Module U: Understanding how effective TLR agonists are for attacking the latent HIV reservoir

Warner C. Greene MD, PhD

MODULE LEADER
HIV Latency

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- Only about 1 cell out of every million CD4 T cells is latently infected (*rare event*)
- This reservoir is comprised of only $10^5$–$10^6$ cells in total (*rather small in size*)
- Because of their long survival and homeostatic proliferation, the latent reservoir persists virtually unchanged for the life of patients despite ART (*durable*)
Is HIV latency an accidental or planned event?
Robert Siliciano, MD, PhD
Principal Investigator, HHMI
Professor
Division of Infectious Diseases
Johns Hopkins University School of Medicine

Leor Weinberger, PhD  
amfAR Scientist and Senior Investigator  
Gladstone Institutes  
Professor, Biochemistry and Biophysics  
University of California, San Francisco

Razooky BS, Pai A, Aull K, Rouzine IM, Weinberger LS.  

Rouzine IM, Weinberger AD, Weinberger LS.  
Any hope for an HIV cure?
Timothy Brown

Proof of Concept:
Two stem cell transplants with CCR5-deficient donor cells for leukemia
No detectable virus since 2007
Considered an example of viral eradication
Our challenge
To go from $n=1$ to $n = 37$ million
An HIV Cure Must Be:

Safe
Effective
Scalable
What will our approach in the amfAR Institute be?
amfAR Institute for HIV Cure Research in San Francisco

Warner Greene
Eric Verdin
Melanie Ott
Leor Weinberger
Matija Peterlin
Steve Reed
Satish Pillai
Lewis Lanier
Peilin Li
Joe Wong
Greg Barton
Romas Geleziunas
Tomas Cihlar
“Shock and Kill”
Multiple Rounds of Shock Will Be Required

- Known Reservoir of Inducible Virus
- New Reservoir of Intact but Non-induced Virus
- Defective Virus

Ho et al, Cell, 2013
How to Achieve Effective Shock and Kill?

We will try to harness the innate immune response for shock and kill turning this normally protective response against the HIV
Toll-like Receptors

2011 Nobel Prize to Drs. Bruce Beutler and Jules Hoffmann
An Overview of the Toll-like Receptor Family and their Ligands
Do the TLR agonists act directly on CD4 T cells?
T Cells + TLRs: Reactivation 3 Days

Spinoculate with: mCherry, Luciferase HIV-1 Saquinavir

Purified CD4 T Cells from Tonsils

Day: 0

On Day 6: Saquinavir Raltegravir with TLRs

Check Virus Reactivation After 72 h
If not direct, does the effect occur through dendritic cells or macrophages?

What is the key cytokine?
T Cells + DCs + TLRs: Reactivation 3 Days

Monocytes
Purified from PBMC

Spinoculate with:
 mCherry
Luciferase HIV-1
Saquinavir

Purified CD4 T Cells
from Tonsils

Day: 0

1 2 3 4 5 6

Check Virus Reactivation
After 72 h

Treated with IL-4 and GM-CFS for 6 days

Monocyte-derived DCs

Stimulate with TLRs 24 h
Add DCs cells and its medium into CD4+ T cells (T:DCs = 10:1)

On Day 6:
Saquinavir
Raltegravir
Conclusion

Consistent, although moderate, activation of latent virus expression when monocyte derived dendritic cells and latently infected tonsil cells are incubated together in the presence of TLR4 and TLR7 activators.
Any synergy or additivity?
Conclusion

No evidence for clear synergy or even additivity when the TLR4 and TLR7 activators are combined
How active are these TLR agonists as latency-reversing agents in “gold standard” cells from HIV-infected individuals on long term ART?
Conclusion

Overall, several TLR agonists exhibit moderate “shocking” activity in cells from HIV infected individuals on ART. However, these agents appear more active in *in vivo* probably because they also act as adjuvants boosting the adaptive immune response against HIV.
Putting the Kill in “Shock and Kill”

- Latently Infected CD4 T Cell
- Purging Latent Reservoir
- Productively Infected CD4 T Cell
- HIV-induced Cell Death
- Shocking Agent
- Budding
- Assembly
- 1-2 Days
- HAART
- Uninfected CD4 T Cells

amfAR Institute
FOR HIV CURE RESEARCH
Can we harness another component of the innate immune response for the kill?
Stimulating the RIG-I pathway to kill cells in the latent HIV reservoir following viral reactivation

Peilin Li¹,², Philipp Kaiser¹, Harry W Lampiris¹,², Peggy Kim¹, Steven A Yukl¹,², Diane V Havlir²,³, Warner C Greene²,⁴,⁵ & Joseph K Wong¹,²

Dr. Peilin Li
Dr. Joe Wong
Acitretin *Both* Induces RIG-I and Acts as a Weak LRA Activating Latent HIV Proviral Gene Expression
The RIG-I Pathway Can Induce Apoptosis
Acitretin +/- SAHA Significantly Lowers HIV DNA Concentration in HIV Patient Cell Cultures
Harnessing the Innate Immune Response to Selectively Kill Reactivated Reservoir Cells

Latently HIV-infected Cell

Acitretin

HIV RNA

HATs p300

HIV-LTR

RIG-I

MAVS

IRF-3

BAX

Caspase-3

Interferon (IFN)

Apoptosis

Reducing Proviral DNA

Antiviral Signalling