An *In Vitro* Compound Screen for Selective Apoptosis Induction In HIV-1 Infected Resting CD4⁺ T Cells

Adam M Spivak, Alberto Bosque, Vicente Planelles
University of Utah, Salt Lake City, UT, USA

Contact Information
Adam Spivak MD
Division of Infectious Diseases
University of Utah School of Medicine
30 North 1900 East 4B319 SOM
Phone: 801 585 7433
Fax: 801 585 3377
adam.spivak@hsc.utah.edu

Background

- Despite the ability of antiretroviral therapy to suppress viral replication, transcriptionally silent proviral HIV-1 persists in resting memory CD4⁺ T cells (known as the latent reservoir)

- Current "shock and kill" strategies to target the latent reservoir may induce proviral transcription *in vivo* [1] (the 'shock'), however this is unlikely to lead to reservoir depletion [2] (the 'kill') without an additional stimulus [3]

- We hypothesize that resting central memory CD4⁺ T cells (T_{cm}) expressing HIV-1 genes are phenotypically distinct from their uninfected and latently infected counterparts and can be selectively targeted for apoptosis

Methods

- We designed an *in vitro* screening assay to evaluate for selective apoptosis induction among primary T_{cm} actively expressing HIV-1 genes

- After isolating primary naïve CD4⁺ T cells from healthy donors, cells are activated *in vitro* and allowed to progress toward resting cultured T_{cm} phenotype in IL-2-containing culture (assay shown **above**, based on [4])

- Cells are infected with eGFP-pseudotyped HIV-1 ten days after activation when they demonstrate a resting phenotype

- Compound screening performed with commercial kinase inhibitor library (EMD Millipore). Cells exposed to compounds for 16 hours followed by flow cytometry evaluating for early apoptosis by Annexin V staining among GFP⁺ and GFP⁻ populations

Results

- A majority of protein kinase inhibitors tested showed no effect on apoptosis threshold, and several were found to be highly toxic to all cells in culture over the 16 hour exposure period

- We identified seven compound 'hits' that led to increase in apoptosis (>3SD above apoptosis mean value from negative control wells) among cells expressing HIV-1 genes with minimal bystander apoptosis

- These compounds affect a variety of intracellular pathways that have been associated with modulation of cellular apoptosis threshold including glycogen synthase kinase 3α and β, Rho kinase and extracellular signal-regulated kinases

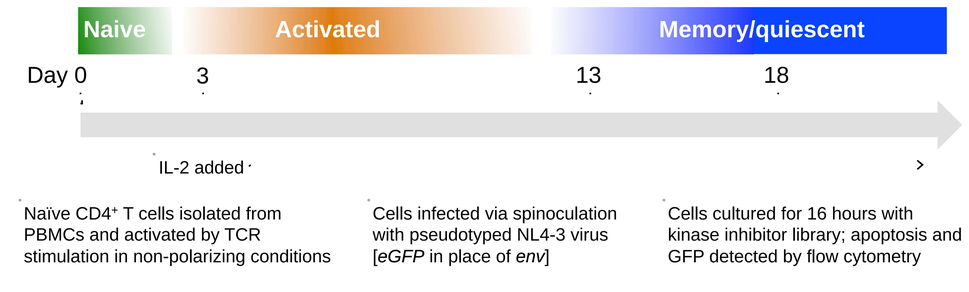
BILL & MELINDA GATES foundation

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References

1. Archin NM. Nature 2012
2. Spivak AM. Clin Infect Dis 2013
3. Shan L. Immunity 2012
4. Bosque A. Blood 2009

Assay Time Line

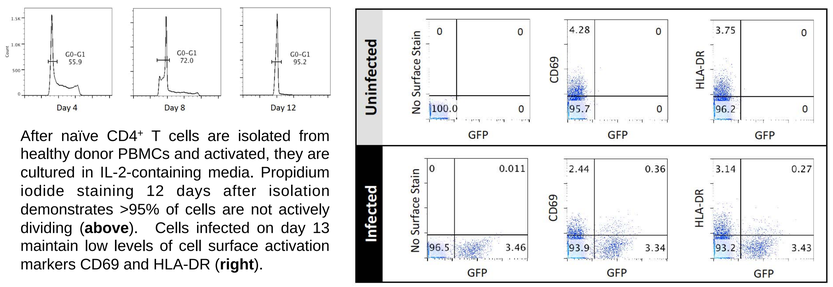


Naïve CD4⁺ T cells isolated from PBMCs and activated by TCR stimulation in non-polarizing conditions

Cells infected via spinoculation with pseudotyped NL4-3 virus [*eGFP* in place of *env*]

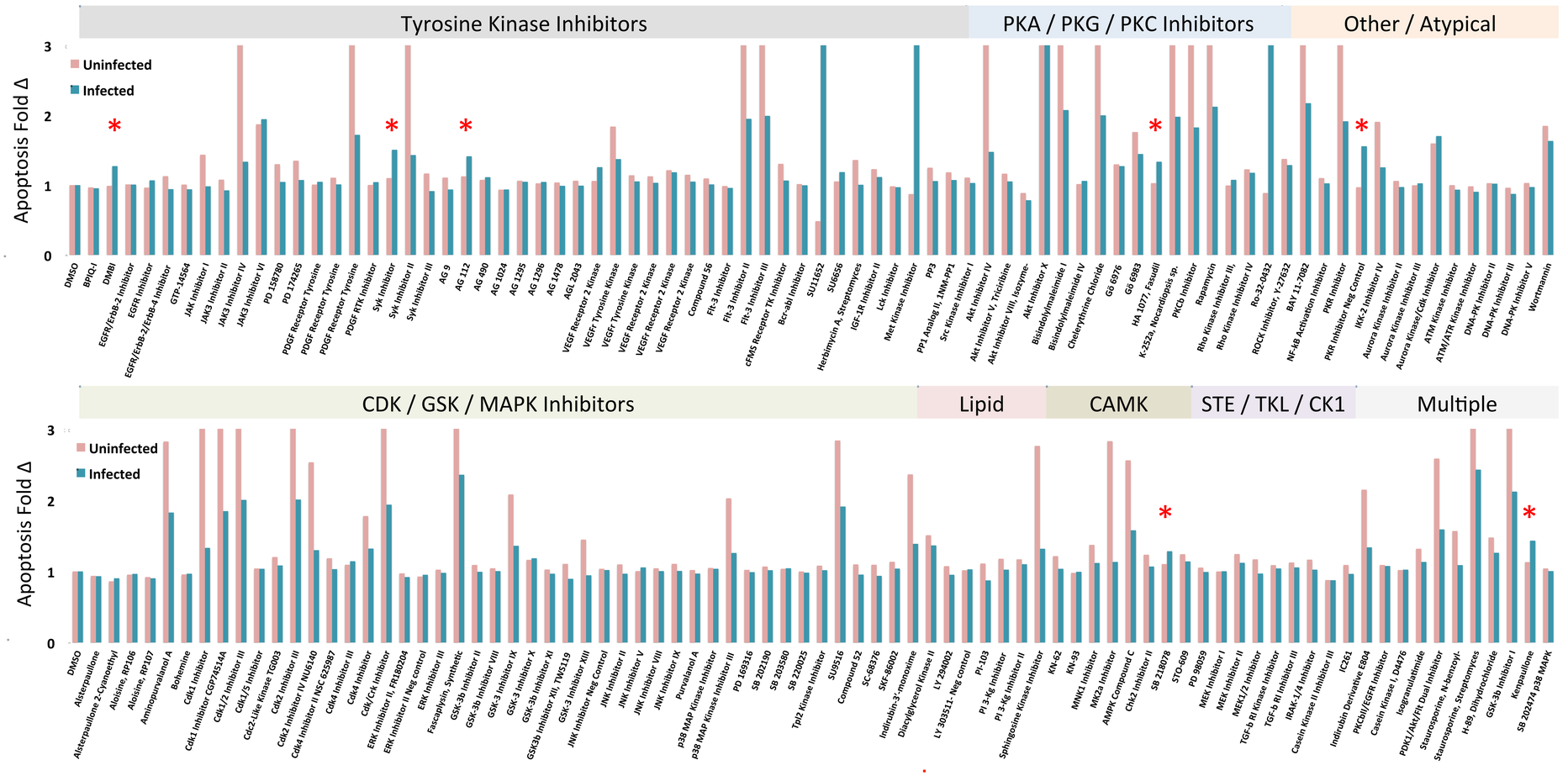
Cells cultured for 16 hours with kinase inhibitor library; apoptosis and GFP detected by flow cytometry

Primary Cell Phenotype

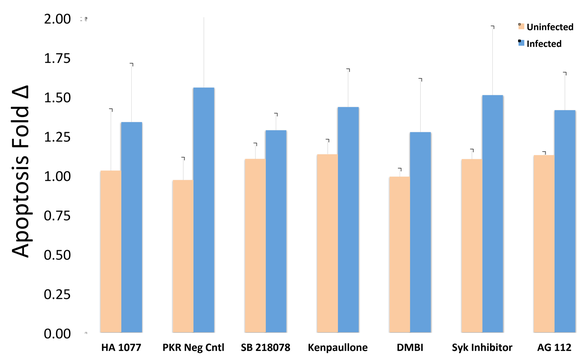


After naïve CD4⁺ T cells are isolated from healthy donor PBMCs and activated, they are cultured in IL-2-containing media. Propidium iodide staining 12 days after isolation demonstrates >95% of cells are not actively dividing (**above**). Cells infected on day 13 maintain low levels of cell surface activation markers CD69 and HLA-DR (**right**).

Kinase Inhibitor Apoptosis Induction



Kinase Inhibitor Screening Hits



Mean apoptosis fold changes (compared to DMSO controls) for three independent experiments are shown **above**. Seven compounds demonstrated selective apoptosis induction in GFP-expressing cells with minimal bystander cell apoptosis (**shown above with * and at left**). Four are protein tyrosine kinase inhibitors (**below**). HA 1077 and Kenpaullone both inhibit extracellular signal-regulated kinases which have been described to modulate apoptosis threshold.

Compound Name	Kinase Inhibitor Family	Intracellular Pathways Affected
HA 1077	AGC	Rho kinase, ERK 1 and 2
PKR Neg Cntl	OTHER	unknown
SB 218078	CAMK	Chk1
Kenpaullone	CMGC, CK1, TK	GSK3α and β, multiple CDKs, ERK1 and 2
DMBI	TK	PDGF β-receptor
Syk Inhibitor	TK	Syk TK
AG 112	TK	EDGF receptor TK

Conclusions and Future Directions

- We developed a primary cell screening assay to identify compounds that selectively induce apoptosis in resting T_{cm} that actively express HIV-1 proviral genes but not in uninfected bystander cells.

- A compound screen using a commercial protein kinase inhibitor library identified several candidate compounds that selectively induce apoptosis in resting T cells expressing HIV-1 genes. These results were reproducible across multiple independent experiments.

- Mechanistic studies are underway to understand the pathways involved in altering the apoptotic threshold of HIV-1 infected resting T cells. Additional screening using this assay may further ongoing eradication efforts.