Gilead HIV Eradication Program

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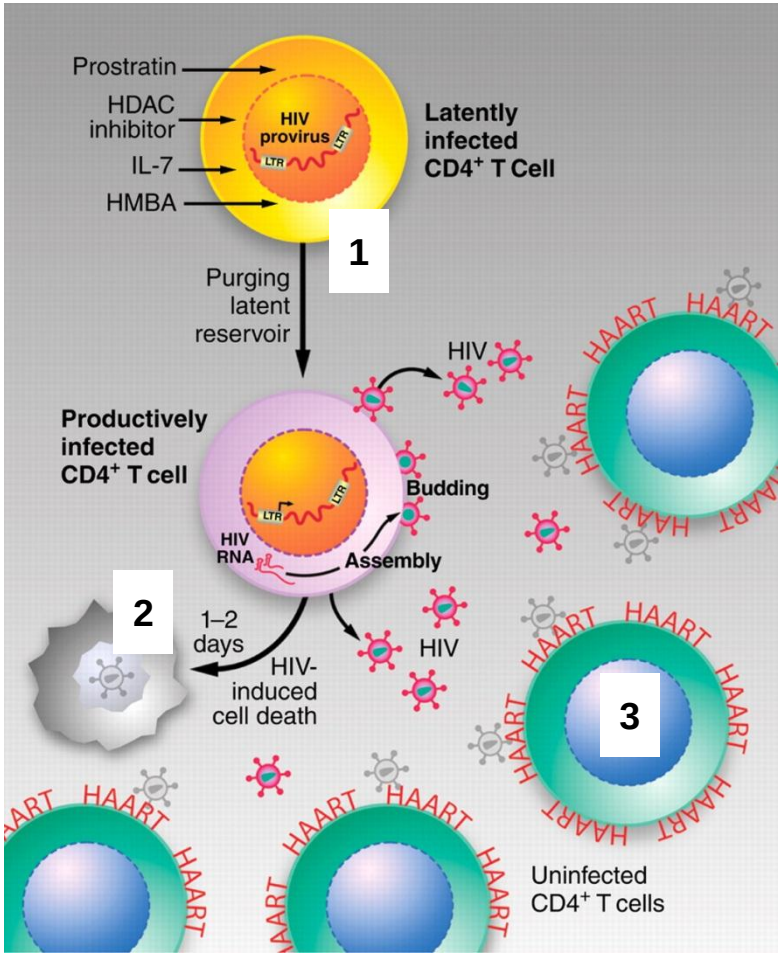
Potential Strategy to Eradicate Latently Infected Cells

1. **Activate HIV expression in latently infected cells**
   - De-repress chromatin: Inhibit HDACs
   - Activate transcription factors (NF-κB)
   - Activate HIV mRNA elongation (PTEF-b)
   - Other (HTS)

2. **Eliminate cells actively replicating HIV**
   - Viral cytopathic effects
   - Virus-specific immune responses
     - Immunomodulators
     - Therapeutic vaccines
   - Virus-directed toxins

3. **Infection by newly produced virus particles blocked by ARVs (Intensify?)**



Richman et al., Science 323 (2009)

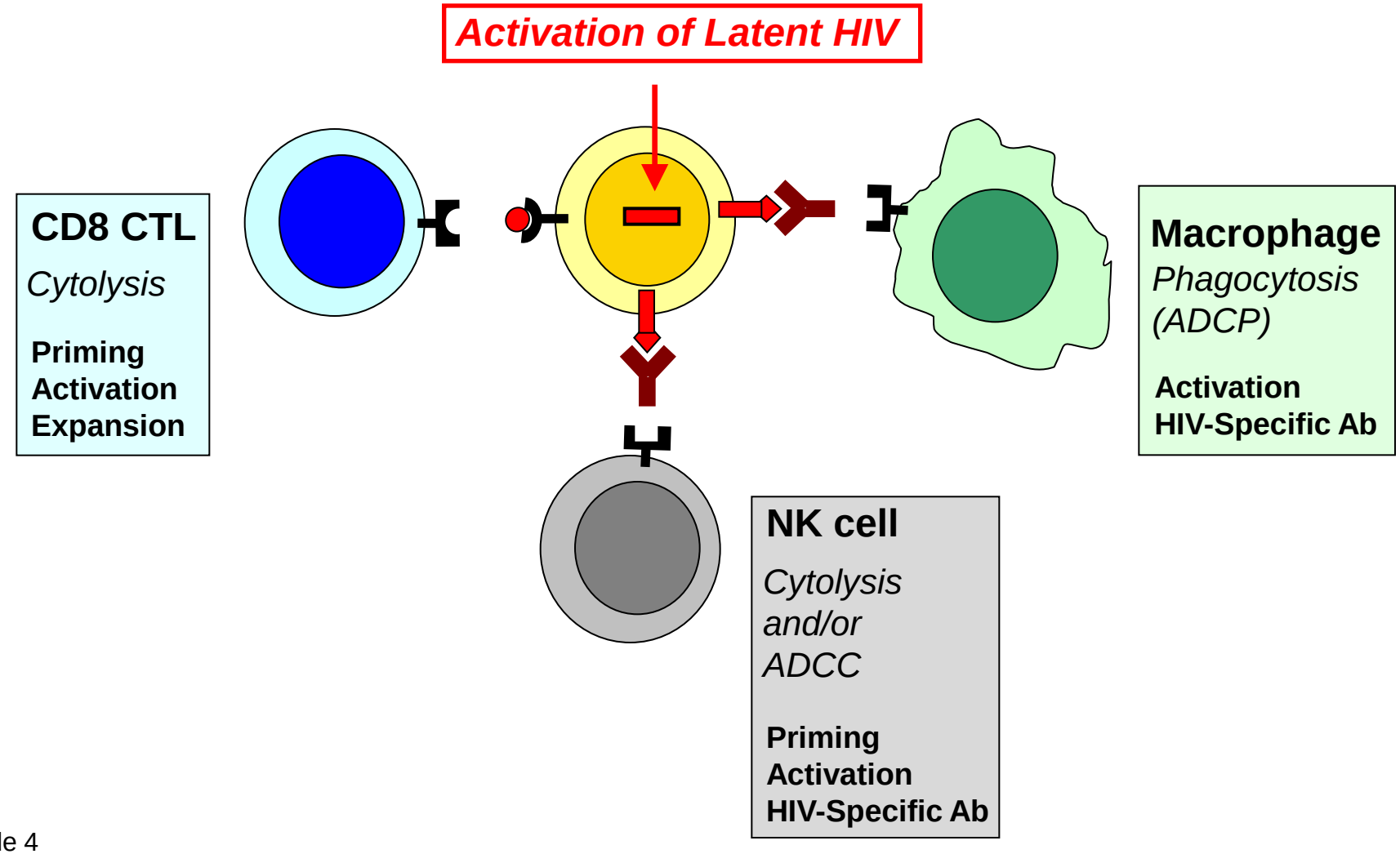
Recent Key Developments

- **David Margolis (University of North Carolina)**
  - CROI & Keystone 2012
  - 400 mg SAHA (Vorinostat) in seven HIV+ subjects (ART)
  - 4 - 6 h post-dose: HIV RNA levels increased (5.2x) in CD4+ T-cells
  - First proof of concept in humans that HDACi activates latent HIV expression
  - SAHA is mutagenic: Multiple dose studies problematic

- **Robert Siliciano (Johns Hopkins University)**
  - Shan *et al*, Immunity 36: 491 (2012)
  - CD8+ CTLs necessary to kill HIV-infected cells treated with SAHA (in vitro)
  - Virus activation alone may not suffice to kill host cell

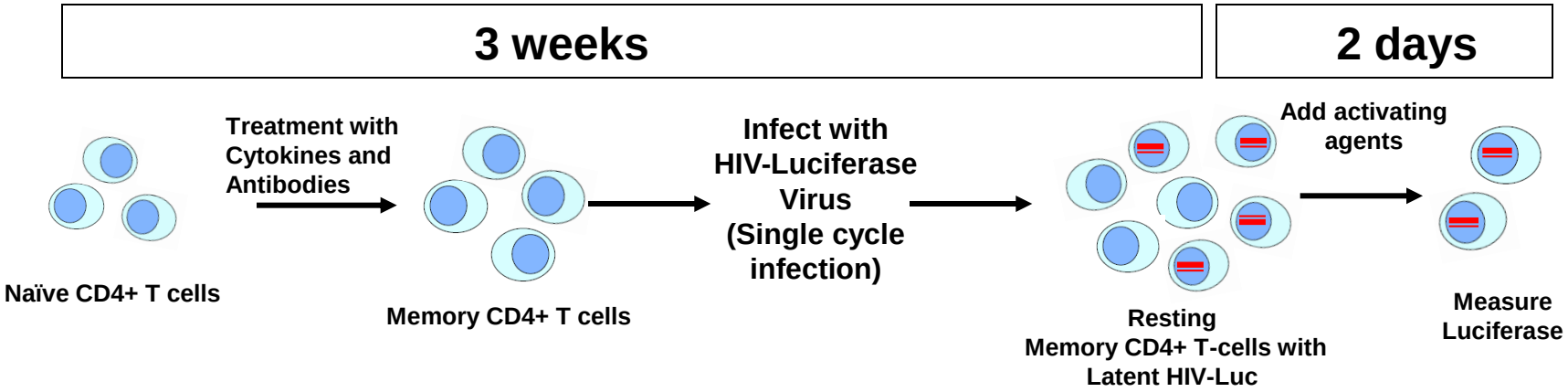
- **NEW FOCUS**
  - Combination approaches
  - Activate latent HIV and kill cells expressing HIV
  - HDAC inhibitors with immune-based strategies
    - Therapeutic vaccines that elicit CD8+ CTLs

Immune-Mediated Killing of Infected Cells Expressing Reactivated HIV



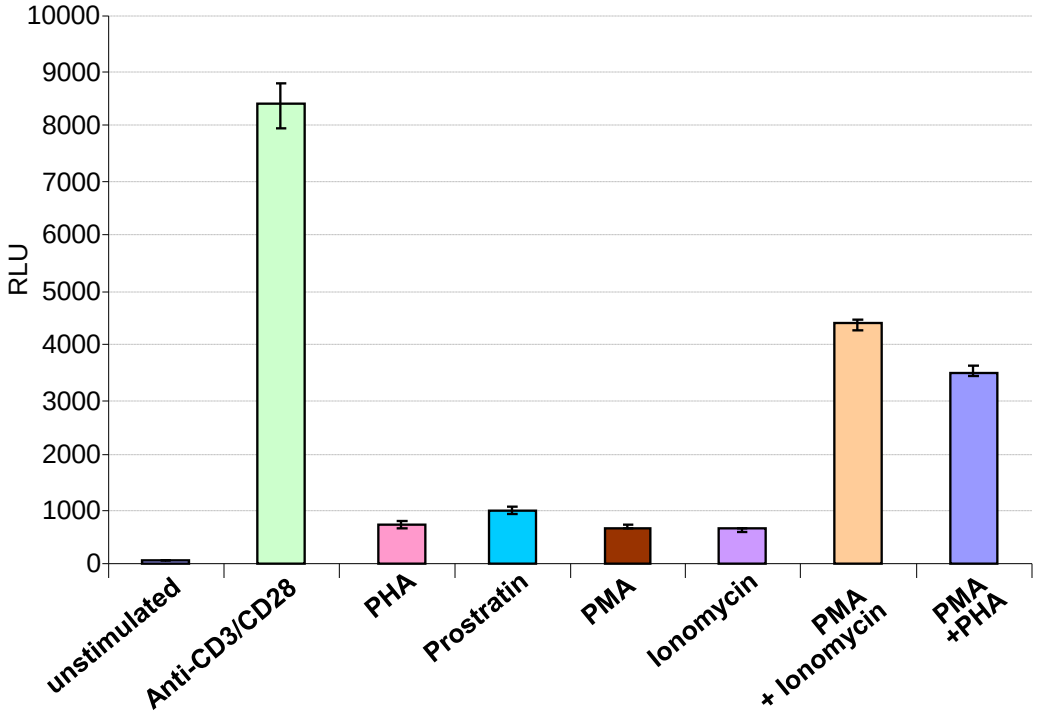
Primary Cell HIV Latency Assay

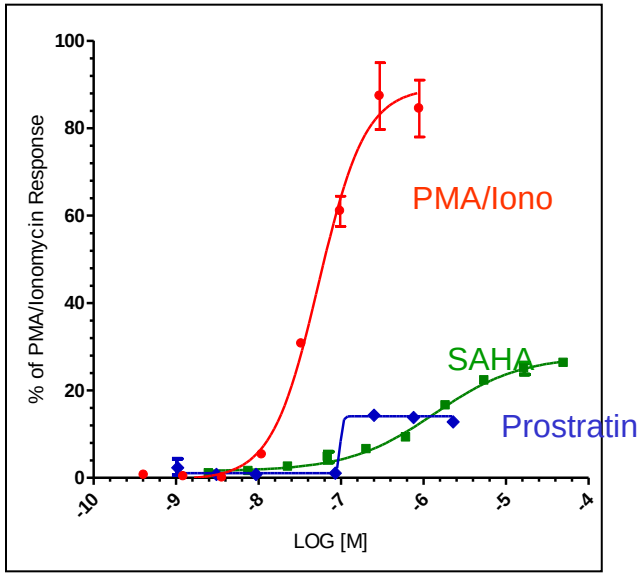
- **Generating Latent HIV-infected CD4+ memory T-cells *in vitro***



- **Modified from Bosque and Planelles (2009)**
- **Greater sensitivity (Cod. Opt. Luciferase / infection protocol)**
- **Validated with agents that activate latent HIV**
- **Miniaturized (384-well plates) and automated for HTS**
  - **10,000 cells / 20 μl**
  - **20 nl compound / acoustic dispenser**

Assay Validation





- CD3/CD28 - TCR signaling
- PHA - Mitogen
- PMA, Prostratin - PKC & NF-κB activation
- Ionomycin - NFAT activation
- SAHA - HDAC inhibitor

Prostratin:

•Robust and good breadth of latent HIV activation in vitro

•Toxicities in preclinical studies

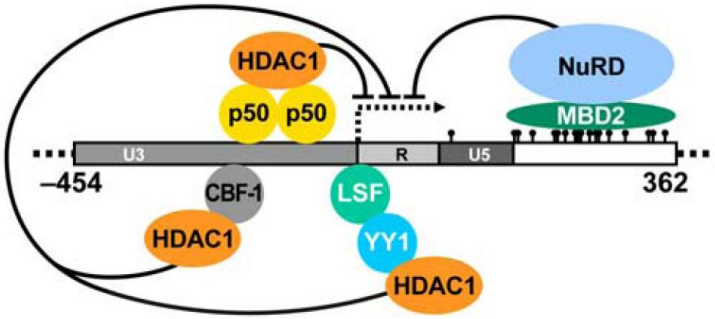
SAHA:

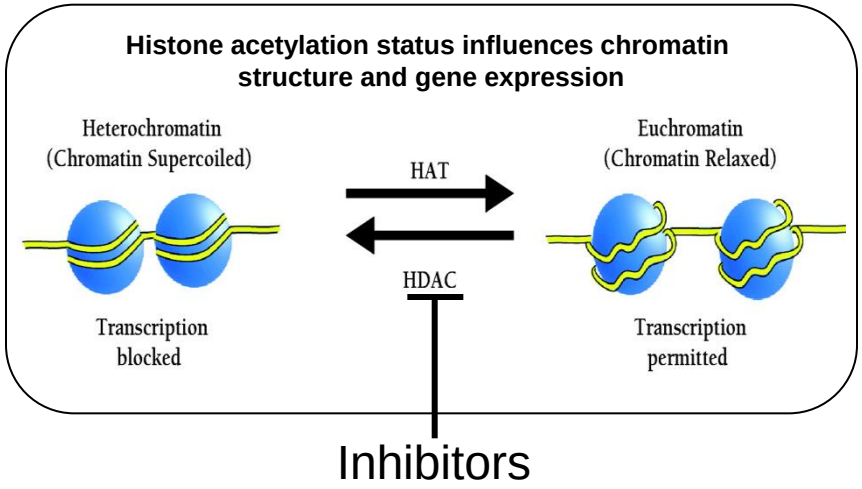
•Clinical studies in HIV+ populations

Histone Deacetylases (HDACs) and Latent HIV

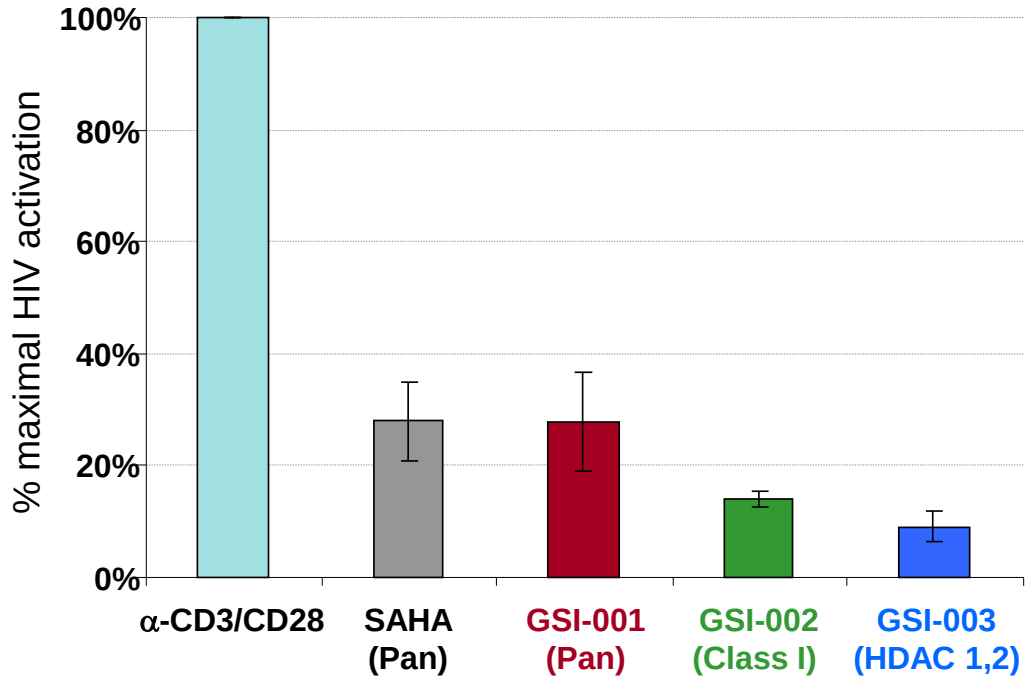
- Family of zinc metalloenzymes
- Catalyze removal of acetyl groups from lysine
- Cellular substrates: Histones, Tubulin, Transcription factors
- HDAC inhibitors activate latent HIV

HDACs are Recruited to the HIV LTR





HDAC Inhibitors From Gilead's Collection Activate Latent HIV



AMES	+	+	-	+

HDACi	EC_{50} [μM] HIV activation
SAHA	0.6
GSI-001	0.06
GSI-002	0.5
GSI-003	0.4

Are HDACis activating

1. A fraction of latent proviruses (vs anti-CD3/CD28) → Combination agent?
2. Same proviruses but less robustly (vs anti-CD3/CD28)→ Multiple rounds ~ CD3/CD28?

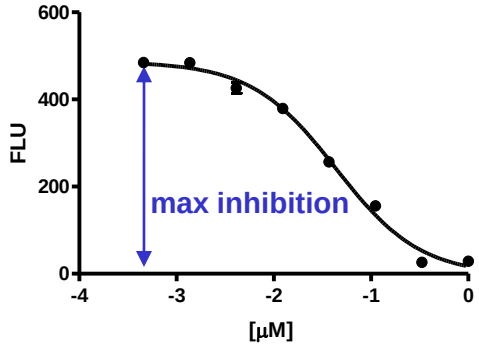
What level/breadth of HIV reactivation will be needed to achieve efficacy (drop in latent reservoirs)?

HIV Activation Correlates with Intracellular HDAC Inhibition

Cellular HDAC inhibition

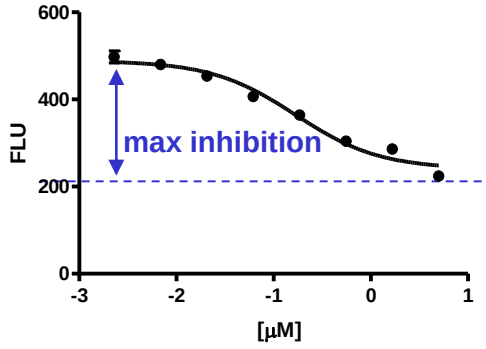
GSI-001 (pan)

EC_{50} = 0.06 μM



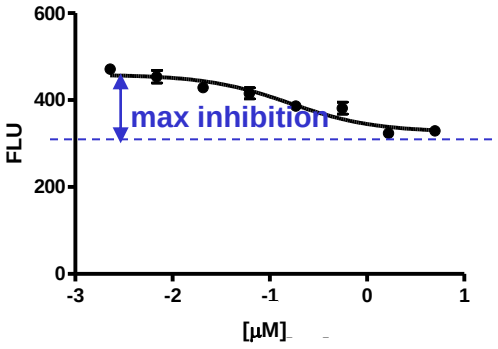
GSI-002 (class I)

EC_{50} = 0.15 μM



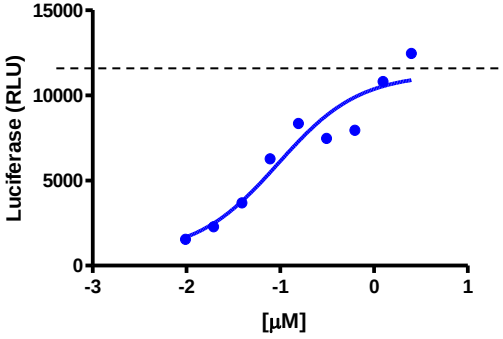
GSI-003 (1,2 selective)

EC_{50} = 0.08 μM

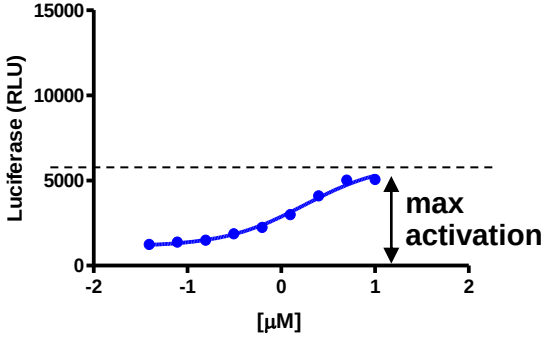


HIV activation

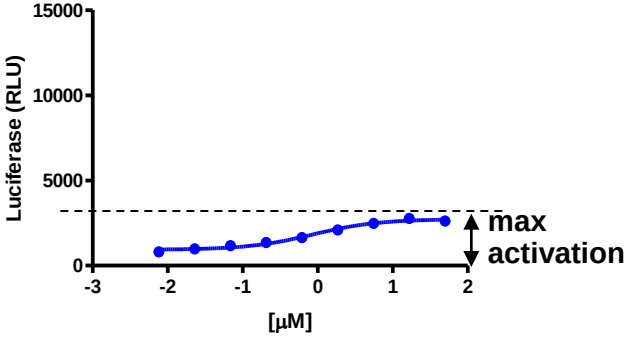
EC_{50} = 0.06 μM



EC_{50} = 0.5 μM

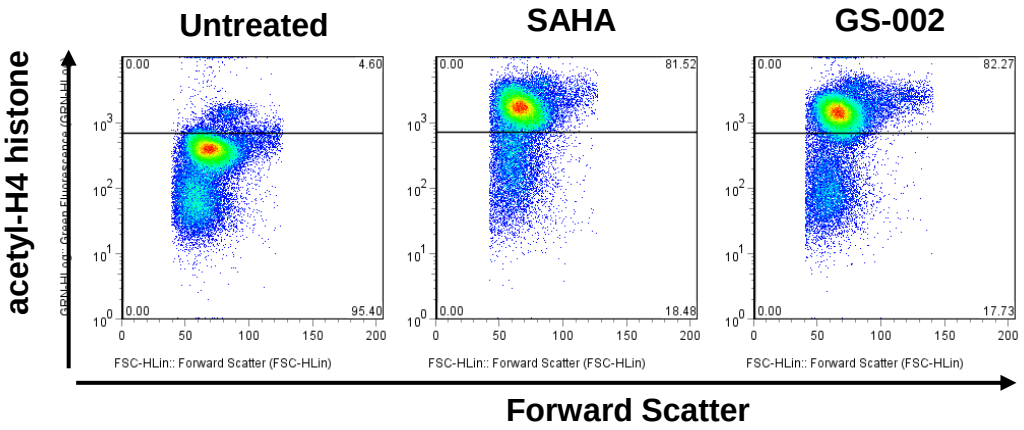


EC_{50} = 0.4 μM

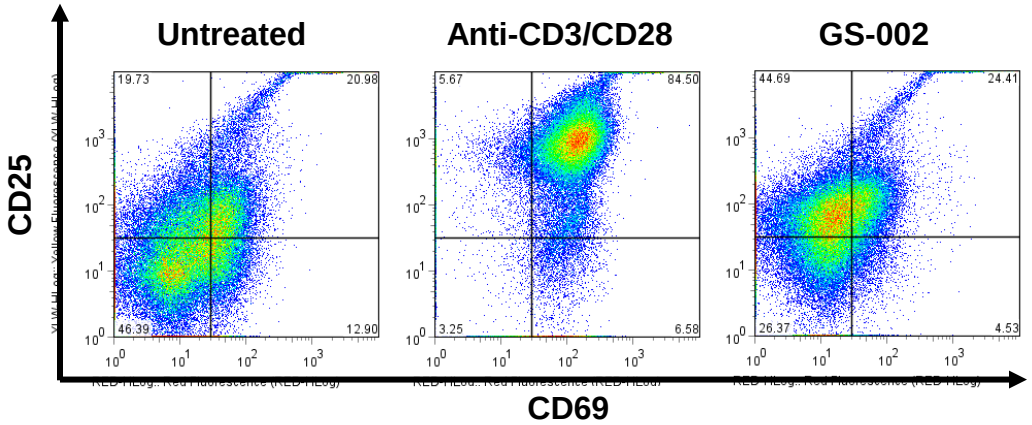


HDACi GSI-002

GSI-002 increases histone acetylation in T-cells



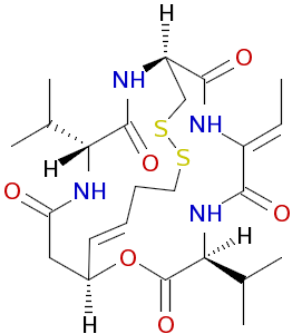
GSI-002 does not activate T-cells



GSI-002: Increased histone acetylation *in vivo* and well tolerated in rats (2-3 weeks dosing)

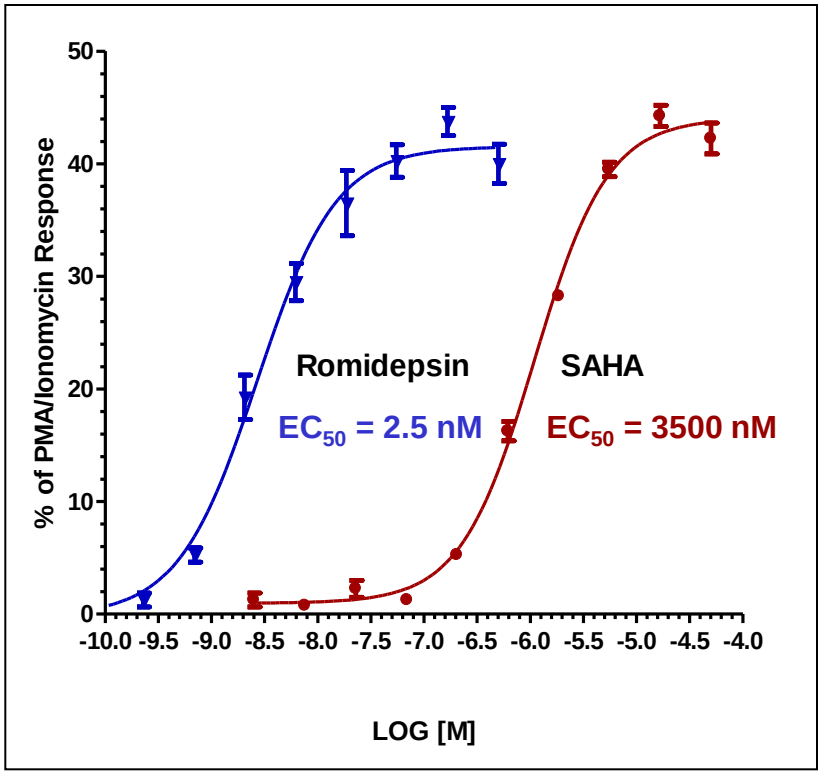
HDACi Romidepsin (RMD)

- FDA-approved for treatment of CTCL in patients who received prior systemic therapy
- Dosed at 14mg/m^2 (MTD) by IV infusion on Days 1, 8, 15 of 28 day cycle; multiple cycles
- AMES(-)
- Linear pharmacokinetics (1-24 mg/m^2)
- Metabolized by CYP3A4
- Common AEs: nausea, fatigue, vomiting
- Toxicities
  - Anemia, thrombocytopenia
  - Risk of QT prolongation (monitor K^+, Mg^{++} levels)



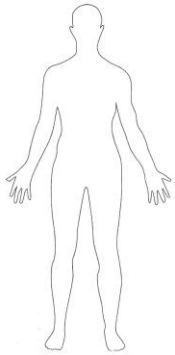
Reactivation of Latent HIV *In vitro* by HDACis Romidepsin and SAHA

HIV Latency Assay



- **Romidepsin is ~ 1000x more potent than SAHA at inducing latent HIV**
- **Romidepsin is a 1000 – 20,000-fold more potent inhibitor in HDAC enzymatic assays**

Ex vivo HIV Activation Assay with Memory CD4+ T-cells from HIV+ Subjects on ART



HIV+ subject on cART

HIV+ Leukopac
~10^{10} PBMCs

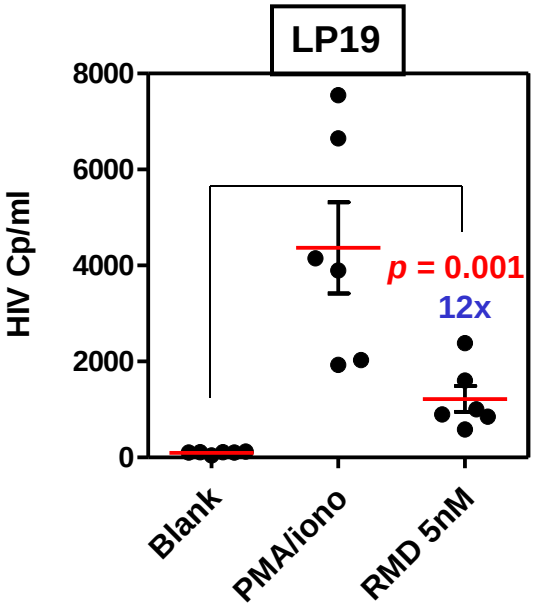
magnetic
bead isolation

PBMCs,
50 - 100 million / well

Memory CD4+ T cells,
8 - 10 million / well

Add compounds + ARV (6 day incubation)

Roche AmpliPrep/Taq: isolates and measures
HIV RNA from virions in culture supernatants



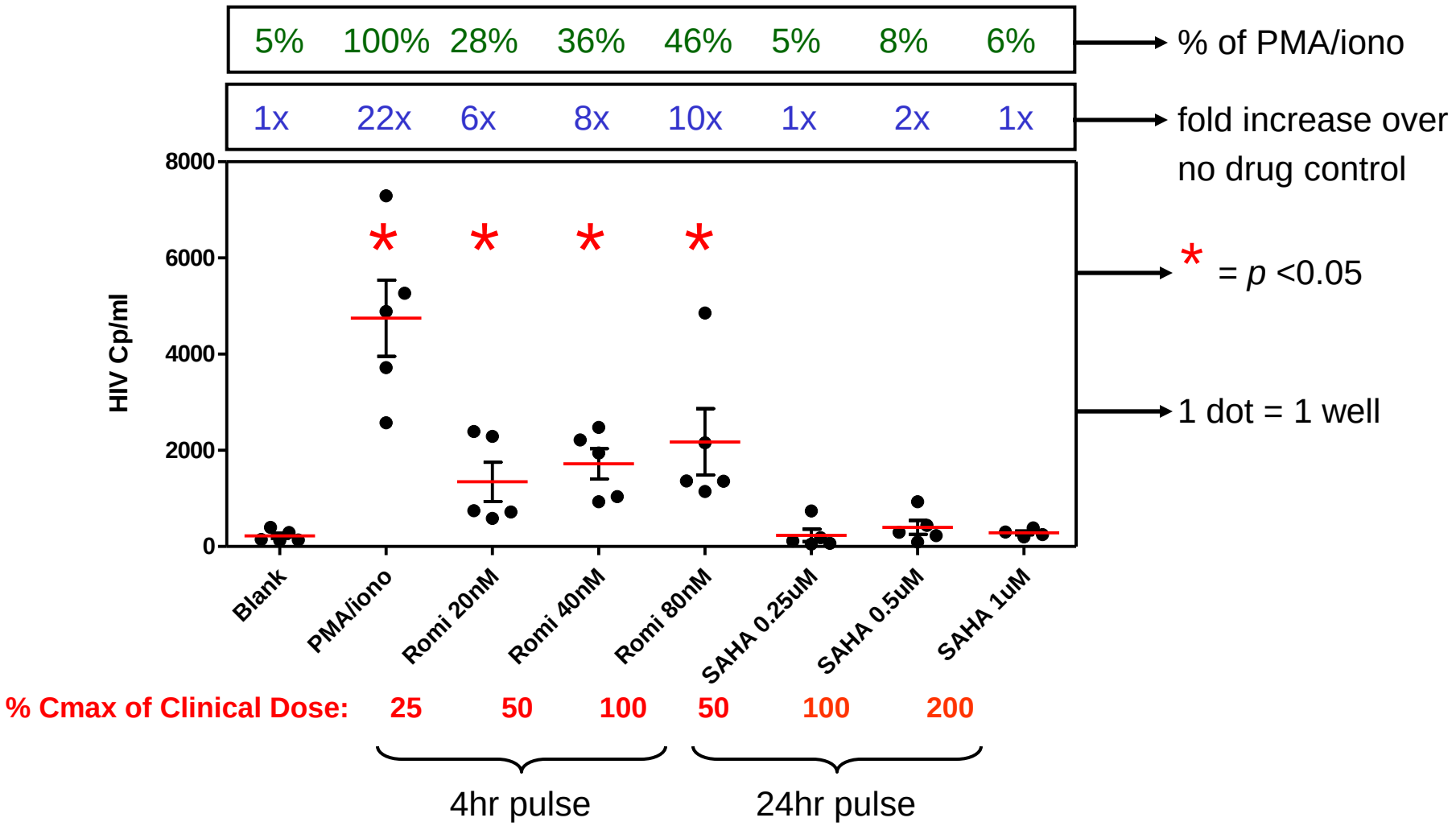
Assay adapted from Reuse *et al* (2009)

Romidepsin (5nM) Activates HIV in Memory $CD4^+$ T-cells from 12/13 HIV+ Subjects on ART

HIV-infected donor	Fold increase over no drug control	Average HIV RNA post-induction (copies/ml)	*p* value vs. no drug control
1	4.8	1,032	0.029
2	5.6	1,740	0.0058
3	14.0	1,435	0.0008
4	3.0	1,299	0.0005
5	5.9	1,685	0.015
6	14.0	315	0.019
7	5.2	599	0.00002
8	2.8	6,659	0.014
9	16.0	1,476	0.00003
10	20.1	10,908	0.0003
11	1.8	6	0.36
12	5.8	148	0.005
13	12.3	1,219	0.001
	Avg = 8.6		

5 nM RMD is equivalent to a C_{max} at 6% of dose for CTCL patients (14 mg/m^2) OR 0.9 mg/m^2

Romidepsin vs. SAHA in Memory CD4+ T-cells from HIV+ Subject (on ART)



Romidepsin vs. SAHA (Vorinostat) Human Drug Levels and Virus Activation

	Virus Activation		Pharmacokinetics		
	EC_{50} (nM)*	**Dose**	**C_{max} (nM)**	**Free Conc. at C_{max} (nM)****	**Free Conc./ EC_{50}**
Romidepsin	0.96	14 mg/m^2	697	49	51
Vorinostat	1,904	400 mg	1200	360	0.2

*In tissue culture medium containing FBS:
Romidepsin EC_{50} is 1.6nM x 60% (free drug) = 0.96 nM
Vorinostat EC_{50} is 2,800nM x 68% (free drug) = 1,904 nM

**In human serum:
Romidepsin is 93% bound, 7% free
Vorinostat is 70% bound, 30% free

- **Romidepsin may be a better inducer of latent HIV *in vivo***
- **Virus induction possible at a small fraction of MTD (for CTCL)**
- **Romidepsin is AMES(-) enabling multiple dose studies**

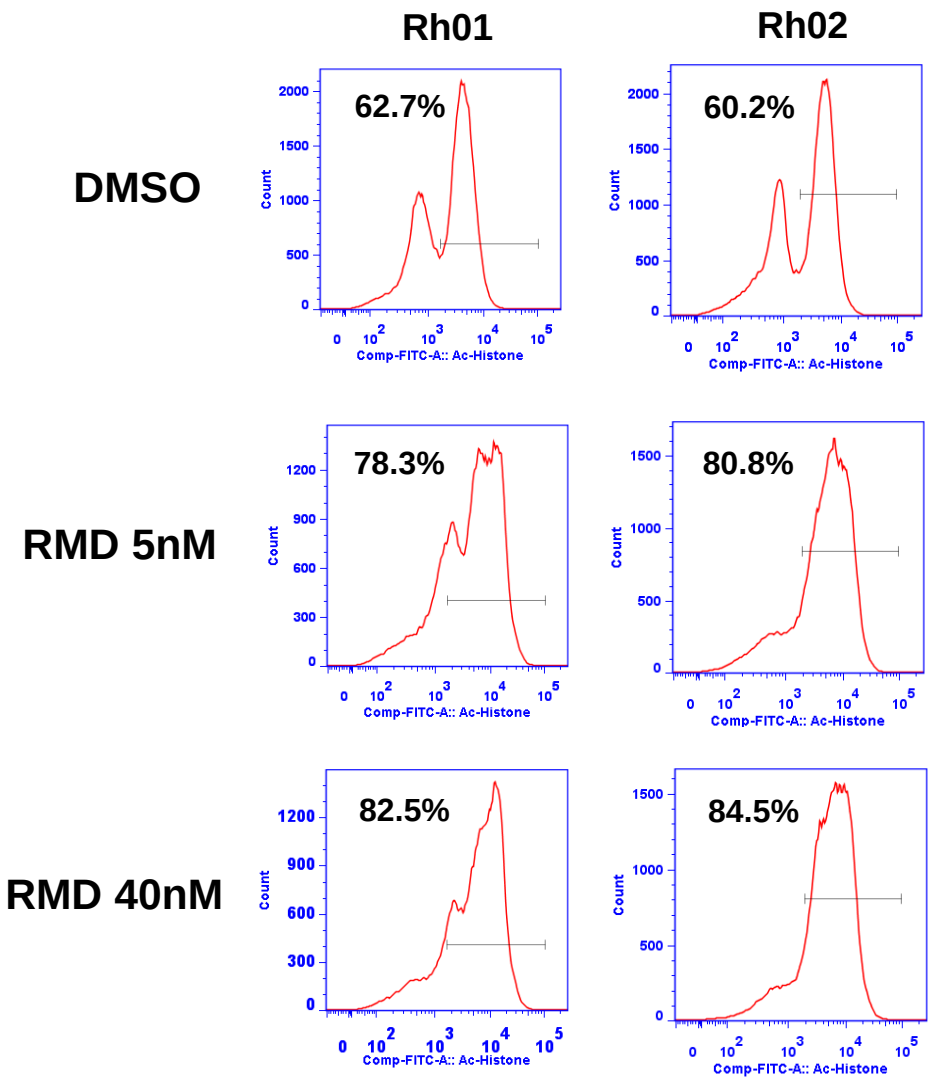
Romidepsin Study in Rhesus Macaques

PK	Dose	C_{max} (nM)	Free Fraction at C_{max} (nM)	Free Fract./ Intrinsic EC_{50}*
Human	14 mg/m^2	697	49	51
Rhesus	10 mg/m^2	245**	96	100

Intrinsic EC_{50} (HIV Latency Assay) = 0.96 nM
***** Berg et al, Cancer Chemother Pharmacol, 2004***

- **Most Significant AE in Rhesus Macaques: Transient leukopenia**

Romidepsin Increases Histone Acetylation in Rhesus Macaque PBMCs (in vitro)



- 4 hour pulse treatment with RMD
- Flow cytometry at 24h

High Throughput Pilot Screen using HIV Latency Assay

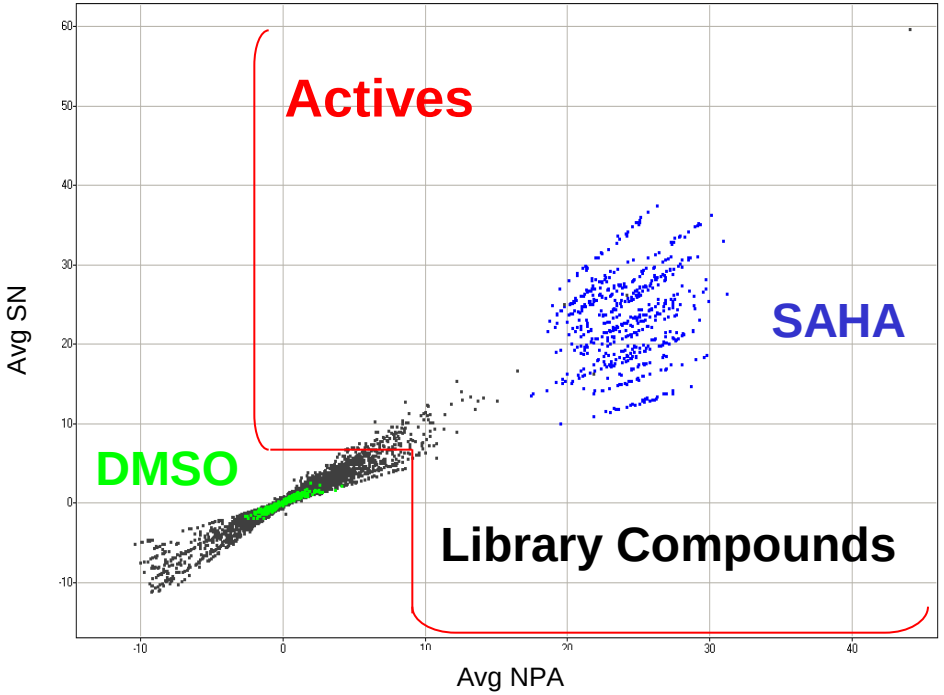
Subset of Gilead Library

- 1% Actives
  - 89 Compounds
  - 15 Substructure Clusters

Commercially available libraries

- 0.8% Actives
  - 16 compounds
  - 5 with cross-donor activity

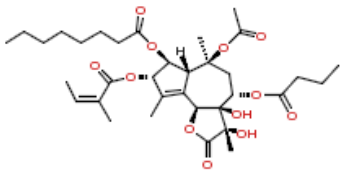
Compound Activity



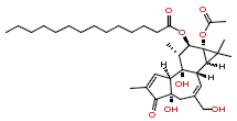
- Actives: 10 Std Dev above DMSO Control
- No DMSO false positives
- All SAHA data points cluster together

Hits from Library Screening

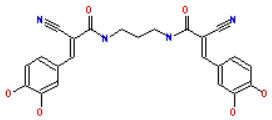
Thapsigargin



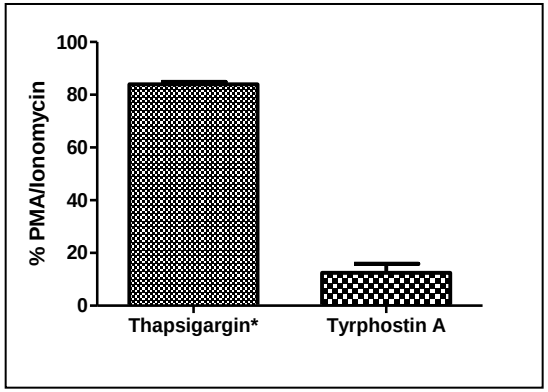
PMA



Tyrphostin A



HIV Reactivation



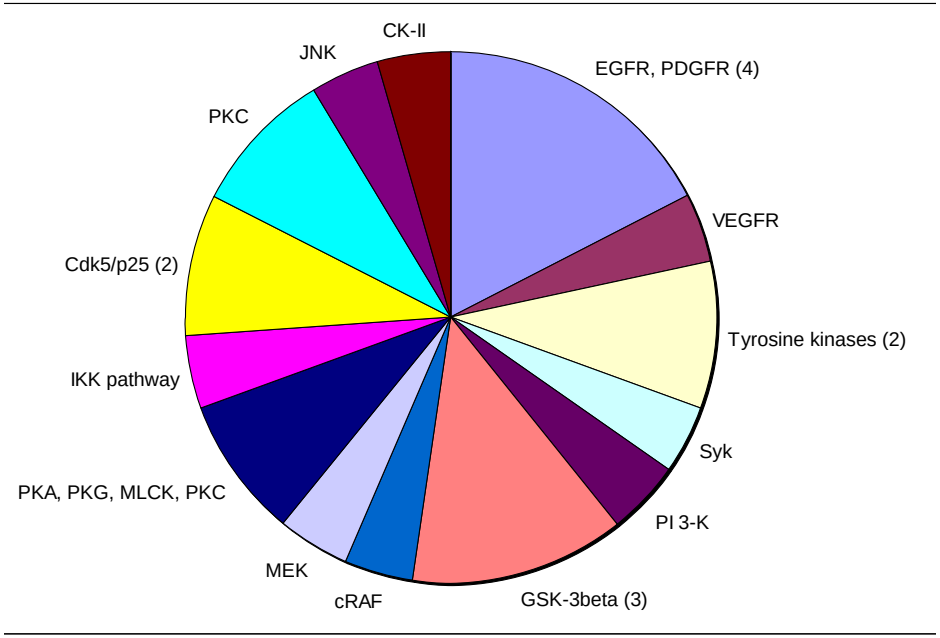
*Papp et al (1995)

	Thapsigargin	PMA	Tyrphostin A
Target	SER Calcium Pump	PKC	Tyr kinases
Mechanism	Inhibitor	Activator	Inhibitor
Pathway	[Ca++]i elevation / NFAT activation?	NFkB activation	?
Donors	6/6	6/6	3/6

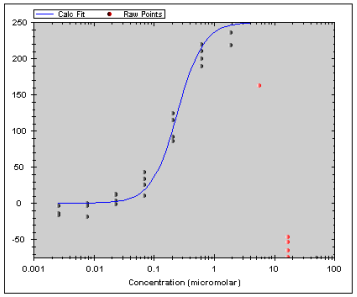
- **Actives reconfirmed as bona fide hits**
- **Potential new targets and mechanisms to explore**

HIV Reactivation with Kinase Inhibitors

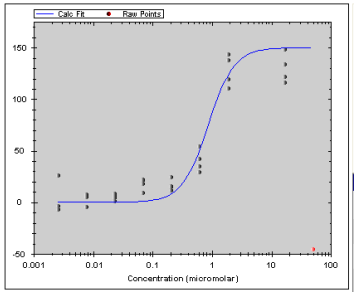
- Screened kinase inhibitors
  - 20% compounds with $EC_{50} < 10\mu M$
- Confirm activity with more selective kinase inhibitors
- Identify signaling pathways and novel targets



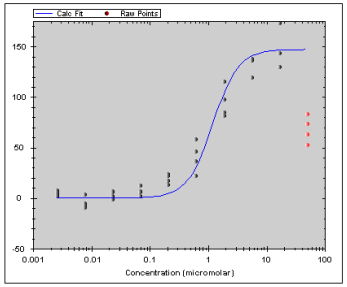
CDKi



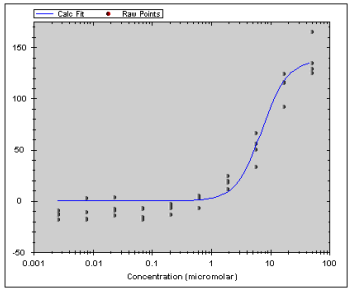
CDKi



Tyrphostin B

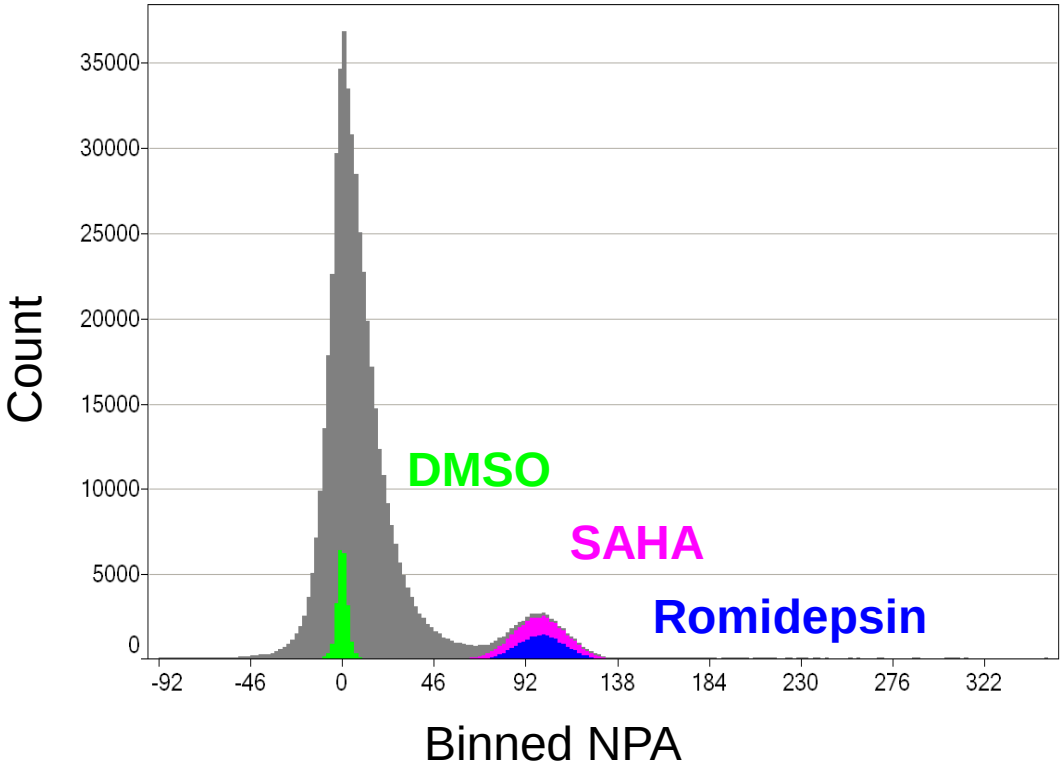


Tyrphostin C

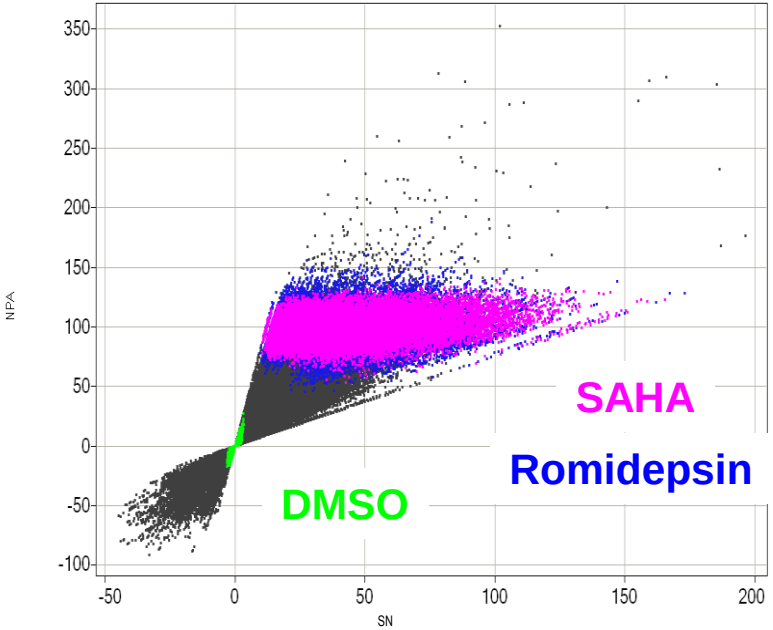


High Throughput Screen of Gilead's Compound Collection in HIV Latency Assay

Actives distribution: Normalized Percent Activity (NPA)



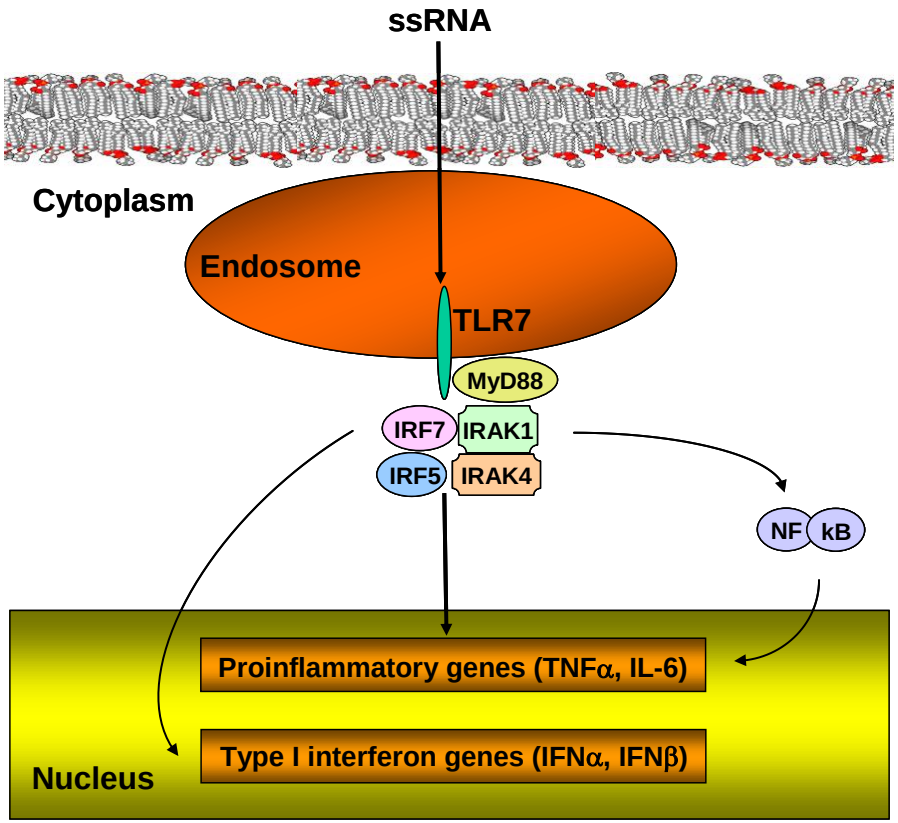
Compound activity by S/N and NPA



- **1% Hit rate**
- **Consistent SAHA and Romidepsin activity in all donors**

Toll-like Receptor 7 (TLR7)

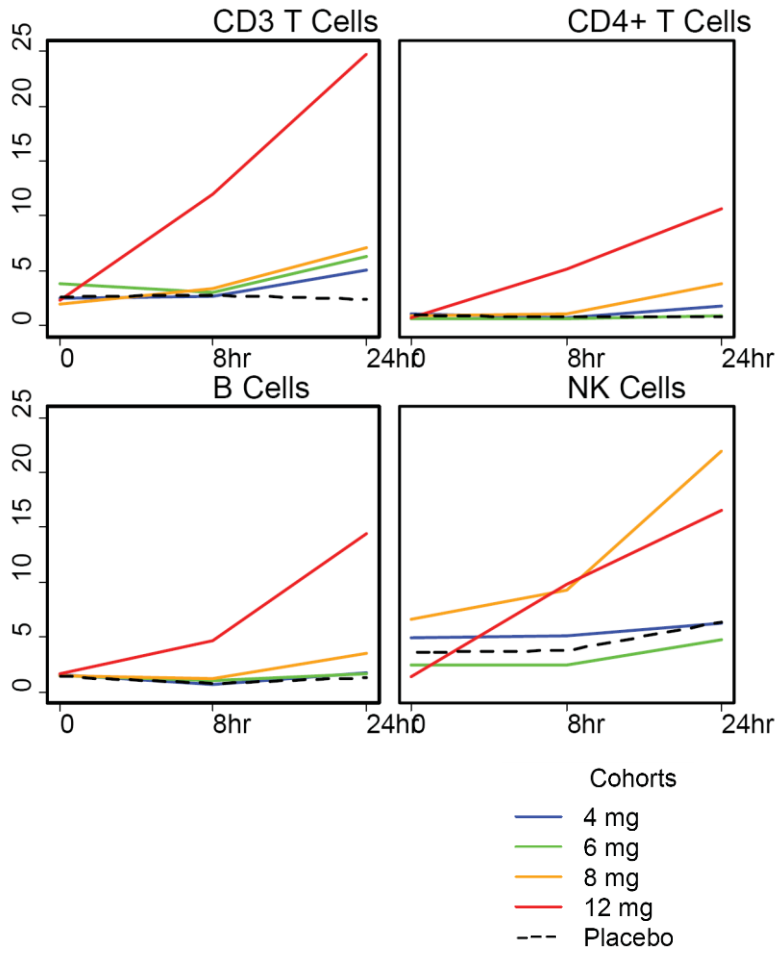
- Toll-like receptors (TLR) sense pathogen patterns
- TLR7 detects single-stranded RNA
- TLR7 activation leads to production of type I interferons (IFNα, IFNβ)



GS-9620 a Potent TLR7 Agonist

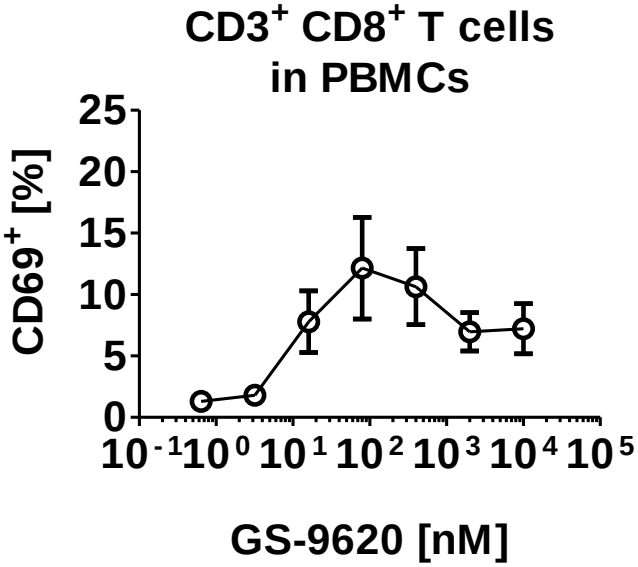
- **HBV-infected Chimps**
  - Treatment: 8 weeks
  - vDNA and HBsAg reduction
  - Dose-dependent IFNα increase
  - Activation of B-, T- and NK cells
- **WHV-infected Woodchucks**
  - Treatment: 4 weeks
  - vDNA and WHsAg reduction
  - Induction of anti-WHsAg Abs
- **Healthy Volunteers**
  - Phase I Study
  - SAD

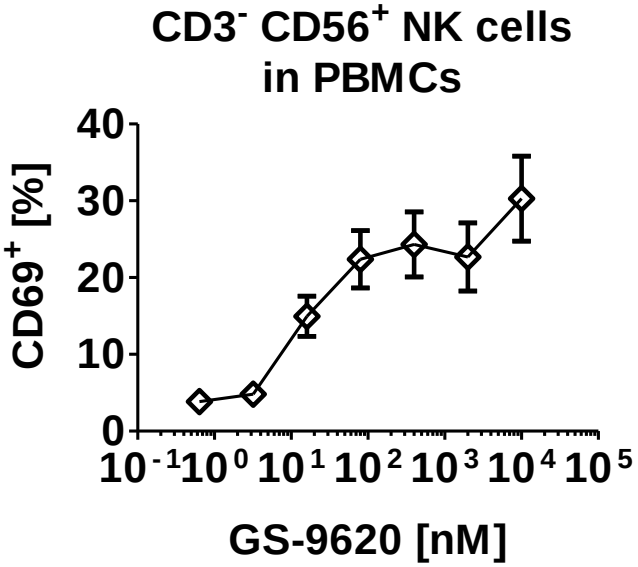
Median Lymphocyte Activation (%CD69$^+$) by Cohort



Lopatin *et al*, Lanford *et al*, Menne *et al* (EASL 2011)

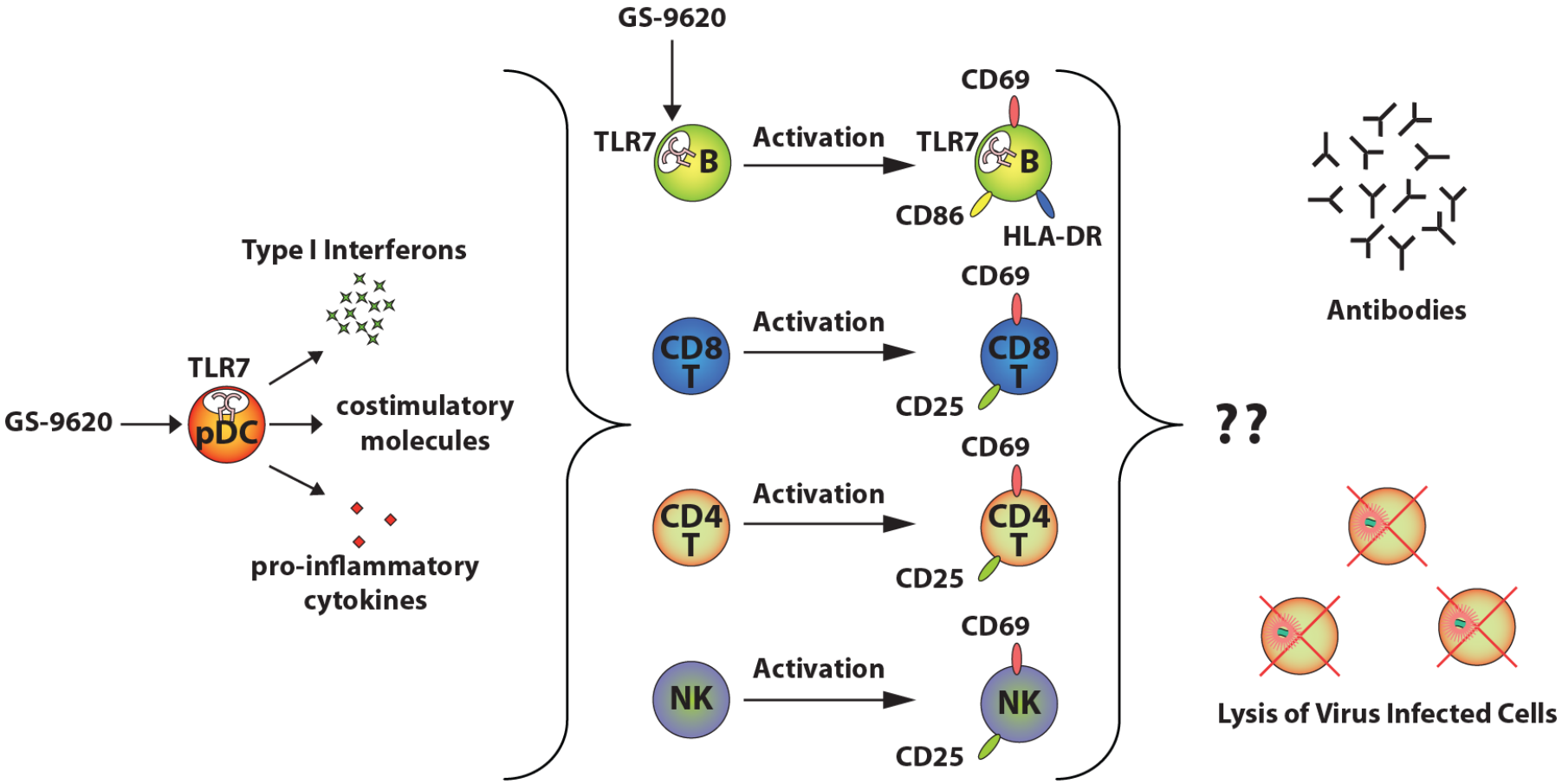
GS-9620 Activates NK and CD8+ T-cells in vitro





- **TLR7 agonist activates CD8+ T-cells and NK cells in human PBMC cultures**
- **No activation using purified cultures (indirect effect)**

Can TLR7 Agonists Enhance Immunity to Eliminate HIV-Infected Cells?



- Can activation of DCs help prime HIV-specific immune responses?
- Can activated CD8+ CTLs and NK cells help clear cells expressing reactivated HIV?

Future Directions

- Test HDACi and TLR7 agonist in animal model of AIDS
- Characterize HTS hits
  - Uncover novel mechanisms governing HIV latency
  - Identify molecular targets for drug discovery
    - Kinases may play a role in establishing HIV latency
  - Discover NCEs

Acknowledgements

Gilead

- Bei Li
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- Michael Graupe
- Randy Halcomb
- Bing Lu
- Jim Zheng
- Tiffany Barnes
- Mini Balakrishnan
- Anne Chester
- Tomas Cihlar
- Gregg Jones
- Paul Duatschek
- Joe Hesselgesser

University of Utah

- Vicente Planelles
- Alberto Bosque

NCI, Frederick

- Jeff Lifson