Baricitinib reverses HIV associated neurocognitive disorders and reservoir seeding in *in vivo, in vitro* and *ex vivo*
Background:
Barriers to an HIV Cure

• Current HAART cannot eliminate HIV-1.
  • Viral reservoirs:
    – Myeloid (including brain/CNS).
    – Lymphoid.
    – Pharmacological sanctuaries.
  • Ongoing inflammation (sCD14, IL-6, TNF-α, IL-7/15, D-dimer, sCD163, IL-1-α/β, others) even in individuals with well-controlled viremia contributes to reservoir:
    – Establishment, maintenance, and expansion.

Unmet clinical need = safe, specific, potent inhibitors of HIV-induced inflammation.
**Hypothesis:**
Blockade of HIV-induced inflammation with a Jak inhibitor could lead to purge of the viral reservoir, resulting in a functional cure or elimination of HIV-1.

Reservoir cell with current HAART

Reservoir persists, divides, expands.

Inability to eliminate HIV-1
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Blockade of HIV-induced inflammation with a Jak inhibitor could lead to purge of the viral reservoir, resulting in a functional cure or elimination of HIV-1.

Reservoir cell with current HAART

- Reservoir persists, divides, expands.
- Inability to eliminate HIV-1

Reservoir cell with ruxolitinib as HAART add on

- Decreased reservoir lifespan.
- Block reservoir expansion, reseeding.
  - Possibility to remove HAART without viral rebound.
  - Functional cure or elimination of HIV-1.
  - Shorter duration of treatment.

Reservoir cells will die and reservoir could be eliminated.
Markers of the Jak-STAT pathway and homeostatic proliferation are associated to HIV reservoir size \textit{in vivo}

\begin{itemize}
\item \textbf{A} Absolute CD4 counts
\item \textbf{B} pSTAT5 (MFI) in CD4 T cells
\item \textbf{C} IL-7R (MFI) in CD4 T cells
\item \textbf{D} PD1 (MFI) in CD4 T cells
\item \textbf{E} % positive DR-38 cells in CD4 T cells
\end{itemize}

HIV DNA (log 10^6 CD4 T cells)

N = 32 HIV infected individuals

Ruxolitinib and tofacitinib inhibit T-cell markers associated with viral persistence, increased reservoir size and reseeding \textit{ex vivo} in CD4+ T cells of viremic donors.

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

\* $p < 0.05$, \** $p < 0.01$, \*** $p < 0.001$, \**** $p < 0.0001$, One Way ANOVA
Jak inhibitors reduce frequency of cells harboring integrated viral DNA ex vivo in CD4 T cells from HIV+ individuals


* p < 0.05, ** p < 0.01, *** p < 0.001, p < 0001, One Way ANOVA
Jak inhibitors block reseeding of the HIV reservoir *ex vivo* in CD4 T cells from HIV+ individuals

CD4+ T cells from ART treated aviremic donors

<table>
<thead>
<tr>
<th>IL-15 +/-</th>
<th>CD3/CD28 +/- 1.0 μM ruxolitinib</th>
</tr>
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<tr>
<td>6 days</td>
<td></td>
</tr>
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Quantify reactivated HIV-1 (extracellular HIV-1 RNA copies)

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Ruxolitinib blocks bystander infection in primary CD4+ T cells

Gavegnano et al, PLOS ONE, submitted 2017

**** p < 0.0001, One Way ANOVA
Jak inhibitors block reservoir establishment, maintenance, and expansion in primary monocytes/macrophages \textit{in vitro}

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<th>Drug</th>
<th>EC$_{50/90}$ in PBM cells, µM</th>
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- No observed toxicity ≥ 50 µM across all cells tested.
- Therapeutic window > 100 for all measures reported.
- All concentrations that block pro-HIV events are physiological.

Gavegnano and Schinazi et al, AAC, 2013 and unpublished work.
Jak inhibitors block reservoir establishment, maintenance, and expansion in primary monocytes/macrophages *in vitro*

Reservoir reseeding from potential pharmacological sanctuary sites

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<th>Reduction of non-dividing latent CD4 T cells, µM</th>
<th>Inhibition of HIV-induced HLA-DR and CD163 (macrophages, µM)</th>
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• Therapeutic window > 100 for all measures reported.
• All concentrations that block pro-HIV events are physiological.

Gavegnano and Schinazi et al, AAC, 2013 and unpublished work.
Question

What about Baricitinib for HIV Associated Neurocognitive Disorder (HAND)?
Baricitinib for HAND

- HAND “HIV Associated Neurocognitive Disorder”.
  - > 50% of HIV+ individuals with well controlled viremia have HAND.
  - Significant percentage of HIV+ individuals without well controlled viremia have HAND.
  - No FDA approved treatments.
Baricitinib for HAND

- HAND “HIV Associated Neurocognitive Disorder”.
  - > 50% of HIV+ individuals with well controlled viremia have HAND.
  - Nearly all HIV+ individuals without well controlled viremia have HAND.
  - No FDA approved treatments.

- Major driver of HAND is HIV-induced inflammation:
  - HIV infection in microglia inflames the brain, inducing cell death and damage to neurons and astrocytes.
  - CNS infection promotes infiltration of activated monocytes and macrophages to the brain.
  - Chronic inflammation results in neurocognitive impairment and is not addressed by existing HAART.
Baricitinib for HAND

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- Baricitinib is a Jak 1/2 inhibitor with potent anti-inflammatory properties, including *in vivo* blockade of:
  - IL-6
  - TNF-α
  - D-dimer
  - IL-1-α/β
  - IL-10
  - CRP
  - IL-7, IL-15
Advantages of baricitinib versus ruxolitinib

**Jak 1/2 inhibitors that are nanomolar in vivo inhibitors of IL-6, IL-1 α/β, TNF-α, CRP, D-Dimer, other inflammatory markers.**

- **FDA approved for myelofibrosis (2011).**
- **FDA approved for polythemia vera (2014).**
- **Orally available bid dosing (10-15 mg).**
- **Hepatic clearance.**
- **No approval for pediatric population.**
- **ACTG sponsored multi-site Phase 2A study “A Randomized, Pilot Study of Ruxolitinib in Antiretroviral-Treated HIV-Infected Adults in HIV-infected subjects” (n = 60; underway).**

- **EU and Japan approval for rheumatoid arthritis.**
- **FDA approval pending in the United States.**
- **Orally available qid dosing (1, 2, 4 mg).**
- **Renal clearance.**
- **Second generation Jak inhibitor with reduced toxicity profile.**
- **Approved in pediatric populations (EU, Japan).**
Our group and more recently others demonstrated that Jak inhibitors can block HIV replication and associated inflammation in macrophages and T cells \textit{in vitro, ex vivo, and in vivo}. 

\textbf{Novel mechanisms to inhibit HIV reservoir seeding using Jak inhibitors.}

Gavegnano C\textsuperscript{1}, Brehm JH\textsuperscript{2}, Dupuy FP\textsuperscript{3}, Tallia A\textsuperscript{2}, Ribeiro SP\textsuperscript{2}, Kulpa DA\textsuperscript{1}, Cameron C\textsuperscript{2}, Santos S\textsuperscript{4}, Hurwitz SJ\textsuperscript{1}, Marconi VC\textsuperscript{5}, Routy JP\textsuperscript{6}, Sabbagh L\textsuperscript{7}, Schinazi RF\textsuperscript{1}, Sékaly RP\textsuperscript{2}.

\textbf{Ruxolitinib and tofacitinib are potent and selective inhibitors of HIV-1 replication and virus reactivation in vitro.}

Gavegnano C\textsuperscript{1}, Detorio M, Montero C, Bosque A, Planelles V, Schinazi RF.

\textbf{The Janus kinase inhibitor ruxolitinib reduces HIV replication in human macrophages and ameliorates HIV encephalitis in a murine model.}

Halle WB\textsuperscript{1}, Gavegzano C\textsuperscript{2}, Tao S\textsuperscript{2}, Jiang Y\textsuperscript{3}, Schinazi RF\textsuperscript{4}, Tyor WR\textsuperscript{5}.

\textbf{Janus kinase inhibition suppresses PKC-induced cytokine release without affecting HIV-1 latency reversal ex vivo}

Adam M. Spivak\textsuperscript{11}, Erin T. Larragoite\textsuperscript{21}, McKenna L. Coletti\textsuperscript{2}, Amanda B. Macedo\textsuperscript{2}, Laura J. Martins\textsuperscript{2}, Alberto Bosque\textsuperscript{1} and Vicente Planelles\textsuperscript{2}.
Hypothesis:
Baricitinib can reverse HAND in the murine HAND model (and potentially in humans) due to its potent anti-inflammatory properties, and its ability to block HIV replication and reactivation in primary macrophages and microglia.
HAND model: 
*Human macrophages in a SCID mouse*

ORT = Object Recognition Test; quantifiable measure of cognitive function.

Questions

1. Does baricitinib cross the blood-brain-barrier (BBB)?

2. Does baricitinib reverse HIV-induced neurocognitive impairments?
   1. Object recognition measurements (memory).
   2. Cellular markers:
      » p24⁺/CD163⁺ cells (human macrophages).
      » Activated microglia (MHCII⁺/CD45⁺).
      » MAP-2 (neuronal death).
      » GFAP (astrogliosis).
Methods

• N = 5 per cohort C57/black mice were administered a single dose of 10 or 50 mg/kg baricitinib subcutaneously.
• At 1, 4, 8 and 24 h, plasma and brain samples were collected and assayed with LC-MS/MS to quantify concentrations of baricitinib.
Baricitinib crosses the BBB and concentrations at 1 hr are similar to ruxolitinib

Baricitinib concentrations in the brain

Linear pharmacokinetic dynamics

Baricitinib (ng/g)
Questions

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HAND model:  
*Human macrophages in a SCID mouse*

**Focusing on object recognition:**

ORT = Object Recognition Test; quantifiable measure of cognitive function.

- **Baricitinib subcutaneous injection q.d.**
  - Group 1: 10 mg/kg baricitinib
  - Group 2: 50 mg/kg baricitinib
Object Recognition: 
*Did I see that before?*

1. **Training Phase:** 10 minutes to “acclimate” to the area and objects.

2. **Preference Test:**
   - One object is replaced with a new object.
   - 5 min observation of time spent exploring objects after 5 minute or 2 hr delay from original exploration period.

3. **Discrimination Index Calculation:**
   - \[
   \frac{\text{time spent with object A} - \text{time spent with object B}}{\text{total time exploring both objects}}
   \]
   - Discrimination indices of 0 indicate equal exploration of both objects.
10 mg/kg and 50 mg/kg dose baricitinib results in reversal of HAND

N = 40 total mice; 10 per group

* Significant difference versus HIV infected mice

D13 - 2hr Delay

- Uninfected control
- HIV
- HIV + 10 mg/kg baricitinib
- HIV + 50 mg/kg baricitinib

* p - 0.014
* p - 0.013
* p - 0.003
Questions

1. Does baricitinib cross the blood-brain-barrier (BBB)?

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       » p24⁺/CD163⁺ cells (human macrophages).
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10 mg/kg and 50 mg/kg baricitinib block HIV infection and HIV-induced activation and inflammation in the HAND model in a dose dependent manner.

**Activated murine microglia (MHCII/CD45)**

Uninfected control  | HIV  | HIV + low dose baricitinib  | HIV + high dose baricitinib

**HIV-infected human macrophages (p24/CD163)**

Uninfected control  | HIV  | HIV + low dose baricitinib  | HIV + high dose baricitinib

* p < 0.01; significant difference versus HIV infected mice.
** p < 0.001; significant difference versus HIV infected mice.
*** p < 0.05; significant difference versus HIV infected mice.
Additional \textit{in vitro} information: Baricitinib blocks key events driving HIV persistence in myeloid cells

- Summary of data not shown today:
- Baricitinib blocks:
  - HIV replication in primary human macrophages and microglia-like cells.
  - HIV reactivation from macrophages harboring latent HIV.
  - HIV-induced activation of primary monocytes and macrophages:
    - CD14$^+$/CD16$^+$ (monocytes).
    - HLA-DR$^+$/CD163$^+$ (macrophages).
Conclusions

• Baricitinib crosses the BBB.

• Baricitinib is markedly more potent in the murine HAND model *versus* data published from our group on ruxolitinib (2016).

• 10 mg/kg and 50 mg/kg baricitinib reverse HAND in the murine HAND model.

• 10 mg/kg and 50 mg/kg baricitinib block p24 production and HIV-induced activation in macrophages/microglia in the HAND model.
  
  • Dose dependence observed; both doses confer statistical significance, lower p-values for high dose baricitinib.

• Baricitinib blocks key events that drive viral persistence in the CNS, including HIV infection, HIV-induced activation, and HIV reactivation in primary macrophages and microglia-like cells.

• Baricitinib as a treatment for HAND needs to be evaluated in humans.
Team members

- Raymond F. Schinazi, PhD, DSc
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- Vincent Marconi, MD
- William R. Tyor, MD
- Woldeab Haile, PhD
- Selwyn J. Hurwitz, PhD
- Sijia Tao, PhD

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