Ruxolitinib-Mediated HIV-1 Reservoir Decay in A5336 Phase 2a Trial

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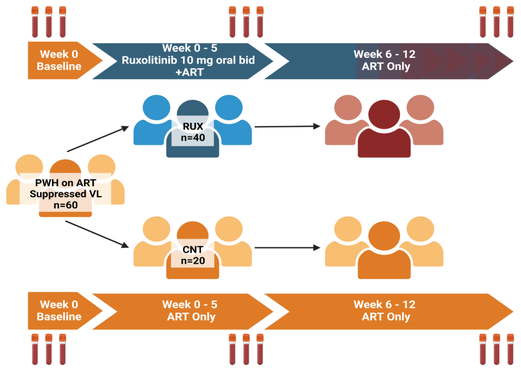
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Background

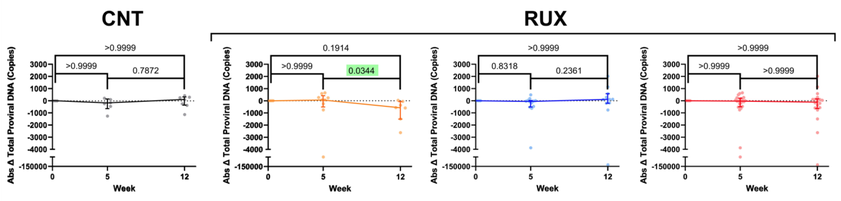
- For 38 million people living with HIV (PWH), there are no approved therapeutics that target the HIV reservoir, the greatest barrier to HIV cure
- PWH experience chronic inflammation and immune dysregulation due to chronic infection
- Chronic inflammation is largely driven by the Jak STAT pathway which is both activated by and produces pro-inflammatory and pro-pathogenic factors
- This uncontrolled systemic inflammation contributes to reservoir maintenance and phenomena such as inflammaging, immune exhaustion
- **Here, we aim to determine the effect of FDA approved ruxolitinib on immune dysregulation and the HIV-1 reservoir in PWH on ART in the phase 2a multi-site human trial (A5336)**

Trial Design

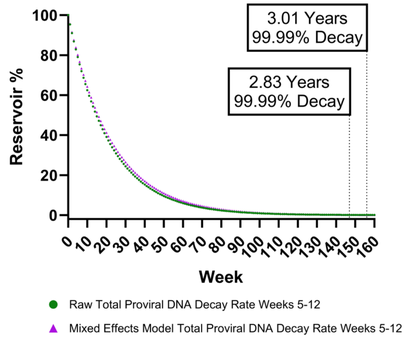
- Ruxolitinib, an orally bioavailable Jak 1/2 selective inhibitor, is FDA approved
- Recruited 60 PWH aged 18-75 on ART ≥ 2 years, continuously virologically suppressed, CD4 > 350 cells/mm^3, with no significant medical condition besides HIV or hypertension
- 40 PWH received ruxolitinib (10 mg bid) in addition to ART **[RUX]** while 20 PWH received only ART from week 0 through 5 **[CNT]**
- Peripheral blood samples were collected in each group at baseline (week 0), week 5, and week 12
- We further classify individuals in the RUX group with high (top 1/3) and low (bottom 2/3) baseline reservoir measurements to assess if ruxolitinib differently impacts patients dependent on baseline reservoir size



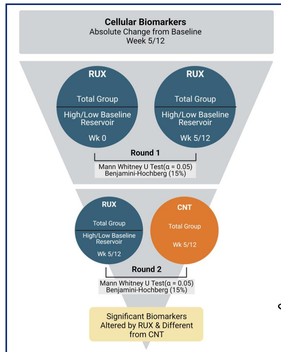
Reservoir Decay

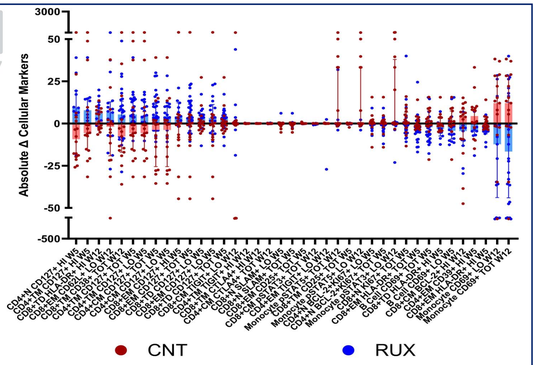


- We measured proviral HIV DNA (IPDA) and examined the change in total, intact, and defective (3′ or 5′) copies over time **(top)**
- We found a significant decay in total proviral DNA copies from week 5 to 12 in high baseline reservoir PWH on RUX
- Using the reservoir decay rate (week 5-12), we estimate 99.99% clearance of the HIV-1 reservoir in 2.86 years
- Model limited to estimating the peripheral reservoir and not sanctuary sites such as the CNS or gut



Altered Biomarkers



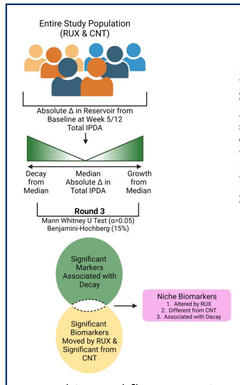


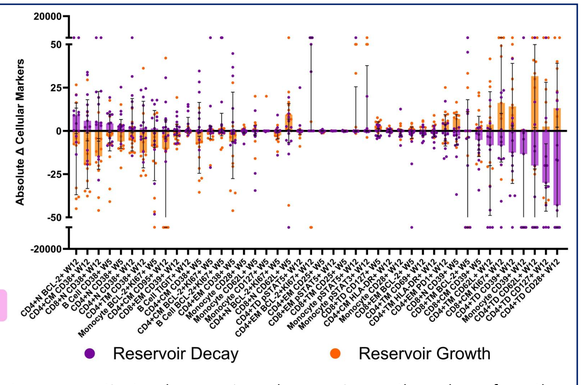
- As shown in the workflow funnel **(left)** absolute change in cellular markers was determined for all biomarkers from their baseline in each patient
- RUX was analyzed both as the entire group and stratified between high and low baseline reservoir
- Non-parametric Mann Whitney U tests (α=0.05) were used to assess significant changes in RUX markers from patient baseline and further to determine if those significant changes were also significantly different from CNT **(right)**
- Affected markers relate to activation, reservoir expansion, cell survival, and immune dysregulation

Ruxolitinib administered for 5 weeks (10 mg *bid*) provides a proof of principle demonstrating that Jak 1/2 selective inhibitors can reverse immune dysfunction that prevents HIV-1 cure and also significantly decays the reservoir in a subset of PWH

HIV-1 reservoir decay associated with increased CD127+ and reduced BCL-2+Ki-67+, pSTAT5+, pSTAT3+, and CD25+ expression

Reservoir Decay Correlates

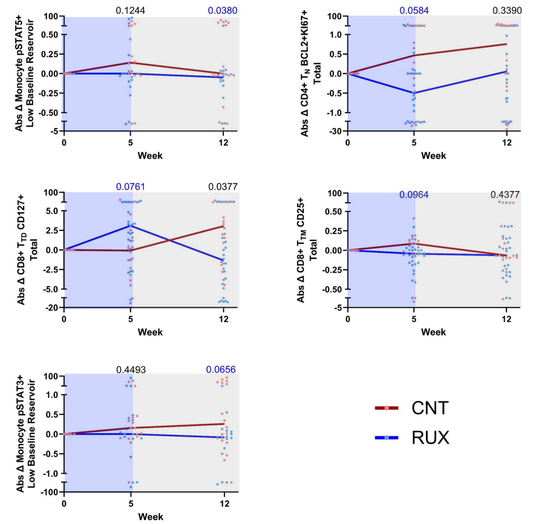


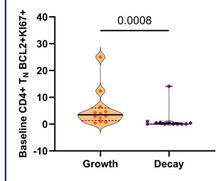


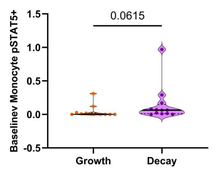
- In this workflow, we aim to assess patients who experienced reservoir growth or decay from the median **(left)** and associated biomarkers
- We compared these markers with the significant biomarkers (previous section) to achieve a highly specific list of biomarkers altered by ruxolitinib, significant from control, and also associated with reservoir decay **(niche biomarkers)**
- PWH who experienced reservoir decay have an opposite expression profile of those who experienced growth **(right)**

Niche Biomarkers

- BCL2/KI67, in CD4+ T_N, indicated productively infected cells with an increased lifespan (Bahbouhi et al. PMID: 14764521)
- CD127, in CD8+ T_{TD}, indicates a contained viral load (Lv et al. PMID: 20807412), and otherwise is inversely correlated to homeostatic proliferation
- pSTAT5 and pSTAT3, in monocytes, indicates activation of the Jak STAT pathway and is an upstream factor of several HIV persistence factors (Reece et al. PMID: 36569952)
- CD25, in CD8+ T_{TM}, is indicative of cellular activation and proliferation (Bajnok et al. PMID:28555090, Cauda et al. PMID:2978456, Boyman et al. PMID:22343569)







- 2 of these markers are predictive of decay:
- At baseline (all participants), PWH with lower expression of BCL-2/KI67 are more likely to experience reservoir decay
  - RUX reduced BCL-2/KI67 expression in CD4+ T_N
- At baseline (all participants), PWH with higher expression of pSTAT5 are more likely to experience reservoir decay
  - RUX reduced pSTAT5 expression in monocytes

Conclusions & Future Work

- We have demonstrated that:
  - Ruxolitinib treatment results in significant decay of HIV-1 reservoir in PWH with high baseline reservoir (week 5-12)
  - Our reservoir decay model predicts 99.99% clearance of peripheral reservoir in 2.86 years
  - Many significant biomarkers altered by ruxolitinib relate to immune activation, reservoir establishment, maintenance, and expansion, cell survival (reservoir lifespan), and immune dysregulation
- The Δmagnitude in marker expression associated with reservoir maintenance (control group) is reversed with ruxolitinib intervention
- PWH are more likely to experience reservoir decay if BCL-2/KI67 expression is low and pSTAT5 expression is high
- Biomarkers that 1) were significantly altered by ruxolitinib, 2) significantly different from control, and 3) associated with reservoir decay were identified (CD4+ T_N BCL2/KI67+, CD8+ T_{TD} CD127+, CD8+ T_{TM} CD25+, Monocyte pSTAT5+, and Monocyte pSTAT3+)

- **Ruxolitinib, a Jak 1/2 selective inhibitor, administered for 5 weeks (10 mg *bid*) provides a proof-of-principle demonstrating that Jak 1/2 selective inhibitors such as ruxolitinib or baricitinib (second generation agent) can reverse immune dysfunction that prevents HIV-1 cure, and also significantly decays the reservoir in a subset of PWH**
- **Longer duration studies with a Jak 1/2 selective inhibitor are warranted and underway, and may merit ruxolitinib or baricitinib as a backbone for cure-based eradication strategies**

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