

A5336

A Randomized, Pilot Study of Ruxolitinib in Antiretroviral-Treated HIV-Infected Adults

A Multicenter Trial of the AIDS Clinical Trials Group (ACTG)

Sponsored by:

**The National Institute of Allergy
and Infectious Diseases**

IND # 125,580

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APPENDIX I: SAMPLE INFORMED CONSENT

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STUDY MANAGEMENT

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Protocol E-mail Group

Sites should contact the Computer Support Group at the Data Management Center (DMC) as soon as possible to have the relevant personnel at the site added to the actg.protA5336 e-mail group. Include the protocol number in the e-mail **participant** line.

- Send an e-mail message to actg.user.support@fstrf.org

Clinical Management

For questions concerning entry criteria, toxicity management, concomitant medications, and enrollment, contact the protocol team.

- Send an e-mail message to actg.corea5336@fstrf.org. Include the protocol number, patient identification number (PID), and a brief relevant history.

Laboratory

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- Send an e-mail message to actg.corea5336@fstrf.org (ATTN: Michael Lederman, Athe Tsibris and Charles Flexner).

Data Management

For nonclinical questions about transfers, inclusion/exclusion criteria, case report forms (CRFs), the CRF schedule of events, randomization/registration, delinquencies, and other data management issues, contact the data manager. CRFs can be downloaded from the FSTRF website at www.fstrf.org.

- For transfers, reference the Patient Transfer from Site to Site SOP 119, and contact **Dave Nichols** directly.
- For other questions, send an e-mail message to actg.corea5336@fstrf.org (ATTN: **Dave Nichols**).
- Include the protocol number, PID, and a detailed question.

Randomization

For randomization/**participant** registration questions or problems and study identification number SID lists.

- Send an e-mail message to rando.support@fstrf.org. Call the Statistical and Data Analysis Center (SDAC)/DMC Randomization Desk at 716-834-0900 x7301.

Computer and Screen Problems

Contact the SDAC/DMC programmers.

- Send an e-mail message to actg.support@fstrf.org or call 716-834-0900 x7302.

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- Send an e-mail message to actg.corea5336@fstrf.org (ATTN: Suria Yesmin).

Copies of the Protocol

To request a hard copy of the protocol, send an email message to ACTGNCC@s-3.com (ATTN: Diane Delgado). Electronic copies can be downloaded from the ACTG Web site (<https://www.actgnetwork.org>).

Product Package Inserts and/or Investigator Brochures

To request copies of product package inserts or investigator brochures, contact the DAIDS Regulatory Support Center (RSC) at RIC@tech-res.com or call 301-897-1708.

Protocol Registration

For protocol registration questions, send an e-mail message to Protocol@tech-res.com or call 301-897-1707.

Protocol Activation

For questions related to protocol activation, contact the clinical trials specialist (**Suria Yesmin**, syesmin@s-3.com) or ACTG Site Coordination group at actgsitecoordination@s-3.com.

Study Product

For questions or problems regarding study product, dose, supplies, records, and returns, call Ana Martinez, protocol pharmacist, at 301-435-3734.

Study Drug Orders

Call the Clinical Research Products Management Center (CRPMC) at 301-294-0741.

IND (Investigational New Drug) Number or Questions

The IND number will be available on the PSWP within 30 days of the submission to the FDA. For any questions related to the IND submission, contact the DAIDS RSC at Regulatory@tech-res.com or call 301-897-1706.

Expedited Adverse Event (EAE) Reporting/Questions

Contact DAIDS through the RSC Safety Office at DAIDSRSCSafetyOffice@tech-res.com or call 1-800-537-9979 or 301-897-1709; or fax 1-800-275-7619 or 301-897-1710.

Phone Calls

Sites are responsible for documenting any phone calls made to A5336 team members.

- Send an e-mail to actg.corea5336@fstrf.org.

Protocol-Specific Web Page

Additional information about management of the protocol can be found on the protocol-specific web page (PSWP).

GLOSSARY OF PROTOCOL-SPECIFIC TERMS

3TC	lamivudine
ABC	abacavir
ACR	American College of Rheumatology
ALT	alanine aminotransferase
AML	acute myeloid leukemia
ANC	absolute neutrophil count
AST	aspartate aminotransferase
ATP	adenosine triphosphate
BBB	blood brain barrier
CMV	cytomegalovirus
CNS	central nervous system
CSF	cerebrospinal fluid
CVD	cardiovascular disease
CWRES	conditional weighted residual
EBV	Epstein Barr virus
EFV	efavirenz
ET	essential thrombocythemia
FOCE INT	first order conditional estimation with η - ω interactions
FTC	emtricitabine
G-CSF	granulocyte colony stimulating factor
GM-CSF	granulocyte-macrophage colony stimulating factor
HBM	human biological materials
HHV	human herpes viruses
HSV	herpes simplex viruses
INSTI	integrase strand transfer inhibitors
JAK	Janus activating kinase
LTBI	latent tuberculosis infection
LTR	long terminal repeat sequences
mCSF	macrophage colony stimulating factor
MDM	monocyte-derived macrophages
NHP	non-human primate
NNRTIs	nonnucleoside reverse transcriptase inhibitors
NRTIs	nucleoside reverse transcriptase inhibitors
PML	progressive multifocal leukoencephalopathy
PV	polycythemia vera
RA	rheumatoid arthritis
ROS	reactive oxygen species
SAE	serious adverse events
SIV	simian immunodeficiency virus
STAT	signal transducer and activator of transcription
SUSARs	serious unexpected serious adverse reactions
TCR	T cell receptor
TDF	tenofovir
TNF- α	tumor necrosis factor alpha
T _{scm}	stem-cell memory T-cells

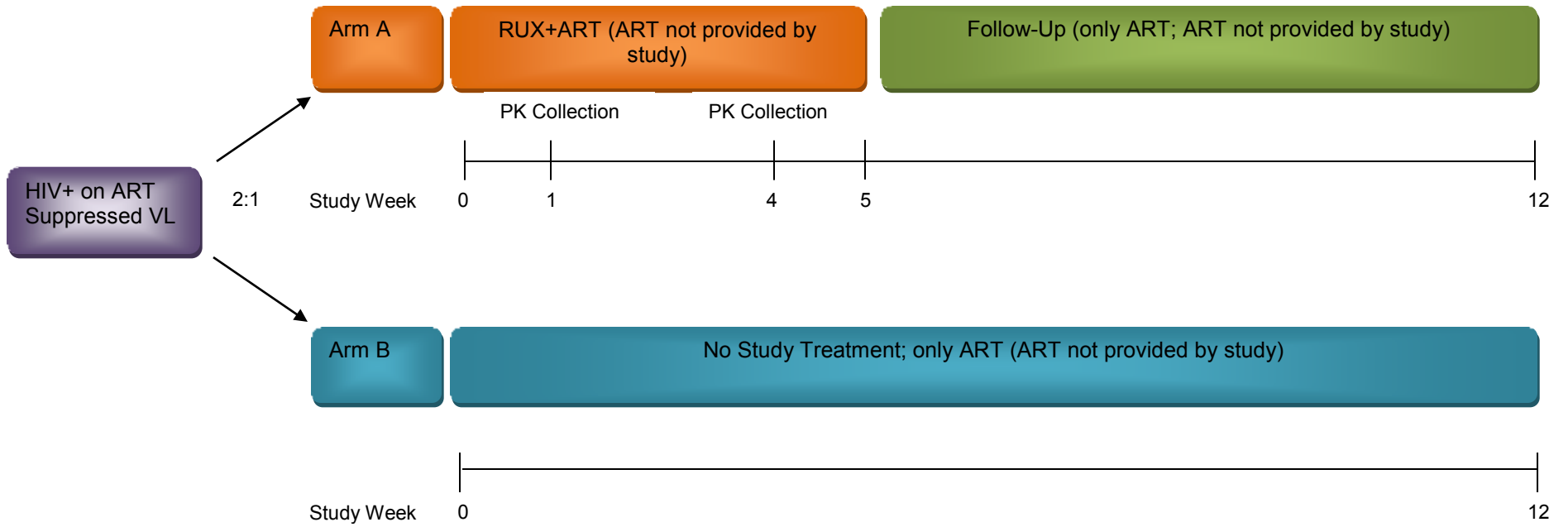
SCHEMA
A5336

A Randomized, Pilot Study of Ruxolitinib in Antiretroviral-Treated HIV-Infected Adults

- DESIGN** This is a multi-center, randomized, open-label, two arm (phase IIa) study. Eligible **participants** on select antiretroviral therapy (ART) with virologic suppression will be randomized to receive ruxolitinib or no study treatment to measure safety and tolerability and to determine whether there are changes in systemic immune activation and inflammation after 5 weeks of treatment with ruxolitinib.
- DURATION** **Participants** will participate in the study for up to 12 weeks after randomization.
- SAMPLE SIZE** A total of 60 **participants** will be enrolled in the study (40 on ruxolitinib [Arm A]; 20 on no study treatment [Arm B]).
- The study aims to enroll at least 15 **participants** on efavirenz (EFV) in order to have at least 8 ruxolitinib-treated **participants** to evaluate pharmacokinetic (PK) interactions with EFV.
- POPULATION** HIV-1 infected males and females ≥ 18 and < 75 years of age on stable ART for at least **12 weeks** prior to study entry containing either tenofovir/emtricitabine (TDF/FTC) or abacavir/lamivudine (ABC/3TC) plus a nonnucleoside reverse transcriptase inhibitor or integrase strand transfer inhibitor (NNRTI or INSTI, not containing cobicistat) who:
- Are on ART for at least 2 years
 - Have virologic suppression defined as: (a) at least two HIV-1 RNA measurements < 50 copies/mL, one of which was obtained within 336 to 31 days, inclusive, prior to study entry and one obtained between 337 and 730 days, inclusive, prior to entry, (b) no HIV-1 RNA values ≥ 50 copies/mL within 336 days, inclusive, prior to study entry, and (c) not more than one HIV-1 viral load equal to or greater than 50 and less than or equal to 200 copies/mL from a result obtained between 337 and 730 days, inclusive, prior to study entry (and no HIV-1 viral load above 200 copies/mL)
 - **Have a plasma HIV-1 quantitative RNA with a lower limit of quantification < 50 copies/mL (e.g., < 40 copies/mL) obtained ≤ 30 days prior to study entry**
 - Have CD4+ T cell count > 350 cells/mm³ within 30 days prior to study entry
 - Have no other medical conditions, or concomitant medications, prohibiting the use of a janus activating kinase-signal transducer and activator of transcription (Jak-STAT) inhibitor
- STRATIFICATION** To ensure balance by treatment arm for EFV, **participants** will be stratified at randomization based on their EFV status (i.e., whether they are on an EFV-containing regimen).
- REGIMEN** TDF/FTC or ABC/3TC + (NNRTI or INSTI, not containing cobicistat), with one of the following:

ARM A: Treatment with ruxolitinib 10 mg orally twice daily for 5 weeks.

ARM B: No study treatment.



1.0 HYPOTHESES AND STUDY OBJECTIVES

1.1 Hypotheses

- 1.1.1 The use of ruxolitinib for 5 weeks with antiretroviral therapy (ART) consisting of: tenofovir/emtricitabine (TDF/FTC) or abacavir /lamivudine (ABC/3TC) + a nonnucleoside reverse transcriptase inhibitor or integrase strand transfer inhibitor (NNRTI or INSTI) will be safe for virologically suppressed **participants**.
- 1.1.2 The use of ruxolitinib for 5 weeks with ART will be tolerable for virologically suppressed **participants**.
- 1.1.3 The use of ruxolitinib for 5 weeks with ART for virologically suppressed **participants** will reduce systemic immune activation and inflammation.

1.2 Primary Objectives

- 1.2.1 To evaluate the safety of ruxolitinib in ART-treated HIV-1 infected virologically suppressed **participants** during 5 weeks of treatment.
- 1.2.2 To evaluate the tolerability of ruxolitinib in ART-treated HIV-1 infected virologically suppressed **participants** during 5 weeks of treatment.
- 1.2.3 To compare changes in interleukin-6 (IL-6) levels between baseline and week 5 in ART-treated HIV-1 virologically suppressed **participants** who are randomized to ruxolitinib and those randomized to receive no study treatment.

1.3 Secondary Objectives

- 1.3.1 To evaluate the safety of ruxolitinib in ART-treated HIV-1 infected virologically suppressed **participants** during all on-study visits through week 12.
- 1.3.2 To compare changes in IL-6 levels between baseline and week 12 in ART-treated HIV-1 virologically suppressed **participants** who are randomized to ruxolitinib to changes in those **participants** randomized to receive no study treatment.
- 1.3.3 To assess whether the use of ruxolitinib will decrease levels of other measures of inflammation and immune activation including sCD14, tumor necrosis factor alpha (TNF- α), IL-1 α , IL-1 β , IL-18, macrophage colony stimulating factor (mCSF), neopterin, HLA-DR, CD38, and both the distribution of monocyte subsets (defined by expression of CD14+ and CD16+) and phenotypic indices of their activation during 5 weeks of treatment and at week 12.
- 1.3.4 To examine whether the use of ruxolitinib is associated with changes in HIV-1 plasma viral load levels (by a commercial clinical assay and single-copy assay) and CD4+ T cell counts in HIV-1 infected **participants** at baseline compared to 12 weeks after initiation of treatment for those receiving 5 weeks of ruxolitinib versus those receiving no study treatment.

- 1.3.5 To assess changes from baseline in the HIV-1 reservoir, specifically plasma HIV-1 RNA by single copy assay (as above), cellular HIV-1 RNA and total DNA, 2 long-terminal repeat sequences [LTRs], and integrated DNA.
- 1.3.6 To evaluate drug-drug interactions between ruxolitinib and antiretrovirals (ARVs) and to characterize the association between ruxolitinib pharmacokinetic (PK) parameters and changes from baseline in inflammatory biomarkers in **participants** on the ruxolitinib treatment arm.
- 1.3.7 To evaluate whether the use of ruxolitinib is associated with more frequent reactivation of human herpes viruses (ie, cytomegalovirus [CMV], Epstein Barr virus [EBV], herpes simplex viruses [HSV], human herpes viruses [HHV] 6, 7, and 8) as measured in longitudinally collected oral swabs.**

1.4 Exploratory Objective

To derive and evaluate a summary measure of immune activation and inflammation and how changes in this measure differ between the study treatment arm and the no treatment arm.

2.0 INTRODUCTION

2.1 Background

Although ART presents a viable and well-established strategy for treating individuals living with HIV-1, a limitation of current ART is the inability to completely reduce systemic inflammation to that seen in age-matched controls in the general population. This inflammation has several consequences for individuals on ART including: (1) establishment of a systemic pro-HIV-1 milieu resulting in an increase in HIV-1 target cells, and possibly thereby maintaining HIV-1 viral reservoirs and allowing infection to persist in various microenvironments; (2) less robust immunologic reconstitution [1]; and (3) increased end-organ damage.

Primary myelofibrosis is a myeloproliferative disease whereby the bone marrow is replaced with collagen fibrosis. This fibrosis occurs due to stromal cell overproduction of collagen stimulated by abnormal megakaryocytes. In response to the subsequent pancytopenia, hematopoietic cells migrate out of the bone marrow resulting in extramedullary hematopoiesis and hepatosplenomegaly. A majority of patients with primary myelofibrosis have a somatic mutation in the gene encoding the cytoplasmic tyrosine kinase, Jak1/2, resulting in activation of Jak1/2 signaling. Activation of the janus activating kinase and signal transducer and activator of transcription (Jak-STAT) pathway leads to production of cytokines that cause the abnormal megakaryocytes to proliferate. Ruxolitinib, a commercially available, US Food and Drug Administration (FDA)-approved Jak2 inhibitor, disrupts this pathway. Many of the cytokines produced from Jak-STAT signaling are also central to the pathogenesis of HIV. It is from this observation that ruxolitinib was first considered as a potential therapeutic option for HIV-1 disease.

Ruxolitinib possesses unique qualities distinct from those of traditional ART, including 1) nanomolar *in vivo* inhibition of TNF- α , IL-6, and other pro-HIV inflammatory cytokines, which have been associated with increased risk of morbidity and mortality in ART-suppressed HIV-1 individuals [2, 3], 2) sub-micromolar (0.1 μ M) inhibition of IL-2, IL-6, IL-7, IL-15, or interferon (IFN)- α induced activation of the Jak-STAT pathway in primary CD4+ T cell lymphocytes, 3) sub-micromolar inhibition of HIV-1/2 replication in primary human lymphocytes and macrophages, 4) down-regulation of HIV-1 induced activation in primary human lymphocytes, and 5) direct inhibition of the Jak-STAT pathway, which is activated early in HIV-1 infection [4].

These attributes of ruxolitinib could address many of the limitations of existing ART and provide a novel mechanism to 1) reduce reservoir seeding and homeostatic proliferation, which is driven by IL-7 and IL-15 orchestrated proliferation of stem-cell memory T cells (T_{SCM}) and other latent T cell subsets; 2) reduce systemic inflammation by inhibition of IL-6 and other pro-HIV/pro-inflammatory cytokines, which would otherwise serve to promote pro-HIV-1 events including (a) priming of uninfected cells for infection, (b) recruitment of uninfected cells to the site of infection, (c) reservoir maintenance [5, 6], and to accelerate disease progression (including AIDS and serious non-AIDS events [7, 8]; 3) reduce CD14+/CD16+ monocytes and CD163 macrophages, which can reduce trafficking of these activated, infected monocytes to the brain/central nervous system (CNS) where they would otherwise either differentiate into macrophage-like cells containing HIV-1 or transfer infection to perivascular macrophages within the CNS (this may also lead to a reduction in cardiovascular events via similar processes [8, 9]), and 4) confer anti-HIV-1/2 potency across primary human lymphocytes and macrophages. Point 4 addresses persistent infection in macrophage-derived viral sanctuaries, which is not adequately controlled by current ART due to suboptimal intracellular concentration of ART in these cells [10]. Additionally, as macrophages are long-lived cells that harbor virus, efficient inhibition of viral replication in these cells, and down-regulation of HIV-1 induced activation (CD163) in these cells by a mechanism distinct from direct inhibition of the viral replication cycle may provide a foundation to control inflammation-driven events including increased replication due to pro-inflammatory cytokines, induction of reactivation of virus from these cells, priming of uninfected macrophages by inflammation induced mCSF, cerebrospinal fluid production (which promotes viral replication), and trafficking of infected myeloid lineage cells to the brain (driven by HIV-1 orchestrated activation of macrophages, which is marked by elevated CD163 levels). Together the potential ability of ruxolitinib to address these unmet needs could (a) contribute to control of persistent infection, (b) limit reservoirs maintenance, and (c) prevent ongoing infection across and within tissue compartments. Although adverse effects (AEs) exist, administration of ruxolitinib to patients with CD4+ T cell counts ≥ 350 cells/mm³, platelet counts $> 200,000$ /mm³, absolute neutrophil count (ANC) ≥ 750 /mm³, and hemoglobin ≥ 12 g/dL with close monitoring for AEs should not present any greater risk to safety compared to HIV-1 infected healthy **participants** on ART.

The HIV-1 reservoir is comprised of latently infected cells, predominantly central memory and transitional memory CD4+ T cell lymphocytes [11], that contain integrated HIV-1 DNA. Integrated HIV-1 DNA can remain latent or be actively transcribed; the effect of ruxolitinib on this equilibrium is unknown. To assess the effects of ruxolitinib on the HIV-1 reservoir, markers of HIV-1 production (plasma and cellular HIV-1 RNA levels, 2-LTR circles) and persistence (total and integrated HIV-1 DNA) can be measured and followed longitudinally. HIV-1 2-LTR circles are dead-end, non-integrated DNA species that are

formed when HIV-1 infects a cell but the linear HIV-1 DNA fails to integrate into the human genome. 2-LTR circles can remain detectable for at least 10-12 years during suppressive ART [12], and it remains unclear whether they are a marker of ongoing HIV-1 replication or a byproduct of long-lived episomes maintained by latently-infected cell division.

This study relies on the well-established anti-inflammatory profile of ruxolitinib in patients treated for primary myelofibrosis as a foundation to probe the effect of reducing inflammation on pro-HIV events described above in patients that are virologically controlled on ART, so that the role of inflammation in HIV-1 infection can be more clearly defined. Should ruxolitinib efficiently inhibit inflammation in the HIV-1 suppressed **participant** on ART, it may be feasible that individuals with well-controlled viremia receiving ART could undergo therapy interruption to determine whether viral rebound occurred post-ruxolitinib administration, either in the absence or presence of ruxolitinib. These data would ultimately define whether selective, systemic inhibition of pro-inflammatory/pro-HIV cytokines could result in partial or total inhibition of viral rebound, or a functional cure. The study is designed to address the inflammation-driven facets of ART-suppressed HIV-1 infection, and to explore a novel therapeutic strategy that reduces cellular activation and systemic inflammation, targets Jak-STAT signaling across lymphocytes and macrophages, and ensures viable delivery across the blood-brain barrier (BBB) to eliminate the HIV-1 reservoir of the CNS.

2.2 Rationale

HIV-1 continues to be a global health crisis with more than 34 million people living with HIV-1 worldwide (UNAIDS/WHO). Despite the success of combination ART in suppressing viral replication, HIV-1 continues to persist. Although several mechanisms likely contribute to persistence during ART, the stable integration of HIV-1 DNA within resting CD4+ T cells and perhaps cells of the monocyte/macrophage lineage is generally assumed to be the most important mechanism [13]. All approved ART drugs target steps in actively replicating virus, rendering them ineffective for 1) eliminating latent virus, 2) preventing or reversing reactivation of latent virus, 3) preventing recruitment of uninfected cells to the site of infection, and 4) inhibiting pro-HIV/pro-inflammatory cytokine milieus that drive chronic infection, disease progression, and end-organ damage. To this end, in the era of ART, it has become apparent that inflammation will continue to result in end-organ damage and eradication of HIV-1 cannot occur without elimination of the latent viral reservoir. For these reasons, novel therapies are needed.

Role of Jak-STAT Pathways in HIV-1 Infection

The transcriptional status of HIV-1 is tightly coupled to the activation state of its host cell. The phosphorylation state of Jak-STAT pathways is known to influence HIV-1 infection in various target cells [14, 15]. HIV-1 orchestrated pro-inflammatory cytokines or envelope gp120 activates Jak and STAT proteins leading to expression of multiple receptors and/or cytokines and generalized immune dysfunction (including activation) in a variety of HIV-1 target cells, including primary human lymphocytes and macrophages [4, 8-9, 13-19]. Cytokines that are upregulated and expressed include IL-6, TNF- α , and IFN-1 α/β [20-23]. Inflammation, most notably associated with IL-6, can contribute to reseeding and maintenance of the viral reservoir because IL-6 stimulates HIV-1 replication by activating viral transcription in synergy with TNF- α and also by targeting a post-transcriptional step [24, 25].

Blocking Jak-STAT signaling pathways could reverse or prevent HIV-induced activation of both macrophages and lymphocytes, thereby reducing HIV-orchestrated systemic immune activation and suppressing chronic inflammation. Consequently, HIV-1 production from latently infected cells and HIV-1 infection of new target cells may decrease. Inhibition of these pathways may also reduce trafficking of virus and infected cells across the BBB, preventing CNS persistence and HIV-associated neurocognitive disease. Since HIV-1 infection of macrophages causes alterations in the cell cycle [26], chemotaxis [27], increased levels of TNF- α and other TH2-type cytokines [28], phagocytosis [29, 30], and antigen presentation [31], inhibition of Jak-STAT pathways might have profound beneficial effects on these cells.

Consequently, we anticipate that by blocking Jak-STAT signaling, the CD4+ T cell count could also increase owing to two primary mechanisms: 1) a reduction in HIV-1 replication-dependent cell death and 2) a reduction in bystander apoptosis-induced cell death independent of HIV-1 replication. The first mechanism relies upon the theory that there is ongoing HIV-1 replication in sanctuary sites despite suppressive ART [32-34]. In this setting, a reduction in pro-inflammatory cytokines could result in fewer target cells trafficking to sites of infection, decreased expression of chemokine receptor type 5 (CCR5) and other cellular activation markers that would otherwise make uninfected cells more permissive to HIV-1 infection, and fewer virus particles would be produced per HIV-1 infected cell.

Monocytes/Macrophages as a Reservoir for HIV-1

Cells of the monocyte/macrophage lineage have, as of yet, a poorly defined role in HIV-1 persistence during long-term ART. Several studies have shown a correlation between activated, CD14+/CD16+ monocytes and HIV-1-associated neurocognitive impairment, immune activation, higher viral loads, and disease progression [35-38]. In untreated HIV-1 and possibly treated HIV-1 disease, carriage of HIV-1 across the BBB by infected monocytes, activated macrophages, and lymphocytes establishes a persistent infection primarily in perivascular macrophages and microglia as viral reservoirs in the immune-privileged CNS (the "Trojan horse" hypothesis) [22, 36, 39]. In contrast to HIV-1 infected CD4+ T cells, infection in brain macrophages and microglia leads to an extended life span and increased survival in the presence of apoptotic stresses, while concomitantly orchestrating a pro-inflammatory cytokine milieu that drives viral replication in already infected cells, primes uninfected cells for infection, and results in production of reactive oxygen species and other factors that drive HIV-associated neurocognitive impairments [20, 40-44]. In addition, viral proteins expressed early in the viral replication cycle (such as Nef, Tat, and virion-associated Vpr) play a role in the formation of viral reservoirs in macrophages by activating transcription and interfering with apoptotic machinery [19].

The BBB also consists of a number of unique elements that prevent passage of many drugs into the brain (as also seen in lymphoid tissue). Several drug physical properties influence the penetration of drugs through the BBB, including molecular weight, protein binding, lipophilicity, and the action of passive and adenosine triphosphate (ATP)-dependent influx and efflux pumps [45]. Viral populations in the CNS can diverge from those of plasma due to the resulting differences in tissue drug exposures. Furthermore, the dynamic interaction between signaling pathways that promote virus replication, active/infiltrating macrophages, cytokines/chemokines, and secreted neurotoxic products, may interfere with the overall homeostatic regulation required to maintain normal quiescent brain function. The overall mechanism of action of Jak inhibitors and

their role in inhibiting pro-HIV-1 events that are not addressed by current highly active ART are depicted below in Figure 1.

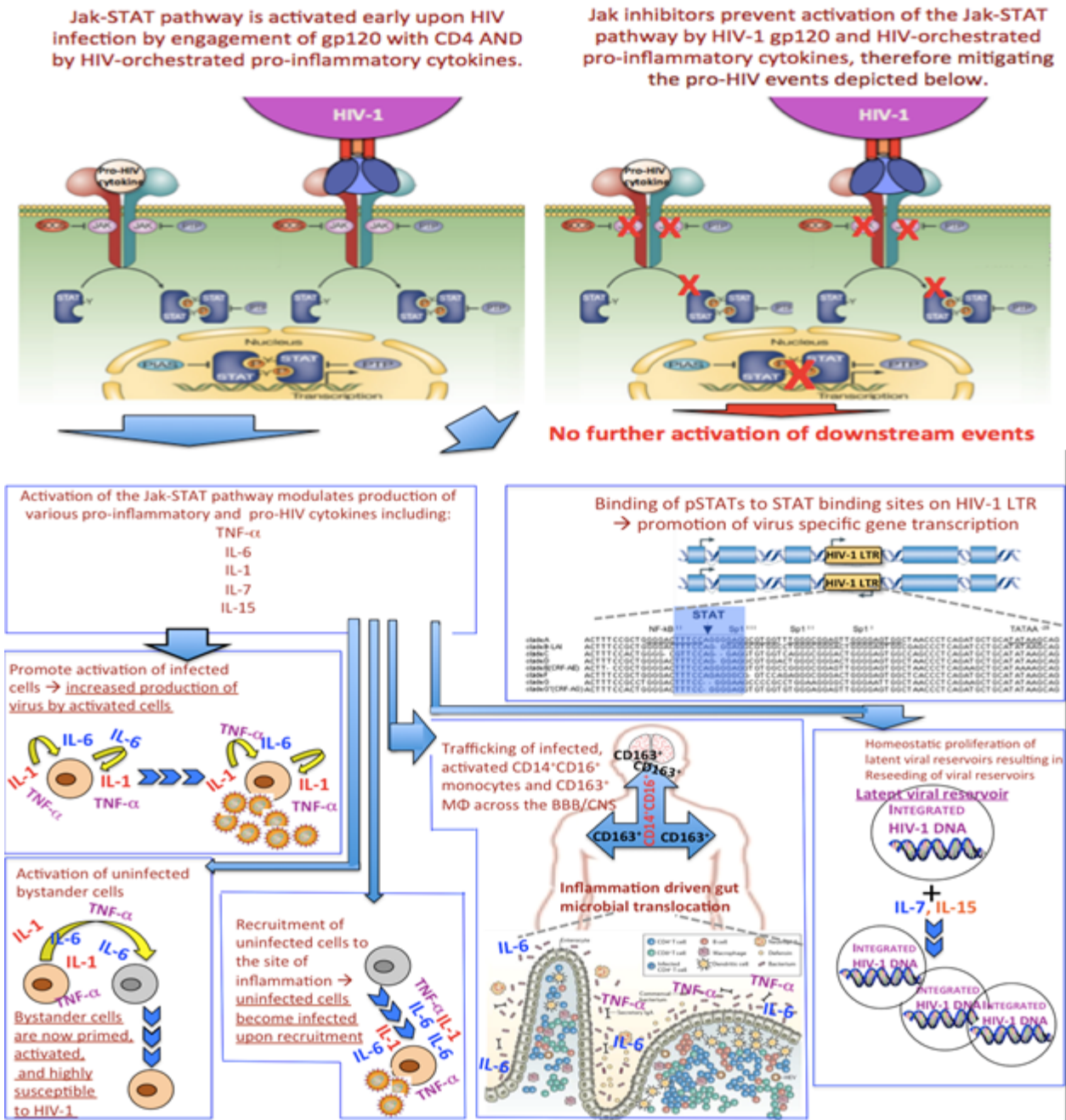


Figure 1. The Jak-STAT pathway is activated by either HIV-orchestrated pro-inflammatory cytokines or HIV-1 gp120 engaging the host cell (upper left panel). Upon activation, multiple pro-HIV-1 events transpire, including 1) increased production of virus in already infected cells; 2) priming of uninfected cells for infection; 3) recruitment of uninfected cells to the site of infection, therefore exposing them to infection and priming them for infection; 4) activation of monocytes and macrophages, which promotes trafficking of these infected cells across and to the CNS/brain; and 5) microbial translocation in the gut (boxes above). Use of Jak inhibitors prevents activation

of the Jak-STAT pathway, mitigating these pro-HIV-1 events (upper right panel) [46, 47].

Table 1: The Proposed Impact of Ruxolitinib on Various Biomarkers Relevant to HIV-1 Pathogenesis, Immunologic Recovery, and the Reservoir

Markers	Role in HIV-1 infection in the absence of ruxolitinib
CD14+/CD16+ monocytes	Trafficking of infected monocytes to the CNS and across the BBB
CD163	A: Trafficking of infected macrophages to the CNS and across the BBB B: Recruitment of macrophages to privileged sites of low level replication (gut, lymph nodes, others)
IL-6, IL-1, TNF-alpha, GM-CSF	A: Prime uninfected cells for infection B: Produce more virus per infected cell C: Drive gut microbial translocation D: Impact end-organ disease such as cardiovascular, neurocognitive and malignancies E: Potential impact on collagen deposition in the bone marrow but this effect was previously implicated in individuals with myelofibrosis
IL-7, IL-15	Drivers of homeostatic proliferation of latently infected memory lymphocytes
pSTATs binding to HIV-1 LTR	Promotes HIV-specific gene transcription

Ruxolitinib

The commercially available Jak 1/2 inhibitor, ruxolitinib ((3R)-3-cyclo-pentyl-3-[4-(7H-pyrrolo [2, 3-d]pyrimidin-4-yl)pyrazol-1-yl]propanenitrile; Jakafi, Incyte Corporation and Novartis), acts by interfering with the binding of ATP to Jaks. Ruxolitinib was approved by the FDA for treatment of persons with intermediate and high-risk myelofibrosis [48] and polycythemia vera. Other clinical trials are currently underway to determine its efficacy and safety for the treatment of rheumatoid arthritis (RA), autoimmune psoriasis, and various malignancies.

In vitro studies performed at Emory University demonstrate antiviral potency of ruxolitinib in HIV-infected monocyte-derived macrophages (MDM) and lymphocytes grown in 20% serum. The EC_{50/90} in macrophages was in the range of 0.09/1.3 to 0.3/4.8 µM with and

IC₅₀ (cellular toxicity) of 13.4 to >100. The EC_{50/90} in lymphocytes ranged from 0.02 - 4.7 μ M with no effect on viability up to 50 μ M [49]. Furthermore, ruxolitinib has a short plasma $t_{1/2}$ (~3 hr), so that the duration of exposure to ruxolitinib is considerably shorter than in vitro exposure (5 days). Maximal plasma concentrations in humans (C_{max}) are between 3.6-10.0 μ M and persist for < 30 min [50, 51] demonstrating a wide therapeutic window of safety.

Ruxolitinib is a nanomolar in vivo inhibitor of TNF- α , IL-6, and other pro-HIV inflammatory cytokines (see Figure 2, based on phase I/II and III clinical studies) [2, 3], and is a direct inhibitor of the Jak-STAT pathways activated early in HIV-1 infection [4]. Data provided by Incyte Corporation on **participants** treated with ruxolitinib for myelofibrosis (Study INCB 18424-256) demonstrated that inhibition of pro-inflammatory and activation markers were observed within 2 weeks of administration of ruxolitinib 25 mg twice a day (BID), and in many cases were observed as early as 3 days post administration (Figure 4). Overall, ruxolitinib is a promising drug for further testing as a modulator of inflammation due to its well-documented safety profile and nanomolar anti-inflammatory action in humans together with its sub-micromolar antiviral activity against HIV-1 in culture [50, 52].

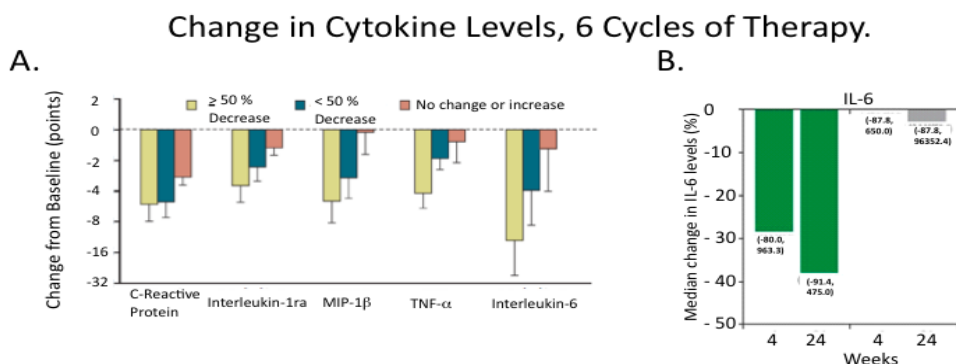


Figure 2 (adapted from [2, 3]). Changes in selected cytokines and C-reactive protein, an acute phase reactant and a marker for inflammation, show in patients with $\geq 50\%$ decrease those with < 50% decrease, and those with no change or an increase in the composite symptom score after 6 cycles of treatment as compared with baseline, where y-axis is reported as “points” which is a change cytokine level versus baseline (A), or median change in IL-6 levels versus baseline (percent) (B).

Safety of Ruxolitinib

Over 9,800 individuals have or are currently taking ruxolitinib, as reported by Incyte Corporation. Post-marketing surveillance for safety of this drug has continued since it was approved for myelofibrosis by the FDA in November 2011, with no recommended changes by the FDA. Additionally, Dr. Srdan Verstovsek, the first author of the clinical trials performed with ruxolitinib for myelofibrosis, stated that their longitudinal study has not identified any new toxicities [50]. In his review, all published reports have shown satisfactory safety data on 1,400 **participants** with no new toxicities [53].

In the initial dose-finding study of ruxolitinib in myelofibrosis (Study INCB 18424-251), the most common AEs observed included hematologic (thrombocytopenia and anemia) and non-hematologic AEs which were infrequent (bruising, dizziness, and headache) after a median of 14.7 months on therapy [2]. There were few Grade 3 or 4 events when

using FDA-approved doses (15 mg BID or 20 mg BID depending on the platelet count and adjusted as needed for efficacy or toxicity). Of the 153 **participants** in the study, serious adverse events (SAEs) occurred in 59 **participants**, of whom only 12 had SAEs that were considered to be possibly related to treatment. The hematologic events varied by the dosing regimen (Table 2a). Diarrhea, fever, and urinary tract infections were described in 5.9%, 2%, and 2.6%, respectively, for all grades with only 0.7% having a Grade 3 or 4 fever. Individuals with HIV-1 were excluded from these studies.

In the phase III COMFORT-1 study in individuals with myelofibrosis (FDA dosing as above), there was a survival advantage when compared to placebo with 13 deaths in the ruxolitinib group (8.4%, N=155) and 24 deaths in the placebo group (15.6%, N=154) during a median follow-up period of 51 weeks [3]. Among the deaths, there was no detectable increase risk of serious infections; pneumonia occurred in two **participants** who received ruxolitinib and one who received placebo, and sepsis occurred in two **participants** from each arm. Although two **participants** developed acute myelogenous leukemia (AML) after receiving ruxolitinib, they were at increased risk for this disease prior to receiving the study drug. Grade 3 or 4 thrombocytopenia occurred in 12.9% of the ruxolitinib arm and 1.3% in the placebo arm with Grade 3 episodes of bleeding in 2.6% of **participants** in the ruxolitinib arm and 2.0% in the placebo arm; 1.3% of **participants** in each arm had Grade 4 episodes of bleeding. There was one Grade 3 event of bruising in the ruxolitinib arm. Thrombocytopenia rarely recurred at a Grade 3 or 4 level after appropriate dose reduction and was not associated with an increase in bleeding events, although bruising was more common in the ruxolitinib group. Grade 3 or 4 anemia peaked at weeks 8 to 12 and then subsequently declined to levels similar to those in placebo-treated **participants**.

A longer term evaluation of 107 **participants** enrolled in the open label, non-randomized phase I-II study of ruxolitinib (INCB 18424-251) showed that 49 (46%) discontinued ruxolitinib by 3 years (24 by year 1, 36 by year 2, and 46 by year 3). Of those who discontinued treatment, only 3 (2.8%) did so for unacceptable toxicity. The others discontinued for progressive disease (12), withdrawal of consent (7), physician decision to discontinue (5), intercurrent illness (3), death (13), or other reasons (6). None of the deaths were considered by the investigators as related to ruxolitinib, see Table 2b. [52]. A cohort description in 120 Asian **participants** treated for myelofibrosis with ruxolitinib was published online in late 2014 [54]. The authors concluded that, "Adverse events were consistent with those seen in the COMFORT studies." The principal AEs were anemia, thrombocytopenia and diarrhea. Of 7 deaths that occurred in the cohort, one death due to sepsis and one due to hepatorenal syndrome were suspected by the investigator to be related to ruxolitinib treatment.

Table 2. (a) Hematologic AEs [2] (*red box) indicates dose similar to that proposed for this study, (b) Rate and Reasons for Discontinuation in Study INCB18424-251 [52].

a. Hematologic Adverse Events

Variable	10 mg Twice Daily	15 mg Twice Daily	25 mg Twice Daily	50 mg Twice Daily	25 mg Once Daily	50 mg Once Daily	100 mg Once Daily	200 mg Once Daily	Total
Thrombocytopenia – no./total no. (%) [*]									
Grade 3	3/29 (10)	1/35 (3)	11/47 (23)	3/5 (60)	0/6 (0)	6/22 (27)	2/6 (33)	0	26/153 (17)
Grade 4	0	0	3/47 (6)	1/5 (20)	0	2/22 (9)	0	1/3 (33)	4/153 (3)
New anemia [‡] , participants who were transfusion independent	3/19 (16)	2/24 (8)	8/30 (27)	0/2 (0)	1/4 (25)	5/15 (33)	1/4 (25)	3/3 (100)	23/101 (23)

* Values for platelet count abnormalities were graded according to the Common Terminology Criteria for Adverse Events (CTCAE), version 3.0 (Grade 3 = 25×10^9 /liter to $< 50 \times 10^9$ /liter; Grade 4 = $< 25 \times 10^9$ /liter).

[‡] Anemia was defined as a decrease in the hemoglobin level of more than 20 g per liter to a Grade 3 or 4 level (as defined according to the CTCAE, version 3.0) in patients who were not transfusion dependent.

b. Rate and reasons for discontinuation in study INCB18424-251, An Open-Label, Nonrandomized Phase I-II Study of Ruxolitinib

	MDACC	Mayo Clinic Rochester
Participants Enrolled, N	107	51
Participants remaining on study, N (%)	58 (54)	5 (10)
Participants who discontinued, N, (%)	49 (46)	46 (90)
Discontinued by 1 y., %	24	51 [‡]
Discontinued by 2 y., %	36	72 [‡]
Discontinued by 3 y., %	46 ^{‡‡}	89 [‡]
Primary reason for discontinuation, [*] N, (%)		
Death	13 (12.1)	4 (7.8)
Progressive disease	12 (11.2)	10 (19.6)
Participant withdrawal of Consent	7 (6.5)	15 (29.4)
Physician decision to Discontinue	5 (4.7)	12 (23.5)
Intercurrent illness	3 (2.8)	0
Unacceptable toxicity	3 (2.8)	1 (2.0)
Other	6 (5.6)	4 (7.8)

Data provided by Incyte Corporation upon MD Anderson Cancer Center request.

* As categorized by defined headings on case report forms (CRFs) filled out by the investigator.

† Discontinuation rates as published in Tefferi et al in 2011[55].

†† NOTE: not all ongoing **participants** have reached year 3; by Kaplan-Meier analysis, the discontinuation ratio at year 3 was 53 %.

Additional safety data on ruxolitinib from studies conducted for other indications are available online at “ClinicalTrials.gov”; however, causality of side effects listed was not determined in these reports. As an example, the “Study to Determine the Safety and Efficacy of Ruxolitinib (INCB018424) in **Participants** With Polycythemia Vera (PV) or Essential Thrombocythemia (ET)” had 13.7% (10/73) of **participants** with SAEs that varied depending on the dose (10 mg BID, 25 mg BID, and 50 mg QD) and underlying disease (PV or ET), and the SAE included one report each of pneumonia, thrombocytopenia, anemia, renal cancer, renal failure, congestive cardiac failure, atrial flutter, headache, variceal bleeding, gastrointestinal bleeding, cholecystitis and bronchitis. In a second metastatic prostate cancer study, out of the 22 **participants** who received ruxolitinib and experienced an SAE, two had anemia, and there was one report each of an overdose, pathologic fracture, cardiac arrest, sepsis, gastrointestinal bleeding, prostate cancer metastasis, metastatic pain, hydronephrosis, and renal failure. In a third multiple myeloma study, out of 20 **participants** who received either ruxolitinib (N=13) or ruxolitinib plus dexamethasone (N=7), there were three SAE reports of pneumonia, two reports each of gastrointestinal bleeding and disease progression, and one report each of anemia, congestive cardiac failure, pericardial effusion, pyrexia, fatigue, pain, pneumococcal sepsis, urinary tract infection, overdose, hyperglycemia, lung cancer, seizure, pulmonary embolism, and hematoma. The incidence of SAE was significantly higher in the ruxolitinib plus dexamethasone group versus ruxolitinib group. Progressive multifocal leukoencephalopathy (PML) [56], reactivation of latent tuberculosis (pulmonary [57, 58] and disseminated [59, 60]), hepatitis B infection [61, 62], herpesvirus infections [63], *Pneumocystis jiroveci* pneumonia [64], toxoplasmosis retinitis [65], and *Cryptococcus neoformans* pneumonia [66] have been reported on **participants** who received ruxolitinib treatment for myelofibrosis.

Incyte Corporation provided unpublished data presented at both the American College of Rheumatology (ACR) meeting as well as unpublished data obtained internally, demonstrating that 10 mg ruxolitinib BID has equivalent efficacy for cytokine reduction, but the average change in hemoglobin and platelet count is negligible. These data demonstrate that 10 mg BID is sufficient to inhibit production of pro-inflammatory and activation markers while concomitantly mitigating any potential detrimental side effects that were observed at higher dosages. Therefore, for this study the 10 mg BID dose has been selected for the treatment arm in order to minimize treatment-related AEs while maximizing efficacy.

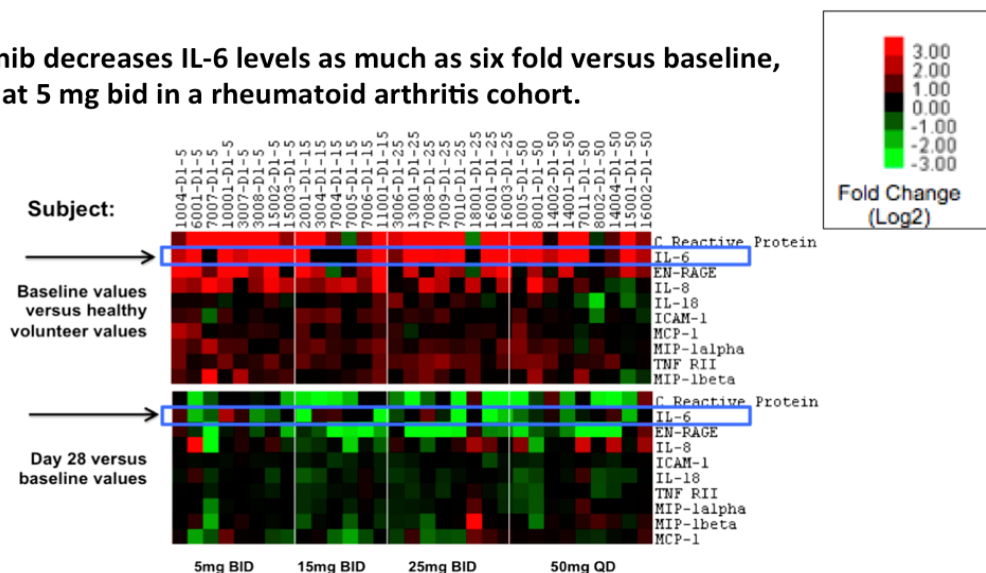
Dose Effects on Biomarkers

Data on cytokine changes in **participants** treated with lower doses of ruxolitinib for myelofibrosis were provided by Incyte Corporation. Additionally, data on cytokine changes in **participants** treated with lower doses of ruxolitinib for RA have been obtained from an oral presentation at the 2008 American College of Rheumatology Annual Scientific Conference. Both of these data sources demonstrate reductions in levels of various biomarkers (see heatmap below, Figure 3), as well as RA and myelofibrosis symptom scores with doses as low as 5 mg BID.

Aggregate data have been provided by Incyte Corporation relative to the dose of 10 or 15 mg BID ruxolitinib in Study INCB 18424-356, "Study of Efficacy and Safety in Polycythemia Vera **Participants** Who Are Resistant to or Intolerant of Hydroxyurea: JAK Inhibitor INC424 (INCB018424) Tablets Versus Best Available Care: (The RESPONSE Trial)". Many **participants** in the study did not demonstrate levels of IL-6 above the lower limit of detection (LLD) of 6 pg/mL at baseline. Therefore, at endpoint measurement, it was not possible to determine whether their IL-6 levels had decreased (Figure 5 below). Considering only those **participants** with elevated levels of IL-6 at baseline, four of six **participants** demonstrated significant decreases in IL-6 levels (Figure 6 below). The **participants** with a low baseline IL-6 level could have had a decline in IL-6 while on ruxolitinib but those changes would not be detected by the assay used. A more sensitive assay for IL-6 will be employed in A5336, with a LLD of 0.2 log₁₀ pg/mL (SD=0.25 log₁₀ pg/mL). This will allow us to measure an effect for any **participants** with a base line IL-6 above approximately 0.5 pg/mL.

There are published data in myelofibrosis showing that symptomatic improvement for symptoms thought to be caused by elevated inflammatory cytokines, including night sweats, pruritus and bone pain are equally improved at doses of 10 mg BID as at higher doses up to 25 mg BID. There is a clear drop off in symptomatic benefit when the dose is lower than 10 mg BID (given as 5 mg BID), but even at that level there is improvement compared to placebo [67]. Reductions in phosphorylated-STATs and Jaks at 10 mg BID are also observed in myelofibrosis and RA studies, each of which are surrogate markers for down regulation of IL-6 levels.

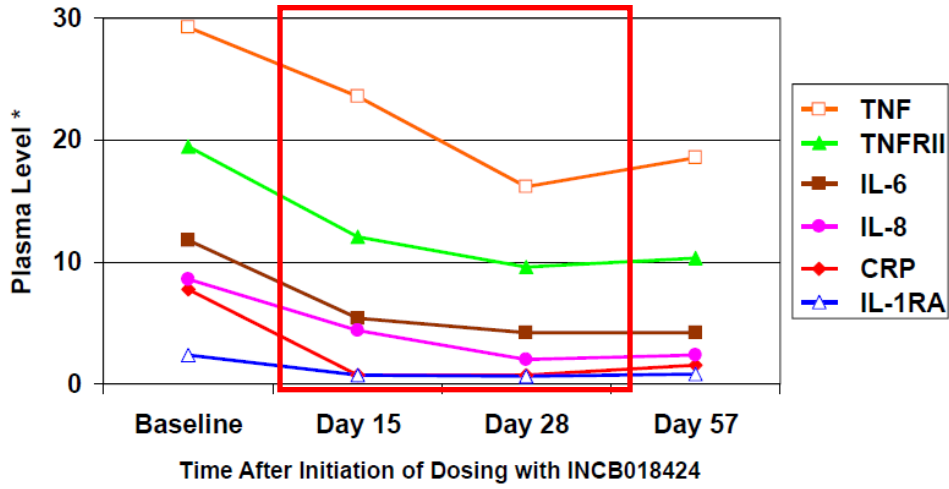
Ruxolitinib decreases IL-6 levels as much as six fold versus baseline, starting at 5 mg bid in a rheumatoid arthritis cohort.



Annals Rheum Dis, 2008, v67SII:62

A Randomized Placebo-Controlled Study of INCB018424, a Selective Janus Kinase1&2 (JAK1&2) Inhibitor in Rheumatoid Arthritis (RA). Presented by Larry Moreland, U. Pitt, William Williams, Peggy Scherle, Jack Shi, Robert Newton, Edward McKeever, Jordan Fridman, Timothy Burn, Kris Vaddi, Richard Levy Incyte Corporation, Wilmington DE.

Figure 3. Ruxolitinib decreases levels of IL-6 biomarker



*Plasma levels are given in pg/ml for TNF and IL-6; in ng/ml for TNFRII, IL-8, and IL-1RA; and in mg/ml for CRP.

Figure 4. Changes in inflammatory markers demonstrate predominant effect at 4 weeks (28 days) for 21 participants with myelofibrosis receiving 25 mg BID (Study INCB 18424-256)

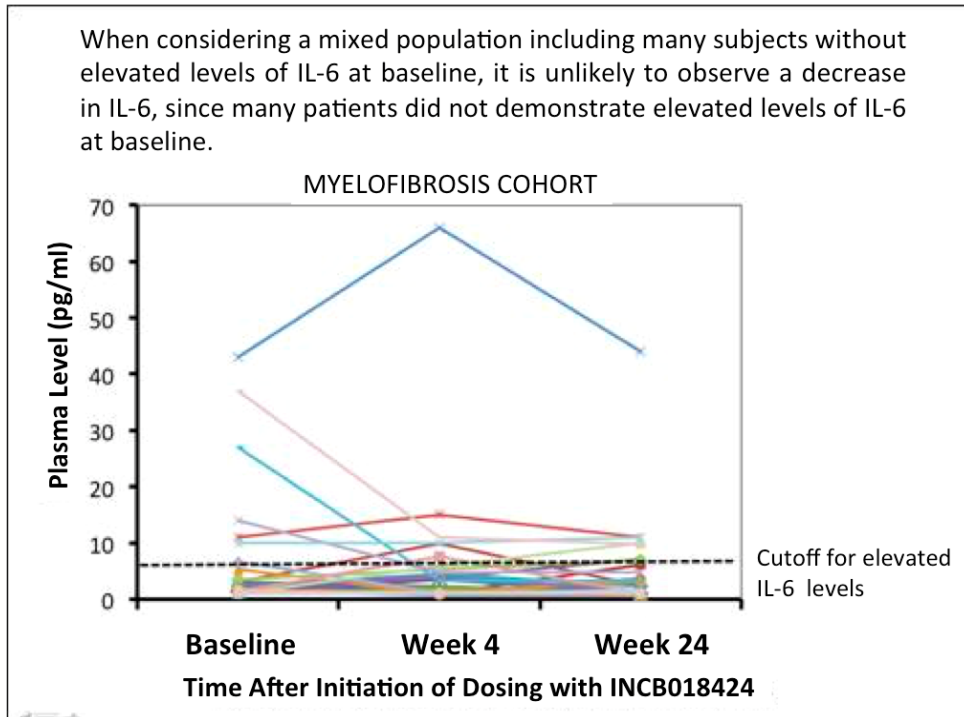


Figure 5. Participants with and without elevated IL-6 at baseline who received 10-15 mg BID for polycythemia vera and essential thrombocythemia (Study INCB18424-356)

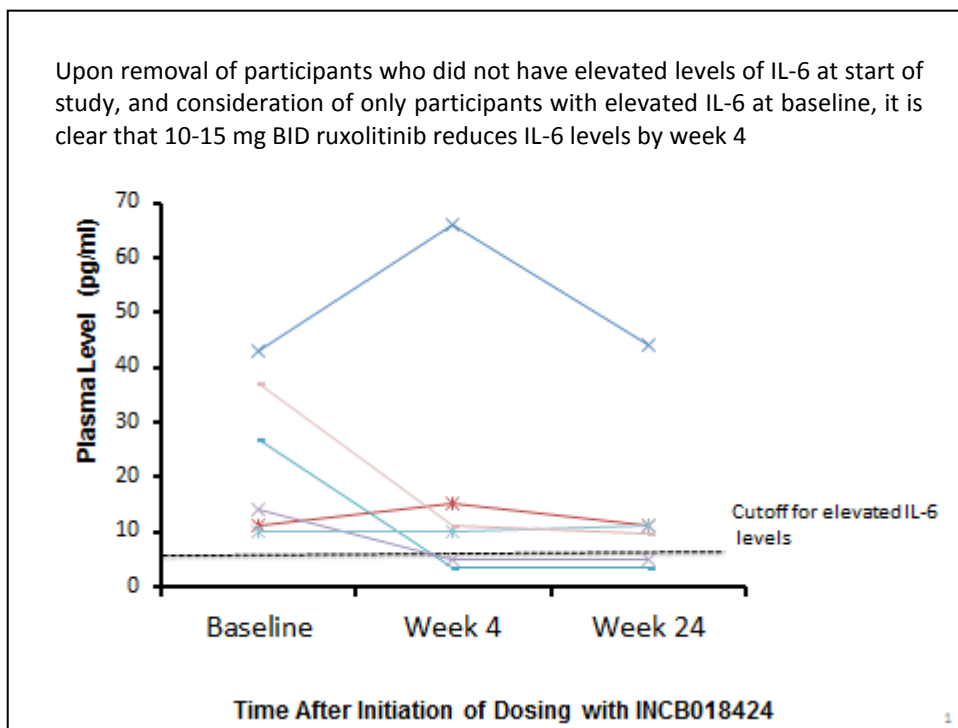


Figure 6: **Participants** with elevated IL-6 at baseline who received 10-15 mg BID for polycythemia vera and essential thrombocythemia (Study INCB18424-356)

Pharmacology of Ruxolitinib

The FDA-approved starting dose is 20 mg given orally (PO) BID for **participants** with a platelet count $> 200,000/\text{mm}^3$, 15 mg PO BID for **participants** with a platelet count of $100,000/\text{mm}^3$ to $200,000/\text{mm}^3$, and 5 mg PO BID for **participants** with a platelet count between $50,000/\text{mm}^3$ and $100,000/\text{mm}^3$. Data from Incyte Corporation have shown that ruxolitinib can be dosed at 10 mg BID with roughly equivalent myelofibrosis efficacy and significantly reduced AEs compared to higher doses. Dose reductions and subsequent dose increases in clinical trials and in the clinical setting are quite common with rapid resolution of AEs (and improvement in clinical indicators). Similarly, titrating dose to effect is both recommended and commonly practiced as well. Therefore, the protocol team has chosen to use a dose of 10 mg BID (without dose escalations) for all **participants** in this study and will exclude **participants** with platelet counts $\leq 200,000/\text{mm}^3$.

Ruxolitinib is a substrate of CYP3A4, but not an inhibitor of this enzyme (see package insert). It is recommended that when administering ruxolitinib with strong CYP3A4 inhibitors, the total daily dose of ruxolitinib should be reduced to 5 mg BID (i.e., approximately 50% of the dose rounding up to the nearest dosage strength). No dose adjustment is necessary for mild or moderate CYP3A4 inhibitors (see package insert). Raltegravir and other INSTI are not substrates for CYP3A4.

Safety and Coadministration of EFV with Ruxolitinib

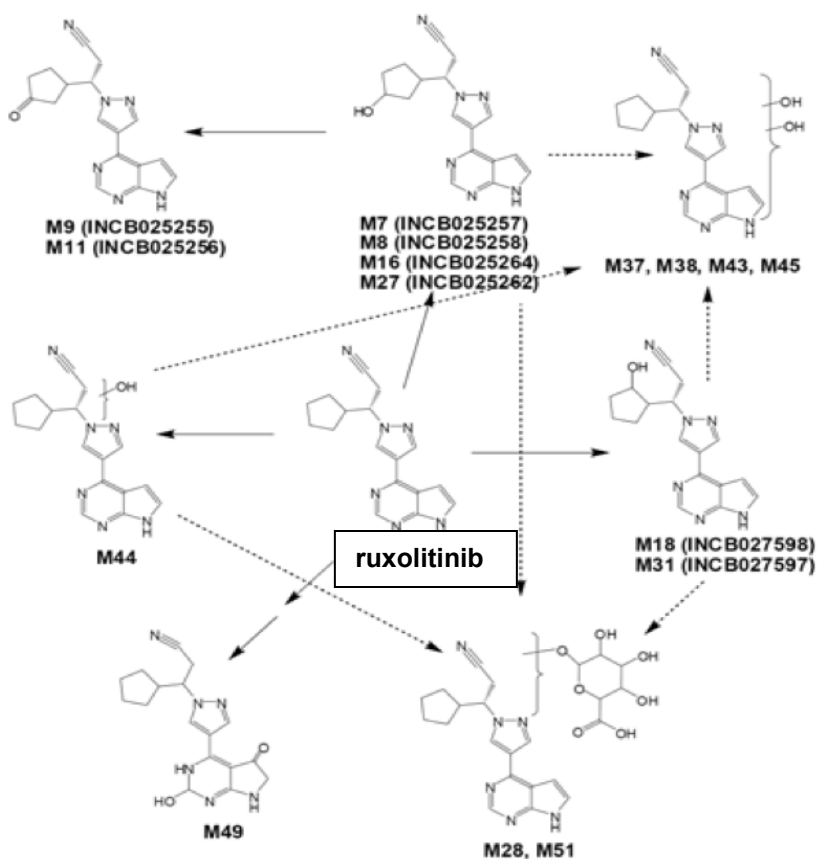
Efavirenz is classified as a moderate CYP3A4 inducer [68]. No dose adjustment is recommended when ruxolitinib is coadministered with a CYP3A4 inducer (see package insert). However, plasma AUC values for ruxolitinib metabolites increased with

increasing severity of renal impairment.

Metabolites of Ruxolitinib

Metabolites of ruxolitinib act on the same pharmacological receptors, however at lower potency (1/5 to 1/2 of the parent compound) and at relatively low concentrations. Therefore, when ruxolitinib is metabolized, the effect of the metabolite is between 20-50% of the original parent compound. Thus, the contribution of toxicity resulting from an increase in drug metabolites would be more than compensated for by a net decrease in concentration of the parent drug. The rates of elimination half-life of ruxolitinib and its metabolites are rapid and similar (approximately 3 – 5.8 hours). Therefore, steady-state accumulation is not expected to occur (see more at: http://www.incyte.com/sites/default/files/Jakafi_PI.pdf?time=20141230135651). The dose selected for this study is 10 mg BID, which is the recommended reduced dose and is below or equivalent to package insert guidelines when considering metabolites of ruxolitinib in the presence of EFV.

The mass balance trial INCB 18424-134 reported that following a single 25 mg dose of ruxolitinib solution (100 μ Ci 14 C-ruxolitinib) parent drug was the predominant entity in circulation, representing 58% to 74% of the total radioactivity between 1 and 6 h post-dose. Metabolite M18 was observed at 17% of the total, circulating, drug-related material based on AUC and the other observed ruxolitinib mono- and di-hydroxylated and ketone metabolites represented less than 10% (Figure 7). Eight of these metabolites (M7, M8, M9, M11, M14, M16, M18, and M27) when added to parent drug, accounted for greater than 90% of the drug-related material in circulation (based on AUC). No metabolites were observed in human plasma after 12 h post-dose.



Source: Study report for study INCB 18424-134

Figure 7: Metabolites of ruxolitinib. In summary, M18 is the major metabolite with 17%; sum of 8 other metabolites $\leq 10\%$.

Justification for Choice of IL-6 as a Primary Endpoint Marker

IL-6 has been the most highly implicated biomarker for end-organ disease and mortality on ART. A number of inflammation-associated biomarkers have demonstrated prognostic significance in well-treated HIV-1 infection. These include IL-6, D-dimers, sCD14, sCD163, anti-TNF-receptor, CRP, monocyte phenotype, and T cell activation. Perhaps the most consistent and well-validated biomarkers are IL-6 and D-dimer. In the SMART study, IL-6 levels were most strongly associated with cardiovascular disease (CVD) and mortality (odds ratio for 4th/1st quartile of 3.5 and 11.8 for CVD and mortality, respectively) [69, 70]. These results were confirmed in a large analysis of all treated **participants** in the INSIGHT families (which included ESPIRIT and SILCAAT, $n=3766$), a single determination of IL-6 was associated with disease progression over the subsequent 5 to 8 years. The prognostic significance of IL-6 in treated HIV-1 disease was confirmed in a longitudinal analysis of **participants** in ALLRT and a case-control study from **participants** enrolled in SOCA/SCOPE [71, 72]. A number of other studies have confirmed that IL-6 is higher than expected in treated HIV-1 disease [73-75], and associated with poor prognosis [8, 76, 77]. The impact of IL-6 on prognosis is generally not attenuated after controlling for viral load, CD4+ T cell count, and other factors. To our knowledge, there is no other biomarker which has been so consistently associated with disease progression in the context of treated HIV-1 disease. Furthermore, mutations in the IL-6 gene have been associated with increased IL-6 levels coincident with an

increase in CVD, placing IL-6 in the casual pathway, not just as a surrogate marker of inflammation [7] but as an earlier predictor of disease progression [78].

In order to assess feasibility, we examined **participants** who were analyzed as part of NWCS 329, wherein **participants** from several ACTG trials were followed for cardiovascular and malignant endpoints. Cases and controls were selected based on these particular outcomes. Among the 171 controls with viral load < 50 copies/mL and CD4+ T cell > 350 cells/mm³ at 1 year after ART initiation, the following IL-6 quartiles were identified:

Quartile 1 (25%):	IL-6 < 0.86
Quartile 2 (26%):	IL-6 = 0.86 to 1.42
Quartile 3 (20%):	IL-6 = 1.43 to 2.0
Quartile 4 (29%):	IL-6 ≥ 2.1

Based on these data, we believe that a reasonable number of **participants** will have levels of IL-6 within a range conducive for appreciating a reduction in the presence of ruxolitinib.

Justification for the Choice of Assay to Measure Changes in IL-6

We have measured plasma levels of IL-6 using the R&D high sensitivity assay. This is a solid-phase sandwich ELISA that can be performed on serum or plasma. In this assay, the LLD is usually around 0.16 pg/mL, and the assay range is 0.11 pg/mL-10 pg/mL. The manufacturer reports that there is < 0.5% cross-reactivity observed with molecules related to IL-6. Using this assay, it was reported [63] that among 60 **participants** with virologic control and immune failure (CD4+ T cell < 350/uL), 20 **participants** with virologic control and immune success (CD4+ T cell > 500/uL) and 21 healthy controls, none had IL-6 levels in plasma lower than 0.46 pg/mL. Samples are obtained in EDTA and processed and cryopreserved within 6 hours of blood draw. Assays are performed on cryopreserved samples using manufacturer's instructions and inter-assay consistency is evaluated by running an aliquot of the same sample on each plate. Thus we are confident that our assay is sensitive enough to measure IL-6 levels in plasma in our study **participants**.

HIV-1 Persistence

Recent *in vitro* data have provided further insight into the potential role for ruxolitinib to affect the HIV-1 reservoir.

Overall, the potential mechanisms impacted by ruxolitinib include the following:

1. Seeding of the HIV-1 reservoir: Recent data (Figure 8) and others show that γ -C receptor cytokines (IL-2, IL-7, IL-15) which are triggered by chronic T cell receptor (TCR) activation and bystander immune activation (bacterial translocation, alarmins) can upregulate HIV-1 transcription and production. This is because the sequence of the HIV-1 LTR includes STAT binding sites [5, 79]. This ultimately results in increased numbers of infected cells, increased production of viral particles and an enhanced seeding of the HIV reservoir. Inhibition of Jak-STAT signaling pathways should decrease the number of HIV-1 infected cells and ultimately the HIV-1 reservoir.

2. Maintenance of the HIV-1 reservoir: We have shown that IL-7 (a γ -C receptor cytokine) maintains and expands the latent HIV-1 reservoir by triggering homeostatic proliferation [5]. Inhibition of homeostatic proliferation downstream of cytokines such as IL-7 and IL-15 should result in a decreased expansion/maintenance of the latent HIV-1 reservoir.
3. Inhibition of cytokine induced inflammation driven HIV-1 replication and T cell dysfunction/exhaustion: Several pro-inflammatory cytokines, which are upregulated in HIV-1 infection, enhance HIV-1 replication as they can activate T cells thereby increasing expression of CCR5 and trafficking of these cells to sites of infection; they also inhibit T cell and innate immune function as these cytokines upregulate type I interferons (IL-7, IL-15) and several negative regulators of T cell activation (PD-1, Lag-3) and their ligands (PDL-1, MHC II) in individuals with HIV-1. This should induce HIV-1 latency in cells expressing these molecular factors [5, 22, 79, 80].
4. Inhibition of Jak-STAT may prevent the induction of an HIV-1 latent pool by preventing expression of negative regulators on T cells and their ligands on innate immune cells. This could result in the restoration of innate and adaptive immune function, two critical steps in controlling or eliminating latently infected cells and a functional cure [81].

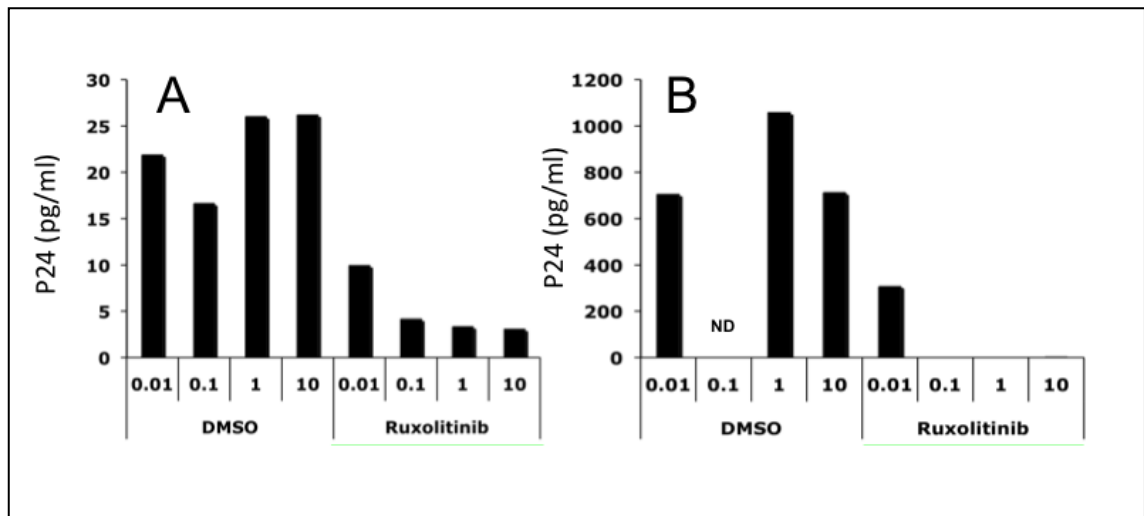


Figure 8. Inhibition of HIV-1 Replication by Ruxolitinib. Viral production as measured by p24 ELISA in primary CD4⁺ T cells isolated from a viremic donor stimulated for 3 (A) and 6 days (B) with α CD3/28 in the presence of increasing concentration of ruxolitinib or DMSO (vehicle) only, reported in μ M (x-axis).

Infectious HIV-1 reservoir measurements using a quantitative virus outgrowth assay (Q-VOA) may underestimate reservoir size [82] and require larger volume blood draws than are feasible in this study. We plan to use total and integrated HIV-1 DNA levels as markers of HIV-1 persistence and plasma HIV-1 RNA and peripheral blood mononuclear cells (PBMC)-associated HIV-1 RNA and 2-LTR circles as markers of HIV production. Measurement of plasma virus load, cellular RNA and 2-LTR circles will examine two different components of HIV-1 replication: the amount of virion production that may occur during ruxolitinib therapy and the degree to which virions may infect cells and yet fail to

form proviruses. STAT-5-like sequences are present in the HIV-1 LTR and suggest a mechanism whereby ruxolitinib treatment may modulate HIV-1 latency. 2-LTR circle quantification will corroborate changes that may be observed in total HIV-1 DNA, relative to integrated HIV-1 DNA, during ruxolitinib therapy.

Activation of Viral Co-infections

Previous studies reported reactivation of herpes viruses among participants receiving ruxolitinib for clinical indications; however, the use of other immunosuppressant therapies makes it impossible to assess the individual contribution of ruxolitinib [83, 84]. As part of this study we will determine whether the use of ruxolitinib is associated with more frequent reactivation of human HHV as measured in longitudinally collected oral swabs. Shedding of HHV DNA is frequently detected in saliva of healthy individuals and more frequently in HIV-infected adults and persistent replication of HHV (in particular CMV) was repeatedly associated with increased systemic inflammation and cellular activation [85, 86, 87]. As an alternative hypothesis, since persistent inflammation can also be a trigger for HHV reactivation, reduction in inflammation after ruxolitinib administration might be associated with decrease in HHV replication.

If treatment with ruxolitinib is associated with increased oral shedding of HHV, we will determine whether the presence of enhanced sub-clinical HHV replication might in turn affect the ability of this immuno-modulatory intervention to reduce inflammation during suppressive ART. Also, if increased reactivation occurs, then measures of asymptomatic HHV replication should be considered as a possible safety measure for future studies.

Potential Impact of this Clinical Trial

Chronic inflammation and immune activation promote end-stage organ disease leading to decreased survival in virologically suppressed individuals on ART. To date, interventions to mitigate this process are severely lacking. Inhibition of the Jak-STAT pathway using a FDA-approved agent, ruxolitinib, provides an unprecedented opportunity to significantly attenuate levels of inflammation and such an approach may restore the immunologic balance. We hypothesize that Jak-STAT inhibitor treatment will result in higher CD4+ T cell counts as well as lower levels of immune activation and chronic inflammation. We expect to observe decreased immune activation that will persist following discontinuation of ruxolitinib. We also posit that by “resetting” the immunologic balance in favor of less immune activation and inflammation, this immunologic milieu will persist in the absence of ruxolitinib. Since lower levels of immune activation and chronic inflammation have been associated with fewer AIDS and serious non-AIDS clinical events, we anticipate that long term use of ruxolitinib could provide a tangible clinical benefit, i.e., increased event-free survival. Furthermore, we have shown in vitro that ruxolitinib has specific anti-HIV-1 properties even in resting CD4+ T cells and monocyte/macrophages, and can reduce homeostatic proliferation, which we expect will result in a decline of the HIV-1 reservoir. If successful, this study will be the first of its kind to demonstrate both lasting immunologic enhancement and impact on the HIV-1 reservoir.

Justification for Proceeding with Studies in Humans with HIV-1

Presently studies of ruxolitinib are being conducted on non-human primates (NHP) infected with simian immunodeficiency virus (SIV) (both viremic and virologically

suppressed on ART). However, there is no guarantee that the PKs, safety profile, and efficacy of ruxolitinib in these models will correlate with humans infected with HIV-1.

The dose selected for the NHP/SIV study is significantly lower than that which has already been dosed in other NHP studies (Incyte Corporation data). The dose being used in the NHP/SIV study is also calculated to mirror the 10 mg BID dosing in humans, which is well tolerated and lower than commonly prescribed doses of 15, 20, or 25 mg BID. To that end, studies with prolonged dosing periods of ruxolitinib in the NHP have not been conducted previously. The dose selected herein for the NHP study is designed to provide a balance between what we know is well tolerated in humans, and a safe level relative to what has been dosed in NHP previously. More specifically, the dose to be used in the NHP study has been calculated using the published single dose PK of ruxolitinib in NHP, allowing for mathematical scaling of NHP dosing to match plasma PKs observed in humans for 10 mg BID dosing.

The data obtained could provide a proof-of-principle for reduction in SIV or HIV induced inflammation and activation, but species and virus differences dictate that results should not be used to modify human clinical studies. Differences between the SIV infected NHP model and humans, such as virus (HIV versus SIV), cellular factors, coreceptors/receptors, and generalized cross-species differences, dictate that this pilot NHP study will probably not yield information resulting in modification of the human clinical protocol.

The NHP pilot study included a total of four rhesus macaques with viral loads > 200,000 copies/mL, which were treated with 2.2 mg/kg ruxolitinib BID via oral administration for a total of 6 weeks. Two monkeys were administered add-in HAART [(-)-FTC, PMPA, and Kaletra] starting at week 4 of ruxolitinib administration. During the first dose of ruxolitinib, blood samples were collected at baseline (before drug), and 1, 2, 4, and 12 hour post administration for PKs. Blood samples were tested once a week, thereafter, and plasma collected during the remainder of the study (6 weeks total). Samples (3 mL) were aliquoted to collect whole blood (for measuring of integrated viral DNA in PBMCs, monocytes and macrophages and cell associated immunology markers, e.g., D16, CD163, Ki67, HLA-DR, CD25, and CD38. Plasma samples have been stored at -70° C before being assayed to determine concentrations of ruxolitinib, (-)-FTC, PMPA, and Kaletra, and soluble activation markers, e.g., sCD14, IL-6, IL-1/b. CSF was collected once per week to measure drug levels and viral loads.

The safety profile of ruxolitinib in individuals with myelofibrosis has been well-documented and FDA-approved for this indication. Emerging data have shown that even fewer AEs have been reported in individuals without myelofibrosis (solid tumors and RA). We believe that the population of individuals living with HIV-1 who would be eligible for this study is immunologically and hematologically “healthier” than individuals with myelofibrosis and therefore should have few AEs.

Justification for the **Participant** Population Selected

Virologically Suppressed on ART

In order to assess the impact of ruxolitinib on chronic inflammation and systemic immune activation, it was important to exclude the impact of ART on these same markers through mechanisms dependent on and independent of viral replication.

Age and CD4+ T Cell Criteria

In order to minimize AEs associated with ruxolitinib, we chose a **participant** population with an adequate CD4+ T cell -count and within a safe age range, ≥ 18 to < 75 . Furthermore, the immune system of adults and children of ages outside of this range behave differently, which could introduce additional confounders into the efficacy assessment.

Exclusion of Pregnant Women

Ruxolitinib has been designated pregnancy category C. In animal studies, treatment with ruxolitinib resulted in an increase in late resorptions and reduced fetal weights at maternally toxic doses. There are no adequate and well-controlled studies of ruxolitinib in pregnant women.

Exclusion of **Participants** with Hepatitis B Virus (HBV) or Hepatitis C Virus (HCV) Infection

According to Incyte Corporation, there are only five **participants** with HBV infection and no **participants** with HCV infection who received ruxolitinib. Additionally, two case reports of HBV reactivation in individuals receiving ruxolitinib for myelofibrosis were published [61, 62]. Therefore, it was recommended that **participants** with HBV infection or HCV infection be excluded from this study.

Risk Benefit Assessment

Ruxolitinib may have side effects, including thrombocytopenia, anemia, diarrhea, fever, infections, and fatigue and could result in changes of ARV concentrations. There are also risks associated with phlebotomy such as discomfort, bleeding, bruising and/or inflammation plus a small risk of infection. It is not known if ruxolitinib causes harm to the fetus. By using a low dose of ruxolitinib, short treatment duration, and choosing a population that has higher CD4+ T cell counts and that is not pregnant, we feel these risks have been minimized. This study is intended to explore how ruxolitinib could impact the immune system for individuals with virologic suppression on ART. There **is no anticipated benefit for participants participating in this study**. Information learned from this study may help others who have HIV-1 and are experiencing ongoing inflammation and systemic immune activation. For a further description of the potential impact of this study, refer to the section "Potential Impact of This Clinical Trial" (see above).

3.0 STUDY DESIGN

A5336 is a randomized, open-label, prospective, 2-arm (phase IIa) study that will enroll 60 HIV-1 infected **participants** (≥ 18 to < 75 years of age) who have been virologically suppressed for at least 2 years (defined below in section 4.1.3), have CD4+ T cells > 350 cells/mm³ ≤ 30 days prior to study entry, and have plasma HIV-1 RNA below the limit of detection ≤ 30 days prior to entry. **Participants** will be randomized at a 2:1 ratio to either Arm A (treatment with ruxolitinib) or Arm B (no study treatment) (40 and 20 in the ruxolitinib and no study treatment arms, respectively). **Participants** in Arm A will receive treatment with ruxolitinib 10 mg BID for 5 weeks. Based upon the mechanism of action for ruxolitinib, it is not expected that the plasma viral load will increase. However, as a safety precaution, **participants** will be monitored for changes in HIV-1 viral load. If changes in inflammatory markers are observed and/or if there are advances in the field

which might provide further rationale for assessing the virologic endpoints in the absence of achieving the desired immunologic outcomes, we will assess for changes in the HIV-1 reservoir (specifically plasma HIV-1 RNA by single copy assay, cellular HIV-1 RNA and total DNA, 2 LTRs, and integrated DNA). Refer to section 6.1, Schedule of Events (SOE), for specific evaluations and time points. **Participants** in Arm A will be followed for 7 weeks after discontinuation of ruxolitinib, and will be on study for a total of 12 weeks. **Participants** in Arm B will be followed on-study for 12 weeks.

4.0 SELECTION AND ENROLLMENT OF **PARTICIPANTS**

4.1 Inclusion Criteria

- 4.1.1 HIV-1 infection, documented by any licensed rapid HIV test or HIV enzyme or chemiluminescence immunoassay (E/CIA) test kit at any time prior to study entry and confirmed by a licensed Western blot or a second antibody test by a method other than the initial rapid HIV and/or E/CIA, or by HIV-1 antigen, or historical plasma HIV-1 RNA viral load.

NOTE: The term “licensed” refers to a US FDA-approved kit, which is required for all IND studies.

WHO (World Health Organization) and CDC (Centers for Disease Control and Prevention) guidelines mandate that confirmation of the initial test result must use a test that is different from the one used for the initial assessment. A reactive initial rapid test should be confirmed by either another type of rapid assay or an E/CIA that is based on a different antigen preparation and/or different test principle (eg, indirect versus competitive), or a Western blot or a plasma HIV-1 RNA viral load.

- 4.1.2 CD4+ T cell count $> 350 \text{ cells/mm}^3 \leq 30$ days prior to study entry **at** any US laboratory that has a CLIA (Clinical Laboratory Improvement Amendments) certification or its equivalent.
- 4.1.3 Documentation of virologic suppression defined as:
- (a) at least two historical HIV-1 RNA < 50 copies/mL using FDA-approved assay performed by any laboratory that has a CLIA certification or its equivalent while on ART, one of which was obtained between 336 to 31 days, inclusive, prior to study entry and one obtained between 337 and 730 days, inclusive, prior to study entry,
 - (b) no HIV-1 RNA values ≥ 50 copies/mL using a FDA-approved assay performed by any laboratory that has a CLIA certification or its equivalent within 336 days, inclusive, prior to study entry, and
 - (c) not more than one HIV-1 RNA value equal to or greater than 50 and less than or equal to 200 copies/mL from a result obtained between 337 and 730 days, inclusive, prior to study entry (and no HIV-1 viral load above 200 copies/mL).

- 4.1.4 Screening plasma HIV-1 quantitative RNA below the limit of detection using an FDA-approved assay with a lower limit of quantification < 50 copies/mL (e.g., < 40 copies/mL obtained by the Abbott Real-Time assay or 20 copies/mL by the Roche Taqman v2.0 assay) performed by any laboratory that has a CLIA certification or its equivalent **obtained** ≤ 30 days prior to study entry.
- 4.1.5 **Tuberculosis (TB) screening within 365 days of the screening visit diagnosed by tuberculin skin test or interferon gamma release assay.**
- 4.1.6 Currently on continuous ART for at least 730 days prior to study entry, defined as continuous ART for the 730 days period, inclusive, prior to study entry with no ART interruption longer than 7 consecutive days.
- NOTE: The current regimen must include tenofovir/emtricitabine (TDF/FTC) or abacavir/lamivudine (ABC/3TC) + a nonnucleoside reverse transcriptase inhibitor or integrase strand transfer inhibitor (NNRTI or INSTI, not containing cobicistat) for at least 60 days, inclusive, prior to study entry.
- 4.1.7 The following laboratory values obtained ≤ 30 days prior to entry by any US laboratory that has a CLIA certification or its equivalent:
- Absolute neutrophil count (ANC) ≥ **1000**/mm³
 - Hemoglobin ≥ 12.0 g/dL
 - Platelets ≥ 200,000/mm³
 - Calculated creatinine clearance (CrCl) ≥ **80** mL/min (by Cockcroft Gault equation). NOTE: A calculator for estimating the CrCl can be found at www.fstrf.org/ACTG/ccc.html
 - Aspartate aminotransferase (AST) (SGOT) ≤ **1.5x** ULN
 - Alanine aminotransferase (ALT) (SGPT) ≤ **1.5x** ULN
 - Alkaline phosphatase ≤ **1.5x** ULN
- 4.1.8 For females of reproductive potential (defined as women who have not been post-menopausal for at least 24 consecutive months, i.e., who have had menses within the preceding 24 months, or women who have not undergone surgical sterilization, specifically hysterectomy and/or bilateral oophorectomy or bilateral salpingectomy) must have a negative serum or urine pregnancy test with a sensitivity of 25 mIU/mL within 72 hours, inclusive, prior to study entry.
- 4.1.9 All **participants** must agree not to participate in a conception process (e.g., active attempt to become pregnant or to impregnate, sperm donation, in vitro fertilization).
- 4.1.10 All **participants** of reproductive potential, who are participating in sexual activity that could lead to pregnancy, must agree to use at least one reliable method of contraception while receiving the study drugs and for 7 weeks after stopping the medications. Acceptable forms of contraceptive include:
- Condoms (male or female) with or without a spermicidal agent
 - Diaphragm or cervical cap with spermicide

- Intrauterine device (IUD)
- Hormone-based contraceptive (must contain at least 35 mcg of ethinyl estradiol)

4.1.11 Women who are not of reproductive potential (women who have been post-menopausal for at least 24 consecutive months or have undergone hysterectomy and/or bilateral oophorectomy or salpingectomy) or men who have documented azoospermia or undergone vasectomy are eligible to start study drugs without requiring the use of contraceptives. Acceptable documentation of sterilization and menopause is specified below.

Written or oral documentation communicated by clinician or clinician's staff of one of the following:

- Physician report/letter
- Operative report or other source documentation in the patient record (a laboratory report of azoospermia is required to document successful vasectomy)
- Discharge summary
- Follicle stimulating hormone-release factor (FSH) measurement elevated into the menopausal range as established by the reporting laboratory.

4.1.12 Men and women age ≥ 18 and < 75 years.

4.1.13 Ability and willingness of **participant** or legal representative to provide written informed consent and attend study visits as scheduled at a participating site.

4.2 Exclusion Criteria

4.2.1 A current or past history of progressive multifocal leukoencephalopathy.

4.2.2 Breastfeeding or pregnancy.

4.2.3 Use of strong inhibitors or inducers of CYP3A4 including a protease inhibitor, cobicistat or entry inhibitors as part of the current ART regimen or other concomitant therapy.

4.2.4 Known allergy/sensitivity or any hypersensitivity to components of study drug or their formulation.

4.2.5 Active drug or alcohol use or dependence that, in the opinion of the site investigator, would interfere with adherence to study requirements.

4.2.6 Acute or serious illness or infection requiring systemic treatment and/or hospitalization within 60 days prior to entry.

4.2.7 Vaccinations (other than influenza) ≤ 30 days prior to the study entry visit.

NOTE: Influenza vaccine is permitted. **Participants** are encouraged to get this vaccine ≥ 7 days prior to the study pre-entry visit.

- 4.2.8 Use of immunomodulators (e.g., interleukins, interferons, cyclosporine), systemic cytotoxic chemotherapy or investigational therapy ≤ 60 days prior to study entry.
- 4.2.9 Any **current diagnosis or past history of a significant pulmonary, neurologic, cardiac, renal, or hepatic disorder** prior to study entry, **excluding treated HIV or treated hypertension. Diagnoses that would lead to exclusion include, but are not limited to the following:**
- CDC category C AIDS-indicator conditions
NOTE A: Except HIV encephalopathy, HIV wasting, esophageal candidiasis, or pneumocystis pneumonia without dissemination.
NOTE B: List available:
<http://www.cdc.gov/mmwr/preview/mmwrhtml/00018871.htm>
 - Varicella zoster
 - Lymphoproliferative malignancy
 - **Chronic or severe psychiatric condition**
 - **Chronic liver disease of any etiology and any degree of severity.**
 - **Chronic hepatitis**
 - **Disseminated fungal infection of any type or duration that is not limited to cutaneous or mucocutaneous surfaces**
 - **A medical disorder that predisposes to bleeding**
NOTE: If a site investigator is unsure of whether a history of a significant medical or psychiatric condition should lead to participant exclusion, the investigator should err on the side of safety if s/he believes that an active or clinically resolved disorder may put the participant at risk from participation in the study, influence the results of the study, or affect the participant's ability to participate. If still uncertain, then the site should contact the protocol team (actg.corea5336@fstf.org) to assist in a determination.
- 4.2.10 Change in the ART regimen within 12 weeks, inclusive, prior to study entry or intended modification of ART during the study.

NOTE: Modifications of ART doses during the 12 weeks prior to study entry are permitted. In addition, the change in formulation (e.g., from standard formulation to fixed-dose combination) is allowed within 12 weeks prior to study entry. A within class single drug substitution (e.g., switch from nevirapine to efavirenz or from atazanavir to darunavir) is allowed within 12 weeks prior to study entry, with the exception of a switch between any other NRTI to/from abacavir. No other changes in ART within the 12 weeks prior to study entry are permitted. **However, participants need to be receiving (and tolerating) an allowable (for study purposes) ART regimen for at least 4 weeks prior to study entry.**

- 4.2.11 History of untreated latent tuberculosis infection (LTBI) diagnosed by tuberculin skin test or interferon gamma release assay. LTBI treatment would consist of 9 months of isoniazid or an equivalent therapy **completed at least 4 weeks prior to study entry.**

4.3 Study Enrollment Procedures

- 4.3.1 Prior to implementation of this protocol, and any subsequent full version amendments, each site must have the protocol and the protocol consent form(s) approved, as appropriate, by their local institutional review board (IRB)/ethics committee (EC) and any other applicable regulatory entity (RE). Upon receiving final approval, sites will submit all required protocol registration documents to the DAIDS Protocol Registration Office (DAIDS PRO) at the Regulatory Support Center (RSC). The DAIDS PRO will review the submitted protocol registration packet to ensure that all of the required documents have been received. Site-specific informed consent forms (ICFs) WILL be reviewed or approved by the DAIDS PRO, and sites will receive an Initial Registration Notification when the DAIDS PRO receives a complete registration packet. Receipt of an Initial Registration Notification indicates successful completion of the protocol registration process. Sites will not receive any additional notifications from the DAIDS PRO for the initial protocol registration. A copy of the Initial Registration Notification should be retained in the site's regulatory files.

Upon receiving final IRB/EC and any other applicable RE approvals for an amendment, sites should implement the amendment immediately. Sites are required to submit an amendment registration packet to the DAIDS PRO at the RSC. The DAIDS PRO will review the submitted protocol registration packet to ensure that all the required documents have been received. Site-specific ICF(s) WILL NOT be reviewed and approved by the DAIDS PRO, and sites will receive an Amendment Registration Notification when the DAIDS PRO receives a complete registration packet. A copy of the Amendment Registration Notification should be retained in the site's regulatory files.

For additional information on the protocol registration process and specific documents required for initial and amendment registrations, refer to the current version of the DAIDS Protocol Registration Manual.

Once a candidate for study entry has been identified, details will be carefully discussed with the **participant**. The **participant** will be asked to read and sign the approved protocol consent form. For **participants** from whom a signed informed consent has been obtained, an ACTG Screening Checklist must be entered through the Data Management Center (DMC) **Participant** Enrollment System.

4.3.2 Randomization/**Participant** Registration

Participants who meet enrollment criteria will be registered to the study according to standard ACTG DMC procedures. For **participants** from whom informed consent has been obtained, but who are deemed ineligible or who do not enroll into the initial protocol step, an ACTG Screening Failure Results form must be completed and keyed into the database.

4.4 Coenrollment Guidelines

- Sites are encouraged to coenroll **participants** in A5128, “Plan for Obtaining Informed Consent to Use Stored Human Biological Materials (HBM) for Currently Unspecified Analyses,” and **A5351s**, “**Effect of Immune-Modulatory Interventions on Cytomegalovirus Replication during Suppressive Antiretroviral Therapy**”
- Coenrollment in A5128 and **A5351s** does not require permission from the A5336 protocol chairs.
- For specific questions and approval for coenrollment in other studies, sites should contact the protocol team via e-mail as described in the Study Management section.

5.0 STUDY TREATMENT

5.1 Regimens, Administration, and Duration

Study treatment is defined as ruxolitinib. **Participants** will be randomized at a 2:1 ratio to either 5 weeks of treatment with ruxolitinib or no study treatment as follows:

ARM A: Ruxolitinib 10 mg PO BID with or without food. **Participants must remain on ART regimen (not provided by the study) for the duration of the study.**

ARM B: No study treatment. **Participants should be encouraged to remain on ART regimen for the duration of the study.**

Participants in Arm A should be instructed not to drink grapefruit juice or eat **grapefruit** while taking ruxolitinib. **Participants** will remain on study treatment for five weeks and **be** followed for seven weeks after treatment discontinuation. **Participants** will be on study for **a total of 12 weeks.**

5.2 Study Product Formulation and Preparation

Ruxolitinib tablets 10mg should be stored at room temperature between 20° and 25°C (68° and 77°F). Excursions permitted between 15°C and 30°C (59°F and 86°F). See USP controlled room temperature.

5.3 Pharmacy: Product Supply, Distribution, and Accountability

5.3.1 Study Product Acquisition/Distribution

Ruxolitinib, manufactured by Incyte Corporation, will be provided by Emory University and available through the NIAID Clinical Research Products Management Center (CRPMC). The site pharmacist should obtain the study product(s) for this protocol by following the instructions in the manual Pharmacy Guidelines and Instructions for DAIDS Clinical Trials Networks.

5.3.2 Study Product Accountability

The site pharmacist is required to maintain complete records of all study products received from the NIAID CRPMC and subsequently dispensed. All unused study products in CRSs must be returned to the NIAID CRPMC (or as otherwise directed by the sponsor) after the study is completed or terminated. The procedures to be followed are in the manual Pharmacy Guidelines and Instructions for DAIDS Clinical Trials Networks.

5.4 Concomitant Medications

Whenever a concomitant medication or study agent is initiated or a dose changed, investigators must review the concomitant medication's and study agent's most recent package insert, Investigator's Brochure, or updated information from DAIDS to obtain the most current information on drug interactions, contraindications, and precautions.

Additional drug information may be found on the updated ACTG Drug Interactions Database located at: http://tprc.pharm.buffalo.edu/home/di_search/

5.4.1 Prohibited Medications

A list of prohibited medications can be found on the protocol-specific web page (PSWP), <https://member.actgnetwork.org/study/53292#profile=4>.

5.4.2 Precautionary Medications

A list of precautionary medications is posted on the PSWP, <https://member.actgnetwork.org/study/53292#profile=4>.

6.0 CLINICAL AND LABORATORY EVALUATIONS

6.1 Schedule of Events

Evaluation	Screening	Pre-Entry	Entry	On-Study Evaluations (Weeks)						Confirmation of Virologic Failure	Premature Treatment/Study Discontinuation Evals.
				1	2	4	5	10	12		
Window	≤30 days before entry	≥24 hours after screening and ≤7 days before entry		± 3 days				± 7 days			
Documentation of HIV-1	X										
Medical History	X		X								
Medication History	X		X								
Complete Physical Examination		X									
Targeted Physical Examination	X		X	X	X	X	X	X	X		X
Clinical Assessments			X	X	X	X	X	X	X		X
Concomitant Medications	X		X	X	X	X	X	X	X		X
Study Treatment Modifications	X		X	X	X	X	X	X	X		X
ART Modifications	X		X	X	X	X	X	X	X		X
Hematology	X		X	X	X	X	X	X	X		X
Liver Function Tests	X		X	X	X	X	X	X	X		X
Blood Chemistries	X		X	X	X	X	X	X	X		X
Fasting Lipid Panel			X				X				X (if prior to week 5)
Pregnancy Testing	X		X ¹	If suspected							
Nadir CD4+ T cell Count			X								
Documentation of Pre-ART CD4+ T cell Count			X								
CD4+/CD8+	X	X ²	X		X		X		X		X

Evaluation	Screening	Pre-Entry	Entry	On-Study Evaluations (Weeks)						Confirmation of Virologic Failure	Premature Treatment/Study Discontinuation Evals.
				1	2	4	5	10	12		
Window	≤30 days before entry	≥24 hours after screening and ≤7 days before entry		± 3 days			± 7 days				
Stored PBMCs for Other Immunologic Studies			X				X		X		X
Stored Plasma Soluble Markers		X	X			X	X		X		X
Stored Plasma Soluble Marker:IL-6		X	X			X	X	X	X		X
Documentation of Pre- ART HIV-1 RNA			X								
Plasma HIV-1 RNA	X		X		X		X		X	X	X
Stored Plasma HIV-1 RNA for Single Copy Assay			X				X		X		X
Stored PBMC Reservoir Assays			X				X		X		X
Stored Plasma/PBMC		X	X		X		X		X		X
Stored Oral Swabs for HHV		X	X	X	X	X	X	X	X		X
Plasma PK Collection for Arm A				X		X					X (if prior to week 4)
Medication Diary for Arm A (D=Distribute; C=Collect)			X (D)	X (C)	X (D)	X (C)					X (C) (if prior to week 4)
Adherence Assessment			X	X	X	X	X	X	X		X

¹Refer to section 6.2.2 for pregnancy testing at study entry.

²Only one CD4+/CD8+ is required if screening and pre-entry visits are combined.

6.2 Timing of Evaluations

6.2.1 Screening and Pre-Entry Evaluations

Screening and pre-entry evaluations must occur prior to the **participant** starting any study medications, treatments, or interventions.

Screening

Screening evaluations to determine eligibility must be completed within 30 days prior to study entry unless otherwise specified. In addition to data being collected on **participants** who enroll into the study, demographic, clinical, and laboratory data on screening failures will be captured in a Screening Failure Results form and entered into the ACTG database.

Pre-Entry

Pre-entry evaluations must be completed at least 24 hours after screening evaluations have been completed and within 7 days prior to the entry visit.

The pre-entry and screening visits may be combined in the event the **participant** presents with the following: Documentation of HIV-1 status and the results of an HIV-1 RNA level performed at a CLIA-certified laboratory \leq 30 days prior to study entry.

6.2.2 Entry Evaluations

Entry evaluations must occur at least 24 hours after pre-entry/screening evaluations unless otherwise specified. All entry evaluations must be performed after successful randomization and completed prior to the initiation of study treatment. If needed, the pregnancy testing at screening can be accepted for the entry value if the screening value was obtained within 72 hours of entry. All **participants** must begin treatment within 72 hours after **enrollment**.

6.2.3 Post-Entry Evaluations

On-Treatment Evaluations

Evaluations must occur after randomization. Study visits must be scheduled on the weeks indicated in section 6.1, within the visit windows.

The weeks 1 through 5 study visits must be scheduled per section 6.1, the SOE table \pm 3 days.

Please note **influenza** vaccination is prohibited \leq 7 days prior to all study visits. **The study visit will need to be rescheduled if the influenza vaccine was given \leq 7 days prior to a study visit. All other vaccinations are prohibited throughout the duration of the study.**

Post-Treatment Evaluations

There will be 7 additional weeks of follow-up after 5 weeks of study treatment. **Participants** will undergo evaluations as outlined at weeks 10 and 12 in section 6.1. The week 10 and 12 study visits must be scheduled per the SOE \pm 7days. Week 12 is the final study visit.

NOTE: **Participants** who discontinue treatment prematurely are followed on study/off treatment until study completion, i.e., week 12.

Virologic Failure

For **participants** who have suspected virologic failure, based on an HIV-1 RNA level above the lower limit of quantification, the site should query the **participant** about intercurrent vaccination, acute illness, or identified periods of nonadherence. If one of these conditions is suspected to account for the increased HIV-1 RNA, then no further evaluation is necessary until the next study visit. If none of these factors are present, a confirmatory HIV-1 RNA should be done within 7 days of receipt of the results of the first test. If the repeat HIV-1 RNA is **below the assay limit of quantification**, then no further evaluation is necessary. See section 7.5 for management of confirmed virologic failure.

6.2.4 Discontinuation Evaluations

Evaluations for Randomized or Registered **Participants** Who Do Not Start Study Treatment

All CRFs must be completed and keyed for the period up to and including entry. No further follow up is required for **participants** randomized to Arm A who do not start study treatment.

Premature Treatment Discontinuation Evaluations

Participants who prematurely discontinue the study treatment prior to week 5 should complete the premature treatment discontinuation evaluations within 14 days after discontinuing treatment. **Participants** will then be followed on study/off study treatment.

Site personnel should notify the protocol core team (actg.corea5336@fstrf.org) within 48 hours of any **participant** who prematurely discontinues the study treatment.

Premature Study Discontinuation Evaluations

Participants who prematurely discontinue the study will have the premature treatment/study discontinuation evaluations performed prior to being taken off the study.

Site personnel should notify the protocol core team (actg.corea5336@fstrf.org) within 48 hours of any **participant** who prematurely discontinues the study.

Pregnancy

Pregnancy will result in immediate and permanent discontinuation of the study treatment. Please see section 7.4 for detailed information regarding **participant** management.

6.3 Instructions for Evaluations

All clinical and laboratory information required by this protocol is to be present in the source documents. Sites must refer to the Source Document Guidelines on the DAIDS Web site for information about what must be included in the source document: <http://www.niaid.nih.gov/labsandresources/resources/daidsclinrsrch/documents/sourcedocappndx.pdf>.

All stated evaluations are to be recorded on the CRF and keyed into the database unless otherwise specified. This includes events that meet the International Conference on Harmonisation (ICH) definitions for a SAE:

- Results in death
- Life-threatening
- Requires inpatient hospitalization or prolongation of existing hospitalization
- Results in persistent or significant disability/incapacity
- Congenital anomaly/birth defect
- Other important medical event (may not be immediately life-threatening or result in death or hospitalization but may jeopardize the patient or may require intervention to prevent one of the events listed above).

To grade diagnoses, signs and symptoms, and laboratory results, sites must refer to the DAIDS Table for Grading the Severity of Adult and Pediatric Adverse Events (DAIDS AE Grading Table), Version 2.0, **November 2014**, which can be found on the DAIDS RSC Web site: <http://rsc.tech-res.com/safetyandpharmacovigilance/>.

6.3.1 Documentation of HIV-1

Section 4.1.1 specifies assay requirements for HIV-1 documentation. HIV-1 documentation is not recorded on the CRF.

6.3.2 Medical History

The medical history must include all diagnoses identified by the ACTG criteria for clinical events and other diagnoses. In addition to reporting all diagnoses within the past 30 days, the following diagnoses should be reported regardless of when the diagnosis was made.

- AIDS-defining conditions
- Bone fractures (verbal history accepted)
- Coronary heart disease
- Cancer (exclusive of basal/squamous cell skin cancer)
- Diabetes

- Tuberculosis
- Chronic **or acute** HCV
- Chronic **or acute** HBV
- **HIV encephalopathy**
- **HIV wasting**
- **Esophageal candidiasis**
- **Pneumocystis pneumonia without dissemination**

Any allergies to any medications and their formulations must also be documented.

6.3.3 Medication History

A medication history must be present, including start and stop dates. The table below lists the medications that must be included in the history.

Medication Category	Complete History or Timeframe	Record on CRFs
ART	Complete history (estimated dates if the exact dates cannot be obtained)	Yes
Immune-based therapy	Complete history (estimated dates if the exact dates cannot be obtained)	Yes
HIV-1-related vaccines	Complete history	Yes
Prescription drugs for treatment of opportunistic infections	Within 30 days prior to study entry	Yes
Prescription drugs for prophylaxis of opportunistic infections	Within 30 days prior to study entry	Yes
Prescription drugs: All including statins, anticoagulation therapy, NSAIDS, anti-inflammatory drugs not considered immune based therapy	Within 30 days prior to study entry	Yes
Alternative therapies	Within 30 days prior to study entry	Yes
Dietary supplements	Within 30 days prior to study entry	Yes
Aspirin use	Documentation of any aspirin use over a period of more than 5 days in any given week, taken within 30 days prior to study entry	Yes

6.3.4 Physical Examinations

Complete Physical Exam

A complete physical examination is to include at a minimum an examination of the skin, head, mouth, and neck; auscultation of the chest; cardiac exam; abdominal exam; examination of the lower extremities for edema; and Karnofsky performance score. The complete physical exam will also include signs and

symptoms, diagnoses, and vital signs (temperature, pulse, respiration rate, and blood pressure).

Targeted Physical Exam

A targeted physical examination is to include vital signs (temperature, pulse, respiration rate, and blood pressure) and is to be driven by any previously identified diagnoses or new signs or symptoms that the **participant** has experienced within 30 days prior to entry or since the last visit.

6.3.5 Clinical Assessments

Height

Height (**in cm**) will be measured at the entry visit.

Weight

Weight (**in kg**) will be measured at **screening**, entry and at all subsequent study visits.

Signs and Symptoms

At entry, all grades that occurred 30 days before entry must be recorded; post-entry, only signs and symptoms Grade ≥ 2 must be recorded except record all non-traumatic bruising regardless of grade. All Grade 3 or higher sign/symptom, any sign/symptom regardless of grade that led to a change in treatment, or that met ICH, EAE, or SAE guidelines, are defined by the protocol as reportable events that will require more detailed event reporting and must be reported on the CRF within 2 business days.

Diagnoses

At entry and on-study visits, record all diagnoses identified by the ACTG criteria and any of the following targeted diagnoses occurring since the last study visit:

- New onset seizures; or physician diagnosed CNS abnormality.

Post entry diagnoses must be reported on the CRF within 2 business days.

6.3.6 Concomitant Medications

Record in source documents and CRFs additions or discontinuations of any prescription medications and any medication or supplement included in the precautionary medication list (See PSWP). Record on the **source documents and CRFs** use of aspirin (chronic use only, defined as daily aspirin use over a period of more than 5 days in any given week) since the last study visit, including actual or estimated start dates and stop dates.

6.3.7 Study Treatment Modifications

Record all study drug modifications, including initial doses, **participant**-initiated and/or protocol-mandated modifications, inadvertent and deliberate interruptions, and permanent discontinuation of treatment at each visit. **Participant** missing

greater than 3 doses of study drug per week for two or more weeks results in a study treatment discontinuation and should be recorded on the CRF. The protocol core team (actg.corea5336@fstrf.org) must be notified of any permanent discontinuation of study drug.

6.3.8 ART Modifications

Record all modifications, including initial doses, **participant**-initiated and/or protocol-mandated modifications, inadvertent and deliberate interruption of 7 days or more and permanent discontinuation.

Arm A **participants** who change or discontinue ART must prematurely discontinue study treatment and be followed on study/off study treatment. Refer to protocol section 6.2.4 for premature treatment discontinuation evaluations.

6.3.9 Laboratory Evaluations

All laboratory evaluations at entry and week 5 study visit must be collected in a fasting state (8-hour fast with exception of water and medications). If **participants** are in a non-fasting state at the time of the study visit, they should return to the clinic within 72 hours for a fasting blood draw.

- At screening and entry all laboratory values must be recorded.
- For post-entry assessments, record all Grade ≥ 2 laboratory values, except for estimated CrCl, creatinine, AST (SGOT), ALT (SGPT), ANC, hemoglobin, and platelet count. These values should be recorded at every visit regardless of grade.
- All grade 3 or higher laboratory values, any laboratory value that led to a change in treatment or that met ICH, EAE, or SAE guidelines are defined by the protocol as reportable events that will require more detailed event reporting and must be reported on the CRF within 2 business days.

Although specific virologic and immunologic assays are described below, newer technology may preclude the utility of these assays and, therefore, this protocol will allow for the more advanced assays to be performed instead.

Hematology

Hemoglobin, hematocrit, red blood cells (RBC), mean corpuscular volume (MCV), white blood cells (WBC), differential WBC, absolute neutrophil count (ANC), platelets.

Liver Function Tests

AST (SGOT), ALT (SGPT), alkaline phosphatase, indirect bilirubin, direct bilirubin, and total bilirubin.

NOTE: Indirect bilirubin may be calculated from the total and direct bilirubin values.

Blood Chemistries

Electrolytes (sodium, potassium, chloride, bicarbonate), glucose, creatinine, blood urea nitrogen (BUN) and CrCl as estimated by the Cockcroft-Gault equation.

NOTE: A calculator for estimating the CrCl can be found at:

<https://www.fstrf.org/apps/cfm/apps/common/Portal/index.cfm?event=postLogin>.

Fasting Lipid Panel

Cholesterol, triglyceride, high-density lipoprotein (HDL), low-density lipoprotein (LDL). **If the LDL cannot be calculated due to elevated triglyceride levels, a direct LDL should be measured.**

Pregnancy Testing

For women with reproductive potential: Serum or urine β -HCG (urine test must have a sensitivity of 25 mIU/mL).

6.3.10 Nadir CD4+ T cell Count

The **participant's** prior nadir CD4+ T cell count (absolute value and date) should be documented when possible with a copy of the nadir CD4+ T cell count report. If this documentation is not available, then **participant** recollection will suffice. For **participants** who do not know the exact nadir value and for whom there is no source documentation, then recall of the categorical nadir (e.g., < 50, < 100, < 200 cells/mm³) will suffice.

6.3.11 Documentation of Pre-ART CD4+ T cell Count

Record on the CRF the documented date and level of the **participant's** pre-ART CD4+ T cell count that was obtained most immediately prior to a **participant's** initiating ART. If documentation is not available, then **participant** recollection will suffice.

6.3.12 Immunologic Studies

CD4+/CD8+

At screening, pre-entry and for all on-study evaluations obtain absolute CD4+/CD8+ count and percentages from a laboratory that possesses a CLIA certification or equivalent and is certified for protocol testing by the DAIDS Immunology Quality Assurance (IQA) Program. **Note only one CD4+/CD8+ value is needed if the screening and pre-entry visits are combined.**

Because of the diurnal variation in biomarkers that may be measured as part of the study (e.g., CD4+ and CD8+ T cell counts and other biomarkers that may be measured on stored samples), blood draws for individual **participants** should be performed consistently in either the morning or the afternoon throughout the study, if possible.

6.3.13 Stored PBMCs for Other Immunologic Studies

PBMCs will be collected and stored at the indicated visits per section 6.1 (see Laboratory Processing Chart [LPC] for more information) for potential comparative assessment of:

- At specified time points, cellular (macrophage/monocytes and lymphocytes) markers of immune activation and inflammation in the peripheral blood including but not limited to HLA-DR, CD16+, CD38, CD56, CD69, and CD14+CD16+ monocyte levels will be determined using flow cytometry and using stored specimens at the end of the study.
- Lymphocyte markers of immune activation in the peripheral blood including but not limited to HLA-DR, CD38, CD69, and CD14+/CD16+ monocyte levels using microarray panels for both mRNA and protein expression.
- Macrophage/monocyte markers of immune activation and inflammation in the peripheral blood including but not limited to HLA-DR, CD38, CD69, and CD14+CD16+ monocyte levels using microarray panels for both mRNA and protein expression.

6.3.14 Stored Plasma Soluble Markers

Plasma for stored soluble markers will be collected and stored at the indicated visits per section 6.1 (see LPC for more information) for comparative assessment of:

- Soluble markers of immune activation and inflammation in the peripheral blood including but not limited to sCD14, TNF- α , IL-1 α , IL-18, IL-1 β , mCSF, and neopterin will be determined using enzyme-linked immunoassay using stored specimens at the end of the study.
- Soluble markers of immune activation and inflammation in the peripheral blood including but not limited to sCD14, TNF- α , IL-1 α , IL-1 β , mCSF, and neopterin using microarray panels.

6.3.15 Stored Plasma Soluble Marker: IL-6

Plasma for stored soluble marker IL-6 will be collected and stored at the indicated visits per section 6.1.

6.3.16 Documentation of Pre-ART HIV-1 RNA

Record on the CRF the documented date and level of the **participant's** pre-ART viral load that was obtained immediately prior to a **participant's** initiating ART. If documentation is not available, then **participant** recollection will suffice.

6.3.17 Virologic Studies

Plasma HIV-1 RNA

Screening HIV-1 RNA must be performed within 30 days prior to study entry. All HIV-1 RNA determinations must be done **using an FDA-approved assay with a lower limit of quantification < 50 copies/mL (eg, < 40 copies/mL obtained by the Abbott real-time assay or < 20 copies/mL by the Roche Taqman v2.0 assay) performed** by a laboratory that possesses a CLIA certification or equivalent. Eligibility will be determined based on the screening value.

After the screening visit, HIV-1 RNA quantification assay will be performed at the protocol-designated laboratory. See the LPC for processing, shipping, and storage information.

6.3.18 Stored Plasma for HIV-1 RNA Single Copy Assay (SCA)

Plasma will be obtained and stored for determination of plasma HIV-1 RNA by SCA at the indicated visits per section 6.1. This assay will be performed if significant changes in the immunologic markers are observed and/or if there are advances in the field that might provide further rationale for assessing the virologic endpoints in the absence of achieving the desired immunologic outcomes. See the LPC for details on processing plasma samples for the SCA test.

6.3.19 Stored PBMC Reservoir Assays

Samples will be collected and stored as indicated in section 6.1 for potential comparative assessments of change in HIV-1 reservoir size as measured by:

- a. Total and integrated HIV-1 DNA
- b. HIV-1 cell-associated RNA
- c. 2 LTR circles

Reservoir assays will be performed if significant changes in the immunologic markers are observed and/or if there are advances in the field that might provide further rationale for assessing the virologic endpoints in the absence of achieving the desired immunologic outcomes.

6.3.20 Stored Plasma/PBMC

Stored Plasma/PBMC will be collected and stored as indicated in section 6.1 for future HIV-1 related studies and shipped according to the A5336 LPC.

6.3.21 Stored Oral Swabs Collection

Oral swabs will be collected as indicated in section 6.1 to measure levels of HHV DNA. It is recommended that participants abstain from drinking coffee 1 hour prior to collection. Samples will be stored and shipped according to the A5336 LPC.

6.3.22 Plasma PK Sample Collection for Arm A **Participants**

Samples will be collected as indicated in section 6.1 for assessment of the PK of ruxolitinib and ARV medicines. Apart from arriving at the clinic in a fasted state, **participants** may eat and drink throughout the day as they wish. Each PK visit requires that the **participants** take their morning dose of ruxolitinib and their ARV medications as observed doses administered in the clinic. **Participants** should be contacted the night before and reminded not to take their morning dose of ruxolitinib or their ARV medications until instructed during their clinic visit, and to bring their study and ARV medications (including EFV) with them in the morning. The timing of the previous drug administration must be documented. If **participant** skipped previous day's medication for the weeks 1 and 4 visits or took ruxolitinib before arriving at clinic, then baseline PK sampling should not be drawn and an alternative visit should be scheduled to measure PK. The exact time (hour and minute) of administration of the observed dose of ruxolitinib and ARV must be recorded on the CRF, along with the exact time at which each blood sample was obtained. Samples will be collected at the following time points during each visit:

Hour	Activity
-0.5 – 0.0	Pre-dose (baseline) PK sample
0.0	Observed Administration of study drug and ARV medications; start clock; record administration time
1.0 – 1.5	PK sample
2.5 – 4.0	PK sample
4.0 – 6.0	Lunch
6.0 – 8.0	PK sample

The use of an intravenous (IV) catheter is optional depending on **participant** preference. Please refer to the LPC for sample collection, processing, storage and shipping information.

6.3.23 Medication Diary for Arm A **Participants**

At the weeks before the PK assessment, a medication diary will be given to **participants** and collected from **participants** at the PK visits as indicated in section 6.1. **Participants** will be instructed to record the date and time they took their ruxolitinib and their ARV medications for the 3 days preceding the PK study visit.

6.3.24 Adherence Assessment

Self-reported adherence for ruxolitinib and ARV will be performed at the indicated visits per section 6.1.

7.0 CLINICAL MANAGEMENT ISSUES

7.1 Toxicity

Only toxicities related to the study **drug**, (ruxolitinib,) will be considered in the toxicity management section. The grading system is located in the Division of AIDS Table for Grading the Severity of Adult and Pediatric Adverse Events (DAIDS AE Grading Table), Version 2.0, **November 2014**, located at the DAIDS RSC Web Site: <http://rsc.tech-res.com/safetyandpharmacovigilance/>

NOTE: The study core team must be notified by e-mail regarding toxicities that result in a change in regimen or discontinuation of study treatment (actg.corea5336@fstf.org).

7.1.1 Grade 1 or 2

Participants who develop a Grade 1 or 2 AE or toxicity may continue study drug without alteration of the dosage, except as stated in sections 7.2.1 to 7.2.8. For **participants** experiencing Grade 1 or 2 AEs who choose to discontinue study drug, the site investigator should complete premature treatment discontinuation evaluations, and the **participant** should be encouraged to complete evaluations off treatment/on study.

NOTE: If **participants** discontinue study drug due to experiencing Grade 1 or 2 AEs, this should be noted in the CRF as the reason for discontinuation.

7.1.2 Grade 3

Participants who develop a Grade 3 AE thought to be related to the study drug should have their study medication **discontinued**. If the investigator has evidence that the AE has NOT been caused by study drug, **study drug should be held until the AE or clinically significant laboratory abnormality is Grade \leq 1 in severity**, except as stated in sections 7.2.1 to 7.2.8 below.

If the same Grade 3 AE recurs at any time within 3 weeks after restarting treatment, the study drug must be permanently discontinued.

Participants experiencing Grade 3 AEs requiring permanent discontinuation of study drug should be followed weekly until resolution of the AE and should complete the premature treatment discontinuation evaluations and should be encouraged to complete off treatment/on study evaluations.

7.1.3 Grade 4

Participants who develop a Grade 4 symptomatic AE will have study drug permanently discontinued, except as stated in sections 7.2.1- 7.2.8 below. **Participants** experiencing Grade 4 AEs requiring permanent discontinuation of study **drug** should be followed weekly until resolution of the AE and should complete the premature treatment discontinuation evaluations and the

participant should be encouraged to complete off treatment/on study evaluations.

7.2 Other Toxicities

7.2.1 Platelet Count Reductions

7.2.1.1 If a **participant** develops a platelet count decrease of >25% from baseline with an absolute value remaining above 200,000/mm³, then dosing can continue.

7.2.1.2 If the platelet count decreases from baseline by $\geq 25\%$, and the absolute value is between 150,000-200,000/mm³, then dosing of the study drug should be reduced to 10 mg QD. The CBC should then be repeated weekly until the platelet count either returns to > 200,000/mm³ or remains less than 10% below the value that led to the dose reduction for two consecutive values. If the platelet count returns > 200,000/mm³, and is > 1.10 times the value that led to the dose reduction, then BID study drug dosing can resume. The **participant** will then have weekly monitoring until the platelet count remains at a value that is greater than the value at BID dose resumption for at least two consecutive values. After resumption of the BID dosing if the platelet count decreases to < 200,000/mm³, then the dose will be decreased to 10 mg daily and remain at that dose except as noted in section 7.2.3.

7.2.1.3 If the platelet count decreases by $\geq 25\%$, and an absolute value below 150,000/mm³, then the study drug should be discontinued. The CBC should then be repeated weekly until the platelet count returns to > 150,000/mm³.

7.2.2 Anemia/Neutropenia

If a **participant** experiences treatment-limiting **Grade 3 or higher decrease** (in the opinion of the site investigator) in anemia or neutropenia, then study drug should be withheld. **Anemia should not** be managed by transfusions or recombinant erythropoietin, and neutropenia **should not** be managed with granulocyte-colony stimulating factor (G-CSF) or granulocyte-macrophage colony-stimulating factor (GM-CSF). Once the anemia or neutropenia has returned to Grade ≤ 2 , the study drug can be restarted.

7.2.3 Creatinine Clearance

Any **participant** experiencing a calculated CrCl < **60** mL/min should have the value confirmed within 7 days. For those **participants** who experience a confirmed (2 consecutive) CrCl < **60** mL/min, study drug should be withheld. If the site investigator determines that another condition caused the decrease in CrCl, then study drug may be restarted if approved by the protocol team. **Participants** should be followed as medically indicated until the serum creatinine

returns to Grade \leq 2 or baseline.

7.2.4 Bruising and Bleeding

For **participants** who report **generalized bruising and/or symptoms of unexplained or abnormal bleeding, hematoma or epistaxis**, the **study drug should be discontinued**.

7.2.5 Nausea (with or without vomiting) and/or Diarrhea

With the onset of Grade \geq 1 nausea advise **participants** to take study drug with food. **Participants** may also be treated as needed with antiemetics given PO or by suppository.

In **participants** with persistent nausea, consideration should be given for evaluation of pancreatitis, hepatitis, or hyperlactatemia/lactic acidosis, with evaluation for each if clinically indicated.

For Grade 1 or 2 diarrhea, therapy should be continued with symptomatic treatment with antimotility agents. If Grade \geq 3 diarrhea occurs and is unresponsive to antimotility agents, and for which an alternative etiology (e.g., infectious diarrhea) is not established **and judged related to study drug**, study drug should be withheld until resolution of diarrhea to Grade \leq 1 or return to baseline. If Grade \geq 3 diarrhea recurs upon the resumption of the study drug, study drug should be discontinued.

7.2.6 Hypertriglyceridemia/Hyperlipidemia

If elevated triglyceride or lipid levels are from a non-fasting blood draw, repeat the draw after an 8-hour fast. Only levels done in a fasting state should be used to determine toxicity management. **Participants** with asymptomatic Grade 3 **or higher increases in** triglyceride, total cholesterol, or LDL elevations **must discontinue** study drug. Please see the recent review adapted from the original recommendations of the ACTG Cardiovascular Disease Focus Group (<http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2753589>) for full discussion of management of hyperlipidemia in the context of HIV-1 disease.

7.2.7 Hyperglycemia

Participants with Grade 3 **or higher** hyperglycemia **must discontinue** their study drug **unless judged unrelated to the study drug. If judged unrelated, study drug must be held until the glucose abnormalities are Grade \leq 1. Oral hypoglycemic and insulin should not be used in order to allow continued study drug administration.** A confirmatory fasting glucose must be obtained within 2 weeks and prior to the institution of medical therapy.

7.2.8 AST or ALT Elevations

Study **drug must** be **discontinued** for asymptomatic Grade 3 **or higher** AST/ALT elevations **unless judged unrelated to study drug**. Careful assessments should be done to rule out the use of alcohol, non-study drug-related drug toxicity, or viral hepatitis as the cause of Grade 3 elevations. **If judged unrelated, study drug should be held until AST and/or ALT levels are Grade ≤ 1.**

7.3 Other Diseases

For unexplained confusion, seizures, or other CNS abnormality site investigators should assess the **participant**, in conjunction with the primary physician to determine the cause of the symptoms. If no cause can be identified, or other physician suspects PML, then study drug should be discontinued. The **participant** should then be followed on study until another cause is established, or PML is considered confirmed or probable according to criteria in Appendix 100, ACTG Criteria for Clinical and Other Events. For any infection, site investigators should assess the **participant**, in conjunction with the primary physician to determine relatedness to the study drug. If the infection is considered related to the study drug, then the study drug should be discontinued.

7.4 Pregnancy

Female **participants** who become pregnant while on study treatment must immediately discontinue study treatment. **Participants** should have the Premature Treatment/Study Discontinuation Evaluations completed within 2 weeks after stopping study treatment. Pregnancies that occur on study should be reported prospectively to The Antiretroviral Pregnancy Registry. More information is available at www.apregistry.com. Phone: 800-258-4263; Fax: 800-800-1052.

Participants will be encouraged to remain in the study to be followed on study/off study treatment until study completion and will be followed by telephone contact thereafter to determine the pregnancy outcome. If a **participant** does not wish to remain on study follow-up at the time of study discontinuation, site staff should request permission to contact her regarding pregnancy and infancy outcomes at the end of the pregnancy. If the information is obtained, pregnancy outcomes for the **participant** and infant will be submitted on a CRF at the end of pregnancy.

7.5 Confirmed Virologic Failure

In the event of confirmed virologic failure, defined as the occurrence of two sequential plasma HIV-1 RNA values above the lower limit of quantification **in the absence of an interruption in ART**, the study drug must be stopped **for participants in Arm A**. The **participant** will be followed on study with all evaluations performed through week 12. If the **participant** does not agree to continue on study, then the premature study discontinuation evaluations should be completed.

8.0 CRITERIA FOR DISCONTINUATION

8.1 Premature Treatment Discontinuation

- Drug-related toxicity (see section 7.1 Toxicity).
- Requirement for prohibited concomitant medications (see section 5.4).
- Changes to or discontinuation of ART **for Arm A participants**.
- **Participant** repeatedly noncompliant (missing greater than 3 doses of study drug a week for 2 or more weeks).
- Confirmation of virologic failure (see section 7.5).
- Pregnancy or breastfeeding.
- Request by **participant** to terminate treatment.
- Clinical reasons believed life threatening by the physician, even if not addressed in the toxicity section of the protocol.

8.2 Premature Study Discontinuation

- Failure by the **participant** to attend 2 consecutive clinic visits.
- Request by the **participant** to withdraw.
- Request of the primary care provider if s/he thinks the study is no longer in the best interest of the **participant**.
- **Participant** judged by the investigator to be at significant risk of failing to comply with the provisions of the protocol as to cause harm to self or seriously interfere with the validity of the study results.
- At the discretion of the Institutional Review Board/Ethics Committee (IRB/EC), FDA, NIAID, Office for Human Research Protections (OHRP), other government agencies as part of their duties, or the investigator.

9.0 STATISTICAL CONSIDERATIONS

9.1 General Design Issues

A5336 is a randomized (2:1), prospective, open-label, 2-arm pilot clinical trial designed to assess the safety and tolerability of ruxolitinib, and to evaluate whether 5 weeks of treatment with ruxolitinib decreases IL-6 levels in HIV-1 infected **participants** virally suppressed on ART. **Participants** will be randomized 2:1 to receive ruxolitinib or no study treatment. **Participants** receiving ruxolitinib will be followed on treatment for 5 weeks, then for an additional 7 weeks off treatment, resulting in a total 12 weeks of follow-up; **participants** receiving no study treatment will be followed on-study for 12 weeks. The total sample size will be 60 **participants**.

9.2 Objectives

9.2.1 Primary Outcome Measures

9.2.1.1 Safety

Occurrence of any one of the following safety milestones (from entry to week 5).

- For **participants** with entry CD4+ T cell count < 700 cells/mm³, confirmed CD4+ decline > 33% of baseline and to < 350 cells/mm³.
- For **participants** with entry CD4+ T cell count ≥ 700 cells/mm³, a confirmed CD4+ T cell decline > 50 % of baseline.
- Confirmed HIV-1 RNA level **above the lower limit of quantification** in the absence of an interruption in ART.
- New or recurrent CDC category C AIDS-indicator condition.
- HIV-1 associated infection including Varicella zoster.
- Lymphoproliferative malignancies.
- **Discontinuation of ruxolitinib due to** thrombocytopenia.
- Grade 4 or recurrence of Grade 3 anemia/neutropenia.
- Any Grade 4 or recurrence of Grade 3 toxicity related to study drug.
- New diagnosis of pneumonia, sepsis, or bacteremia.

9.2.1.2 Tolerability

- Occurrence of premature discontinuation of study treatment.

9.2.1.3 IL-6

Participant-specific change in the level of plasma IL-6 (using geometric means of two ELISA measurements at pre-entry and entry, and weeks 4 and 5).

9.2.2 Secondary Objective Measures

9.2.2.1 Proportion of **participants** who experienced any one of the primary safety milestones during total follow-up (from entry to week 12).

9.2.2.2 Summary of safety laboratories at baseline and weeks 1, 2, 4, 5, 10, and 12.

9.2.2.3 Change in the level of plasma IL-6 from baseline to weeks 4, 5, 10, and 12.

9.2.2.4 Changes in soluble markers of immune activation and inflammation in the peripheral blood (sCD14, TNF- α , IL-1 α , IL-1 β , mCSF, and neopterin) from baseline to weeks 4, 5, and 12.

- 9.2.2.5 Changes in cellular markers of immune activation and inflammation in the peripheral blood (HLA-DR, CD38, CD69 and CD14+/CD16+ monocyte levels) from baseline to weeks 5 and 12.
- 9.2.2.6 Changes in plasma HIV-1 RNA and CD4+ T cell counts from baseline to weeks 2, 5, and 12.
- 9.2.2.7 Changes in HIV-1 reservoir (HIV-1 RNA by SCA, cellular HIV-1 total RNA and DNA, 2-LTRs, integrated DNA) from baseline to weeks 5 and 12.
- 9.2.2.8 **Level of HHV shedding (CMV, EBV, HHV 6, 7, 8) at pre-entry, entry, and weeks 1, 2, 4, 5, 10, and 12.**

9.2.3 Exploratory Objective Measures

Summary measure of immune activation and inflammation and how changes in this measure differ between treatment groups.

9.3 Randomization and Stratification

At study entry, **participants** will be randomized at a 2:1 ratio (ruxolitinib:no study treatment) using permuted blocks, without institutional balancing. **Participants** will be stratified based on whether they take EFV or do not take EFV at screening, in order to ensure balance by treatment arm for EFV.

9.4 Sample Size and Accrual

The sample size for A5336 was determined based on the primary endpoint for change in IL-6, while at the same time ensuring the sample size was feasible for an early phase investigation of the safety of ruxolitinib.

9.4.1 Changes in IL-6

A total sample size of 60 **participants** will be randomized at a 2:1 ratio to ruxolitinib:no study treatment, allowing for 20% lost to follow-up and treatment discontinuation, is anticipated to yield 48 evaluable **participants** (see section 9.6.1). This will provide 80% power to detect a treatment effect of 0.20 log₁₀ pg/mL, that is a difference between treatment groups in (change in plasma IL-6 from entry to week 5), assuming that the standard deviation (SD) of within-**participant** differences in IL-6 is 0.25 log₁₀ pg/mL, using a two-sided t-test at a 0.10 alpha level.

To minimize variability and maximize statistical power, at each key time point markers will be measured and analyzed as the average of samples taken 1-2 weeks apart (pre-entry and entry; at weeks 4 and 5; at weeks 10 and 12).

The treatment effect of 0.20 log₁₀ scale pg/mL corresponds to average decreases of 37% and 0% in plasma IL-6 levels in the ruxolitinib and no study treatment arms, respectively. This choice of effect size is based on prior data examining plasma IL-6 levels in HIV-1 infected populations and changes in IL-6 levels in ruxolitinib treated populations. Specifically, a 27% (95% confidence interval [CI]: 11% to 39%) reduction in IL-6 levels after 4 weeks and a 37% (95% CI: 13% to 53%) reduction after 24 weeks of treatment was seen in the Phase III COMFORT-1 study [3]. The effect size of 37% is selected because it is contained in the 95% CI for IL-6 reduction at 4 weeks, and balances the interest in the efficacy endpoint with safety considerations as well as feasibility for this early phase investigation of the effects of the study treatment in the HIV-1 population.

The assumed SD for within-**participant** differences in IL-6 of 0.25 log₁₀ is a pooled estimate based on estimates of the between-**participant** SDs and within-**participant** correlation from other ACTG studies. ACTG 5248 estimated the between-**participant** SD of IL-6 at weeks 24 and 48 (after starting ART treatment); the SD was 0.335 at week 24 (based on 35 **participants**) and 0.331 at week 48 (based on 29 **participants**). The SD estimate from 311 controls from NWCS 329 at week 48 was 0.306 based on a single measure [71]. In addition, ACTG 5286 reported the within-**participant** correlation between baseline and week 4 IL-6 measures to be 0.68 (n=43). Two versions of the pooled SD were calculated assuming the same SD at baseline and weeks 5, one assuming both SDs = 0.306, and the other assuming both SDs = 0.331. Each set of SDs was then pooled by accounting for the correlation between time points (r = 0.68), which resulted in SD=0.2448 and SD = 0.2648, respectively. The pooled 0.25 SD is the average of these values.

9.4.2 Safety

The total sample size of 60 **participants** will be randomized at a 2:1 ratio to ruxolitinib:no study treatment ratio, results in 40 **participants** receiving ruxolitinib and 20 receiving no study treatment. The 40 ruxolitinib-treated **participants** will provide 90% probability of observing an AE that would occur in 6% or more of treated **participants**.

We assume the rate of events (outlined in 9.2.1.1) in the no study treatment arm will be no more than 2%. Assuming this rate in the no study treatment arm, we will have 80% power to detect a significant difference between the two arms, via a one-sided Fisher's exact test with 5% alpha, if the rate in the ruxolitinib arm is 27%.

9.4.3 Accrual

It is anticipated that **participants** will accrue to the study at a rate of 7-8 **participants** per month. At this rate, the study should fully accrue within 7-9 months after the first **participant** enrolls.

9.5 Monitoring

Accrual and summary of all Grade ≥ 2 signs and symptoms, laboratory abnormalities and diagnoses, and all reported AEs will be reviewed by the team monthly. This summary will be pooled over the study arms. Baseline characteristics, early study treatment and study discontinuations, and virologic failures, pooled over study arms, will be reviewed regularly by the protocol team. In addition, data and specimen availability will be also regularly reviewed. The Statistical and Data Analysis Center (SDAC) will also prepare quarterly reports of all Grade ≥ 2 events, and all reported AEs by study arm, to be reviewed by the DAIDS clinical representative or designee.

An ACTG-appointed Study Monitoring Committee (SMC) will review accrual, toxicity summaries, off-treatment and off-study rates, and reasons broken down by study arms. An interim safety review by the SMC is planned at the earliest of **6 participants** with 3 weeks of follow-up, **2 participants** with Grade 3/4 toxicities, **3 participants** discontinuing study treatment due to toxicities, or 3 months after the first **participant** enrolls. Subsequent SMC reviews are planned at approximately 6 month intervals, **or in the event of 2 participants with Grade ≥ 3 toxicities or 3 participants discontinuing study treatment due to toxicities in the interim.**

In the event the SMC review is triggered due to toxicities (2 participants with Grade ≥ 3 toxicities or 3 participants discontinuing study treatment due to toxicities), accrual will be paused while the SMC review takes place. In addition, during the course of the study if any participant develops PML, or neurologic condition or dies of a drug-related adverse event, or if more than 2 participants, randomized to Arm A, experience confirmed virologic failure (see section 7.5 for definition) then dosing for all study participants will be stopped.

The team has requested the SMC consider the grade of the events, the time to resolution (if any), **the temporal relationship to drug administration**, and the site clinician's judgment of the relationship to treatment. The team would recommend particular review by the SMC if there were high occurrences of the safety events described in section 9.2.1.1.

Complementing the SMC reviews, the team will also notify the SMC of all drug related SAEs, Grade 4 toxicities, as well as all drug-related Grade 3 toxicities. In addition, the core team or the SMC may ask for the SMC, at any time, to independently review all available safety data (unblinded to treatment arms) and make a recommendation to the End-Organ Disease and Inflammation Transformative Science Group on whether to modify or stop the study. The team will also monitor (monthly) all Grade ≥ 2 signs and symptoms, laboratory abnormalities, and diagnoses, as well as all primary events with summaries pooled over study arms.

9.6 Analyses

9.6.1 Primary Analyses

As this is a pilot study of biologic activity, our primary analysis of efficacy will be an as-treated analysis, limited to **participants** who meet all of the following conditions: (a) have data at baseline and week 5, (b) for the ruxolitinib arm, remain on study treatment through week 5 and miss no more than 6 doses cumulatively, (c) did not change ART, use prohibited medications, or have a virologic failure during this time period.

Changes from baseline to week 5 in plasma IL-6 (on log₁₀ scale) will be summarized with the mean and a 90% confidence interval (CI) for the mean change in each treatment arm, and compared between arms with a t-test. The baseline value will be calculated as the geometric mean of pre-entry and entry, and the week 5 value as the geometric mean of weeks 4 and 5. If missing one of the pre-entry or entry values, then the available measure will be used as the baseline value, and similarly, if missing one of the week 4 or week 5 values, then the available measure will be used as the week 5 value.

Safety will be evaluated by **estimating** the proportion of participants **in the ruxolitinib arm who** experiencing the primary safety outcome measure 9.2.1.1. **In addition, safety will be compared between the treatment arms** via a one-sided Fisher's exact test at 5% alpha level, **in terms of 9.2.1.1, but with this composite safety outcome modified to address the A5336 design where Arm B does not receive any study treatment. Specifically "discontinuation of ruxolitinib due to thrombocytopenia" will be modified to "occurrence of Grade 2 or higher thrombocytopenia" and "any Grade 4 or recurrence of Grade 3 toxicity related to study drug" will be modified to "any Grade 4 or recurrence of Grade 3 toxicity."** Tolerability will be evaluated by summarizing treatment modifications and discontinuations.

9.6.2 Secondary Analyses

Participant-specific changes from baseline to week 5 in other measurements of immune activation and inflammation, and CD4+ T-cell counts will be summarized similarly to the primary efficacy measure, via means and 90% CIs for the mean change for each of the study arms. The log₁₀ transformation is planned for various soluble and cellular markers. Comparisons between study arms will be done via the Wilcoxon rank sum test. HIV-1 RNA will be summarized by the number of **participants** with confirmed HIV-1 RNA > 40 copies/mL and > 200 copies/mL. **Due to an anticipated high proportion of lower limit of detection censoring, levels of HHV shedding will be compared between arms at week 5 using a two-group Wilcoxon rank-sum test with 2-sided alpha=0.10 level. Values below the lower limit of detection will be analyzed as the lowest rank. In the event of limited lower limit of detection censoring, repeated measure modeling will be used to explore longitudinal changes in HHV shedding.**

Safety events (signs and symptoms, laboratory values, diagnoses, and other SAEs) will also be evaluated by summarizing the nature and rate of AEs within

each study arm. Comparisons of overall and toxicity-specific grades between study arms will be shown descriptively.

Longitudinal changes from baseline to week 12 in all measures will be examined via descriptive summaries and plots.

Statistical significance will be tested using a two-sided alpha = 0.10 with no adjustment for multiple testing.

10.0 PHARMACOLOGY PLAN

10.1 Pharmacology Objectives

10.1.1 Primary Objective

10.1.1.1 To measure the PK of ruxolitinib in this population of **participants** with well-controlled HIV-1, and to characterize the relationship between ruxolitinib post-hoc estimated exposures (e.g., steady-state AUC = dose/CL/F) and immunological biomarkers of the study drug.

10.1.2 Secondary Objectives

10.1.2.1 To compare ruxolitinib PK parameters in study **participants** on specific ARV regimens to those reported for historical controls (no coadministered drugs) to assess the potential effect of ARV administration on ruxolitinib PK.

10.1.2.2 To compare PK parameters of selected ARV medications (NNRTI's and INSTI's) when coadministered with ruxolitinib to historical controls (no coadministered drugs) in order to explore possible effects of ruxolitinib on ARV concentrations.

10.1.2.3 To evaluate the relationship between ruxolitinib concentrations in individual **participants** with the time course of changes in biomarkers of immune response (hysteresis analysis).

10.2 Pharmacology Study Design

Participants randomized to Arm A will undergo blood sampling during two study visits as indicated in section 6.1 for assessment of the PK of ruxolitinib and selected ARV drugs.

Each PK visit requires that the study **participant** take their morning dose of ruxolitinib as a timed observed dose administered in the clinic. During each of the two PK visits, four blood samples will be collected for PK analysis.

10.3 Primary and Secondary Data, Modeling, and Data Analysis

The PK of ruxolitinib following oral administration will be modeled using population methods with the NONMEM computer program (7.3 or later, ICON Development Solutions, Leopardstown, Ireland) [88], using the PLT Tools statistical interface (5.1 or later, PLT Soft, San Francisco, CA). Resulting model parameters will be compared to a model recently reported for ruxolitinib without coadministered drugs in patients with myelofibrosis [89]. One and 2-compartmental models, assuming first-order absorption into the plasma compartment will be fitted as they were reported to adequately describe ruxolitinib PK in the myelofibrosis population PK study. Between-**participant** variances (IIV, ω^2) of PK parameters (η) will be modeled as log-normally distributed, and an appropriate residual error model (e.g., proportional and/or additive, ϵ) will be used. Also, since PK samplings are planned on two different occasions, we will also explore inter-occasional variations in model parameters (e.g., oral systemic clearance (CL/F) and distribution volume (V/F, where F=bioavailability, assumed=1, no IV dose administered)), using established methods [90]. Only concentrations > LOQ were included in the analysis. Criteria for model convergence will include convergence to > 3 decimal places using an appropriate convergence method (e.g., First Order Conditional Estimation with η - ω interactions). Model diagnostics of goodness of fit will include the minimum value the NONMEM objective function (OFV= -2 log-likelihood), and visual inspection of graphics, e.g., correlation/symmetry of observations versus predictions (population and post-hoc), conditional weighted residual versus time and predictions [91-93].

Based on the planned 12 hour dosing regimen and reported ruxolitinib plasma concentrations over a 12 hour dose interval, we anticipate that a 1-compartment model will adequately describe the PK of ruxolitinib in this study. However, if the fit of the model to the data is improved using a 2-compartment model, based upon the dispersion of residuals about the respective lines of identity of observed versus predicted plasma concentrations (population and post-hoc), and by a decrease in OFV, then the 2-compartment model will be selected for further modeling. We will also test the significance of body-weight as possible continuous covariate on V/F and CL/F and creatinine clearance (calculated using the Cockcroft-Gault formula) as a covariate of CL/F [94]. The ability of the final model to simulate without bias will be tested using a “visual predictive check” method, in which > 1,000 individuals are simulated using the sampling scheme (to ensure reproducible predictions), and used to compute 5% - 95% percentile ranges of concentration versus time. Actual data will then be superimposed to visually assess model bias [95]. Post-hoc fitted parameters from **participants** administered ruxolitinib alone or with EFV will be compared using ANCOVA. A value < 0.05 will be considered statistically significant for all tests. A similar model will be attempted for EFV and other ARVs provided enough **participants** taking a particular ARV are recruited. Alternatively, their PK will be compared informally with published PK studies [50, 51, 96, 97].

Concentrations of ARVs (NNRTIs and INSTIs) obtained at the same time as ruxolitinib concentrations will be compared to historical controls in order to explore the possible effect of ruxolitinib on ARV concentrations. This is considered an exploratory analysis only.

10.4 Anticipated Outcomes

Based on what is known about the clearance of ruxolitinib, NNRTIs, and INSTIs, we do not expect a clinically significant effect of ruxolitinib on ARV concentrations, nor a clinically significant effect of INSTIs on ruxolitinib concentrations. EFV is a P450 enzyme inducer, and could decrease ruxolitinib concentrations as a result, although the magnitude of this effect is expected to be modest, and since concentrations of ruxolitinib would decrease rather than increase, this should not produce any safety concerns for **participants**.

11.0 DATA COLLECTION AND MONITORING AND ADVERSE EVENT REPORTING

11.1 Records to Be Kept

CRF will be provided for each **participant**. **Participants** must not be identified by name on any CRFs. **Participants** will be identified by the patient identification number (PID) and study identification number (SID) provided by the ACTG DMC upon randomization.

11.2 Role of Data Management

11.2.1 Instructions concerning the recording of study data on CRFs will be provided by the ACTG DMC. Each CRS is responsible for keying the data in a timely fashion.

11.2.2 It is the responsibility of the ACTG DMC to assure the quality of computerized data for each ACTG study. This role extends from protocol development to generation of the final study databases.

11.3 Clinical Site Monitoring and Record Availability

11.3.1 Site monitors under contract to the NIAID will visit participating clinical research sites to review the individual **participant** records, including consent forms, CRFs, supporting data, laboratory specimen records, and medical records (physicians' progress notes, nurses' notes, individuals' hospital charts), to ensure protection of study **participants**, compliance with the protocol, and accuracy and completeness of records. The monitors also will inspect sites' regulatory files to ensure that regulatory requirements are being followed and sites' pharmacies to review product storage and management.

11.3.2 The site investigator will make study documents (e.g., consent forms, drug distribution forms, CRFs) and pertinent hospital or clinic records readily available for inspection by the local IRB, the site monitors, the FDA, the NIAID, the OHRP, and designee for confirmation of the study data.

11.4 Expedited Adverse Event Reporting to DAIDS

11.4.1 Adverse Event Reporting to DAIDS

Requirements, definitions and methods for expedited reporting of AEs are outlined in Version 2.0 of the DAIDS EAE Manual, which is available on the RSC website at <http://rsc.tech-res.com/safetyandpharmacovigilance/>.

The DAIDS Adverse Events Reporting System (DAERS), an internet-based reporting system, must be used for expedited AE reporting to DAIDS. In the event of system outages or technical difficulties, expedited AEs may be submitted via the DAIDS EAE Form. For questions about DAERS, please contact DAIDS-ES at DAIDS-ESSupport@niaid.nih.gov. Site queries may also be sent from within the DAERS application itself.

Sites where DAERS has not been implemented will submit expedited AEs by documenting the information on the current DAIDS EAE Form. This form is available on the RSC website: <http://rsc.tech-res.com/safetyandpharmacovigilance/>. For questions about EAE reporting, please contact the RSC (DAIDSRSCSafetyOffice@tech-res.com).

11.4.2 Reporting Requirements for this Study

- The SAE Reporting Category, as defined in Version 2.0 of the DAIDS EAE Manual, will be used for this study.
- The study agent for which expedited reporting are required is ruxolitinib.
- In addition to the EAE Reporting Category identified above, other AEs that must be reported in an expedited manner are:
 - Platelet count decreases by $\geq 25\%$ from baseline
 - Platelet count with an absolute value below 150,000/mm³
 - New onset leukemia

11.4.3 Grading Severity of Events

The Division of AIDS Table for Grading the Severity of Adult and Pediatric Adverse Events (DAIDS AE Grading Table), Version 2.0, **November 2014**, must be used and is available on the DAIDS RSC Web site at <http://rsc.tech-res.com/safetyandpharmacovigilance/>.

11.4.4 Expedited AE Reporting Period

- The expedited AE reporting period for this study is as per the EAE manual.
- After the protocol-defined AE reporting period, unless otherwise noted, only suspected, unexpected serious adverse reactions (SUSARs), as defined in Version 2.0 of the EAE Manual, will be reported to DAIDS if the study staff become aware of the events on a passive basis (from publicly available information).

12.0 PARTICIPANTS

12.1 Institutional Review Board (IRB) Review and Informed Consent

This protocol and the informed consent document (Appendix I) and any subsequent modifications will be reviewed and approved by the IRB or EC responsible for oversight of the study. A signed consent form will be obtained from the *participant* (or person with power of attorney for **participants** who cannot consent for themselves). The consent form will describe the purpose of the study, the procedures to be followed, and the risks and benefits of participation. A copy of the consent form will be given to the **participant**, or legal representative, and this fact will be documented in the **participant's** record.

12.2 Participant Confidentiality

All laboratory specimens, evaluation forms, reports, and other records that leave the site will be identified by coded number only to maintain **participant** confidentiality. All records will be kept locked. All computer entry and networking programs will be done with coded numbers only. Clinical information will not be released without written permission of the **participant**, except as necessary for monitoring by the ACTG, IRB/EC, FDA, NIAID, OHRP, other government agencies as part of their duties to ensure that research **participants** are protected, or designee.

12.3 Study Discontinuation

The study may be discontinued at any time by the ACTG, IRB/EC, FDA, NIAID, OHRP, or government agencies as part of their duties to ensure that research **participants** are protected.

13.0 PUBLICATION OF RESEARCH FINDINGS

Publication of the results of this trial will be governed by ACTG policies. Any presentation, abstract, or manuscript will be made available for review by the industry supporter(s) prior to submission.

14.0 BIOHAZARD CONTAINMENT

As the transmission of HIV and other blood-borne pathogens can occur through contact with contaminated needles, blood, and blood products, appropriate blood and secretion precautions will be employed by all personnel in the drawing of blood and shipping and handling of all specimens for this study, as currently recommended by the Centers for Disease Control and Prevention and the National Institutes of Health.

All dangerous goods and materials, including diagnostic specimens and infectious substances, must be transported using packaging mandated by CFR 42 Part 72. Please refer to instructions detailed in the International Air Transport Association (IATA) Dangerous Goods Regulations.

15.0 REFERENCES

1. Hunt PW, Cao HL, Muzoora C, et al. Impact of CCR5+ T-cell activation on CD4+ T-cell recovery and mortality in HIV-infected Ugandans initiating antiretroviral therapy. *AIDS*. 2011 Nov 13;25(17):2123-31.
2. Verstovsek S, Kantarjian H, Mesa RA, et al. Safety and efficacy of INCB018424, a JAK1 and JAK2 inhibitor, in myelofibrosis. *N Engl J Med*. 2010;363:1117-27.
3. Verstovsek S, Mesa RA, Gotlib J, et al. A double-blind, placebo-controlled trial of ruxolitinib for myelofibrosis. *N Engl J Med*. 2012;366:799-807.
4. Kohler JJ, Tuttle DL, Coberley CR, et al. Human immunodeficiency virus type 1 (HIV-1) induces activation of multiple STATs in CD4+ cells of lymphocyte or monocyte/macrophage lineages. *J Leukoc Biol*. 2003;73:407-16.
5. Chomont N, El-Far M, Ancuta P, et al. HIV reservoir size and persistence are driven by T cell survival and homeostatic proliferation. *Nat Med*. 2009;15:893-900.
6. Chun TW, Engel D, Mizell SB, et al. Induction of HIV-1 replication in latently infected CD4+ T cells using a combination of cytokines. *J Exp Med*. 1998 Jul 6;188(1):83-91.
7. Interleukin-6 Receptor Mendelian Randomisation Analysis (IL6R MR) Consortium, Hingorani AD, Casas JP. The interleukin-6 receptor as a target for prevention of coronary heart disease: a mendelian randomization analysis. *Lancet*. 2012 Mar 31;379(9822):1214-24.
8. Borges A, Silverberg MJ, Wentworth D, et al. Predicting risk of cancer during HIV infection: the role of inflammatory and coagulation biomarkers. *Clin Science*. 2013; 27(9):1433-1441.
9. Baker J, Huppler HK, Sing A, et al, CDC SUN Study Investigators. Monocyte activation, but not T cell activation, predicts progression of coronary artery calcium in a contemporary HIV cohort. 20th Conference on Retroviruses and Opportunistic Infections, March 3-6, 2013. Atlanta, GA, USA.
10. Gavegnano C, Detorio Ma, Schinazi RF, et al. Cellular pharmacology and potency of HIV-1 nucleoside analogs in primary human macrophages. *Antimicrob Agents Chemother*. 2013 Mar;57(3):1262-9.
11. Chomont N, El-Far M, Ancuta P, et al. HIV reservoir size and persistence are driven by T cell survival and homeostatic proliferation. *Nat Med*. 2009 Aug;15(8):893-900.
12. Besson GJ, Lalama CM, Bosch RJ, et al. HIV-1 DNA decay dynamics in blood during more than a decade of suppressive antiretroviral therapy. *Clin Infect Dis*. 2014 Nov 1;59(9):1312-21.
13. Cobos-Jimenez V, Booiman T, Hamann J, et al. Macrophages and HIV-1. *Curr Opin HIV AIDS*. 2011;6:385-90.

REFERENCES (Cont'd)

14. Trono D, Van Lint C, Rouzioux C, et al. HIV persistence and the prospect of long-term drug-free remissions for HIV-infected individuals. *Science*. 2010;329:174-80.
15. Rivera-Rivera L, Perez-Laspiur J, Colon K, et al. Inhibition of interferon response by cystatin B: implication in HIV replication of macrophage reservoirs. *J Neurovirol*. 2012;18:20-29.
16. McArthur JC, Steiner J, Sacktor N, et al. Human immunodeficiency virus-associated neurocognitive disorders: Mind the gap. *Ann Neurol*. 2010;67:699-714.
17. Anthony IC, Ramage SN, Carnie FW, et al. Influence of HAART on HIV-related CNS disease and neuroinflammation. *J Neuropathol Exp Neurol*. 2005;64:529-536.
18. Bachis A, Avdoshina V, Zecca L, et al. Human immunodeficiency virus type 1 alters brain-derived neurotrophic factor processing in neurons. *J Neurosci*. 2012;32:9477-84.
19. Mocchetti I, Bachis A, Avdoshina V. Neurotoxicity of human immunodeficiency virus-1: viral proteins and axonal transport. *Neurotox Res*. 2012;21:79-89.
20. Dunfee R, Thomas ER, Gorry PR, et al. Mechanisms of HIV-1 neurotropism. *Curr HIV Res*. 2006;4:267-78.
21. Fischer-Smith T, Bell C, Croul S, et al. Monocyte/macrophage trafficking in acquired immunodeficiency syndrome encephalitis: lessons from human and nonhuman primate studies. *J Neurovirol*. 2008;14:318-26.
22. Alexaki A, Liu Y, Wigdahl B. Cellular reservoirs of HIV-1 and their role in viral persistence. *Curr HIV Res*. 2008;6:388-400.
23. Gavegnano C, Schinazi RF. Antiretroviral therapy in macrophages: implication for HIV eradication. *Antivir Chem Chemother*. 2009;20:63-78.
24. Herbein G, Varin A. The macrophage in HIV-1 infection: from activation to deactivation? *Retrovirology*. 2010;7:33.
25. Poli G, Bressler P, Kinter A, et al. Interleukin 6 induces human immunodeficiency virus expression in infected monocytic cells alone and in synergy with tumor necrosis factor alpha by transcriptional and post-transcriptional mechanisms. *J Exp Med*. 1990;172:151-58.
26. Wahl SM, Greenwell-Wild T, Peng G, et al. Viral and host cofactors facilitate HIV-1 replication in macrophages. *J Leukoc Biol*. 2003;74:726-35.
27. Albin A, Benelli R, Giunciuglio D, et al. Identification of a novel domain of HIV that involved in monocyte chemotaxis. *J Biol Chem*. 1998;273:15895-15900.
28. Fantuzzi L, Conti L, Gauzzi MC, et al. Regulation of chemokine/cytokine network during in vitro differentiation and HIV-1 infection of human monocytes: possible importance in the pathogenesis of AIDS. *J Leukoc Biol*. 2000;68:391-99.

REFERENCES (Cont'd)

29. Koziel H, Eichbaum Q, Kruskal BA, et al. Reduced binding and phagocytosis of *Pneumocystis carinii* by alveolar macrophages from persons infected with HIV-1 correlates with mannose receptor downregulation. *J Clin Invest.* 1998;102:1332-44.
30. Reardon CC, Kim SJ, Wagner RP, et al. Phagocytosis and growth inhibition of *Cryptococcus neoformans* by human alveolar macrophages: effects of HIV-1 infection. *AIDS.* 1996;10:613-18.
31. Macatonia SE, Gompels M, Pinching AJ, et al. Antigen-presentation by macrophages but not by dendritic cells in human immunodeficiency virus (HIV) infection. *Immunology.* 1992;75:576-81.
32. Cory TJ, Schacker TW, Stevenson M, et al. Overcoming pharmacologic sanctuaries. *Curr Opin HIV AIDS.* 2013 May;8(3): 190-5.
33. Buzon MJ, Massanella M, Llibre JM, et al. HIC-1 replication and immune dynamics are affected by raltegravir intensification of HAART-suppressed participants. *Nat Med.* 2010;16(4):460-65.
34. Hatano H, Strain MC, Scherzer R, et al. Increase in 2-long terminal repeat circles and decrease in D-dimer after raltegravir intensification in patients with treated HIV infection: A randomized, placebo-controlled Trial. *J Infect Dis.* 2013;208(9):1436-42.
35. Williams DW, Eugenin EA, Calderon TM, et al. Monocyte maturation, HIV susceptibility, and transmigration across the blood brain barrier are critical in HIV neuropathogenesis. *J Leukoc Biol.* 2012 Mar;91(3):401-15.
36. Buckner CM, Calderon TM, Williams DW, et al. Characterization of monocyte maturation/differentiation that facilitates their transmigration across the blood-brain barrier and infection by HIV: Implications for NeuroAIDS. *Cell Immunol.* 2011;267(2):109-23.
37. Fischer-Smith T, Croul S, Sverstiuk AE, et al. CNS invasion by CD14+/CD16+ peripheral blood-derived monocytes in HIV-dementia: perivascular accumulation and reservoir of HIV infection. *J Neurovirol.* 2001 Dec;7(6):528-41.
38. Valcour VG, Shiramizu BT, Shikuma CM. HIV DNA in circulating monocytes as a mechanism to dementia and other HIV complications. *J Leukoc Biol.* 2010 Apr;87(4):621-6.
39. Ganau M, Prisco L, Pescador D, et al. Challenging new targets for CNS-HIV infection. *Front Neurol.* 2012;3:43.
40. Chugh P, Fan S, Planelles V, et al. Infection of human immunodeficiency virus and intracellular viral Tat protein exert a pro-survival effect in a human microglial cell line. *J Mol Biol.* 2007;366:67-81.
41. Eggers C, Hertogs K, Sturenburg HJ, et al. Delayed central nervous system virus suppression during highly active antiretroviral therapy is associated with HIV encephalopathy, but not with viral drug resistance or poor central nervous system drug penetration. *AIDS.* 2003;17:1897-1906.

REFERENCES (Cont'd)

42. Kaul M, Zheng J, Okamoto S, et al. HIV-1 infection and AIDS: consequences for the central nervous system. *Cell Death Differ*. 2005;12 Suppl 1:878-92.
43. Kazmierczak K, Potash MJ. Host and virus strain dependence in activation of human macrophages by human immunodeficiency virus type 1. *J Neurovirol*. 2007;13:452-61.
44. Rumbaugh JA, Nath A. Developments in HIV neuropathogenesis. *Curr Pharm Des* 2006;12:1023-44.
45. Yilmaz A, Price RW, Gisslen M. Antiretroviral drug treatment of CNS HIV-1 infection. *J Antimicrob Chemother*. 2012;67:299-311.
46. Sandler NG, Douek Daniel C. Microbial translocation in HIV infection: causes, consequences and treatment opportunities. *Nat Rev Microbiol*. 2012 Sep;10: 655-66.
47. Shuai Ke, Liu Bin. Regulation of JAK/STAT signaling in the immune system, *Nature Reviews Immunology*. 2003 Nov;3:900-11.
48. Deisseroth A, Kaminskas E, Grillo J, et al. U.S. Food and drug administration approval: ruxolitinib for the treatment of patients with intermediate and high-risk myelofibrosis. *Clin Cancer Res*. 2012;18:3212-17.
49. Gavegnano, C, Detorio, M, Montero, C, et al. Ruxolitinib and tofacitinib are potent and selective inhibitors of HIV-1 replication and virus reactivation in vitro. *Antimicrob Agents Chemother*. 2014;58:1977-1986.
50. Shi JG, Chen X, McGee RF, et al. The pharmacokinetics, pharmacodynamics, and safety of orally dosed INCB018424 phosphate in healthy volunteers. *J Clin Pharmacol*. 2011;51:1644-54.
51. Verstovsek S, Kantarjian HM, Estrov Z, et al. Long-term outcomes of 107 patients with myelofibrosis receiving JAK1/JAK2 inhibitor ruxolitinib: survival advantage in comparison to matched historical controls. *Blood*. 2012;120:1202-09.
52. Shi JG, Chen X, Emm T, et al. The effect of CYP3A4 inhibition or induction on the pharmacokinetics and pharmacodynamics of orally administered ruxolitinib (INCB018424 phosphate) in healthy volunteers. *J Clin Pharmacol*. 2012;52:809-818.
53. Tam CS, Verstovsek S. Investigational Janus kinase inhibitors. *Expert Opin Invest Drugs*. 2013;22(6):687-99.
54. Jung CW, Shih LY, Ziao Z. Efficacy and safety of ruxolitinib in Asian patients with myelofibrosis. *Leuk Lymphoma*. 2014 Nov 20:1-8.
55. Tefferi A, Litzow MR, Pardanani A. Long-term outcome of treatment with ruxolitinib in myelofibrosis. *N Engl J Med*. 2011 Oct 13;365(15):1455-57.
56. Wathes R, Moule S, Milojkovic D. Progressive multifocal leukoencephalopathy associated with ruxolitinib. *N Engl J Med*. 2013 Jul 11;369(2):197-8.

REFERENCES (Cont'd)

57. Chen YH, Lee CH, Pei SN. Pulmonary tuberculosis reactivation following ruxolitinib treatment in a patient with primary myelofibrosis. *Leuk Lymphoma*. 2014 Nov 5:1-2.
58. Palandri F, Poverilli N, Catani L, et al. Ruxolitinib-associated tuberculosis: a case of successful ruxolitinib rechallenge. *Ann Hematol*. 2014 Aug 30.
59. Hopman RK, Lawrence SJ, Oh ST. Disseminated tuberculosis associated with ruxolitinib. *Leukemia*. 2014 Aug;28(8):1750-1.
60. Colomba C, Rubino R, Siracusa L, et al. Disseminated tuberculosis in a patient treated with a JAK2 selective inhibitor: a case report. *BMC Res Notes*. 2012 Oct 5;5:552.
61. Caocci G, Murgia F, Podda L, et al. Reactivation of hepatitis B virus infection following ruxolitinib treatment in a patient with myelofibrosis. *Leukemia*. 2014 Jan;28(1):225-7.
62. Shen CH, Hwang CE, Chen YY, et al. Hepatitis B virus reactivation associated with ruxolitinib. *Ann Hematol*. 2014 Jun;93(6):1075-6.
63. Tong LX, Jackson J, Kerstetter J, et al. Reactivation of herpes simplex virus infection in a patient undergoing ruxolitinib treatment. *J Am Acad Dermatol*. 2014 Mar;70(3):e59-60.
64. Lee SC, Feenstra J, Georghiou PR. Pneumocystis jiroveci pneumonitis complicating ruxolitinib therapy. *BMJ Case Rep*. 2014 Jun 2;2014.
65. Goldberg RA, Reichel E, Oshry LJ. Bilateral toxoplasmosis retinitis associated with ruxolitinib. *N Engl J Med*. 2013 Aug 15;369(7):681-3.
66. Wysham NG, Sullivan DR, Allada G. An opportunistic infection associated with ruxolitinib, a novel janus kinase 1,2 inhibitor. *Chest*. 2013 May;143(5):1478-9.
67. Verstovsek, S, Gotlib, J, Gupta, V, et al. Management of cytopenias in patients with myelofibrosis treated with ruxolitinib and effect of dose modifications on efficacy outcomes. *OncoTargets and Therapy*. 2014;7:13–21.
68. Ward BA, Gorski JC, Jones DR, et al. The cytochrome P450 2B6 (CYP2B6) is the main catalyst of efavirenz primary and secondary metabolism: implication for HIV/AIDS therapy and utility of efavirenz as a substrate marker of CYP2B6 catalytic activity. *J Pharmacol Exp Ther*. 2003;306:287-300.
69. Kuller LH, Tracy R, Belloso W, et al. Inflammatory and coagulation biomarkers and mortality in patients with HIV infection. *PLoS Med*. 2008;5(10):e203.
70. Duprez DA, Neuhaus J, Kuller LH, et al. Inflammation, coagulation and cardiovascular disease in HIV-infected individuals. *PLoS one*. 2012;7(9):e44454.
71. Tenorio, A, Zheng Y, Bosch RJ, et al. Soluble markers of inflammation & coagulation, but not T-Cell activation, predict non-AIDS-defining events during suppressive

REFERENCES (Cont'd)

- antiretroviral therapy (Abstract #790). 20th Conference on Retroviruses and Opportunistic Infections, 2013. Atlanta, GA.
72. Hunt PW, Cao HL, Muzoora C, et al. Impact of tryptophan catabolism on CD4+ T Cell recovery and mortality in HIV-infected Ugandans initiating antiretroviral therapy (Abstract # MOAA0105). 6th International AIDS Society Conference on HIV Pathogenesis, Treatment, and Prevention. July 17-20, 2011. Rome, Italy.
73. Neuhaus, J, Jacobs DR Jr, Baker JV, et al. Markers of inflammation, coagulation, and renal function are elevated in adults with HIV infection. *J Infect Dis.* 2010;201(12):1788-95.
74. Justice, AC, Freiberg MS, Tracy R, et al. Does an index composed of clinical data reflect effects of inflammation, coagulation, and monocyte activation on mortality among those aging with HIV? *Clin Infect Dis.* 2012;54(7):984-94.
75. Lederman MM, Calabrese L, Funderburg NT, et al. Immunologic failure despite suppressive antiretroviral therapy is related to activation and turnover of memory CD4 cells. *J Infect Dis.* 2011;204(8):1217-26.
76. Bjerk, SM, Baker JV, Emery S, et al. Biomarkers and bacterial pneumonia risk in patients with treated HIV infection: a case-control study. *PLoS one.* 2013;8(2):e56249.
77. Ledwaba, L, Tavel JA, Khabo P, et al. Pre-ART levels of inflammation and coagulation markers are strong predictors of death in a South African cohort with advanced HIV disease. *PLoS one.* 2012;7(3):e24243.
78. Hamlyn E, Fidler S, Stohr W, et al. Interleukin-6 and D-dimer levels at seroconversion as predictors of HIV-1 disease progression. *AIDS.* 2014 Mar 27;28(6):869-74.
79. Della Chiara G, Crotti A, Liboi E, et al. Negative regulation of HIV-1 transcription by a heterodimeric NF-kappaB1/p50 and C-terminally truncated STAT5 complex. *J Mol Biol.* 2011;410:933-943.
80. Lassen K, Han Y, Zhou Y, et al. The multifactorial nature of HIV-1 latency. *Trends Mol Med.* 2004;10:525-53.
81. Deeks SG, Tracy R, Douek DC. Systemic effects of inflammation on health during chronic HIV infection. *Immunity.* 2013 Oct 17;39(4):633-45.
82. Ho YC, Shan L, Hosmane NN, et al. Replication-competent noninduced proviruses in the latent reservoir increase barrier to HIV-1 cure. *Cell.* 2013 Oct 24;155(3):540-51.
- 83. Wysham NG, Sullivan DR, Allada G. An opportunistic infection associated with ruxolitinib, a novel janus kinase 1,2 inhibitor. *Chest* 2013; 143(5): 1478-9.**
- 84. Tong LX, Jackson J, Kerstetter J, Worswick SD. Reactivation of herpes simplex virus infection in a patient undergoing ruxolitinib treatment. *JAAD ONLINE* 2014.**

REFERENCES (Cont'd)

85. Gianella S, Massanella M, Richman DD, et al. Cytomegalovirus replication in semen is associated with higher levels of proviral HIV DNA and CD4+ T cell activation during antiretroviral treatment. *J Virol* 2014; 88(14): 7818-27.
86. Gianella S, Strain MC, Rought SE, et al. Associations between virologic and immunologic dynamics in blood and in the male genital tract. *J Virol* 2012; 86(3): 1307-15.
87. Gianella S, Massanella M, Wertheim JO, et al. The Sordid affair between human herpesvirus and human immunodeficiency virus. *J Infect Dis.* 2015 Mar 6. pii: jiv148. [Epub ahead of print] Review.
88. Beal S, Sheiner LB, Boekmann A, et al. NONMEM's User's Guides. ICON Development Solutions, Ellicott City, MD, USA, 2009.
89. Chen X1, Williams WV, Sandor V, et al. Population pharmacokinetic analysis of orally-administered ruxolitinib (INCB018424 Phosphate) in patients with primary myelofibrosis (PMF), post-polycythemia vera myelofibrosis (PPV-MF) or post-essential thrombocythemia myelofibrosis (PET MF). *J Clin Pharmacol.* 2013 Jul;53(7):721-30.
90. Karlsson MO, Sheiner LB. The importance of modeling interoccasion variability in population pharmacokinetic analyses. *J Pharmacokinet Biopharm.* 1993 Dec;21(6):735-50.
91. Hooker A, Staats CE, Karlsson MO. Conditional weighted residuals, an improved model diagnostic for the FO/FOCE methods. Annual Meeting of the Population Approach Group in Europe, 2012, Venice, Italy. Abstract #1001.
92. Hooker AC, Staats C, Karlsson MO. Conditional weighted residuals (CWRES): a model diagnostic for the FOCE method. *Pharm Res.* 2007;24:2187-97.
93. Karlsson MO, Savic R. Diagnosing Model Diagnostics *Pharmacology & Therapeutics.* 2007;82:17-20.
94. Wurtz R, Itokazu G, Rodvold K. Antibiotic dosing in obese patients. *Clin Infect Dis.* 1997;25:112-8.
95. Bergstrand M, Hooker AC, Wallin JE, et al. Prediction-corrected visual predictive checks for diagnosing nonlinear mixed-effects models. *AAPS J.* 2011 Jun;13(2):143-51.
96. Kakuda T, Sekar V, Vis P, et al. Pharmacokinetics and pharmacodynamics of darunavir and etravirine in HIV-1-infected, treatment-experienced patients in the gender, race, and clinical experience (GRACE) trial. *AIDS Res Treat.* 2012:186987.
97. Abdelhady AM, Desta Z, Jiang F, et al. Population pharmacogenetic-based pharmacokinetic modeling of efavirenz, 7-hydroxy- and 8-hydroxyefavirenz. *J Clin Pharmacol.* 2014 Jan;54(1):87-96.

APPENDIX I

DIVISION OF AIDS AIDS CLINICAL TRIALS GROUP (ACTG) SAMPLE INFORMED CONSENT

For protocol A5336: A Randomized, Pilot Study of Ruxolitinib in Antiretroviral-Treated HIV-Infected Adults

SHORT TITLE FOR THE STUDY: (A5336) Trial of Ruxolitinib in Antiretroviral-Treated HIV-Infected Adults (**FINAL** Version 2.0, Dated **05/21/15**)

INTRODUCTION

When a person becomes infected with HIV (the virus that causes AIDS), his/her immune system (the system that helps fight infection) is weakened (partly because the number of CD4+ cells [the number of white blood cells that fight infection] goes down). Despite successful treatment with antiretroviral therapy (ART), latent reservoirs (infected cells that are not actively producing HIV) remain present in the blood and contribute to ongoing immune system activation (immune system becomes active in response to an infection) and inflammation (reaction to an infection or irritation) in the body.

You are being asked to take part in this research study because you:

- are infected with HIV-1
- have a very low viral load (amount of virus in the blood)
- have CD4+ T cell count of more than 350 cells
- **are on stable ART for at least 3 months**

This study is sponsored by the National Institutes of Health (NIH) for the AIDS Clinical Trials Group (ACTG). The doctor in charge of this study at this site is: (insert name of Principal Investigator). Before you decide if you want to be a part of this study, we want you to know about the study.

This is a consent form. It gives you information about this study. The study staff will talk with you about this information. You are free to ask questions about this study at any time. If you agree to take part in this study, you will be asked to sign this consent form. You will get a copy to keep.

WHY IS THIS STUDY BEING DONE?

The purpose of this study is to find out more about the safety and tolerability (how well your body accepts the study drug) of the use of ruxolitinib in persons with HIV-1 infection who are also being treated with ART. We want to learn whether ruxolitinib will decrease inflammation and immune activation in the body; whether ruxolitinib will change the level of HIV-1 in your blood; and how ruxolitinib interacts with ART in the blood.

APPENDIX I (Cont'd)

Ruxolitinib is approved by the U.S. Food and Drug Administration (FDA) to treat myelofibrosis, a disorder in which bone marrow is replaced by scar (fibrosis) tissue. Many of the cytokines (regulators of the body's reaction to infection, immune response, and inflammation) affected by myelofibrosis are also affected by HIV. Because ruxolitinib acts on these cytokines, it is proposed that it may also be a possible treatment for HIV by reducing inflammation. Inflammation has been associated with organ disease in people living with HIV.

WHAT DO I HAVE TO DO IF I AM IN THIS STUDY?

If you decide to take part in this research study, you will be asked to sign this consent form and schedule a screening visit to determine if you can join the study. **If so, you will be randomly assigned (as if by the toss of a coin) to either one of the two groups, Group A or Group B (see below).** The screening visit will take about 1.5 hours.

Screening visit

- Your HIV-1 infection will be confirmed. If there is no record available, you will have another HIV-1 test. You may have to sign a separate consent form before having this test.
- You will have a physical exam and will be asked about your health and medicines you have taken in the past or are taking now.
- You will have about 1 tablespoon of blood drawn for routine safety blood tests and to measure HIV-1 viral load. You will be given the test results when they are available.
- If you are a woman able to become pregnant, you will be asked to give a urine or blood sample to see if you are pregnant. You will not be able to enroll in this study if you are pregnant. You will be told the result of the test when it becomes available.

If you do not enroll into the study

If you decide not to take part in this study or if you do not meet the eligibility requirements, we will still use some of your information. As part of this screening visit, some demographic (for example, age, sex, race), clinical (for example, disease condition, diagnosis), and laboratory (for example, CD4+ T cell count, viral load) information is being collected from you so that ACTG researchers may help determine whether there are patterns or common reasons why people do not join a study.

Pre-Entry

If you qualify for the study, you will come to the clinic at least one day after the screening visit for the pre-entry visit. Please note it is possible that the pre-entry and screening visits may be combined. If not, at the pre-entry visit you will have a complete physical exam. You will also have blood drawn for CD4+ T cell count and for samples to be stored for future HIV-related testing for this study. A total of 2 tablespoon of blood will be drawn at this visit. **Saliva and cells from your cheeks will be collected with an oral swab and tested for the presence of human herpes viruses (HHV; a virus causing sores most often found around the mouth or the genitals).** The visit will last about 30-60 minutes.

Entry visit

If you are eligible for the study, you will come in for an entry visit. You will need to fast before your entry visit. Fasting means nothing to eat or drink other than water and medication for 8 hours before your visit. The visit will last about 60-90 minutes.

APPENDIX I (Cont'd)

During this visit:

- You will have a physical exam performed. You will be asked about medical history and any medicines you have taken since the last visit.
- If you are a woman able to get pregnant, you will be asked to provide blood or urine for a pregnancy test. You cannot start treatment if you are pregnant. You will be given the results of your pregnancy test as soon as it becomes available.
- You will have 7 tablespoons of blood drawn for routine safety blood tests including lipid testing, CD4+ T cell count, viral load, immune tests along with other tests. In addition, some of your blood will be stored for future testing required by the study.
- **Saliva and cells from your cheeks will be collected with an oral swab and tested for the presence of HHV.**
- You will be asked questions about adherence to antiretroviral (ARV) medicines.
- If you are in Group A, you will be given a medication diary to record the date and time of ruxolitinib, and ARV medicines for the 3 days preceding the week 1 study visit. The medication diary will be collected at the week 1 study visit.

Once all these tests are done, you will be randomly assigned to either one of the two groups (Group A or Group B). However, because of the way the study is designed, there will be a greater chance of your being in Group A.

- Group A: Takes ruxolitinib by mouth twice a day for the next 5 weeks.
- Group B: No study treatment.

If you are randomized to Group A, at this visit study staff will give you enough study drug to last until the next study visit. You will be asked not to drink grapefruit juice **or eat grapefruit** while taking the study drug. At the week 5 visit, you must return any remaining study drug.

On Study Visits after Entry

Regardless of which group you are assigned to, you will come to the clinic one week after your initial entry visit (week 1) and then study weeks 2, 4, 5. For the week 5 visit, you will be asked to fast, which means that for eight hours before your visit, you cannot eat or drink anything except water and your medication. Your next visit will not be until week 10 on study and your final visit will be at week 12. Visits will take about 30-60 minutes except weeks 1 and 4 study visits if you are in Group A (see 5th bullet below). If you are in Group A, at the week 2 study visit you will be given a medication diary to record the date and time of ruxolitinib, and ARV medicines for the 3 days preceding the week 4 study visit. The medication diary will be collected at the week 4 study visit.

During these visits:

- You will have a brief physical exam performed.
- You will be asked about any new medications since your last visit including your HIV-1 medications.
- You will have 1 tablespoon of blood drawn for routine safety blood tests, including lipid testing at week 5, and immune tests. At weeks 2, 5, and 12 study visits, blood will be drawn for CD4+ T cells count and viral load tests.
- You will have approximately from 2 to 6 tablespoons of blood drawn and stored for future protocol-related testing (except at the week 1 visit).

APPENDIX I (Cont'd)

- **Saliva and cells from your cheeks will be collected with an oral swab and tested for the presence of HHV.**
- If you are in Group A, at weeks 1 and 4 study visits you will have about 2 tablespoons of blood drawn over 6-8 hours to measure ruxolitinib and **ARV medicines** levels in your blood. You will be contacted the night before each pharmacokinetic (PK) visit to remind you not to take your morning dose of ruxolitinib or ARV medicines (including efavirenz) until instructed during your clinic visit in the morning. You will be asked to bring your study drug and ARV medicines to the clinic. You will have blood drawn 4 times during this time period to determine the levels of ruxolitinib and ARV medicines in your blood. For the multiple blood samples required, an indwelling catheter (a small, thin tube) will be put into an arm vein and left in place during your stay in the clinic if preferred. An IV tube will be placed at the end of the catheter for the PK blood draws. This will allow the blood samples to be drawn without any additional needle sticks. You may eat or drink as you wish during this period.
- You will be asked questions about adherence to the study drug (Group A) and your ARV medicines.

Virologic Failure

If the study staff sees that your viral load has gone up, and there is no other known **explanation** such as the flu, you will be asked to have another test done within 7 days. If your viral load is still up and you are still taking study drug, you will be asked to stop the study drug and come to the clinic within 2 weeks. You will be followed on study/off study treatment until the final study visit, week 12.

Pregnancy

If you become pregnant, **study drug will be discontinued immediately, and** you will be encouraged to remain in the study to be followed on study/off treatment until study completion. You will be followed by telephone contact thereafter to determine the pregnancy outcome.

Premature Treatment/Study Discontinuation

If you stop taking the study drug before the study-defined 5-week treatment period, you will be asked to return to the clinic to complete some evaluations.

- You will have a brief physical examination performed.
- You will be asked about any new medications since your last visit including your HIV-1 medications.
- You will have approximately 6 tablespoons of blood drawn for routine safety blood tests including lipid panel, CD4+ T cells count, viral load, immune tests along with other tests. Also, some of your blood will be stored for future protocol-related testing.
- **Saliva and cells from your cheeks will be collected with an oral swab and tested for the presence of HHV.**
- If you are in Group A, you will have 2 tablespoons of blood drawn for measuring ruxolitinib and HIV-1 drug levels in your blood if you are discontinuing study treatment prior to week 4.
- You will be asked questions about adherence to the study drug (Group A) and your ARV medicines.

Other

If you agree, some of your blood that is left over after all required study testing is done may be stored (with usual protectors of identity) and used for ACTG-approved HIV-1 related research. Please check the appropriate response if you agree to storing samples for future use.

APPENDIX I (Cont'd)

Please indicate below “yes” or “no” and initial and date whether you approve the use of these stored samples. Note that you can withdraw your consent for research on stored specimens at any time you want and the specimens will be discarded.

_____ Yes, I agree. _____ No, I do not agree.

WHAT ARE THE RISKS OF THE STUDY?

The drug used in this study may have side effects, some of which are listed below. Please note that this list does not include all the side effects seen with this drug. This list includes the more serious, **life-threatening** or common side effects with a known or possible relationship. If you have questions concerning the additional study drug side effects please ask the medical staff at your site. The most common side effects observed in the past ruxolitinib clinical studies include:

- Thrombocytopenia (decreased cells in the blood that are needed to stop bleeding)
- **Neutropenia (an abnormally low count of white blood cells that help fight off infections)**
- Diarrhea
- Fever
- Urinary tract infections
- Anemia
- Bruising
- Dizziness
- Headache
- **Transaminase elevations (increased liver enzymes that may indicate liver disease)**
- **Cholesterol elevation**
- **Infections**

It is not known whether the study drug passes through breast milk and whether it can produce adverse effects in the infant. Additionally, it is not known whether taking the study drug will reduce the risk of passing HIV to the baby while breastfeeding. You must tell the study doctor or nurse whether you are breastfeeding before enrolling in the study. **You cannot participate in the study if you are breastfeeding.**

There is a risk of serious and/or life-threatening side effects when non-study medications are taken with the study drug. For your safety, you must tell the study doctor or nurse about all medications you are taking before you start the study and also before starting any new medications while on the study. Also, you must tell the study doctor or nurse before enrolling in any other clinical trials while on this study.

Risk of Blood Draw

Taking blood may cause some discomfort, bleeding, bruising, and/or swelling where the needle enters the body and in rare cases it may result in fainting. There is a small risk of infection.

APPENDIX I (Cont'd)

Risk of Social Harm

Although the study site will make every effort to protect your privacy and confidentiality, it is possible that others could find out that you are participating in this study and that social harms may result (because you could become labeled as being infected with HIV). For example, you could be treated unfairly or discriminated against by family members, friends, and/or the community.

HOW MANY PEOPLE WILL TAKE PART IN THIS STUDY?

About 60 people will take part in this study

HOW LONG WILL I BE IN THIS STUDY?

You will be in this study for about 12 weeks.

WHY WOULD THE DOCTOR TAKE ME OFF THIS STUDY EARLY?

The study doctor may need to take you off the study early without your permission if:

- You miss 2 visits in a row.
- The study is canceled.
- The study doctor or your regular doctor thinks the study is no longer in your best interest.

The study doctor may also need to take you off the study drug without your permission if:

- You have confirmed virologic failure **if you are in Arm A.**
- You miss more than 3 doses of study drug a week for at least two weeks.
- You change or stop your anti-HIV-1 medicines **if you are in Arm A.**
- Continuing the study drug may be harmful to you.
- You need a treatment that you may not take while on the study.
- You are not able to take the study medicine as required by the study.
- You become pregnant.

If you must stop taking the study drug before the study is over, the study doctor may ask you to continue to be part of the study and return for some study visits and procedures.

If you must permanently stop taking ruxolitinib before your study participation is over, the study staff will discuss other options that may be of benefit to you. After you have completed your study participation, the study will not be able to continue to provide you with ruxolitinib that you received on the study. If continuing to take this or a similar drug/agent would be of benefit to you, the study staff will discuss how you may be able to obtain ruxolitinib.

APPENDIX I (Cont'd)

ARE THERE RISKS RELATED TO PREGNANCY?

It is not known if the drug or drug combinations in this study harm unborn babies. If you are having sex that could lead to pregnancy, you must agree not to become pregnant or make a woman pregnant.

Because of the risk involved, you and your partner must use one form of birth control that you discuss with the study staff. You must start one method of birth control when you are taking your study drug. You must continue to use this method until 7 weeks after you stop the study drug.

- Condoms (male or female) with or without a spermicidal agent. Condoms are recommended because their appropriate use is the only contraceptive method effective for preventing HIV-1 transmission.
- Diaphragm or cervical cap with spermicide
- Intrauterine device (IUD)
- Hormone-based contraceptive

If you can become pregnant, you must have a pregnancy test before you enter this study. The test must show that you are not pregnant. If you think you may be pregnant at any time during the study, tell your study staff right away. The study staff will talk to you about your choices. Pregnancy will result in immediate discontinuation of the study drug for the pregnant participant. You will be followed on study until study completion and will be followed by telephone contact thereafter to determine the pregnancy outcome. Pregnancies that occur on study will be reported prospectively to The Antiretroviral Pregnancy Registry.

ARE THERE BENEFITS TO TAKING PART IN THIS STUDY?

This study is intended to gather information about how ruxolitinib acts on your immune system, not to treat your HIV-1 infection. **There is no anticipated benefit for taking part in this study.** Information learned from this study may help others who have HIV.

WHAT OTHER CHOICES DO I HAVE BESIDES THIS STUDY?

Instead of being in this study you have the choice of:

- Treatment with prescription drugs available to you.
- Treatment with other experimental drugs, if you qualify.
- No treatment

Please talk to your doctor about these and other choices available to you. Your doctor will explain the risks and benefits of these choices.

APPENDIX I (Cont'd)

WHAT ABOUT CONFIDENTIALITY?

We will do everything we can to protect your privacy. In addition to the efforts of the study staff to help keep your personal information private, we have gotten a Certificate of Confidentiality from the U.S. Federal Government. This certificate means that researchers cannot be forced to tell people who are not connected with this study, such as the court system, about your participation. Also, any publication of this study will not use your name or identify you personally.

People who may review your records include the ACTG, Office for Human Research Protections (OHRP) or other government agencies as part of their duties, Food and Drug Administration (FDA), (insert name of site) Institutional Review Board (IRB) (a group that protects the rights and well-being of people in research), National Institutes of Health (NIH), study staff, study monitors, and their designees. Having a Certificate of Confidentiality does not prevent you from releasing information about yourself and your participation in the study.

Even with the Certificate of Confidentiality, if the study staff learns of possible child abuse and/or neglect or a risk of harm to yourself or others, we will be required to tell the proper authorities. A description of this clinical trial will be available on www.ClinicalTrials.gov, as required by U.S. law. This Web site will not include information that can identify you. At most, the Web site will include a summary of the results. You can search this Web site at any time.

WHAT ARE THE COSTS TO ME?

Taking part in this study may lead to added costs to you and your insurance company. In some cases it is possible that your insurance company will not pay for these costs because you are taking part in a research study. There will be no cost to you for study drug, study-related visits, physical examinations, laboratory tests, or other study procedures. Note that ARV medicines will not be provided by the study.

WILL I RECEIVE ANY PAYMENT?

You will be compensated for participating in the study regardless of **the group you were** assigned to.

WHAT HAPPENS IF I AM INJURED?

If you are injured as a result of being in this study, you will be given immediate treatment for your injuries. The cost for this treatment will be charged to you or your insurance company. There is no program for compensation either through this institution or the National Institutes of Health. You will not be giving up any of your legal rights by signing this consent form.

APPENDIX I (Cont'd)

WHAT ARE MY RIGHTS AS A RESEARCH PARTICIPANT?

Taking part in this study is completely voluntary. You may choose not to take part in this study or leave this study at any time. Your decision will not have any impact on your participation in other studies conducted by NIH and will not result in any penalty or loss of benefits to which you are otherwise entitled.

We will tell you about new information from this or other studies that may affect your health, welfare, or willingness to stay in this study. If you want the results of the study, let the study staff know.

WHAT DO I DO IF I HAVE QUESTIONS OR PROBLEMS?

For questions about this study or a research-related injury, contact:

- name of the investigator or other study staff
- telephone number of above

For questions about your rights as a research participant, contact:

- name or title of person on the Institutional Review Board (IRB) or other organization appropriate for the site
- telephone number of above

APPENDIX I (Cont'd)

SIGNATURE PAGE

If you have read this consent form (or had it explained to you), all your questions have been answered and you agree to take part in this study, please sign your name below.

Participant's Name (print)

Participant's Signature and Date

Participant's Legal Representative (print)
(As appropriate)

Legal Representative's Signature and Date

Study Staff Conducting
Consent Discussion (print)

Study Staff's Signature and Date

Witness's Name (print)
(As appropriate)

Witness's Signature and Date