 latency is an heterogeneous process…how many shocks will it take?
- Suboptimal tissue ART concentrations may lead to reinfection!
- Risky! The brain reservoir has poor immune surveillance…no kill… neurocognitive disorders?

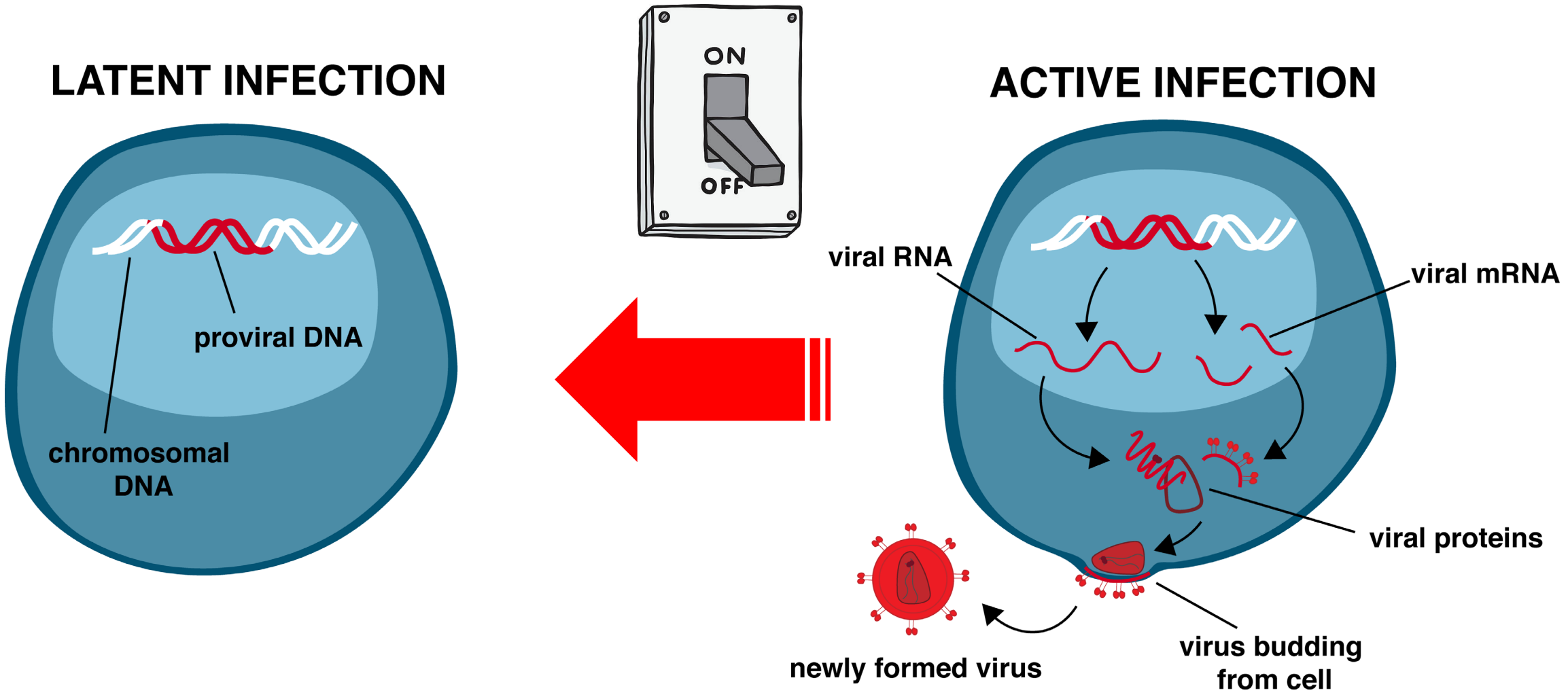
We need better strategies!

Strategies that keep the virus in hibernation need to be explored

→ **Functional cure**

The “Block-and-Lock” approach for a functional cure





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It is not such an odd idea…

In human cells the default state of gene expression is "off" rather than "on"

- Of the estimate ~ 20,000 genes in a cell, only ~ 8,000 are expressed.

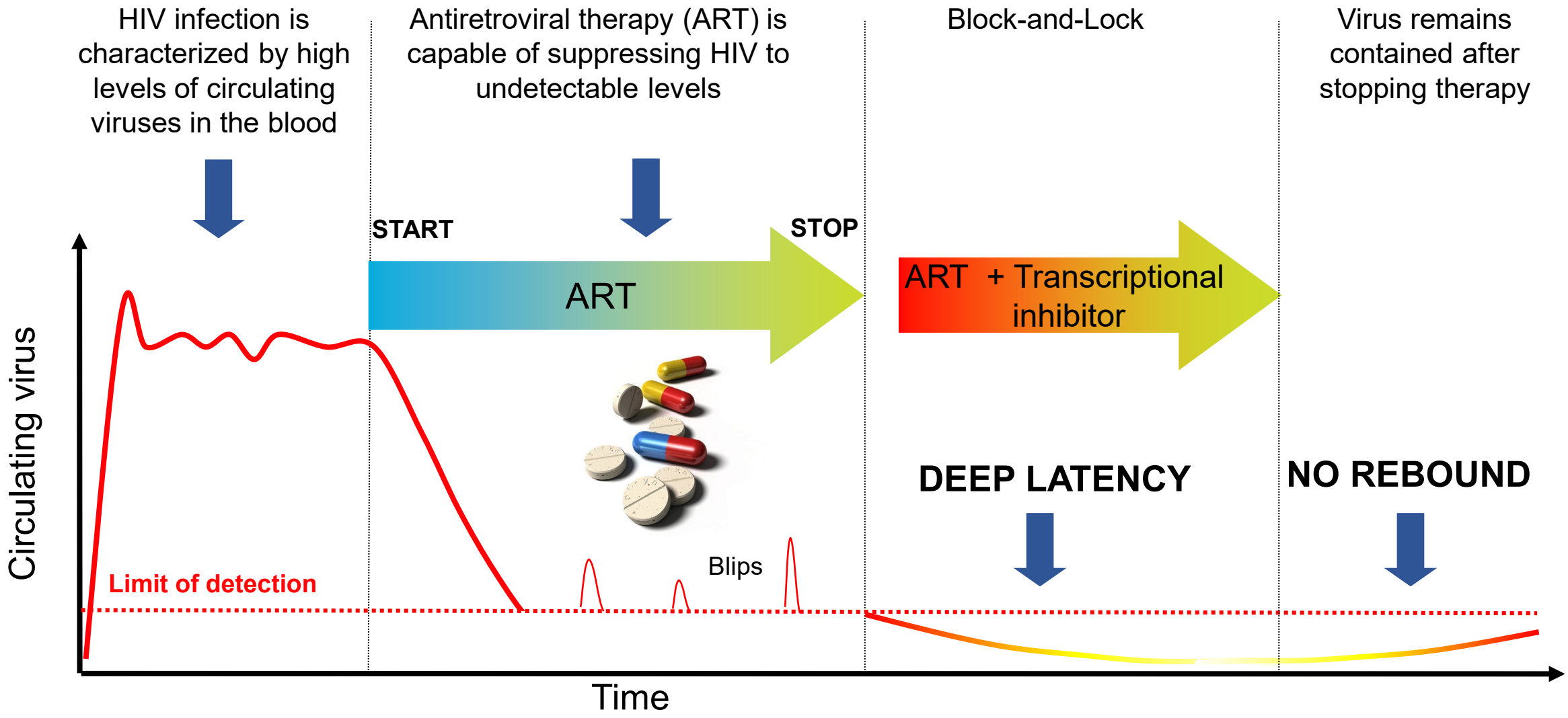
  For instance, only the pancreas produces insulin

- Why is this the case?

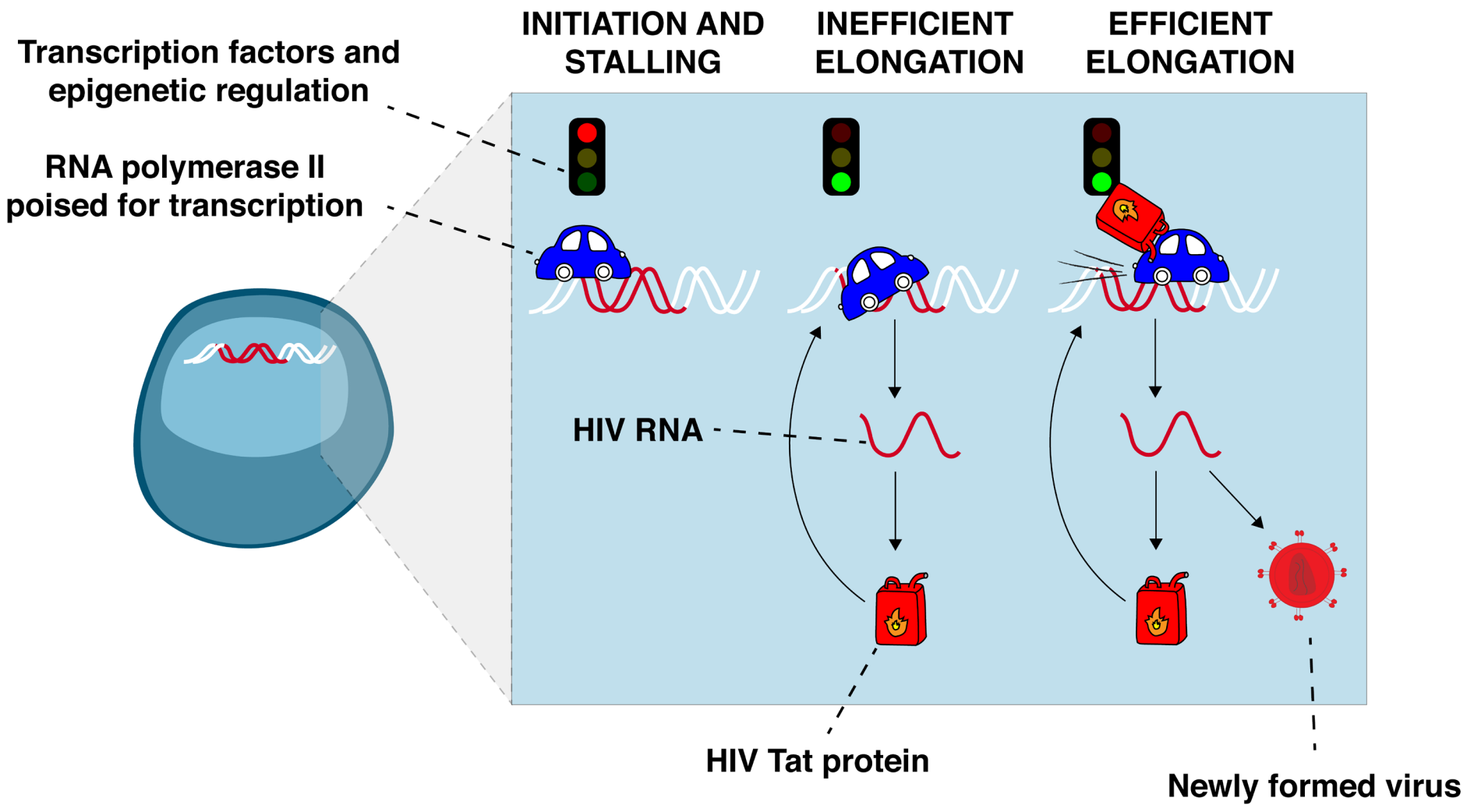
  The secret lies in chromatin which can repackage DNA in more open or close configurations

→ **Epigenetic control**

How would the “Block-and-Lock” approach work exactly?



How do we go about blocking HIV transcription?



Our current strategies aimed at blocking HIV-1 reactivation:

1- Inhibition of the viral Tat protein with the small molecule didehydro-Cortistatin A (dCA)

2 - Degradation of XPB protein with the FDA approved drug Spironolactone

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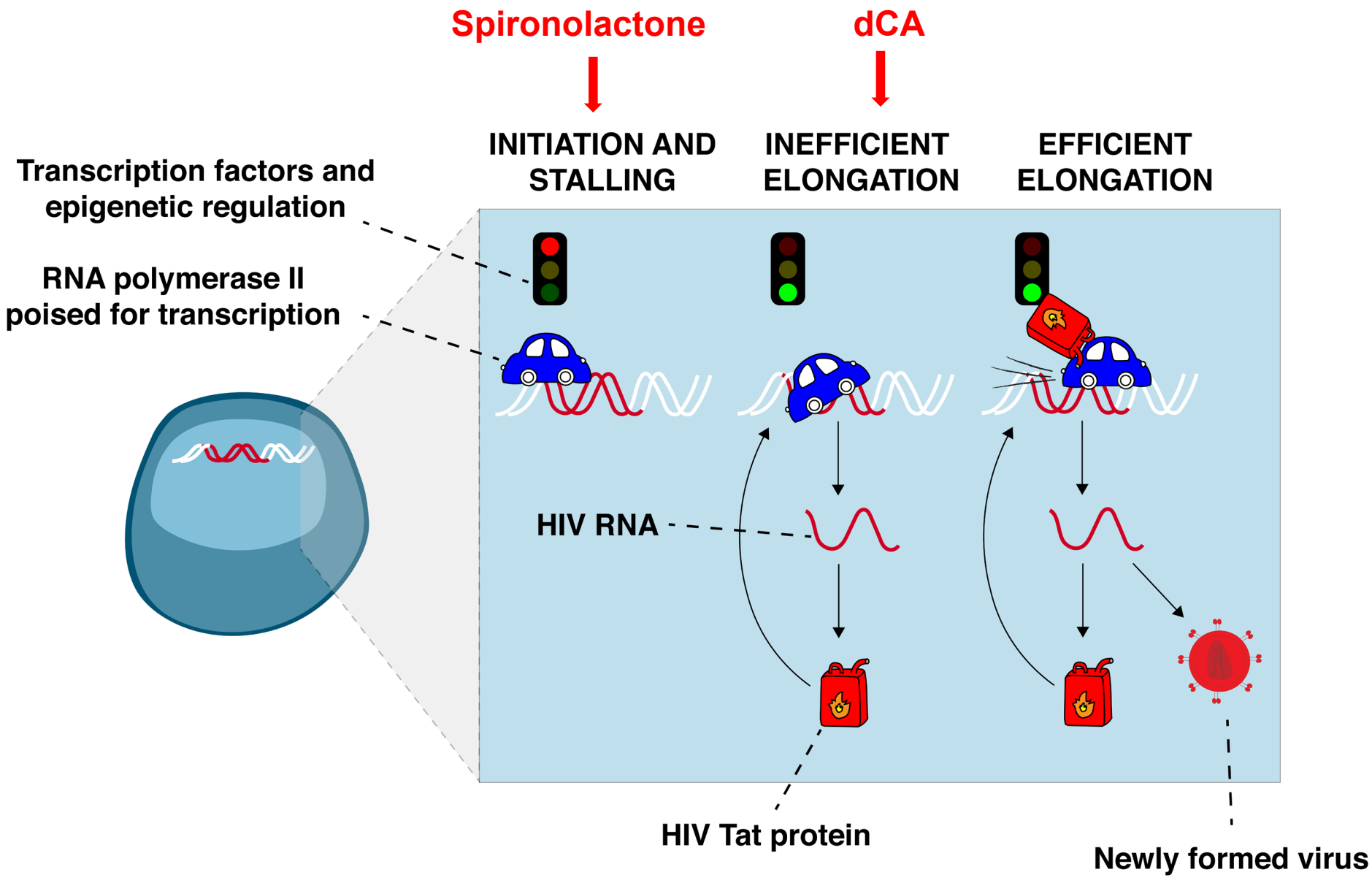
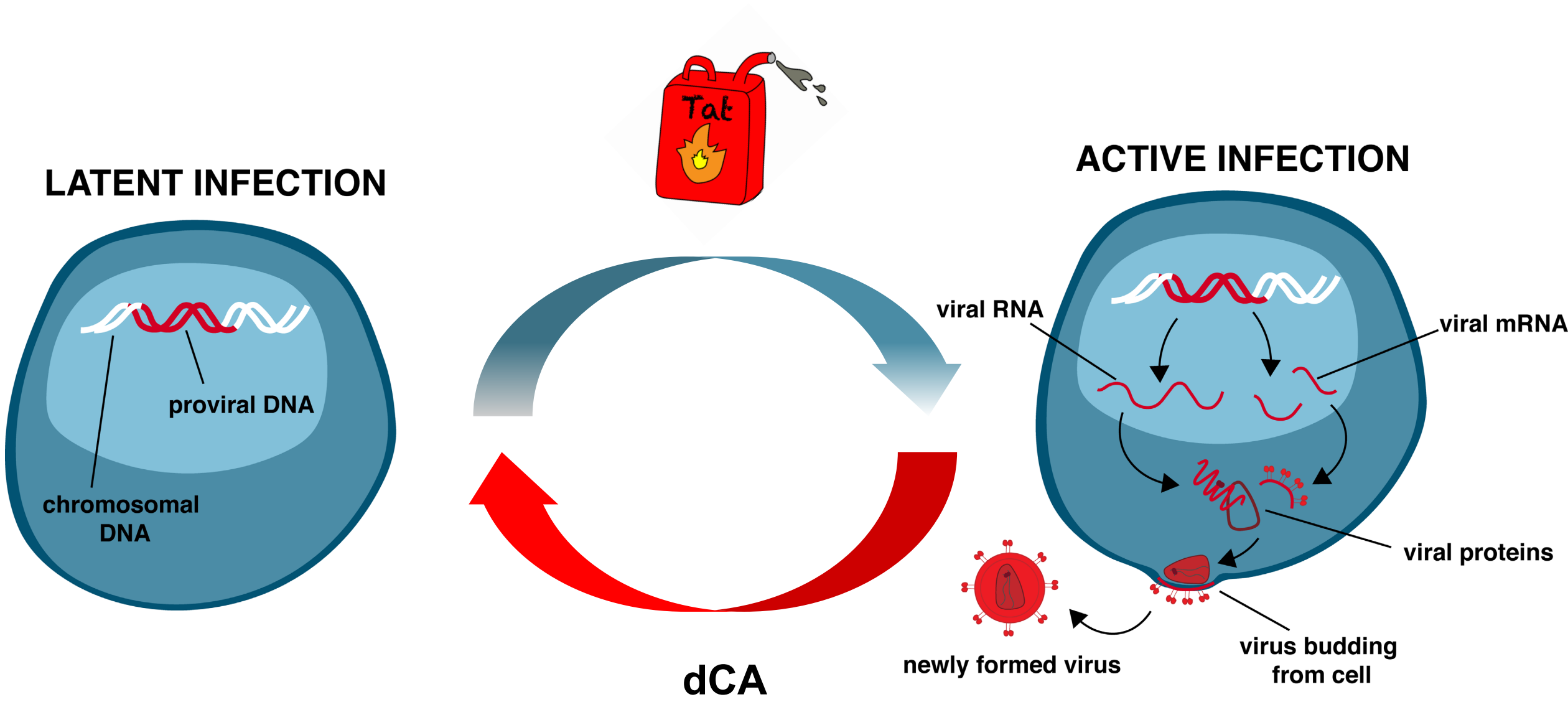
How do we go about blocking HIV transcription?





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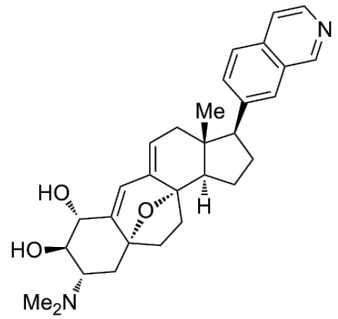
Cortistatin A

dCA is a very potent Tat inhibitor (EC_{50}= 1 nM).

- Steroidal alkaloid isolated from Southeast Asia *Corticium simplex* sponge.

Cortistatin A

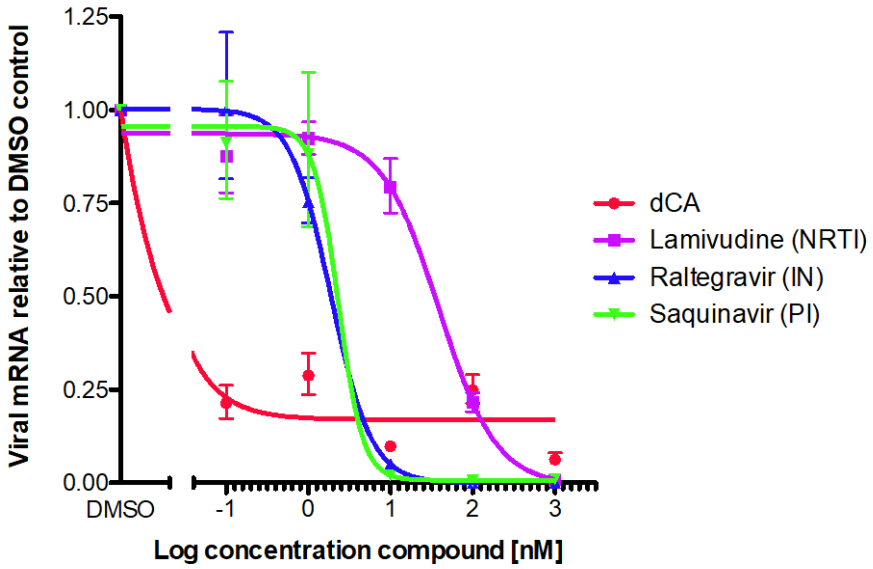


- Anti-tumor activity against in cells.
- Hard to isolate: a total of 1.5 Kg of dried sponge yielded 22 mg of Cortistatin A.
- The Baran lab in Scripps California showed how to synthesize grams of the functional analogue dCA from prednisone in 13 steps.

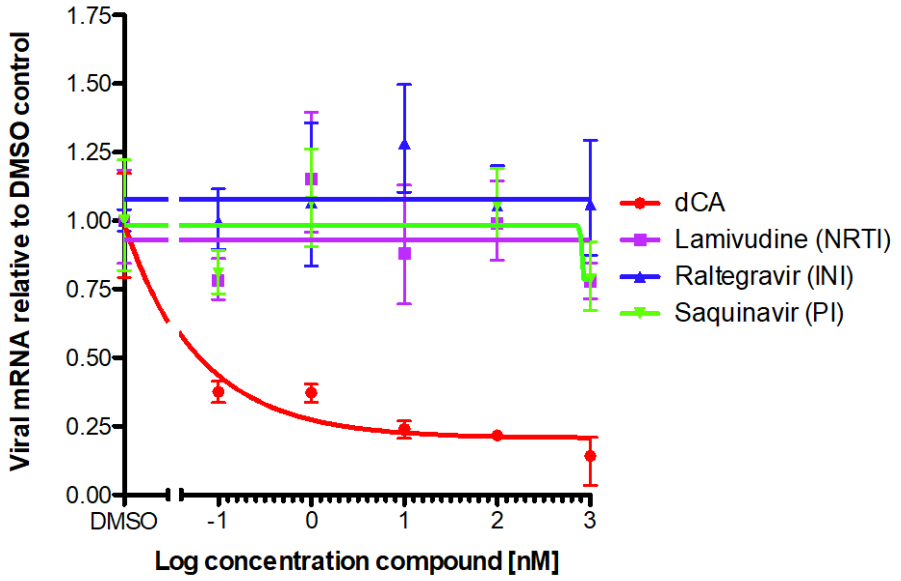
dCA block HIV-1 transcription

Acute infection HeLa-CD4

EC_{50} = 1 nM

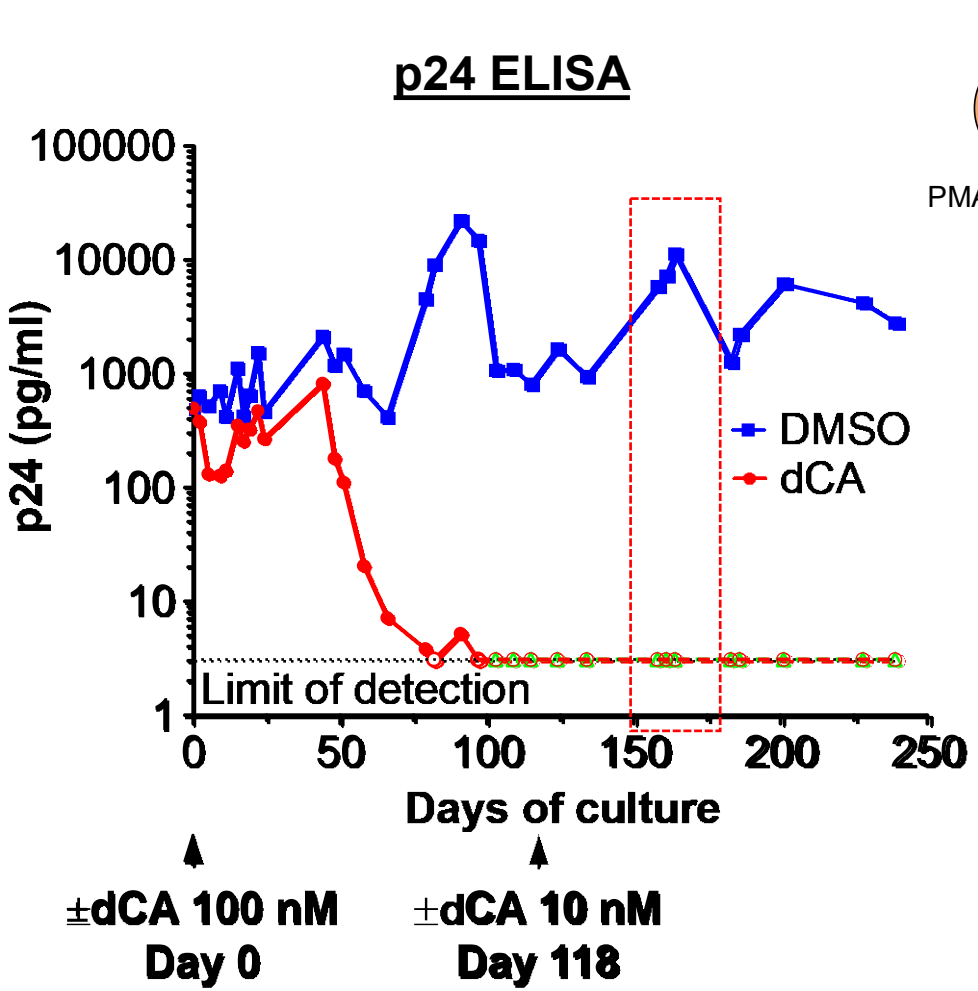


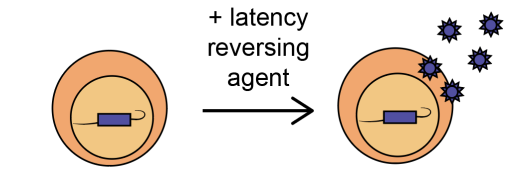
CEM-SS
Chronically infected pNL4-3



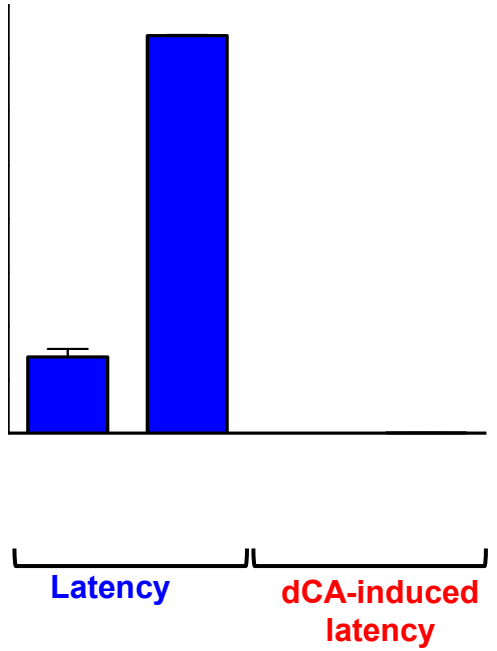
dCA inhibits HIV from infected cells - very different mechanism than clinically available drugs

dCA mediates a state of “deep-latency”





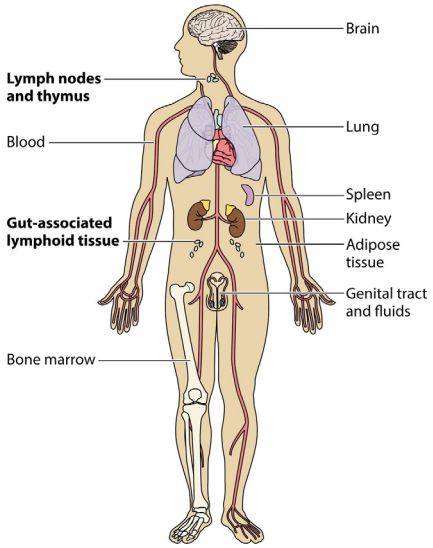
PMA + ionomycin = latency reversing agent



Mousseau et al., 2015, mBio

dCA lowers the viral expression below the limits of detection
No viral rebound upon treatment interruption or stimulation

Can dCA suppress viral reactivation in cells from infected subjects ?



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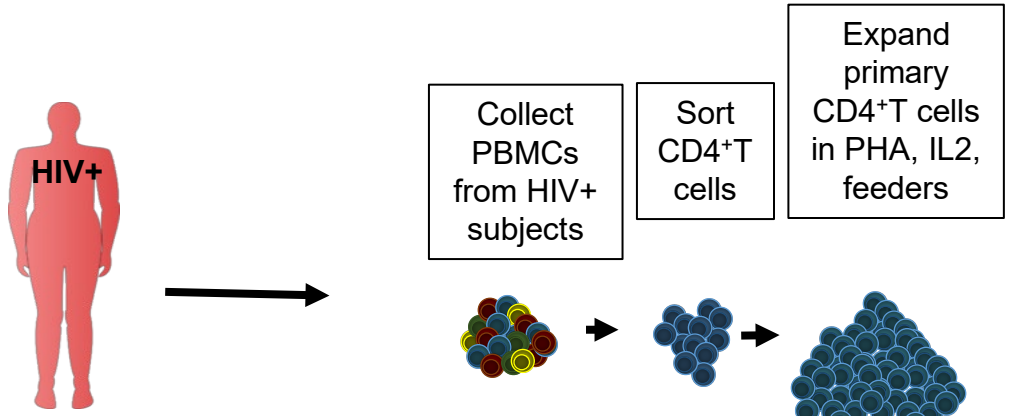
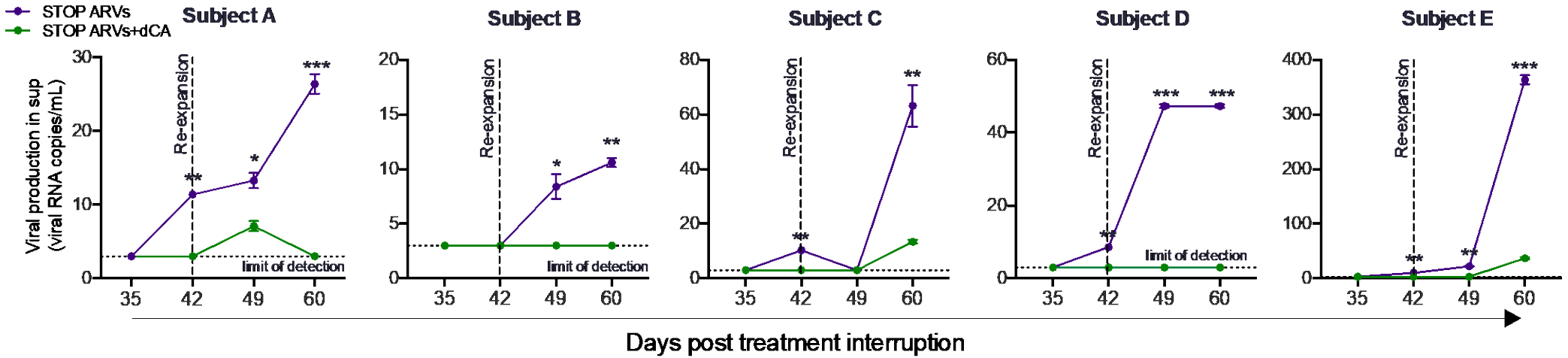
dCA blocks Viral rebound upon treatment interruption



Kessing et al., 2017, Cell Reports



- dCA drastically inhibits viral rebound up to 25 days post treatment interruption, even during strong cellular activation.
- dCA contributes to long lasting epigenetic repression of the HIV promoter.

dCA efficacy in BLT humanized mice?

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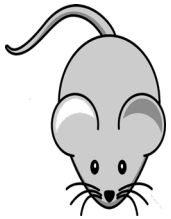
dCA reduces viral mRNA in BLT humanized mouse models

dCA was added to ART for a period of 14 days

BLT

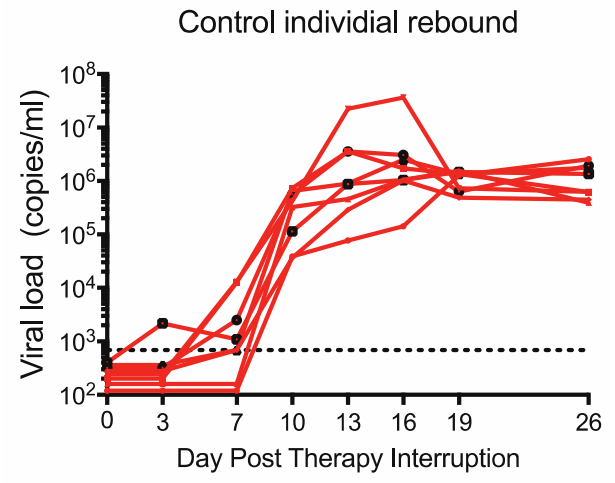


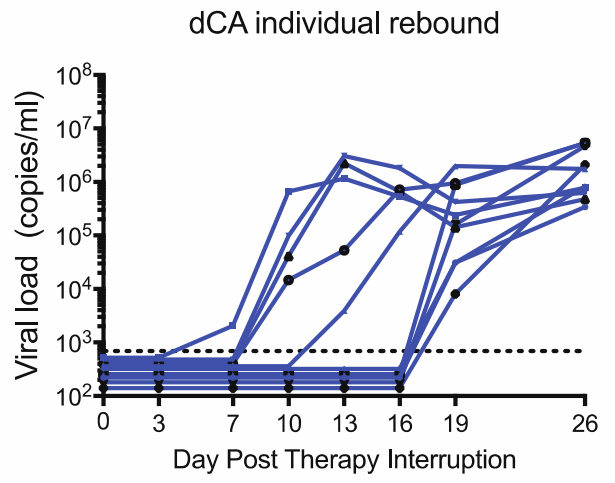
NOD/SCID,NSG,
NOG,TKO

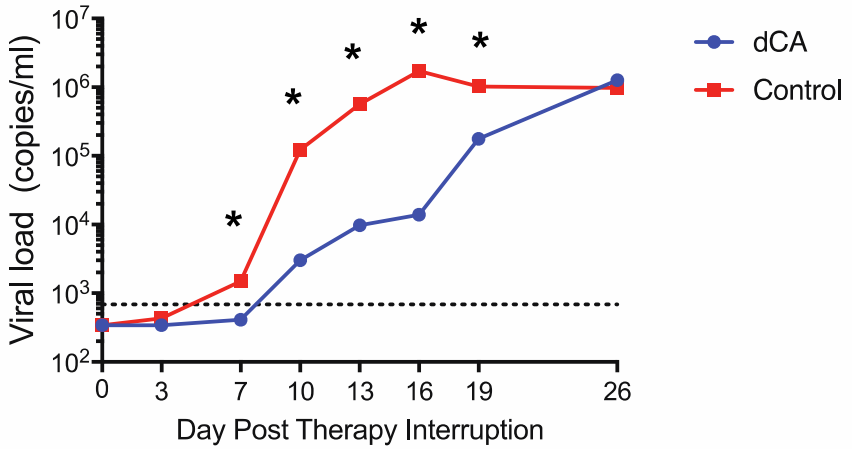
Kessing et al., 2017, Cell Reports

- **Two weeks of dCA treatment results in approximately 1 log reduction in HIV RNA**
- **Importantly in the brain dCA decreases by 7-fold HIV-1 mRNA production compared to control mice**

dCA efficacy in BLT humanized mouse models







Kessing et al., 2017, Cell Reports

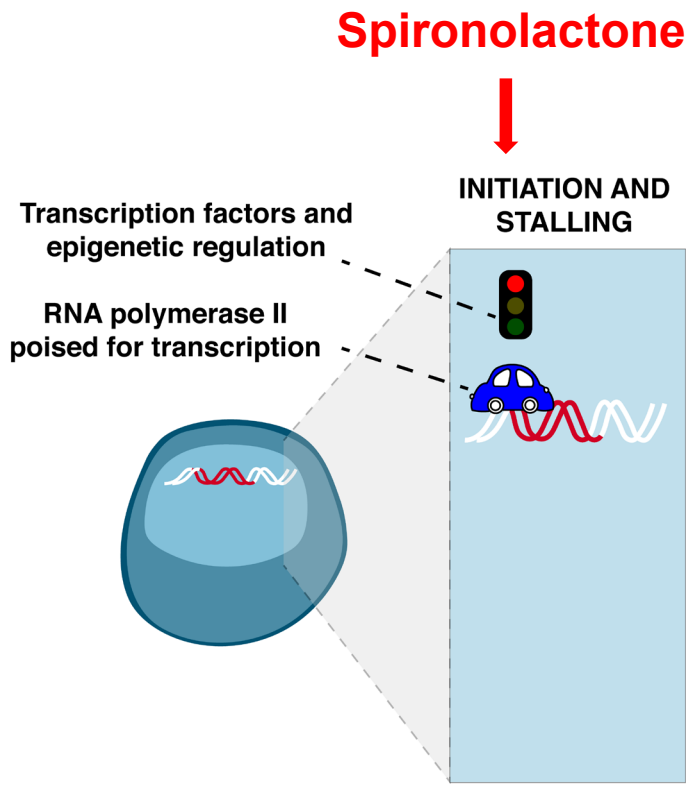
dCA significantly delays and reduces viral rebound levels

Additional benefits of Tat inhibitors

- **Reduction of Tat mediated HIV-1-associated neurocognitive disorders**
- **Reduction of chronic immune activation that comes from ongoing viral production even during suppressive therapy**
- **Virus may develop less resistance since these types of inhibitors (transcriptional inhibitors) uses both viral and cellular components.**

Spironolactone

- FDA approved since 1959 to treat high blood pressure, heart failure, strokes, and kidney disease.
- SP competes with Aldosterone for mineralocorticoid receptor (MR) binding
- SP treatment also causes degradation of the XPB subunit of TFIIH
- Eplerenone (EPL), a more selective MR antagonist analogue, does not degrade XPB



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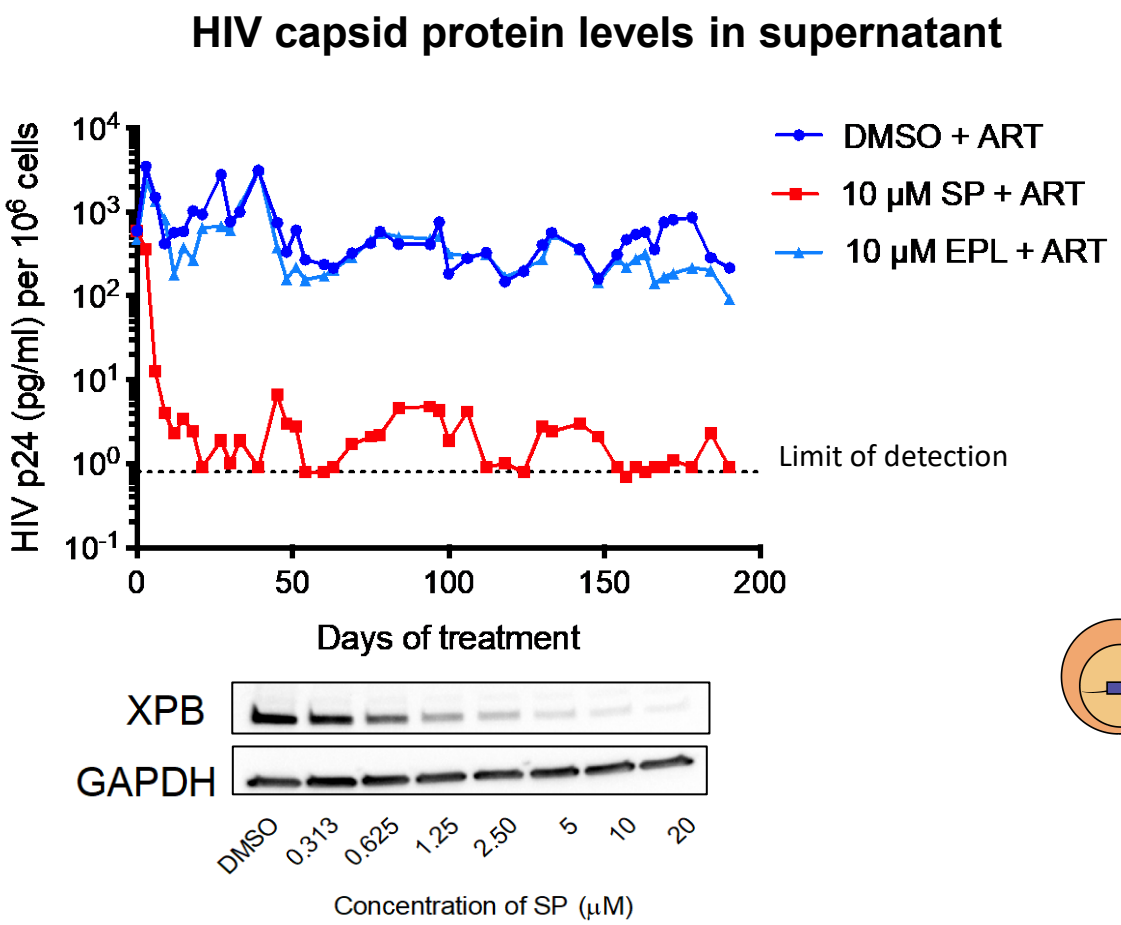
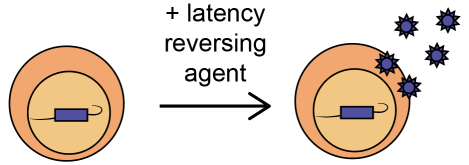
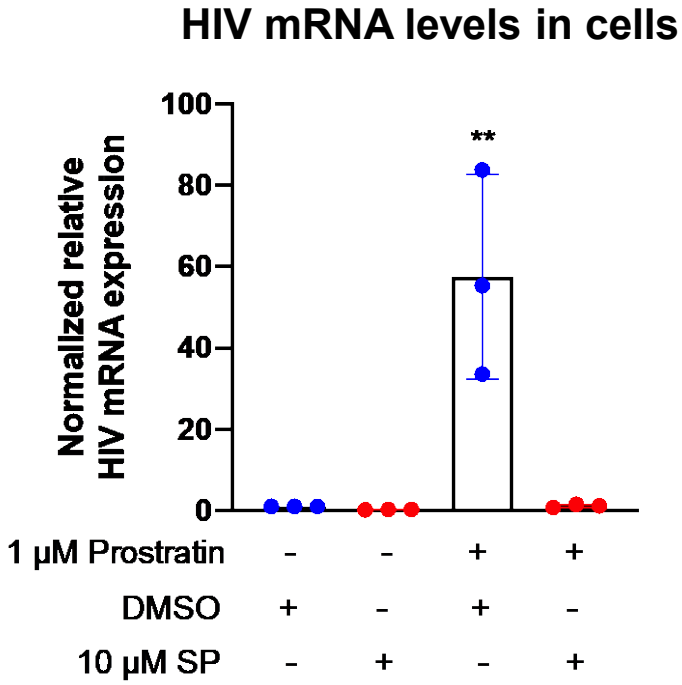
SP treatment suppresses HIV transcription and block viral reactivation





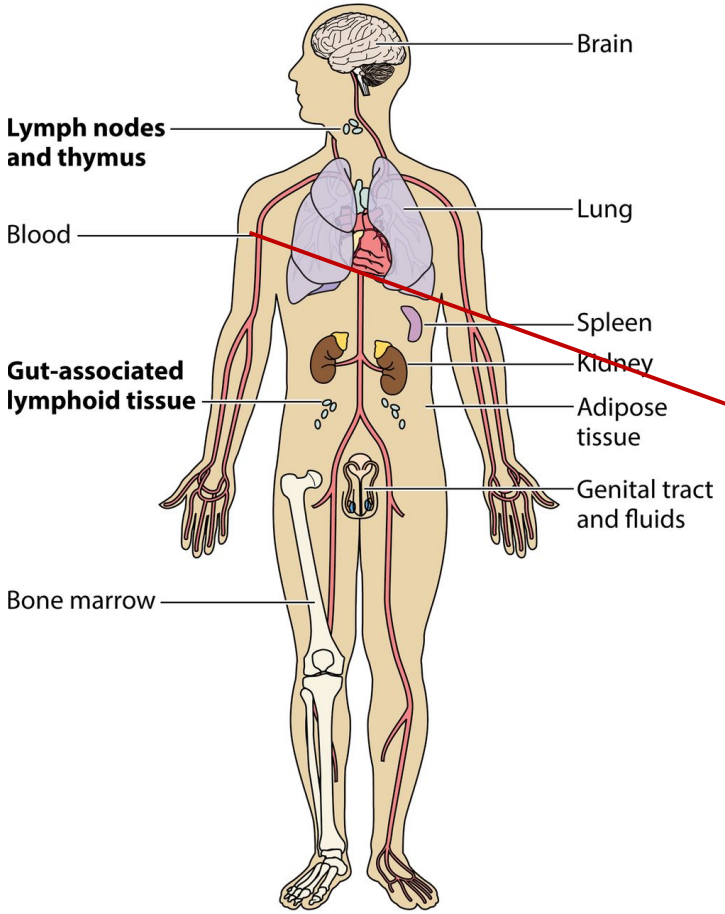


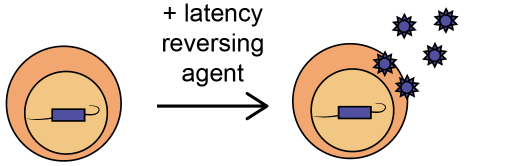
SP inhibits the viral expression to the limit of detection

Treatment with SP blocks reactivation of virus when cells are activated with different latency reversing agents.

Mori et al., 2020, Journal of Virology

SP blocks HIV reactivation in cells from people living with HIV





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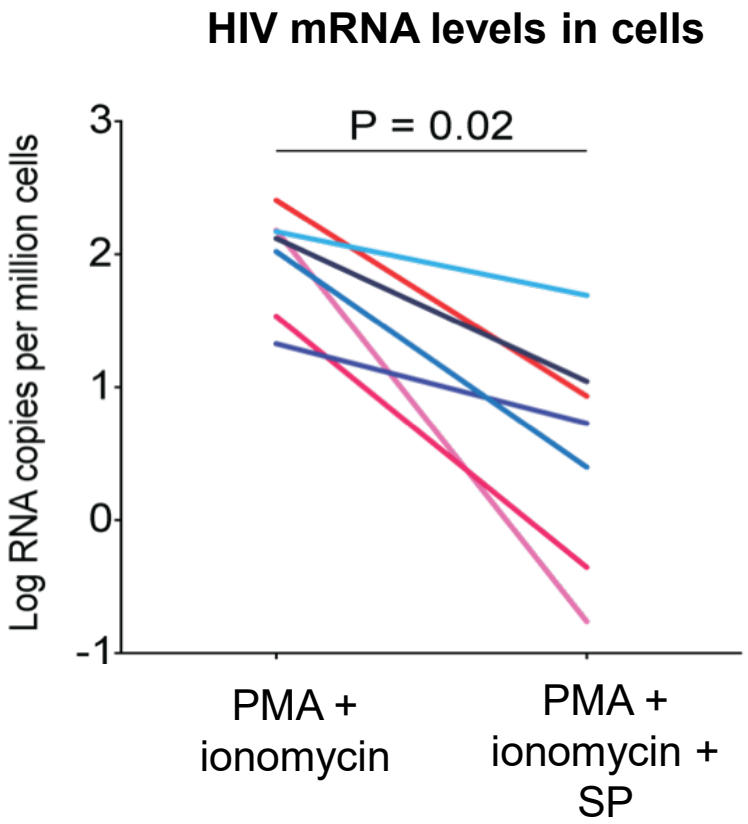

Mori et al., 2020, Journal of Virology

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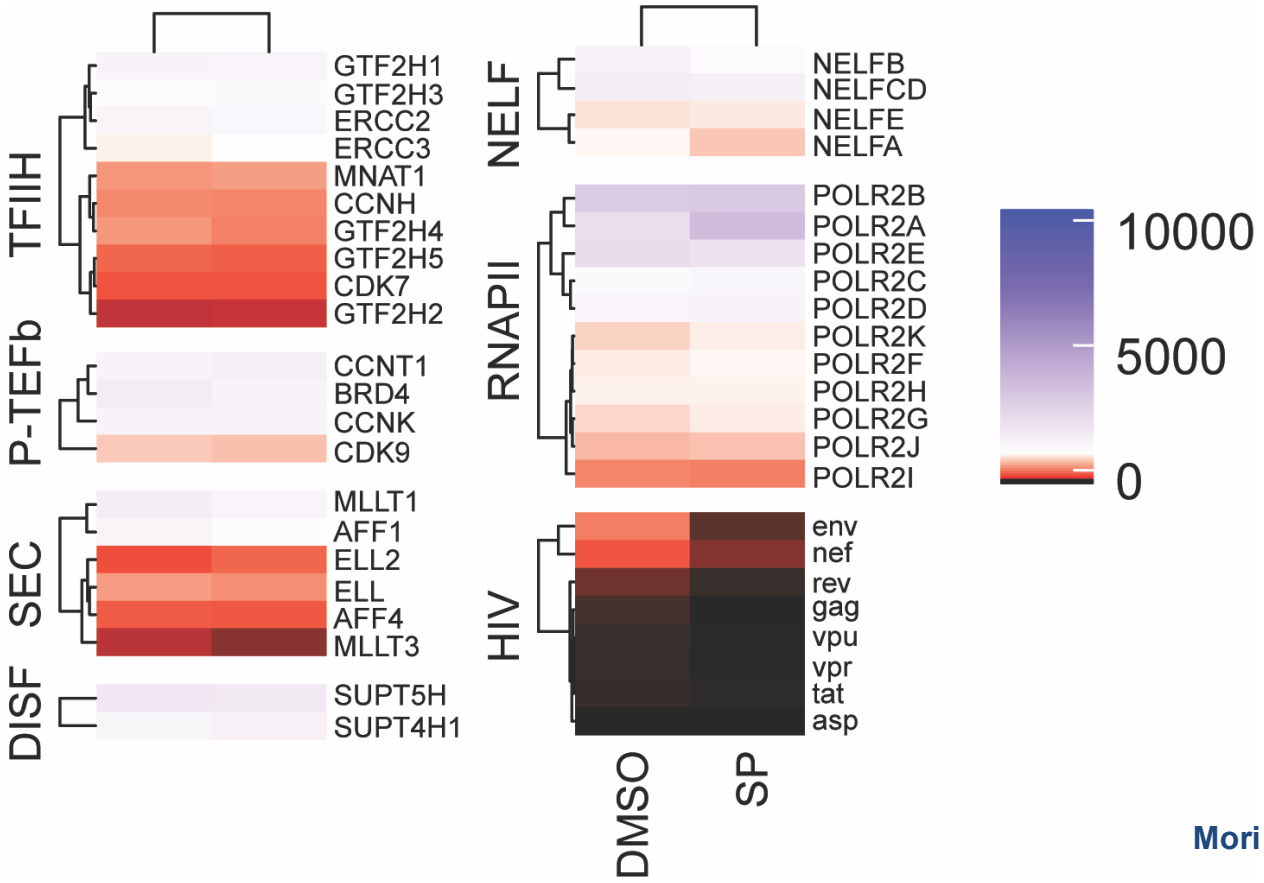
General effects of SP on cell transcription, important effects on HIV

Transcripts per million (TPM) values of HIV and cellular transcription related genes



Mori et al., 2020, Journal of Virology

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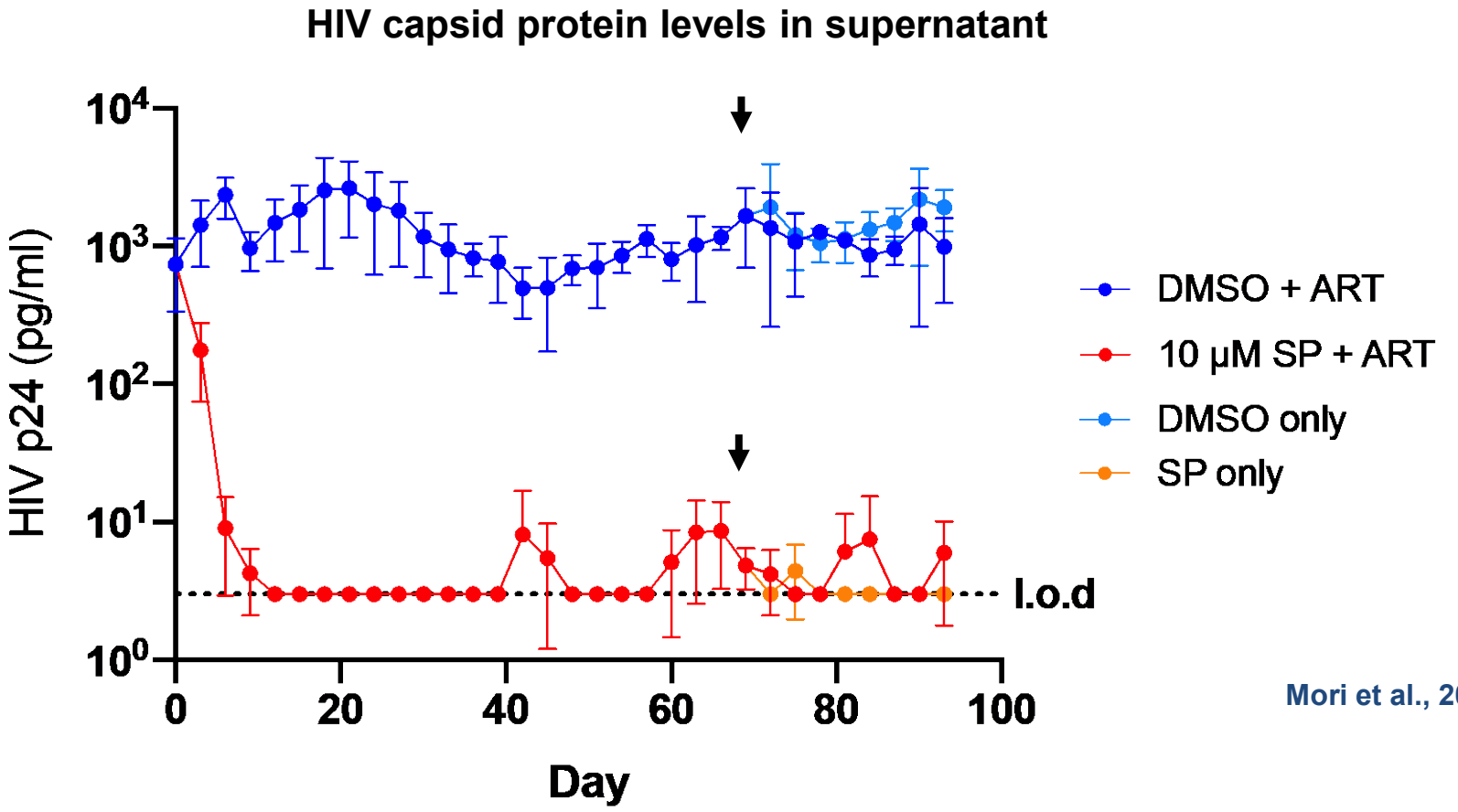
SP treatment potently inhibits HIV gene expression, while having more modest effects on certain on cellular genes, suggesting SP treatment selectively inhibits HIV.

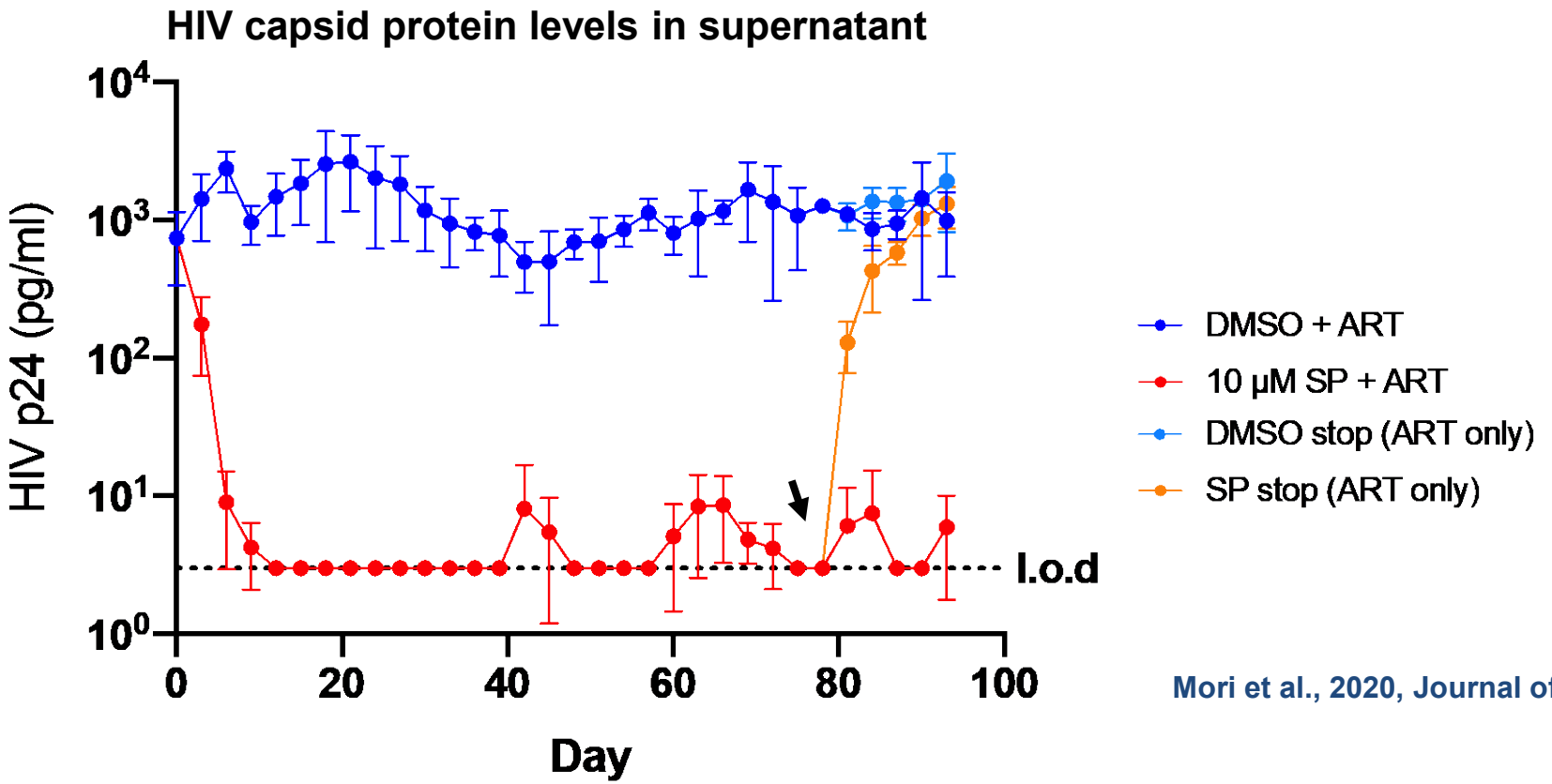
Can SP inhibit HIV expression as a single drug?



Mori et al., 2020, Journal of Virology

Even when all antiretroviral therapy is removed, SP alone maintain transcriptional suppression and no viral rebound occurs if SP is maintained.

Can pre-treatment with SP prevent viral rebound when all therapy is stopped?



Mori et al., 2020, Journal of Virology

Unfortunately, when SP treatment is removed there is rapid viral rebound that correspond to a rapid return of XPB protein levels.

Conclusions

- “Block-and-lock” is a viable approach to an HIV-1 functional cure
- Both dCA and Spironolactone significantly reduce HIV expression from infected cells
- In cells from people living with HIV dCA and Spironolactone suppress HIV
- In humanized mice dCA significantly delays viral rebound by epigenetically silencing the provirus
- SP alone controls viral rebound from latency
- dCA reverts Tat mediated inflammation
- More Tat inhibitors are on the way

Many questions remain to be addressed….

- **Transcriptional inhibitors are unlike any other HIV inhibitor**, as duration of treatment impacts the outcome, because of the feedback nature of the Tat activity and because epigenetic marks at the HIV-1 promoter accrue over time.
- It is **essential to understand the full clinical potential of transcriptional inhibitors**:
  - How long should the treatment be to totally inhibit residual viral production?
  - What is the relationship between HIV inhibition and time to rebound after treatment interruption?
  - Can we remove all therapy altogether or should the transcriptional inhibitor be maintained to keep viral production undetectable?
  - Can it become a permanent block?
  - Do they bring benefits if added to front-line therapy?

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