Silencing the HIV reservoir

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Human immunodeficiency virus (HIV)

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

- **Primary infection**
- **Acute HIV syndrome**
  - Wide dissemination of virus
  - Seeding of lymphoid organs
- **Clinical latency**
- **Symptoms of AIDS**
- **Death**
- **Opportunistic diseases**
- **Constitutional symptoms**
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

- Entry and fusion inhibitors
- Reverse transcriptase inhibitors
- Integrase inhibitors
- Protease inhibitors
The success of antiretroviral therapy (ART)

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

Antiretroviral therapy (ART) is capable of suppressing HIV to undetectable levels. However, the virus rebounds after cessation of therapy.

HIV infection is characterized by high levels of circulating viruses in the blood. Current anti-HIV drugs do not eradicate HIV.
Comparison of latent and active infection

**LATENT INFECTION**
- proviral DNA
- chromosomal DNA

**ACTIVE INFECTION**
- viral RNA
- viral mRNA
- viral proteins
- newly formed virus
- virus budding from cell
Mechanisms and sites of HIV-1 persistence

Persistence of latently infected cells

Ongoing viral replication at low levels

Sites of HIV persistence:
- Brain
- Lymph nodes
- Peripheral blood
- Gut
- Bone marrow
- Genital tract
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)
“Shock-and-Kill” approach to reservoir eradication

SHOCK
Latency reversing agents

KILL
Cytopathic effects and immune response
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)

Panobinostat (multiple doses)

Romidepsin (multiple doses)

Disulfiram (multiple doses)

... but do not significantly reduce the size of the latent reservoir

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

BLOCK-AND-LOCK

Viral transcription inhibitor + epigenetic control
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?

HIV infection is characterized by high levels of circulating viruses in the blood.

Antiretroviral therapy (ART) is capable of suppressing HIV to undetectable levels.

Block-and-Lock:
- ART + Transcriptional inhibitor
- DEEP LATENCY
- NO REBOUND

Virus remains contained after stopping therapy.

Limit of detection: Circulating virus

Time

START STOP
How do we go about blocking HIV transcription?

- Transcription factors and epigenetic regulation
- RNA polymerase II poised for transcription

INITIATION AND STALLING
- HIV RNA

INEFFICIENT ELONGATION
- HIV Tat protein

EFFICIENT ELONGATION
- Newly formed virus
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
How do we go about blocking HIV transcription?

Transcription factors and epigenetic regulation

RNA polymerase II poised for transcription

Spironolactone

dCA

INITIATION AND STALLING

INEFFICIENT ELONGATION

EFFICIENT ELONGATION

HIV RNA

HIV Tat protein

Newly formed virus
Cortistatin A

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

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

- 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”

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

Mousseau et al., 2015, mBio
Can dCA suppress viral reactivation in cells from infected subjects?
dCA blocks Viral rebound upon treatment interruption

- 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.

Kessing et al., 2017, Cell Reports
dCA efficacy in BLT humanized mice?
dCA reduces viral mRNA in BLT humanized mouse models

dCA was added to ART for a period of 14 days

- 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

Kessing et al., 2017, Cell Reports
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
SP treatment suppresses HIV transcription and block viral reactivation

**HIV capsid protein levels in supernatant**

![Graph showing HIV capsid protein levels in supernatant over days of treatment, with DMSO + ART, 10 μM SP + ART, and 10 μM EPL + ART conditions.]

**HIV mRNA levels in cells**

![Graph showing normalized relative HIV mRNA expression, with 1 μM Prostratin, DMSO, and 10 μM SP conditions.]

SP inhibits the viral expression to the limit of detection

Mori et al., 2020, Journal of Virology

Treatment with SP blocks reactivation of virus when cells are activated with different latency reversing agents.
SP blocks HIV reactivation in cells from people living with HIV

Mori et al., 2020, Journal of Virology
General effects of SP on cell transcription, important effects on HIV

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

SP treatment potently inhibits HIV gene expression, while having more modest effects on certain cellular genes, suggesting SP treatment selectively inhibits HIV.
Can SP inhibit HIV expression as a single drug?

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?

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

Mori et al., 2020, Journal of Virology
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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