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Amanda Michael March 29, 2024

Effect of Ruxolitinib on Cardiovascular Disease-Associated Immunomodulatory Biomarkers in People Living With HIV

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Abstract

Effect of Ruxolitinib on Cardiovascular Disease-Associated Immunomodulatory Biomarkers in People Living With HIV

By Amanda Michael

People living with HIV (PWH), even with peripheral viral suppression with antiretroviral therapy (ART), are at an increased risk for cardiovascular disease (CVD) and CVD-related comorbidities, including sudden cardiac death and acute myocardial infarction (MI). While PWH may appear virally suppressed in peripheral blood samples, viral reservoirs persist in gut-associated lymphoid tissue (GALT) and are associated with CVD-related morbidity and mortality. This project aimed to determine if Jak 1/2 inhibitor ruxolitinib, which our group and others have demonstrated confers significant reduction *in vitro* and across *ex vivo* patient samples and murine models to block HIV-associated inflammatory events, could confer efficacy in treating HIV-driven inflammation associated with CVD development and related comorbidities in PWH. Our team performed an AIDS Clinical Trial Group (ACTG) sponsored Phase 2a human study (n = 60, 2:1 ruxolitinib to open label control), including 5 weeks of ruxolitinib (10 mg BID), with additional follow up through week 12. Mixed effects analysis and a Spearman rho correlation test were performed to determine the impact of ruxolitinib on the following biomarkers known to be associated with CVD: IFN-β, IFN-γ, IL-1β, and TNF-α. Significant changes in IFN-β and IFN-γ were observed, indicating that a 5-week course of ruxolitinib can confer modulation of these two cytokines, both of which are involved in innate immune control and harnessing of viral persistence. The same biomarkers were also assessed for correlation with HIV reservoir levels, a key barrier to systemic eradication of HIV-1 independent of ruxolitinib treatment. Correlation between the replication competent reservoir (quantified with the integrated proviral DNA assay; IPDA) and IFN-β demonstrated a strong trend towards significance ($p=0.088$), wherein more IFN-β correlated with a smaller reservoir size. Examining the RUX arm, no significance was observed, collectively underscoring that IFN responses are associated with reservoir control. Ruxolitinib can modulate these responses in PWH, but longer duration treatment is likely needed to observe any meaningful impact on reservoir decline conferred by this mechanism. Future directions include analysis of second-generation Jak inhibitors such as baricitinib for efficacy in treating HIV-driven CVD and related biomarkers in longer duration studies, leveraged from our team's existing trials.

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Background

Most of the morbidity and mortality amongst people with HIV (PWH) on peripherally suppressive antiretroviral therapy (ART) is due to non-AIDS related conditions, including cardiovascular disease (CVD). It has been found that CVD was the cause of death in 23% of PWH less than 40 years old, and 20% of PWH 50 and older (1). It has become increasingly clear that PWH are at a greater risk for experiencing CVD-related events than HIV seronegative persons, and for having more severe outcomes from such events. PWH are 4.5 times more likely to experience sudden cardiac death (2), 2 times as likely to experience acute myocardial infarction (AMI) (3), and 1.6 times more likely to develop new carotid plaque (4). HIV infection increases CVD incidence far beyond traditional risk factors such as smoking and diet. There is a major unmet clinical need to understand the fundamental mechanisms that drive CVD mortality in PWH, with a long-term goal of developing safe, specific inhibitors of these events to mitigate disease.

Mechanisms of Viral Persistence

Standard HIV-tracking practices focus on peripheral blood, which contains only 2 to 5% of the body's lymphocytes. In contrast, gut-associated lymphoid tissue (GALT) contains more than 80% of the body's lymphocytes (5). Viruses have been found to accumulate in GALT, making it a hotspot for acute infections and chronic immune activation and dysfunction (6).

Upon initial HIV-1 infection, CD4+ T-cells are depleted in both GALT and peripheral blood. When highly active antiretroviral therapy (HAART) is initiated CD4+ T-cells typically rebound to normal levels in peripheral blood yet remain depleted in GALT (5). This depleted CD4+ T-cell concentration in GALT persists even in asymptomatic PWH. The failure of T-cell

concentrations to return in GALT even after peripheral viral suppression is thought to be a major contributor to the existence of a viral reservoir (7).

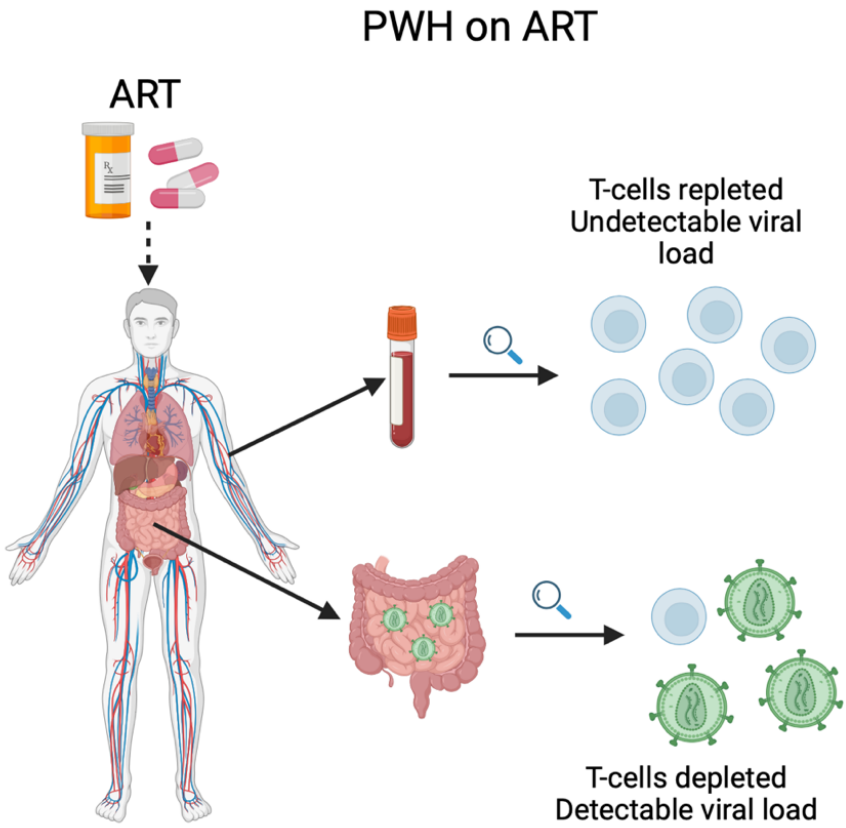

Figure 1: In PWH on ART, viral reservoirs persist in the gut and T-cells are not repleted. Figure created using BioRender.com.

Also of possible contribution to immune cell depletion in GALT is the inability of ART to penetrate GALT. Dolutegravir (DTG), a second-generation integrase strand transfer inhibitor (INSTI) frequently included in an ART regimen, has been found in lower concentrations in rectal tissue than in plasma. This discrepancy is due to the low molecular weight and low octanol water partition coefficient of DTG which makes it more difficult to be absorbed by GALT. Moreover, both HIV-1 infection and ART impact expression of intestinal drug transporters, specifically p-

glycoprotein. P-glycoprotein substrates, such as DTG, may have reduced transport into GALT which inhibits optimal absorption (8).

HIV infection has also shown to have an impact on regulatory T cells (Tregs), which maintain the activity of effector lymphocytes, including TH17 cells. However, there is conflicting literature about whether this impact is net positive or negative. Some studies have found Tregs to be helpful in PWH by suppressing chronic inflammation, while others have found them to be harmful by suppressing immune activity during infection.

Despite conflicting findings, there is overwhelming support that Treg levels are impacted by viral infection, and that they consequently impact viral pathogenesis. This dysregulation of Tregs has been demonstrated to impact the development of CVD by leading to an increase in atherosclerosis. Atherosclerosis, a disease in which lipids and other fibrous elements accumulate under the endothelial lining of arterial tissue, is a leading cause of CVD (9, 10). Impaired Treg function has been associated with the worsening of atherosclerosis (11), and subsequently an increase in incidence of CVD in PWH.

Research has found elite controllers (PWH who naturally control their viral replication) to have lower levels of Tregs. Elite controllers also naturally maintain a proper ratio between Tregs and the TH17 cells they suppress (12). The imbalanced ratios of such T cells in PWH without viral control has an impact on the occurrence of intestinal permeability (leaky gut). Homeostatic T cells, including TH17, are involved in the maintenance of the epithelial barrier. As these cells get depleted during HIV infection, the epithelial barrier becomes less robust. This increases permeability of the barrier, making microbes of the GALT more susceptible to entering circulation. This phenomenon, known as microbial translocation, increases chronic inflammation, decreases proper immune function, and is associated with HIV pathogenesis. As

ART does not eliminate the viral reservoir in GALT, PWH are vulnerable to microbial translocation even after peripheral viral suppression (13).

Immunological Biomarkers

T cell activation is triggered by many immunological biomarkers. Interestingly, and regardless of a CVD history, these biomarkers have been shown to predict cardiac related events in PWH. Levels of HsCRP, IL-6, and D-Dimer are elevated in PWH who develop CVD as opposed to PWH who do not develop CVD. Moreover, the concentrations of these three biomarkers are higher in the plasma of PWH who experience fatal CVD in comparison to PWH who experience nonfatal CVD (14). HIV patients who had high levels of CRP were also at an increased risk of acute myocardial infarction (15). Immunological biomarkers are not eradicated through ART for PWH.

In women whose HIV had been treated with ART, CD4+ and CD8+ T cell activity remained high. CD4+ and CD8+ T cell activations are more elevated in women with HIV who have carotid lesions compared to women with HIV who do not have these lesions. Furthermore, T cell activation was not higher in women without HIV who had carotid lesions. The presence of carotid lesions was further impacted by immunosenescence of T cells. Immunosenescence is a cellular aging process which is expedited by HIV infection, even after completion of ART (16).

Selected Biomarkers

Gut reservoirs of HIV infection result in chronic inflammation for PWH. This inflammation is indicated by elevated levels of inflammatory markers and markers of endothelial dysfunction and hypercoagulability, including but not limited to IL-6, IFN-α/β, IL-1α/β, TNF-α,

CRP, and D-dimer (17-19). For the purposes of this thesis, I have elected to analyze the following biomarkers: IL-6, IFN-β, IFN-γ, IL-1β, and TNF-α.

IL-6

Interleukin-6 (IL-6) is a cytokine that causes oxidative stress and inflammation throughout the body (20). IL-6 can activate inflammatory pathways, notably the Janus Kinase-Signal Transducers and Activators of Transcription (Jak-STAT) pathway, which perpetuates inflammation throughout the body. Expression of IL-6 is associated with cardiovascular events such as atherosclerosis, heart failure, and MI, and higher IL-6 levels after an MI are associated with poor cardiac outcomes (20, 21). IL-6 was analyzed in the initial A5336 study and found to be trending downwards. However, the decrease in concentration was not statistically significant (22). Statistical analysis of IL-6 was not repeated.

TNF-α

Tumor necrosis factor alpha (TNF-α) is a potent pro-inflammatory cytokine and is a popular target of anti-inflammatory drugs. TNF-α antagonists are received by roughly 1 million patients (23). This class of drugs has not been shown to be beneficial in treating heart failure with reduced ejection fraction (HFrEF) and is not recommended for treating such patients. However, research suggests that TNF-α antagonists may be beneficial for treating CVD patients without HFrEF. When tested in patients with rheumatoid arthritis, these drugs have been associated with decreased risk of MI, arterial stiffness, and other CVD-related symptoms. Additionally, TNF-α antagonists protect against the development of atherosclerosis (23-25).

IL1-β

Interleukin-1 beta (IL1-β) is part of the Interleukin-1 family, the primary family of cytokines associated with both chronic and acute inflammation (26). In pigs, increased levels of IL1-β have been demonstrated to increase production of atherosclerotic plaque, while a decrease in IL1-β has been demonstrated to decrease such production (27). This demonstrates the importance of studying IL1-β in the development of CVD.

IFN-β

Interferon beta (IFN-β) is a type-I interferon that is naturally produced in the body (28, 29). Increased levels of type-I IFNs are associated with atherosclerosis and CVD progression (29, 30).

IFN-γ

Interferon gamma (IFN-γ) is the sole type-II IFN. IFN-γ is notable for the study of CVD because it has been observed during multiple stages in the development of atherosclerosis. IFN-γ has also been detected in atherosclerotic lesions, underscoring its importance in the development of atherosclerotic plaque (31).

Unmet Clinical Need

As discussed, HIV is frequently undetectable in peripheral blood despite viral loads existing throughout the body. Available anti-retroviral therapies (ART), which are the standard of care for PWH, target HIV in peripheral blood. Patients with undetectable levels of plasma HIV are still left susceptible to non-AIDS comorbidities, such as CVD. Activation of the Jak-STAT

pathway, which is both reported during initial HIV infection and associated with HIV persistence, has been shown to increase levels of inflammation throughout the body (22, 32-35).

While there are effective treatment options available to treat CVD in HIV seronegative individuals, there is a clear unmet clinical need for therapies that target the underlying drivers of CVD in PWH. Colchicine, lamivudine, and atorvastatin are commonly prescribed to target inflammation. Research has found that colchicine does have some utility in reducing pro-inflammatory cytokines, however, it has demonstrated a narrow therapeutic window of safety *in vivo* and is not FDA approved for use in the United States. Neither lamivudine nor atorvastatin have conferred efficacy in inhibiting HIV-induced inflammation despite their abilities to block viral replication. This underscores the fact that neither ART nor statins (a common therapeutic intervention for CVD) can mitigate harmful inflammation in PWH (36, 37). While there are therapeutics that are effective in reducing CVD, including statins, novel therapeutics are needed to target the elevated inflammation levels among PWH.

Agents that inhibit the Jak-STAT pathway can address this unmet clinical need for safe, potent, and specific inhibitors of chronic inflammation in ART-peripherally suppressed PWH. Such an agent could prevent CVD and other end-organ diseases, as well as decay the HIV reservoir found in the gut and brain.

Ruxolitinib is a promising candidate. Studies have demonstrated that ruxolitinib confers significant anti-HIV-1 effects in macrophages and primary CD4+ T cells (37-43). Ruxolitinib has been observed to reduce the concentration of cells with integrated HIV-1 DNA, block reactivation from latent reservoirs, and reduce bystander cell activation and immune dysfunction (44-49). Additionally, studies have shown that ruxolitinib can confer immunosuppressive and anti-inflammatory effects, with cellular targets in both the innate and adaptive immune systems

(50). As a result, ruxolitinib shows promise for treating HIV comorbidities driven by inflammation such as CVD.

A multi-site phase 2a study funded by the AIDS Clinical Trials Group (ACTG) studied the efficacy of ruxolitinib in patients with well-controlled HIV-1 on suppressive ART (A5336; n=60). Data showed that those randomized to ruxolitinib did not experience more adverse safety events than those in the control group but did demonstrate a significant decrease in key markers of HIV-1 persistence such as CD25, HLA-DR/CD38, and cell survival marker bcl-2 (45).

Methods

Specific Aims

Specific aims for this project were as follows; 1: determine the impact of ruxolitinib on biomarker concentration, and 2: determine the correlation between reservoir and cytokine concentrations.

Study Design

A5336 was a phase 2a multicenter, randomized control trial funded by the ACTG and directed by Drs. Vincent Marconi, Jeffrey Lennox, and Christina Gavegnano. The trial was conducted at 14 academic medical centers across the United States. There were two study arms: the intervention group (RUX), who received ruxolitinib, and the control group (CNT) who received no intervention. PWH were randomized 2:1 into the intervention and control groups (total n=60, RUX n=40, CNT n=20). The study aimed to assess the safety and efficacy of ruxolitinib for PWH on ART (22).

Enrollees were between the ages of 18 and 65, virologically suppressed on ART regiment, had a CD4+ T-cell count greater than 350 cells/mm^2, and had no significant medical history aside from HIV and hypertension. 80% (n=48) of enrollees were male (22).

Participants in the RUX group received 10 mg oral ruxolinitib 2 times a day for 5 weeks. After week 5, ruxolitinib was discontinued but patients were followed for 7 additional weeks (through week 12). CNT patients received no study drug. All participants continued with their existing ART regiment throughout the duration of the study. This trial reported no difference in safety events between arms (22).

Peripheral blood samples were obtained before the trial began, and again at weeks 0, 2, 4, 5, 10, and 12. This analysis utilized samples from only weeks 0, 5, and 12.

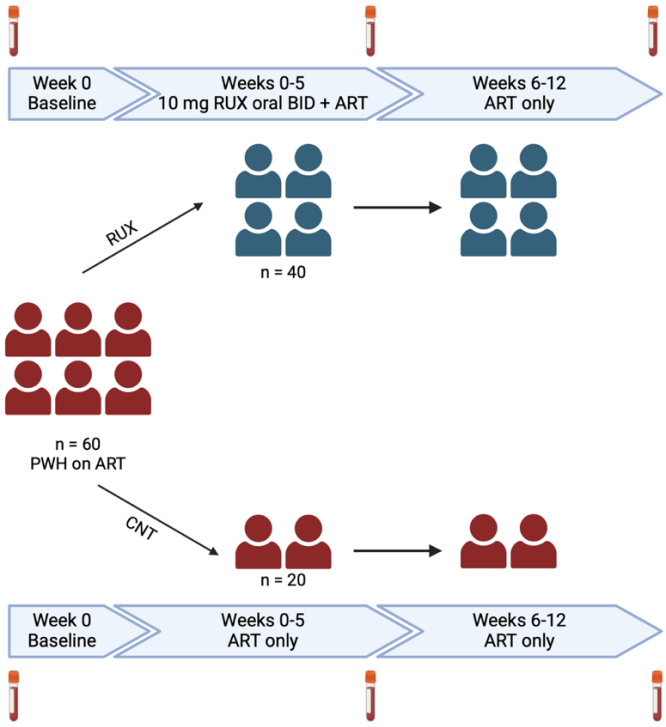

Figure 2: Illustration of A5336 study design. Created using BioRender.com.

Statistical Analysis

I began analysis with a raw data set of cellular marker expression and reservoir measurements obtained from the ACTG for each patient. Reservoir data was assessed using integrated proviral DNA (IPDA), expressed as copies of IPDA per 10^6 CD4+ T-cells.

In Microsoft Excel, cytokine data was analyzed by, first, creating a pseudo set of final concentration data (+ 0.0001 to each value) to avoid zeroes in the fold-change calculation. Patients with multiple reported values for a given timepoint were averaged. Values from all timepoints were then divided by the average concentration from week 0 to determine fold-change. Fold-change values greater than one indicate an increase in biomarker concentration, while a value of less than one indicates a decrease from baseline. A calculated fold-change of 1 demonstrates no change.

Calculated fold-change values were then analyzed using a mixed effects analysis with a Tukey correction for multiple comparisons via GraphPad Prism v10.1.1. Significance was assessed at alpha = 0.05.

To assess the association between cellular markers and reservoir decay, week 5 cytokine and reservoir data for each patient were paired. Only patients with complete data for each measure were included in the analysis. Spearman rho (ρ) correlations (alpha = 0.05) with linear regression trendlines were used to determine correlation (GraphPad Prism v10.1.1). These analyses were stratified by intervention. A ρ value of 1 denotes a perfect positive monotonic relationship, -1 denotes a perfect negative monotonic relationship, and a ρ value of 0 indicates no monotonic relationship between variables.

Additional, supplementary analysis was performed to assess the association between absolute change of IPDA and IFN-γ between weeks 0 and 5. Absolute change was calculated by

subtracting final concentration at week 0 from final concentration at week 5. For patients with multiple concentration values for a single week, reported concentration values were averaged. Analysis was not stratified by intervention, and only patients with complete data for weeks 0 and 5 were included in the analysis. This analysis was performed using spearman rho correlation with a linear regression trendline (GraphPad Prism v10.1.1).

Results

Question 1: Impact of Ruxolitinib on Biomarker Concentration

TNF-α

In the RUX group, there was no significant change in TNF-α concentration between week 0 and week 5 (mean difference (MD) = -0.165, p=0.065), though data were trending towards a significant increase. There was a significant fold-change between weeks 0 and 12 (MD = 0.239, p = 0.0072) and between weeks 5 and 12 (MD = 0.404, p < 0.0001). For CNT, all comparison measures were significant. There was a significant increase between weeks 0 and 5 (MD = -0.286, p = 0.0056), and a significant decrease between weeks 0 and 12 (MD = 0.281, p = 0.021) and weeks 5 and 12 (MD = 0.566, p < 0.0001). As both RUX and CNT groups saw significant change in fold-change measurement, change in TNF-α concentration as a result of ruxolitinib treatment is not considered to be significant.

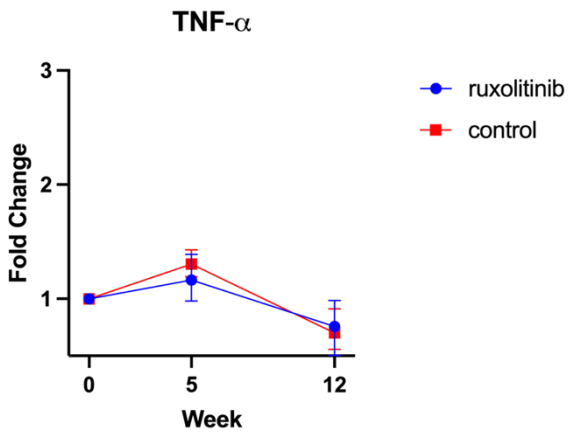

Figure 3: Fold change in TNF-α concentration, compared to week 0. Error bars represent interquartile range (IQR).

IL-1β

In the RUX group, there was a significant increase between weeks 0 and 5 (MD = -10.57, p = 0.001). Changes between weeks 0 and 12 (MD = -0.071, p = 0.965) and weeks 5 and 12 (MD = 10.50, p = 0.512) were insignificant. For CNT, changes between weeks 0 and 5 (MD = -0.610, p = 0.010), weeks 0 and 12 (MD = 0.450, p = 0.030), and weeks 5 and 12 (MD = 1.060, p = 0.002) were all significant. As such, the statistically significant fold-change increase for IL1-β concentration between weeks 0 and 5 was disregarded.

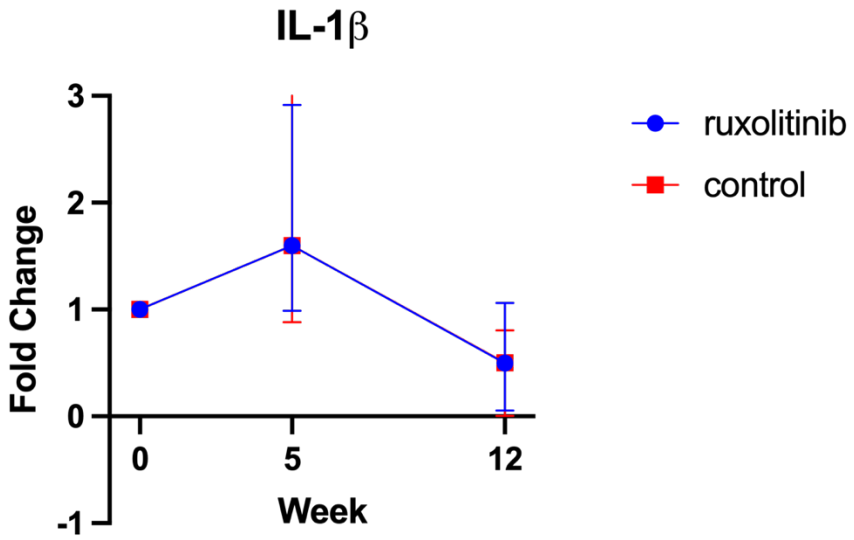

Figure 4: Fold change in IL-1β concentration, compared to week 0. Error bars represent IQR.

IFN-β

For RUX, there were statistically significant fold-change increases for both weeks 0 to 5 (MD = -1.266, p = 0.024) and weeks 0 to 12 (MD = -1.935, p = 0.012). Change between weeks 5 and 12 was not statistically significant (MD = -0.669, p = 0.942). For the CNT group, fold-change between weeks 0 and 5 (MD = -2636, p = 0.053), weeks 0 and 12 (MD = -1915, p = 0.867) and weeks 5 and 12 (MD = 621, p = 0.981) were all statistically insignificant. As such, ruxolitinib intervention resulted in a significant increase in IFN-β concentration between weeks 0 and 5, and a significant decrease between weeks 0 and 12.

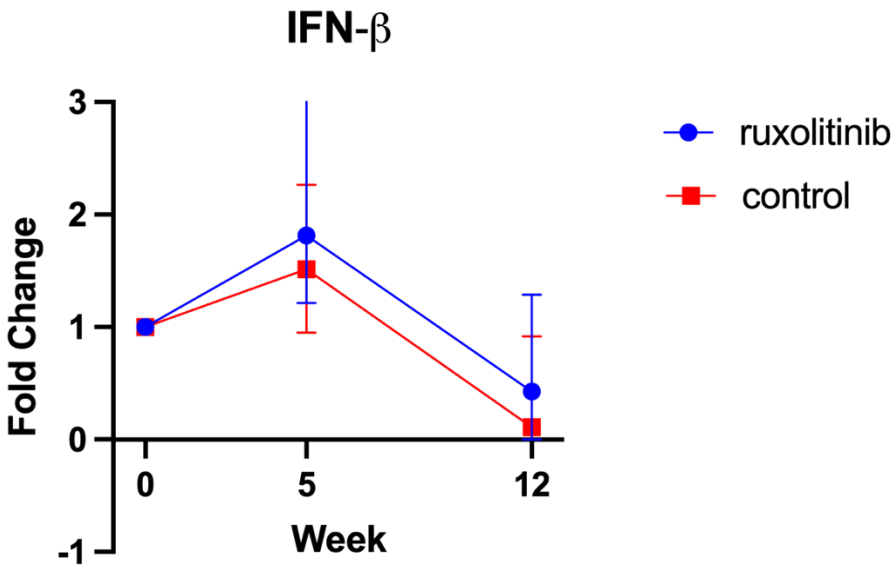

Figure 5: Fold change in IFN-β concentration, compared to week 0. Error bars represent IQR.

IFN-γ

For RUX, there was a statistically significant increase in IFN-γ concentration between weeks 0 and 5 (MD = -1.202, p = 0.0003). Changes between weeks 0 and 12 (MD = -0.528, p = 0.121) and weeks 5 and 12 (MD = 0.674, p= 0.480) were insignificant. For CNT, fold-change between weeks 0 and 5 (MD = -0.336, p = 0.254), weeks 0 and 12 (MD = 0.005, p = 0.999) and weeks 5 and 12 (MD = 0.351, p = 0.482) were statistically insignificant. Therefore, the fold-change increase between weeks 0 and 5 indicates that ruxolitinib did significantly increase IFN-γ concentration.

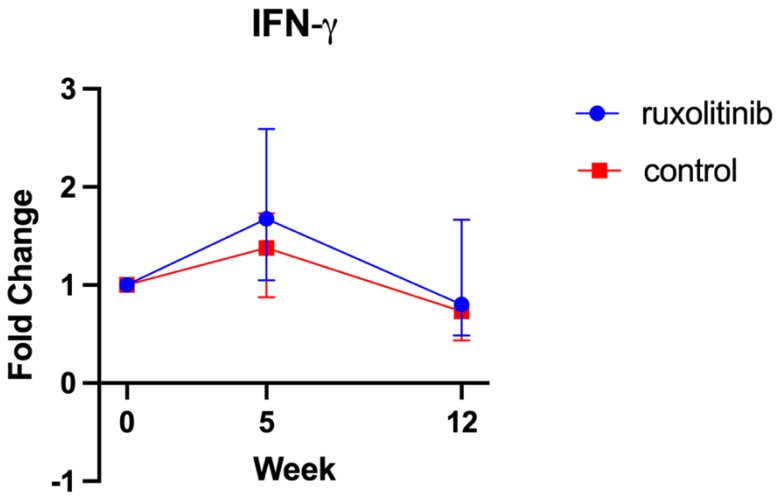

Figure 6: Fold change in IFN-γ concentration, compared to week 0. Error bars represent IQR.

Question 2: Correlation Between Reservoir and Cytokine Concentrations

TNF-α

In a spearman rho analysis of RUX, there was no significant correlation between cytokine and IPDA concentrations ($\rho = 0.102$, $p = 0.620$). Analysis of the CNT group also demonstrated no correlation ($\rho = -0.143$, $p = 0.783$). Combined analysis was also statistically insignificant ($\rho = 0.096$, $p = 0.597$).

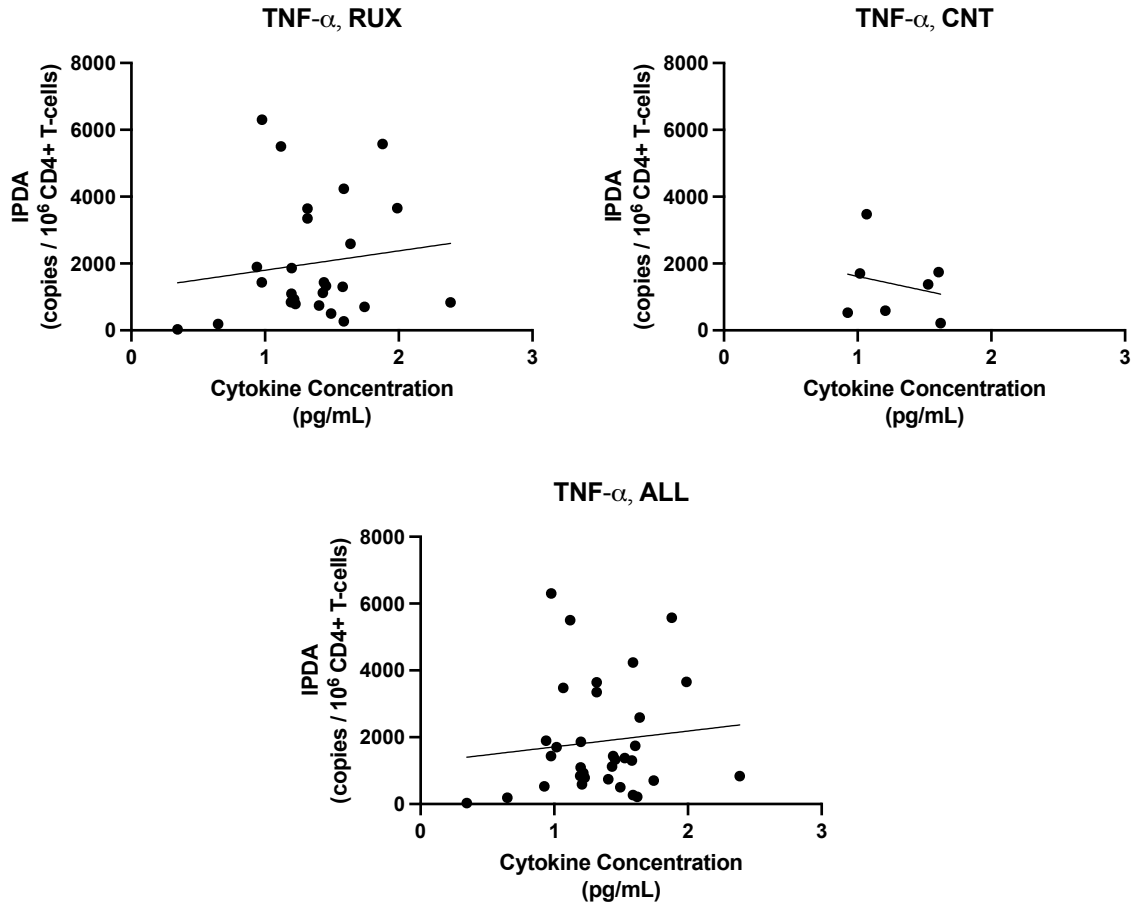

Figure 7: Correlation between TNF-α and IPDA concentrations, stratified by intervention (top) and combined (bottom).

IL-1β

Spearman rho analysis of IL-1β and IPDA concentrations for RUX indicated no significant correlation (ρ = -0.263, p = 0.262). Correlation of the CNT group was also insignificant (ρ = -0.107, p = 0.840). Combined analysis was not significant (ρ = -0.217, p = 0.277).

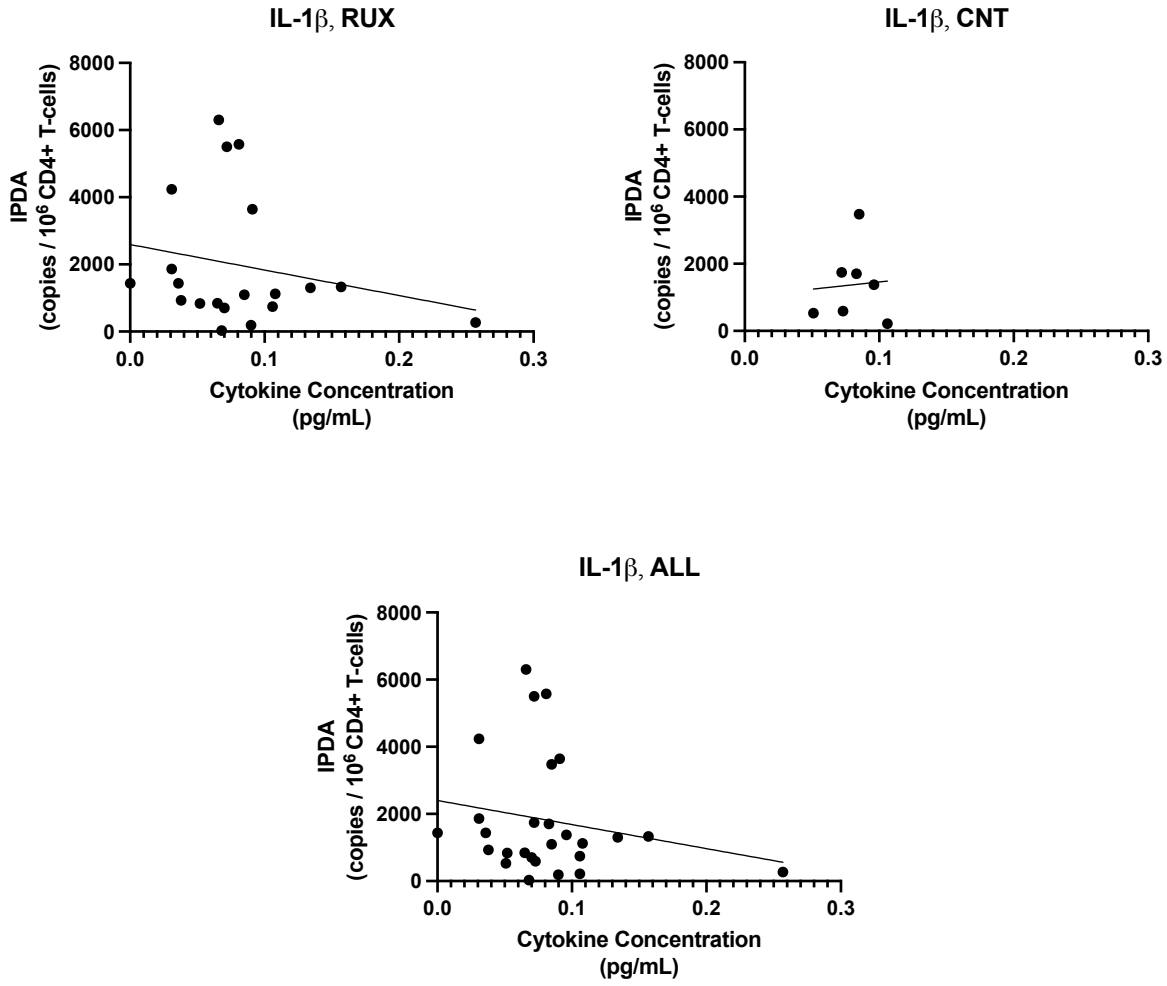

Figure 8: Correlation between IL-1β and IPDA concentrations, stratified by intervention (top) and combined (bottom).

IFN-β

Spearman rho analysis of RUX did not indicate correlation between IFN-β and IPDA concentrations ($\rho = 0.043$, $p = 0.829$). Correlation in the CNT group was not statistically significant, however was trending towards a significant inverse correlation ($\rho = -0.714$, $p = 0.088$). Combined analysis was not statistically significant ($\rho = -0.076$, $p = 0.668$).

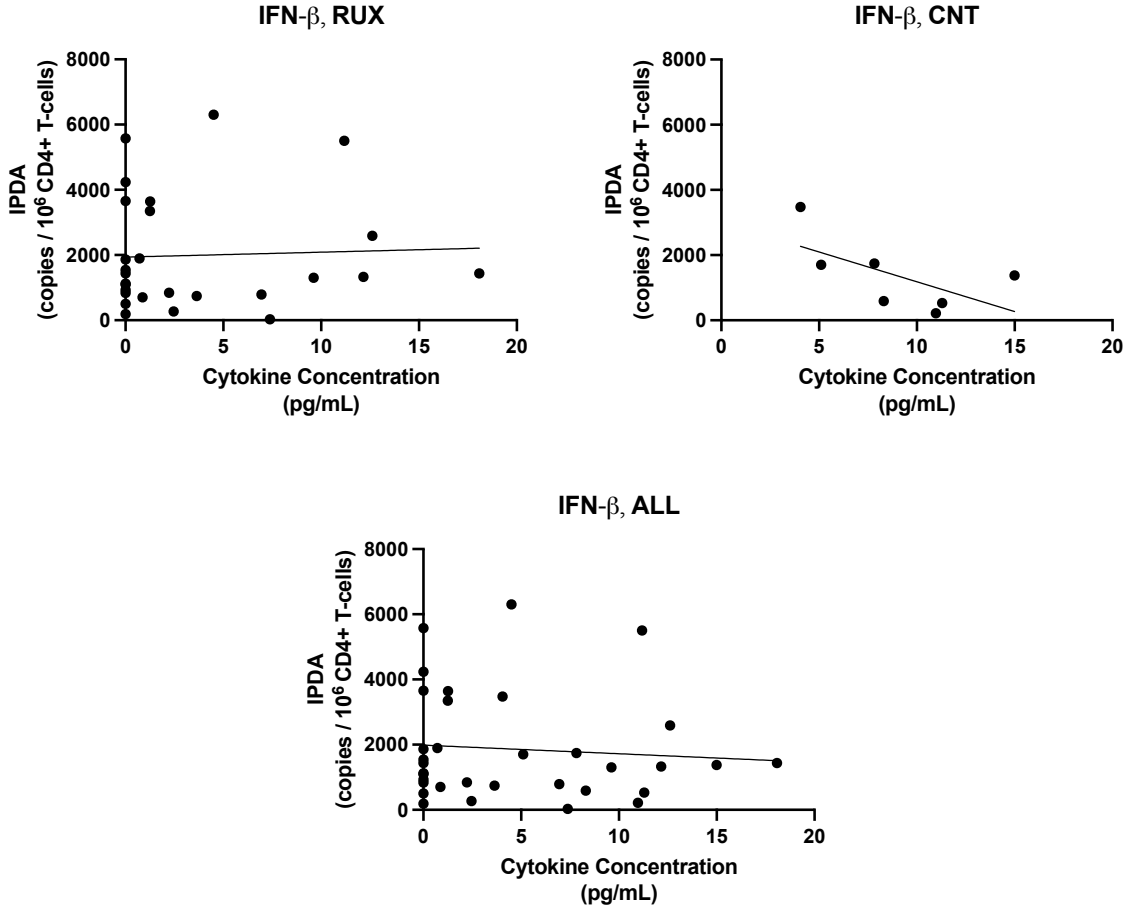

Figure 9: Correlation between IFN-β and IPDA concentrations, stratified by intervention (top) and combined (bottom).

IFN-γ

Spearman rho analysis of RUX indicated no significant correlation between IFN-γ and IPDA concentrations (ρ = 0.189, p = 0.356). Correlation in the CNT group was also insignificant (ρ = -0.071, p = 0.906). Combined analysis also demonstrated no correlation (ρ = 0.171, p = 0.343).

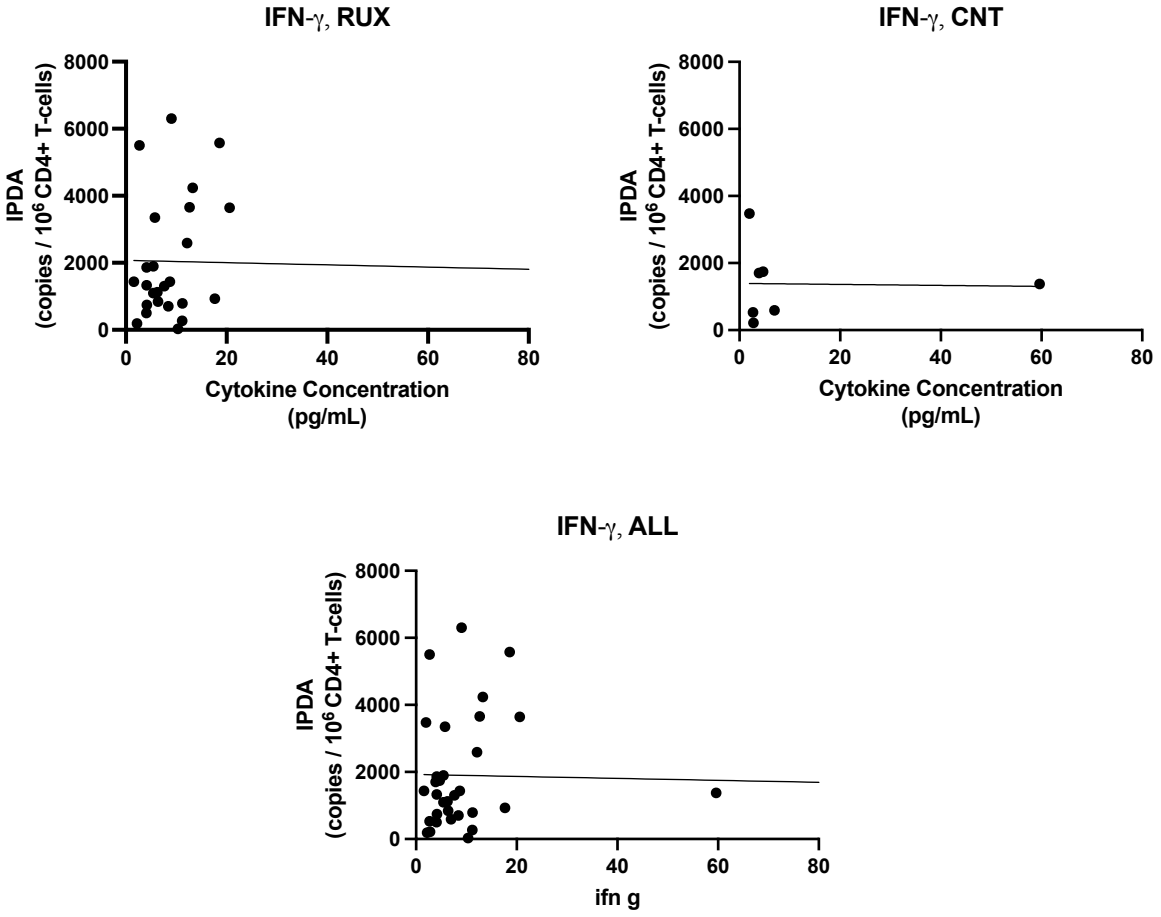

Figure 10: Correlation between IFN-γ and IPDA concentrations, stratified by intervention (top) and combined (bottom).

Supplementary Analysis

Spearman rho analysis of the correlation between absolute change in concentration of IPDA and IFN-γ was not statistically significant ($\rho = 0.078$, $p = 0.743$).

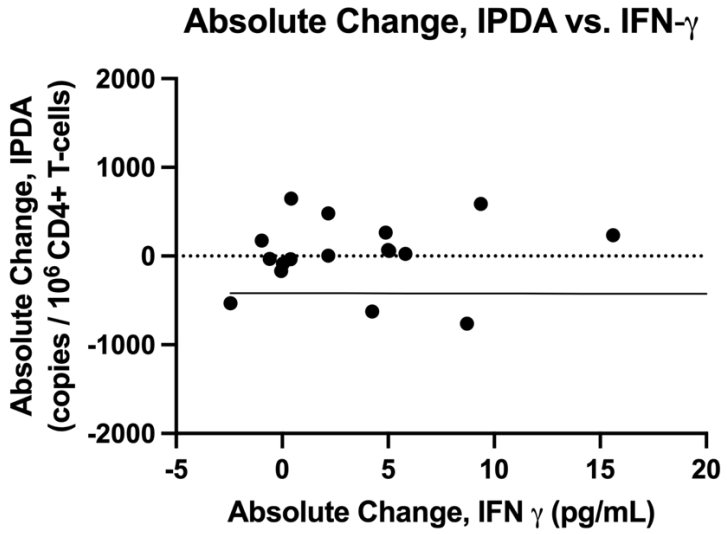

Figure 11: Correlation between absolute change of IFN-γ and IPDA. Absolute change calculated as week 5 – week 0.

Conclusions

Impact of Ruxolitinib on Biomarker Concentration

The mechanism of action of ruxolitinib and other Jak 1/2 inhibitors, along with data from existing studies (50), suggests that the drug confers efficacy as an immunomodulatory agent. As such, we hypothesized significant changes in biomarker concentration for all selected cytokines. However, such an impact was not seen in analysis of TNF-α or IL-1β. This demonstrates that 5-week treatment with 10 mg ruxolitinib BID is not effective in decreasing the concentration of TNF-α nor IL-1β. Significant changes in IFN-β and IFN-γ were observed, indicating that a 5-week course of ruxolitinib at 10 mg BID does confer efficacy in increasing concentrations of these cytokines.

Correlation Between Reservoir and Cytokine Concentrations

Data analysis found no statistically significant correlation between concentrations of IPDA and any of the selected biomarkers. However, correlation between IPDA and IFN-β was trending in the CNT group ($p = 0.088$). Examining the RUX arm, no significance was observed, collectively underscoring that IFN responses are associated with reservoir control independent of ruxolitinib intervention. Ruxolitinib can modulate these responses in PWH, but longer duration treatment is likely needed to observe any meaningful impact on reservoir decline conferred by this mechanism. Lack of significance in all other analyses further demonstrates that 5-week treatment of ruxolitinib at 10 mg BID is not effective in decaying the HIV-1 peripheral reservoir.

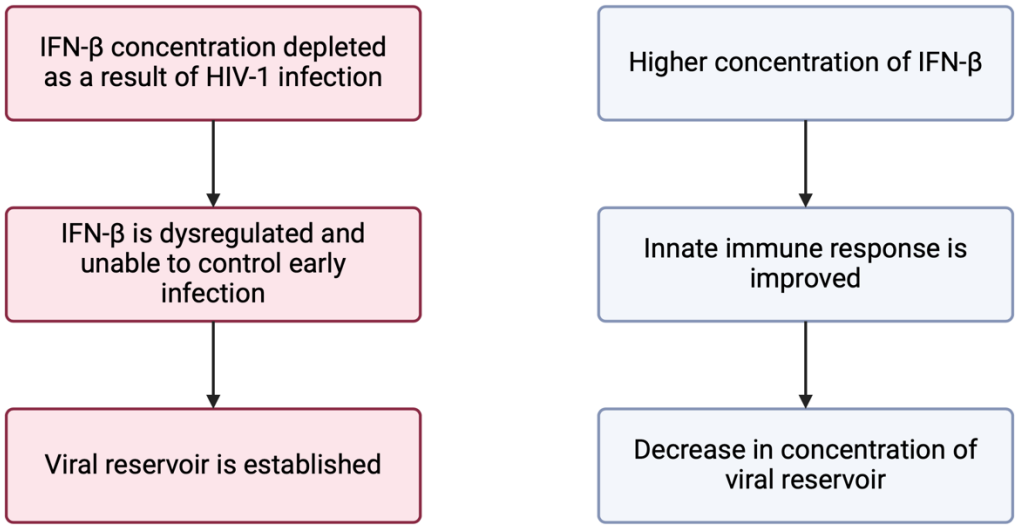

Figure 12: Proposed mechanism for the role of IFN-β in reservoir concentration. Created using BioRender.com

Discussion

While we did not see as much change in biomarker concentration as we had initially anticipated, the A5336 study had significant limitations that likely contributed to the observed results and mitigated our ability to observe quantifiable differences in the biomarkers. These

limitations include, first, the fact that all enrolled individuals were virally suppressed, and the FDA did not allow for the stratification or selection of individuals based on elevated inflammation at baseline. As a result, it is possible that in this small sample of patients, a significant percentage did not have elevated cytokine levels at baseline. Additionally, 10 mg BID is the lowest FDA approved dose of ruxolitinib, and a higher dose may be necessary to observe a sustained and quantifiable modulation of cytokines. Furthermore, enrollees were not equally split between male and female; future studies should disaggregate for sex as a biological variable. Finally, the relatively short duration of the study (only 5 weeks of intervention) could have further limited this modulation. It is possible that chronic immune activation in PWH may require longer than 5 weeks of active treatment of a low dose immunomodulator to observe durable effects on immune activation and inflammation.

Additionally, this study measured biomarker and reservoir concentrations using peripheral blood samples. As discussed earlier, this is not the best way track HIV, as virus often persists in the GALT, leading to inflammation and immune dysregulation not observed in the periphery. However, A5336 was a first-in-class study that provided a springboard for the targeted evaluation of agents that have potential to reduce the reservoir and inflammation in humans safely. More advanced, longer-duration studies targeting tissue sites of inflammation and persistence, including the gut reservoir, are necessary to study this topic further and are underway by our team and collaborators with second generation Jak 1/2 inhibitor baricitinib.

Despite limitations, the study did allow for observation of some significant immunomodulatory impacts of ruxolitinib. Increases in the concentrations of IFN-β and IFN-γ between weeks 0 and 5 indicate that ruxolitinib may have utility for increasing concentrations of both type-I and type-II interferons. There is, however, debate over whether interferons have a

positive or negative impact in PWH, depending upon time of infection and corresponding elevated markers. Interferons are the first line of defense against viruses and are integral in the innate immune response (51). Type-I IFNs (including IFN-β) limit viral replication and express HIV restriction factors. At the same time, in increase in IFN concentration does represent an increase in systemic immune activation and inflammation (52). Type-II interferons (only IFN-γ) function similarly in response to initial HIV-1 infection. IFN-γ is produced in an attempt to clear the virus, but then persists and contributes to the chronic inflammation and immune activation. In contrast to type-I IFNs, IFN-γ does not have a direct antiviral impact on HIV (53). The sustained inflammation resulting from both type-I and type-II IFNs leads to much of the morbidity and mortality associated with HIV, including cardiovascular disease.

IFN-β showed notable changes in both analyses: a statistically significant increase in IFN-β concentration after 5 weeks of ruxolitinib intervention and a trending inverse association between IFN-β and reservoir concentrations in CNT. Due to study constraints, it is not possible to define a full mechanism to explain this phenomenon. However, based on the data available, we propose the following: in PWH, IFN-β is depleted and dysregulated. In turn, innate immune control is dysregulated to a blockade of immune responses. The intrinsic function of IFN-β is to control early infection, so dysregulation at this stage allows the virus to establish a reservoir and overcome the innate immune function intended to clear the virus. When IFN-β concentration increases, the innate immune response is improved, and the immunomodulatory cytokine can help control early establishment of the viral reservoir.

Future Directions

Though the A5336 trial has its shortcomings, it has been instrumental on the path to HIV eradication. The trial demonstrated that ruxolitinib is safe, well-tolerated, and does not negatively interfere with existing ART (22). A subset of patients with a high starting reservoir experienced a decline in the HIV reservoir, which is promising for future studies and bolsters the mechanistic underpinning that Jak 1/2 inhibition can durably impact reduction of the reservoir. Overall, A5336 has become a steppingstone for longer duration studies with advanced generation Jak inhibitors, most notably baricitinib.

A second-generation Jak 1/2 inhibitor, baricitinib works by blocking IFN-α/β, IL-1α/β, TNF-α, CRP, D-dimer, IL-6, IL-7 and IL-15. It is currently FDA approved for the indications of COVID-19, rheumatoid arthritis, and alopecia areata. It is the first and only immunomodulator fully FDA approved for COVID-19 and has monotherapy status. Baricitinib is approved for chronic long-term use in both adults and children with once daily dosing and renal clearance. Renal clearance is critically advantageous as drug-drug reactions with hepatically cleared agents (such as ART) are unlikely. As ruxolitinib is hepatically cleared, this is an important discrepancy. The safety profile of baricitinib is significantly improved compared to ruxolitinib. With improved safety, baricitinib has greater clinical potential in treating the same conditions as ruxolitinib.

Baricitinib shows great promise for HIV-1 eradication and therapeutic intervention. Baricitinib has a less toxic side effect profile than ruxolitinib and is dosed only once daily as opposed to twice. Emerging data from Phase 3 studies suggest that severe side effects are very uncommon and its existing FDA approval for COVID-19, rheumatoid arthritis, and alopecia areata further underscore the safety profile of the agent (46-49, 54, 55). Baricitinib displays

direct-acting antiviral and immunomodulatory effects that target HIV reservoirs. This novel drug shows promise in combatting CVD and other HIV-associated comorbidities.

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