Why ARV treatment doesn’t cure HIV:

- A latent reservoir of long-lived T-cells with integrated HIV DNA is seeded early during acute infection
- Viral persistence results in immune dysfunction

Establishment of Latency

Fate of Latently Infected Cells

Immune dysfunction Prevents Clearance of latently infected cells

HIV Particle

Activated CD4+ T-cell

Resting Memory CD4+ T-cell

Myeloid cell

Minority of cells become latently infected

Majority of cells die or are eliminated

Apoptosis

PD1 and other inhibitory molecules contribute to T-cell dysfunction

Dysfunctional B-cell

Exhausted T-cell

Immune dysfunction reduces the clearance of infected cells
“Flush” and “Kill” Strategy for HIV Eradication

- HIV genome
- Memory CD4+ T cell
- HIV RNA
- HIV proteins
- HIV particles
- Dying infected cell
- Uninfected cell
- Antiretroviral therapy

**FLUSH**

- DNA

**Kill**

- HIV particles

Uninfected cell
HIV DNA transcription prevented by restricted access to needed host enzymes, **chromatin remodeling** and transcriptional interference.

**HDAC inhibitors** (eg TSA, SAHA)
Methyltransferase inhibitors

**NF-κB activators** (Prostratin, PMA)
Akt/HEXIM-1 modulators, (HMBA)
Latency at the HIV Promoter: Epigenetic Silencing and Transcription

Chromatin Structure

Reactivation Step 1: Relaxation of chromatin

HDAC EZH2

HIV TSS

nuc-0

nuc-1

Transcription Regulation

Reactivation Step 2: Stimulate transcription

NFκB

NFAT

P-TEFb

CDK9

HEXIM1

CyclinT1

7S RNA

Phosphorylation

IkB
Oncology HDACIs Being Explored for HIV Latency

Vorinostat (SAHA – Merck):
- Pan HDACI, oral, AMES positive
- Latency clinical POC (Margolis, Lewin)
  - Increase in HIV RNA in resting T-cells
  - Increase in viremia (some pts), no effect vDNA
- Ames(+) = potential safety liabilities?

Panobinostat (Novartis):
- Pan-HDACI; potent/reversible, oral
- Latency clinical POC (Rasmussen; Lichterfeld CROI ‘14)
  - Increase in HIV RNA in resting cells
  - Increase in viremia and decrease in vDNA (some pts)

Romidepsin (Gilead):
- Pan HDACI, potent/irreversible, IV, DDIs
- Clinical POC in progress

Can HDACIs be optimized for HIV latency?
Potential Approaches to Improve Efficacy and Therapeutic Window of HDACIs

► Optimize HDAC activity for HIV latency
  – Increase potency and selectivity profile
  – PK, understand dose/response relationship
    • Differs between reversible and irreversible binders?

► Identify other mechanisms to be used in combination
  – Enhance overall effect and/or
  – Reduce HDACI dose
Next Generation HDAC inhibitors with Improved Selectivity/PK Profile for HIV Latency

<table>
<thead>
<tr>
<th>Criteria</th>
<th>SAHA</th>
<th>Next gen HDACi</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isozyme Selectivity</td>
<td>HDAC3 required</td>
<td>1, 2, 3, 6, 8</td>
</tr>
<tr>
<td><strong>HIV Latency in Jurkat T-cell Model</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$EC_{50}$ (nM)</td>
<td>1000nM</td>
<td>200 nM</td>
</tr>
<tr>
<td>PK profile</td>
<td>very high clearance</td>
<td>Can be tuned</td>
</tr>
</tbody>
</table>

Next gen HDACIs:
Historical chemical matter
Structure-guided design + modeling
Screening
Potential Approaches to Improve Efficacy and Therapeutic Window of HDACIs

► Optimize HDAC activity for HIV latency
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Reactivation Step 1: Relaxation of chromatin

Reactivation Step 2: Stimulate transcription

Transcription Regulation

Phosphorylation
Novel Ultra High Throughput Screen to Identify New Mechanisms and Combinations

Latent Jurkat T-cell model (Jon Karn)

Screened 2.9 million compounds in the presence of 250 nM SAHA

All hits titrated plus and minus 250 nM SAHA

Identified HDACIs plus 2400 unique hits
FTi’s with Differential Binding Modes and Structures


X-ray Crystallographic images of different farnesyl-transferase inhibitor binding modalities. (A) Zinc Binding, (B) Exit Groove Blocker
Correlation Between Farnesyl Transferase Inhibition and HIV-Latency Activation

- Strong Positive correlation between FTi potency (IC$_{50}$ enzyme assay) and HIV latency activation in the presence of 250nM SAHA (EC$_{50}$ in Jurkat T-cell model system)
- Knock-down of FTi-beta subunit leads to activation of HIV LTR in a Jurkat model system
FTIs Increase Emax and Lower SAHA EC50
FTIs Enhance HDACI Effectiveness in Primary T-cells by Stimulating Transcription (J.Karn)

No Stimulation  500nM SAHA  10μM FTi  10μM FTi + 500nM SAHA

- eGFP
- NEF
- Active PTEF-b

Cyclin D3
Validation of FTIs as Inducers of Latent HIV Expression: Summary of data to date

► Farnesyl Transferase (FT) Inhibitors from different structural classes induce latent HIV expression
► Effect on latent HIV expression is reproduced by siRNA knockdown
► Positive correlation between FT enzymatic activity and HIV latency activation in a Jurkat T-cell model system
► FTIs synergize with SAHA in a primary T-cell model and in latently infected memory T-cells isolated from HIV infected patients
► FTIs may increase active pTEFb levels in resting cells
FTIs Synergize with other HIV Latency Activation Mechanisms
Identifying Compounds which Synergize with other Established Mechanisms

~2000 HIV Latency uHTS hits (non-HDACi)
Complete Dose Response,
+/- EC$_{20}$ of SAHA, HDACi (1,2,3), TNF$_{\alpha}$, JQ1, HMBA, Prostratin

Jurkat HIV Latency T-cell Model (Luc reporter) 
N=3

Cytotoxicity of Compounds +/- EC$_{20}$ of known HIV Activators 
N=3

Complete Dose Response of uHTS hits +/- EC$_{20}$ of known HIV Activators 

Re-test of compounds with decreased EC$_{50}$ and/or increased Emax in the presence of known HIV activators
### Synergy Profile of uHTS Hits of Unknown Mechanism

<table>
<thead>
<tr>
<th></th>
<th>EC$_{50}$ Synergy</th>
<th>E$_{max}$ Synergy</th>
<th>EC$<em>{50}$ and E$</em>{max}$ Synergy</th>
</tr>
</thead>
<tbody>
<tr>
<td>HMBA</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>JQ1</td>
<td>12%</td>
<td>72%</td>
<td>7%</td>
</tr>
<tr>
<td>PKC</td>
<td>15%</td>
<td>81%</td>
<td>11%</td>
</tr>
<tr>
<td>HDACi (1,2,3)</td>
<td>14%</td>
<td>78%</td>
<td>10%</td>
</tr>
<tr>
<td>TNFα</td>
<td>13%</td>
<td>80%</td>
<td>9%</td>
</tr>
</tbody>
</table>
Some Key Questions in HIV Latency Drug Discovery

► Can we improve the efficacy/safety of latency activating agents

► Can we increase the extent and spectrum (breadth) of activation through combinations

► Will the same agents/combinations work in all cells
Differential Activity of FTIs in Two Different Jurkat T-cell Models

Jurkat Population Cells (eGFP)
No Synergy Detected

Jurkat 2C4 Cells (Luciferase)
Compounds Synergistic
Some Key Questions in HIV Latency Drug Discovery

► Can we improve the efficacy/safety of latency activating agents

► Can we increase the extent and spectrum (breadth) of activation through combinations

► Will the same agents/combinations work in all cells

► If we increase HIV expression will it lead to cell death or will another modality be required to eliminate these cells

► How much expression is “enough”
gp120 is Expressed on Latently Infected Jurkats After Stimulation w/ TNF or SAHA

*KARN cells were treated with TNF or SAHA for 24 hrs. GFP and gp120 expression were determined at by imaging and flow cytometry.
Approaches for the “Kill”

HDACi with Optimal Selectivity/PK + Synergistic Agent

- Ab-mediated Killing
- IMR/Resurrection of immune response +/-
- Therapeutic Vaccine
# HIV Antibody Conjugates can Eliminate HIV-infected Cells *in vivo*

<table>
<thead>
<tr>
<th>Platform</th>
<th>mAb</th>
<th>MOA</th>
<th>POC (Model)</th>
<th>Pros</th>
<th>Cons</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Bispecific mAb (BsAb)</strong></td>
<td>αgp120/αCD3</td>
<td>Redirected T-Cell killing (CD3)</td>
<td>Yes (Rhesus Macaque)</td>
<td>Highly specific, Does not require high antigen expression/ internalization, Clinical precedence for MOA</td>
<td>Uncertainty around cis/trans effect with CD3 expression, Clinically significant immune activation?</td>
</tr>
<tr>
<td><strong>Antibody Drug Conjugate (ADC)</strong></td>
<td>αgp120</td>
<td>Intracellular cytotoxin delivery (Doxorubicin, PE, ricin)</td>
<td>Yes (BLT Mouse)</td>
<td>Highly specific, Intracellular toxicity, Clinical precedence</td>
<td>Requires internalization, May require high antigen expression</td>
</tr>
<tr>
<td><strong>Antibody Radioisotope Conjugate</strong></td>
<td>αgp41</td>
<td>Localized radiation induced damage (Bi$^{213}$)</td>
<td>Yes (BLT Mouse)</td>
<td>Highly specific, Does not require high antigen expression or internalization, Clinical precedence</td>
<td>Manufacturing and regulatory hurdles, Short t$_{1/2}$ of isotope and commercial impact</td>
</tr>
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</table>
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PD1 and other inhibitory molecules contribute to T-cell dysfunction

Dysfunctional B-cell

Exhausted T-cell

Immune dysfunction reduces the clearance of infected cells
Mechanisms of HIV persistence

**Active reservoir**

Ongoing viral replication

**Latent reservoir**

T cell survival

Homeostatic proliferation

References:

The Latent Reservoir Established Early in Infection… may be Maintained by Multiple Mechanisms

- HIV/CMV persistence
- Immune dysfunction
- Homeostatic proliferation
- Immune activation/Inflammation
Addressing Latency Through Multiple Interventions…

HIV/CMV persistence

Immune activation/Inflammation

Immune dysfunction

Immune reconstitution?

Shock and Kill

DNA

Homeostatic proliferation

Anti-inflammatory?

Therapy intensification
Acknowledgements

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Delaney AIDS Research Enterprise to find a cure

VGTI FLORIDA