Jak inhibitors towards a functional cure or eradication of HIV-1

December 7, 2016
Background

• Major barriers to HIV-1 eradication that are not addressed by existing antiviral agents.

• Preclinical *in vitro, ex vivo, and in vivo* profile of ruxolitinib, tofacitinib and baracitinib that demonstrates potent, specific inhibition of events driving viral persistence.

• Human clinical trials.
  • Currently enrolling multi-site Phase 2a ACTG sponsored study with ruxolitinib.
Inflammation that persists even in individuals with well-controlled viremia is thought to represent:

1) a major contribution to non AIDS and AIDS related morbidity and mortality.
2) a major barrier to eradication.
Data suggest that inflammation is a major contribution to non AIDS and AIDS related morbidity and mortality, and a major barrier to eradication.

**Interleukin-15 (IL-15) Strongly Correlates with Increasing HIV-1 Viremia and Markers of Inflammation.**
PMID: 27880829  Free Article

**Do Biomarkers of Inflammation, Monocyte Activation, and Altered Coagulation Explain Excess Mortality Between HIV Infected and Uninfected People?**

**Inflammatory and coagulation biomarkers and mortality in patients with HIV infection.**
PMID: 18942885  Free PMC Article

**Reservoir expansion by T-cell proliferation may be another barrier to curing HIV infection.**
Kim M, Silicicano RF.
PMID: 26862166  Free PMC Article

**Monocyte Activation is Associated with Worse Cognitive Performance in Virologically Suppressed HIV-Infected Women.**
Imp BM, Rubin LH, Tien PC, Plankey MW, Golub ET, French AL, Valcour VG.
PMID: 27789726
Systemic *in vivo* feedback loop:
Inflammation and immune activation drives viral persistence

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Systemic in vivo feedback loop:
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Systemic in vivo feedback loop: Inflammation and immune activation drives viral persistence

Inflammation that persists even in individuals with well-controlled viremia is thought to represent:

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How are inflammation and immune activation modulating specific cellular events that prevent eradication?
Specific pro-inflammatory events driven by **Jak-STAT** signaling that prevent eradication

- Positive correlation between pSTAT5 and IL-7R, which is associated with increased levels of integrated viral DNA (reservoir size). **Foundation for correlation between pSTATs and reservoirs.**

- PD-1, a marker of reservoir lifespan, correlates with levels of integrated viral DNA, pSTAT5, and homeostatic proliferation (reservoir maintenance/expansion).

- Activation and inflammation (IL-6, IL-7, TNF-α, IL-15, IL-1α/β, others) promotes productive viral replication and increases viral co-receptor, HLA-DR, and CD38, as well as increase in proliferation (reservoir reseeding).

- Pro survival signal Bcl-2 promotes survival of the viral reservoir (reservoir maintenance).

- IL-15, IL-7, and TNF-α induce reactivation of latent HIV-1 (reservoir reseeding).

- Reseeding of reservoirs occurs by ongoing low-level replication in pharmacological sanctuary sites not reached by HAART.

- HIV LTR shows multiple binding sites for pSTAT5, demonstrating that binding of this transcription factor to the LTR could promote pro-HIV transcripts.

- Bystander infection in cells with activated Jak-STAT signaling promotes priming uninfected cells for infection, recruitment of uninfected cells to the site of infection, and reseeding of reservoirs with ongoing low-level replication in pharmacological sanctuary sites.

- **STAT5-driven** homeostatic proliferation could increase absolute numbers of Tregs and lead to further immune dysregulation.

*Closer look at these dynamic events appears on the next slide.*
Inflammatory driven increase in Tregs = further immune dysregulation.

**A.**

Homeostatic Proliferation (Reservoir replication)

Pro-survival signal Bcl-2 can increase the lifespan of the reservoir.

**B.**

pSTATs correlate with reservoir size

Cytokines promote reactivation and reseeding of the viral reservoir.

**C.**

Activation/inflammation promotes increased viral replication and reservoir reseeding.

**D.**

IL-1α/β IL-15 IL-15 IL-15 IL-6 IL-6 pSTAT5 PD-1 HLA-DR CD38 CCR5 Uninfected Cell

IL-1α/β IL-15 IL-15 IL-15 IL-1α/β TNF-α TNF-α TNF-α IL-7 Uninfected Cell

**E.**

pSTAT binding sites in the HIV LTR promote infection and reservoir establishment.

**F.**

Jak-STAT activation, pSTAT binding promotes:
1) bystander cell infection.
2) infection/recruitment of uninfected cells.
**What is the role of Jak inhibitors in blocking these events?**

**A.** Homeostatic Proliferation (Reservoir replication)

Pro-survival signal Bcl-2 can increase the lifespan of the reservoir.

Inflammatory driven increase in Tregs = further immune dysregulation.

**B.** pSTATs correlate with reservoir size

pSTAT binding sites in the HIV LTR promote infection and reservoir establishment.

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**C.**

Jak-STAT activation, pSTAT binding promotes:
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**D.**

IL-1α/β, IL-15, IL-6, TNF-α, CD38, CCR5

IL-1α/β, IL-15, IL-6, TNF-α

**E.**

pSTAT binding sites in the HIV LTR promote infection and reservoir establishment.

**F.**

Uninfected Cell

Uninfected Cell
Blockade of these events with a Jak inhibitor could lead to purge of the viral reservoir, resulting in a functional cure or eradication of HIV-1.

Reservoir cell with current HAART

Reservoir persists, divides, expands.

Inability to eradicate HIV-1

Reservoir cell with ruxolitinib as HAART add on

Reservoir cells will die and reservoir could be eliminated.

- Decreased reservoir lifespan.
- Block reservoir expansion, reseeding.

- Possibility to remove HAART without viral rebound.
- Functional cure or eradication of HIV-1.
  - Shorter duration of treatment.
Can Jak inhibitors block these events?

A. Pro-survival signal Bcl-2 can increase the lifespan of the reservoir.

B. pSTATs correlate with reservoir size

- Homeostatic Proliferation (Reservoir replication)
- Cytokines promote reactivation and reseeding of the viral reservoir.

Inflammatory driven increase in Tregs = further immune dysregulation.

pSTAT binding sites in the HIV LTR promote infection and reservoir establishment.

Jak-STAT activation, pSTAT binding promotes:
1) bystander cell infection.
2) infection/recruitment of uninfected cells.
Markers of the Jak-STAT pathway and homeostatic proliferation are associated to HIV reservoir size \textit{in vivo}.

$N = 32$ HIV infected individuals

Jak inhibitors block cytokine-induced STAT5 phosphorylation and Bcl-2 expression. STAT5 phosphorylation is an anti-apoptotic signal; a decrease indicates a shorter lifespan or reservoir cell.

Ruxolitinib significantly reduces markers for reservoir lifespan in key reservoir subsets from HIV-positive viremic individuals \textit{ex vivo}.

$\text{Bcl-2} = \text{pro survival signal (decrease indicates shorter lifespan or reservoir cell).}$

Can Jak inhibitors block these events?

A. Pro-survival signal Bcl-2 can increase the lifespan of the reservoir.

B. pSTATs correlate with reservoir size

C. Activation/inflammation promotes increased viral replication and reservoir reseeding.

D. IL-1 α/β, IL-15, IL-6, TNF-α, IL-7

E. Jak-STAT activation, pSTAT binding promotes:
   1) bystander cell infection.
   2) infection/recruitment of uninfected cells.

Inflammatory driven increase in Tregs = further immune dysregulation.

pSTAT binding sites in the HIV LTR promote infection and reservoir establishment.
Jak inhibitors block HIV-1 replication and production \textit{ex vivo} and \textit{in vitro}, and inhibit upregulation of HIV co-receptor CCR5 in viremic donors.


***; p < 0.001, One-Way ANOVA
Ruxolitinib and tofacitinib inhibit T-cell markers associated with viral persistence, increased reservoir size and reseeding. 
ex vivo in CD4+ T cells of viremic donors

PD-1 is a major marker for cells undergoing homeostatic proliferation and reservoir size in vivo.

Gavegnano et al, Journal of Clinical Investigation, submitted

* p < 0.05, ** p < 0.01, *** p < 0.001, p < 0.001, One Way ANOVA
Jak inhibitors reduce frequency of cells harboring integrated viral DNA and IL-15-induced reactivation of latent HIV-1 \textit{ex vivo} in CD4 T cells.


* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$, One Way ANOVA
Ruxolitinib inhibits bystander infection


**** p < 0.0001, One Way ANOVA
Considerations for:

1. Safety and specificity
2. Pharmacokinetics *in vivo*
Ruxolitinib does not inhibit normal TCR function and signaling that is independent of HIV-1 infection

Considerations for:

1. Safety and specificity
2. Pharmacokinetics *in vivo*

All block of anti-HIV events occur at concentrations found *in vivo* (pharmacokinetic/dynamic analyses performed).
Ruxolitinib and/or baracitinib studies *ex vivo, in vitro, and in vivo.*

Primary human cells
Baracitinib anti-HIV properties at a glance in primary human cells

<table>
<thead>
<tr>
<th>Drug</th>
<th>EC$_{50/90}$ in PBM cells (µM)</th>
<th>EC$_{50/90}$ in macrophages (µM)</th>
<th>Inhibition of TNF-α induced reactivation (J-lat)</th>
<th>Inhibition of PMA induced reactivation (macrophages)</th>
<th>Reduction of non dividing latent CD4 T cells</th>
<th>Inhibition of HIV-induced HLA-DR and CD163 (macrophages)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baracitinib</td>
<td>0.07/5.9</td>
<td>0.4/7.9</td>
<td>0.7/1.9</td>
<td>0.4/0.7</td>
<td>0.05/0.7</td>
<td>0.1/1.2</td>
</tr>
<tr>
<td>Ruxolitinib</td>
<td>0.1/4.9</td>
<td>0.3/6.1</td>
<td>1.1/3.5</td>
<td>0.3/0.9</td>
<td>0.03/0.7</td>
<td>0.1/1.5</td>
</tr>
<tr>
<td>3TC</td>
<td>0.01/0.9</td>
<td>0.1/1.2</td>
<td>&gt; 50</td>
<td>37.4</td>
<td>37.4</td>
<td>23.7</td>
</tr>
</tbody>
</table>

- No observed toxicity ≥ 50 µM across all cells tested
- Therapeutic window > 100 for all measures reported

Gavegnano and Schinazi et al, AAC, 2013 and unpublished work.
Ruxolitinib and/or baracitinib studies *ex vivo, in vitro, and in vivo.*
The Janus kinase inhibitor ruxolitinib reduces HIV replication in human macrophages and ameliorates HIV encephalitis in a murine model

Woldeab B. Haile c,1, Christina Gavegnano a,c,1, Sijia Tao a,c, Yong Jiang a, Raymond F. Schinazi a,c,*, William R. Tyor b,c,**

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Ruxolitinib and/or baracitinib studies *ex vivo, in vitro, and in vivo.*

Non human primate studies
Schema of sample draws and assays

Week

-1 0 1 2 3 4 5 6 7 8

Collect Sample For viral load

Collect plasma to quantify ruxolitinib levels (PK)

PK sampling 1, 2, 4, 8, 12 hr

2.1 mg/kg oral ruxolitinib bid

PK sampling 1, 2, 4, 8, 12 hr

Add HAART regimen

Collect plasma to quantify ruxolitinib levels (PK)

* To be conducted to ensure that addition of HAART does not modify ruxolitinib PK

Withdraw all drugs
Ruxolitinib does not increase viral loads in pilot study

**Reo-8**
Baseline 346,674 HIV-1 RNA copies/ml

**RWI13**
Baseline 108,171 HIV-1 RNA copies/ml

**RRn-11**
Baseline 400,000 HIV-1 RNA copies/ml

**RHs-13**
Baseline 174,000 HIV-1 RNA copies/ml

= 2.1 mg/kg Jakafi bid
= 2.1 mg/kg Jakafi + HAART (Kaletra + (-)-FTC + PMPA + Raltegravir)
= No drugs
Monkey REO-8; 2 of 4 monkeys responded to ruxolitinib treatment

- MIP-1 beta
- MCP-1
- IL-17 RA
- sCD40L
- IL-10
- IL-6
- IL-4 (all time points below limit of detection)
- IL-1RA
- IL-1 beta
- TNF-alpha
- TNFalpha pg/ml
- IFN-g pg/ml
Summary Conclusions for Preclinical Evaluation of Ruxolitinib/Baracitinib

• Jak inhibitors demonstrate potent, specific inhibition of key events that prevent eradication of HIV-1, which is a unmet clinical need for HIV-infected individuals.
  • Reservoir establishment, maintenance, lifespan, reseeding.
  • Reservoir maintenance.
  • CNS infection and HIV-induced encephalitis and neurocognitive impairments (HAD/HAND).

• Human studies are underway with a phase 2a ACTG sponsored study “Evaluating the safety and tolerability of ruxolitinib in Antiretroviral treated HIV-infected individuals”.

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Team members

- Raymond F. Schinazi, PhD, DSc
- Christina Gavegnano, PhD
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Additional slides
What is the Tyor model and what is it designed to answer?

1. Infect primary human МΦ with HIV-1_{baL}

2. Inject HIV-infected МΦ into a localized site in the murine brain

3. МΦ infection induces:
   - pro-inflammatory cytokines
   - Astrogliosis
   - Astrocyte death
   - Neuron death
   - Microglia death
   - Increased infection in human МΦ implant

4. In situ hybridization to measure:
   - Astrogliaosis
   - Astrocyte death
   - Neuron death
   - Microglia death
   - Increased infection in human МΦ implant
Ruxolitinib crosses the blood-brain barrier in a murine HIV/CNS model

Table 1
Ruxolitinib concentrations in the brain of mice treated with high-dose ruxolitinib (50 mg/kg per injection). Posterior fossa from 6 mice were homogenized and drug levels were measured by tandem mass spectroscopy. All six mice receiving high dose ruxolitinib demonstrated quantifiable concentrations of the drug in the posterior fossa that were harvested during steady-state pharmacokinetics of drug treatment. Concentrations ranged from 34.1–247.2 ng/g of tissue.

<table>
<thead>
<tr>
<th>Sample #</th>
<th>Ruxolitinib (ng/g)</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>133.9</td>
</tr>
<tr>
<td>2</td>
<td>247.2</td>
</tr>
<tr>
<td>3</td>
<td>34.1</td>
</tr>
<tr>
<td>4</td>
<td>139.2</td>
</tr>
<tr>
<td>5</td>
<td>141.8</td>
</tr>
<tr>
<td>6</td>
<td>130.2</td>
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</table>
Before we look at data:
What do we expect for CD45, MAP2, and GFAP in infected versus uninfected mice?

• CD45 is a murine microglial/macrophage marker, and is expected to **INCREASE** in HIV-infected mice due to infiltration of cells from the periphery.
  • Expectation of percentages from Tyor model: 1-5 % positive cells.

• MAP2 is a neuronal marker, and HIV-infection **DECREASES** neuronal expression (death, less dendrites due to inflammation).
  • Expectation of percentages from Tyor model: 30-60 % positive cells.

• GFAP is a marker for astrogliosis (astrocyte activation/inflammation), and HIV infection **INCREASES** this marker.
  • Expectation of percentages from Tyor model: 10-20 % positive cells.
Ruxolitinib decreases *in vivo* markers of HIV-induced encephalitis in a murine CNS HIV model

**A. GFAP**

**B. p24**

**C. CD45**

**D. MAP-2**

Haile and Gavegnano et al, Neurobiology of Disease, 2016
Bcl-2 is up-regulated in HIV infection in lymphocytes and macrophages, and is associated with increased reservoir size in vivo.
Pharmacokinetic simulation for 10 mg and 20 mg bid dosing of ruxolitinib demonstrates that anti-HIV effects occur at physiologically relevant concentrations observed in humans.

Figure 7. Pharmacokinetic simulation for 10 mg and 20 mg bid dosing of ruxolitinib demonstrates that anti-HIV effects occur at physiologically relevant concentrations observed in humans. Simulation of in vivo pharmacokinetics of 10 mg (A) or 20 mg (B) bid ruxolitinib demonstrated that all pro-HIV events that were inhibited by ruxolitinib in vitro occur at or below concentrations within the steady state plasma concentrations observed in vivo for 10 mg bid (A), and 20 mg bid (B). Dotted lines denote IC_{50} at which ruxolitinib confers inhibition in vitro, and notations of A-D denote: CD3 zeta and pSLP76, A; inhibition of Bcl-2 activation, B; inhibition of maintenance and expansion of the T cell reservoir, and antiviral potency against chronic and acute infection, C; inhibition of proliferation/activation (CD38/HLADR, PD1), down regulation of CCR5, inhibition of pSTAT5 by IL-2, IL-7, IL-15, inhibition of bystander infection, D.
