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

Monica D. Reece[1], Zhan Zhang[1], Susan Pereira-Ribeiro[1], Rafick Sékaly[1], Deanna Kulpa[1], Eric White[1], Jack Yoon[1], Raymond F. Schinazi[7], Vincent Marconi[2], Christina Gavegnano[1,3,4,5,6]

@GavegnanoGroup
@MonicaReece
mdreece@emory.edu
www.linkedin.com/in/monica-reece-emory

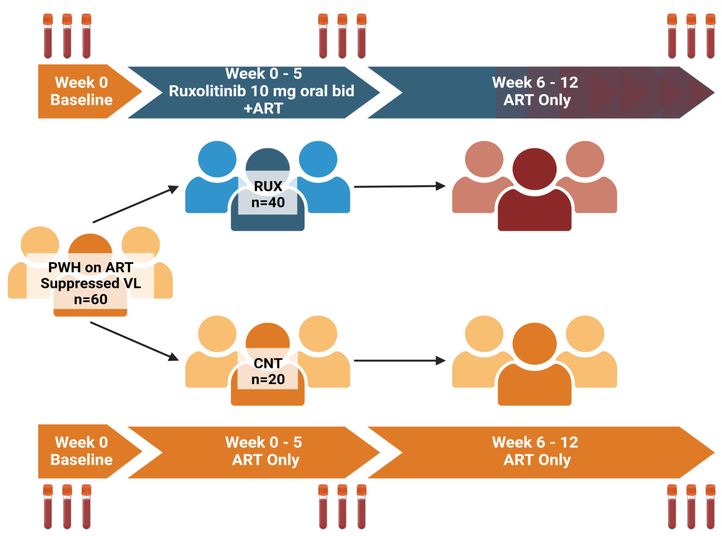
[1]Department of Pathology and Laboratory Medicine, Emory University, Atlanta GA, 30322, USA
[2]Division of Infectious Diseases, Emory University School of Medicine, Atlanta GA, 30322, USA
[3]Department of Pharmacology and Chemical Biology, Emory University, Atlanta GA, 30322, USA
[4]Center for Study of Human Health, Emory University, Atlanta GA, 30322, USA
[5]Atlanta Veterans Affairs Medical Center, Decatur GA, 30033, USA
[6]Center for Bioethics, Harvard Medical School, Boston MA, 02115, USA
[7]Center for ViroScience and Cure, Laboratory of Biochemical Pharmacology, Department of Pediatrics, Emory University School of Medicine, Atlanta GA, 30322, USA

Introduction

- 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 leads to and exacerbates phenomena such as inflammaging, immune exhaustion, and comorbidities like cardiovascular disease (CVD) and HIV-Associated Neurocognitive Dysfunction (HAND)
- **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

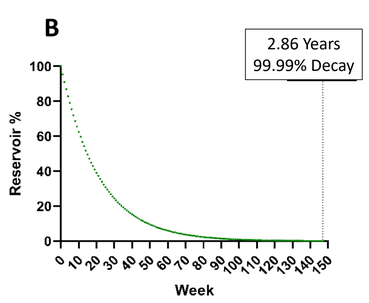
- Ruxolitinib, an orally bioavailable Jak 1/2 selective inhibitor, is FDA approved for the indications of myelofibrosis, polycythemia vera, and graft-versus-host disease
- Exclusion criteria included:
  - ≥18 age ≤75
  - ART regimen containing NNRTI or INSTI without cobicistat for ≥ 2 years
  - Continuously virologically suppressed
  - CD4+ T cell count > 350 cells/mm^3
  - No significant medical condition besides HIV or hypertension
- Trial participants (n=60) were randomized:
  - One group (n=40) received ruxolitinib (10 mg bid) in addition to ART **[RUX]**
  - Another (n=20) received only ART from week 0 through 5 **[CNT]**
  - Both groups were followed until week 12 on ART alone
- Peripheral blood samples were collected in each group at baseline (week 0), week 5, and week 12
- Here, 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

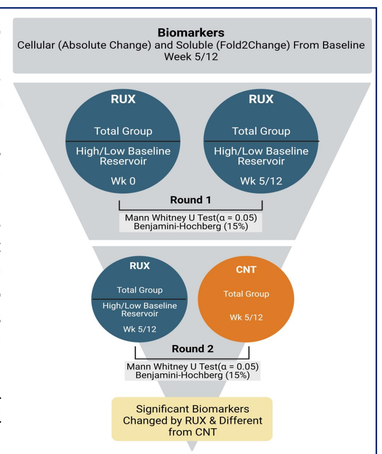
- Our group measured integrated proviral DNA (IPDA) and examined the change in total, intact only, and defective (3' or 5') only proviral DNA copies over time **(A)**
- We found a significant decay in total proviral DNA copies from week 5 to 12 in RUX patients with high baseline reservoir

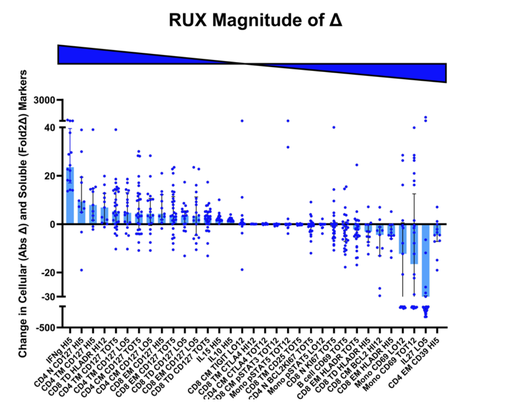
- We calculated the decay rate in reservoir (total IPDA) from weeks 5 to 12
- In our model **(B)**, we estimate 99.99% clearance of the HIV-1 reservoir in 2.86 years
- Our models are limited to estimating the peripheral reservoir and not sanctuary sites such as the CNS or gut, as samples gathered here were peripheral blood
- Total IPDA correlates with integrated HIV DNA measures, allowing interchangeable use of 2 assays



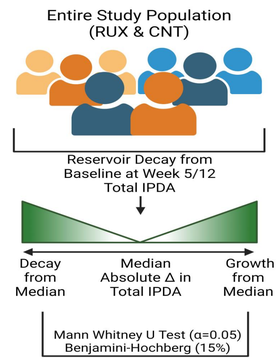
Altered Biomarkers

- As shown in the workflow funnel **(right)** absolute change (cellular markers) and fold 2 change (soluble markers) were determined for all biomarkers from their baseline in each patient
- The RUX group 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 the RUX group markers from patient baseline and further to determine if those significant changes were also significantly different from CNT **(below)**
- Affected markers relate to activation, reservoir expansion, cell survival, and immune dysregulation

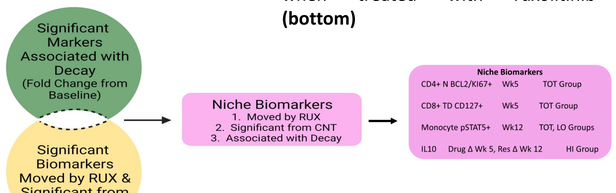


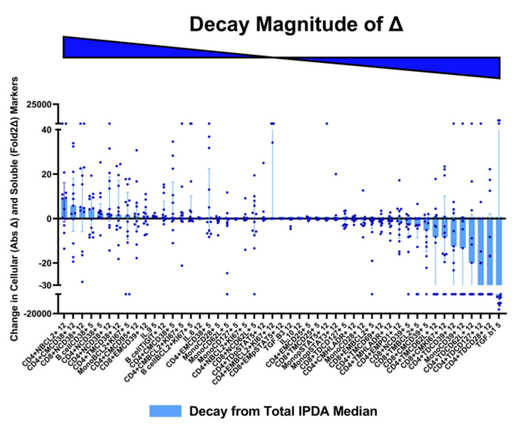


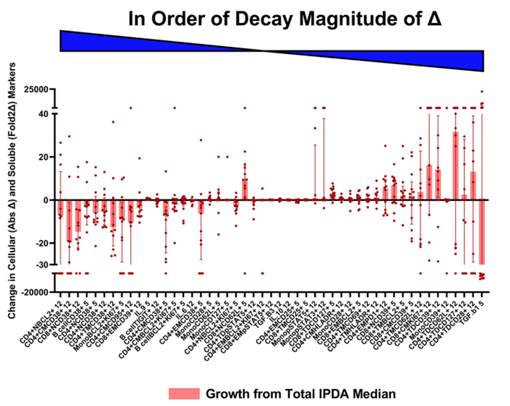
Reservoir Decay Correlates



- 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 **(below, pink square)**
- These biomarkers would serve as baseline predictors for individuals who may experience high reservoir decay when treated with ruxolitinib **(bottom)**

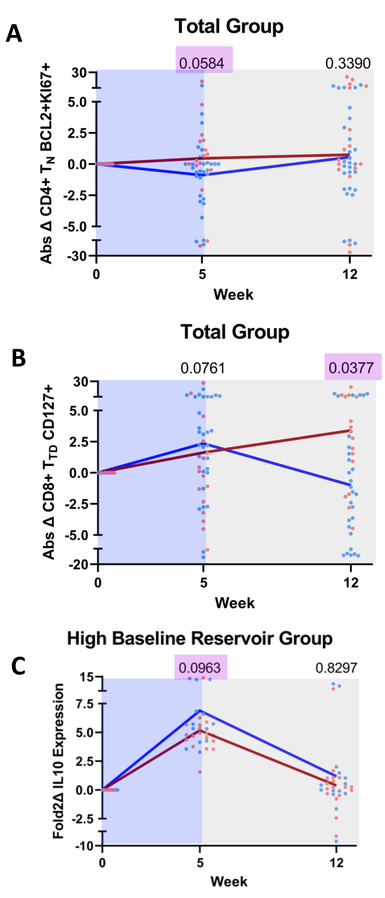




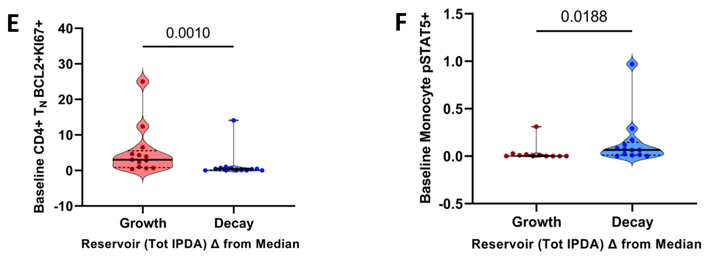


Niche Biomarkers

- Baseline biomarker expression in reservoir growth/decay groups:
  - **(A)** BCL2/KI67
    - In CD4+ T_N, indicated productively infected cells with an increased lifespan (Bahbouhi et al. Blood. 2004. PMID: 14764521)
  - **(B)** CD127
    - In CD8+ T_{TD}, indicates a contained viral load (Lv et al. Virol J. 2010. PMID: 20807412)
    - Potential compensatory effect
  - **(C)** IL10
    - In plasma, interpretation depends on context
  - **(D)** pSTAT5
    - In monocytes, indicates activation of the Jak STAT pathway and is an upstream factor of several HIV persistence factors (Reece et al. Front Immunol. 2022. PMID: 36569952)



- **(E)** 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
- **(F)** 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)
    - 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+
    - Monocyte pSTAT5+
    - IL10
  - **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**

Acknowledgements and References

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