PILOT STUDY OF IXAZOMIB TO REDUCE THE NUMBER OF HIV DNA POSITIVE LYMPHOID CELLS

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PILOT STUDY OF IXAZOMIB TO REDUCE THE NUMBER OF HIV DNA POSITIVE LYMPHOID CELLS

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List of Abbreviations

LIST OF ABBREVIATIONS

AE	Adverse Event/Adverse Experience
ANC	Absolute neutrophil count
ART	Antiretroviral therapy
ARV	Antiretroviral
CFR	Code of Federal Regulations
CRF	Case Report Form
DLT	Dose limiting toxicity
DRESS Syndrome	Drug Reaction (or Rash) with Eosinophilia and Systemic Symptoms
DSMB	Data and Safety Monitoring Board
FDA	Food and Drug Administration
GCP	Good Clinical Practice
hERG	Human ether-à-go-go related gene
HIPAA	Health Insurance Portability and Accountability Act
HIV	Human Immunodeficiency Virus
IB	Investigator's Brochure
IC50	Half-maximal inhibitory concentration
IND	Investigational New Drug Application
IRB	Institutional Review Board
IV	Intravenous
LLN	Lower limit of normal
MLN2238	Ixazomib/NinLaro®
MM	Multiple myeloma
NCI CTCAE	National Cancer Institute Common Terminology Criteria for Adverse Events
NNRTIs	Non-nucleoside reverse transcriptase inhibitors
PHI	Protected Health Information
PI	Principal Investigator
PK	Pharmacokinetic
PN	Peripheral Neuropathy
PO	Oral administration
RBC	Red blood cell
SAE	Serious Adverse Event/Serious Adverse Experience
SCID	Severe combined immunodeficient
SOP	Standard Operating Procedure
TEN	Toxic epidermal necrolysis
ULN	Upper limit of normal
UPP	Ubiquitin-proteasome Pathway

Study Summary

Title	PILOT STUDY OF IXAZOMIB TO REDUCE THE NUMBER OF HIV DNA POSITIVE LYMPHOID CELLS
Running Title	Ixazomib for HIV cure
Protocol Number	16-001938
Phase	Phase I/II
Methodology	Dose escalation, open-label clinical trial.
Overall Study Duration	24 months
Subject Participation Duration	7 months
Single or Multi-Site	Multi-Site
Objectives	The primary objective of the trial is to determine the safety and tolerability of ixazomib in HIV positive persons on antiretroviral therapy. The secondary objective is to determine the effect of ixazomib on HIV reservoir size.
Number of Subjects	Maximum of 17
Diagnosis and Main Inclusion Criteria	Virologically suppressed HIV disease on at least 3 active drug initial or salvage antiretroviral regimens for at least 6 months

Study Product, Dose, Route, Regimen	Patients will be treated in sequential groups after the safety evaluation of each group by the DSMB as follows: Group 1: 3 patients with HIV infection, who meet inclusion criteria, will receive ixazomib 1 mg on days 1, 8 and 15 for three 28 days cycles, then be reviewed by DSMB and based upon their recommendation patients will receive either (i) ixazomib once weekly for 12 weeks or (ii) ixazomib 1 mg on days 1, 8 and 15 for three 28 days cycles. Visits 3-15 will have a window of ± 3 days. Group 2: 3 patients with HIV infection, who meet inclusion criteria, will receive ixazomib 2mg on days 1, 8 and 15 for three 28 days cycles, then be reviewed by DSMB and based upon their recommendation patients will receive either (i) ixazomib once weekly for 12 weeks or (ii) ixazomib 2 mg on days 1, 8 and 15 for three 28 days cycles. Visits 3-15 will have a window of ± 3 days. Group 3: 3 patients with HIV infection, who meet inclusion criteria, will receive ixazomib 3 mg on days 1, 8 and 15 for three 28 days cycles, then be reviewed by DSMB and based upon their recommendation patients will receive either (i) ixazomib once weekly for 12 weeks or (ii) ixazomib 3 mg on days 1, 8 and 15 for three 28 days cycles. Visits 3-15 will have a window of ± 3 days. Group 4: 3 patients with HIV infection, who meet inclusion criteria, will receive ixazomib 4 mg on days 1, 8 and 15 for three 28 days cycles, then be reviewed by DSMB and based upon their recommendation patients will receive either (i) ixazomib once weekly for 12 weeks or (ii) ixazomib 4 mg on days 1, 8 and 15 for three 28 days cycles. Visits 3-15 will have a window of ± 3 days. Group 5: 5 patients with HIV infection, who meet inclusion criteria will receive the maximum tolerated dose of ixazomib per DSMB recommendations. Visits 3-15 will have a window of ± 3 days.
Duration of Treatment Administration for Each Patient	24 weeks
Reference therapy	None

Statistical Methodology	This study is designed to assess the safety and tolerability of ixazomib in an HIV infected patient population, side effects will be graded according to The NCI CTCAE v4.0 Secondary endpoints are 1) Change in HIV DNA in CD4 T cell subsets from Week -1 to Week 24; 2) Change in culturable HIV from Week -1 to Week 24; 3) Change in absolute CD4 T cell count and percentage, CD8 T cell count and percentage, and CD4/CD8 ratio from Week -1 to 24;

1 Introduction

This document is a protocol for a human research study. This study will be carried out in accordance with the applicable United States government regulations and Mayo Clinic research policies and procedures.

1.1 Background

Intense activity is focused on identifying a clinical intervention that results in a long term, drug free remission of HIV-1 infection. Latently infected CD4 T cells harbor transcriptionally silent, replication competent HIV. Because these cells persist long term and are unaffected by current therapies, they represent an HIV reservoir that remains undiminished by current approaches. Pilot clinical trials have tested whether reactivation of HIV-1 from latency will decrease the number of cells containing HIV DNA due to viral cytopathic effect or immune mediated clearance. The latency reversal agents vorinostat, panobinostat and romidepsin result in HIV reactivation as measured by increases in cell associated HIV RNA, yet no change in cell-associated HIV DNA, indicating that the reactivating cells do not die (1-3). Multiple ongoing studies are testing augmentation of the anti-HIV immune response in combination with viral reactivation as a strategy for HIV eradication. **We propose that designing strategies to reduce HIV reservoir size will be informed by a mechanistic understanding of how HIV can kill CD4 T cells in different circumstances of activation, and HIV replication.**

Viral infection is often detected by intrinsic host defense mechanisms that activate cell suicide programs in order to limit the production of progeny virions. In response, viruses have evolved mechanisms to inhibit cell death pathways. Prototypic examples include the human papilloma virus E6 and E7 proteins (4), Baculovirus inhibitor of apoptosis (IAP) and p35 proteins (5), and Adenovirus E1B-55K (6). The role of these viral death inhibitors in viral persistence is well established. In cervical epithelial cells with integrated HPV, for example, chromosomal disruption of either E6 or E7 with CRISPR/Cas9 results in the activation of p53 or retinoblastoma (Rb), respectively, thereby activating cell death cascades (4) and disrupting persistence of the virally infected cell. In baculovirus infected cells, p35 and IAP inhibit apoptosis; and baculoviruses deficient in either of these proteins will induce premature death after infection, resulting in fewer progeny viruses (7). Similarly, deletion of anti-apoptotic EBV BALF1, BHRF1 or both results in premature cell death during EBV infection of primary B cells (8) and deletion of the adenoviral *BCL2* homolog E1B-19K results in premature cell death and loss of persistence of adenoviral infection (9). **Thus, virally encoded apoptosis inhibitors are necessary for infected cells to persist, and enable the persisting cells to produce progeny**

viruses.

Although HIV does not encode an apoptosis inhibitory protein *per se*, HIV-infected cells are nonetheless resistant to apoptosis (reviewed in (10, 11)). This apoptosis resistance reflects the ability of various HIV encoded proteins to upregulate endogenous apoptosis inhibitory proteins and down-regulate pro-apoptotic proteins. For example, Tat upregulates anti-apoptotic Bcl-2, c-FLIP, XIAP and C-IAP2 (12), while Nef causes phosphorylation and inactivation of pro-apoptotic Bad (13), thereby conferring apoptosis resistance (14). This shift in apoptotic balance affords an HIV-infected cell the ability to survive and produce more virions. Experimentally, addition of the pan-caspase inhibitor z-VAD-fmk to primary T cells infected with HIV reduces cell death and augments HIV production because fewer HIV infected cells die (15). Similarly, when primary CD4 T cells transduced and selected for Bcl-2 expression, and then infected with GFP-HIV, some cells revert to a quiescent (GFP low) state, which recapitulates many features of latency, including lack of basal HIV transcription and induction of GFP/HIV by latency reversing agents (16). **Thus there is an established association between apoptosis resistance, Bcl-2 expression and latency during *in vitro* HIV infection**.

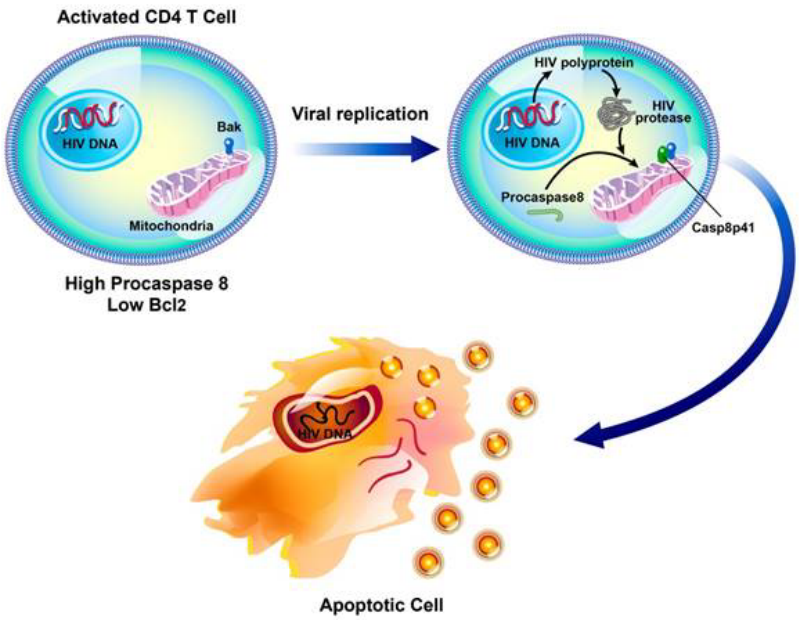


Figure 1: Mechanism of HIV protease induced killing of an activated CD4 T cell. When HIV replicates, HIV protease is active within the cytosol, and it cleaves both viral and host substrates, including procaspase 8. Cleavage of procaspase 8 generates Casp8p41 which translocates to the mitochondrion, where it binds and activates Bak, to induce apoptosis.

While numerous pathways may contribute to the decline of uninfected CD4 T cells during uncontrolled HIV infection (17), fewer pathways have been implicated in the demise of cells directly infected by HIV. Following HIV attachment, at least three distinct pathways can initiate the death of infected cells: RIG-I mediated sensing of HIV RNA (18, 19); IFI-16 sensing of unintegrated HIV DNA (20, 21), and DNA-PK-sensing of HIV integrase nicking of host DNA (22). Once integrated into host DNA, HIV can remain in a latent state for years, or reactivate and replicate. When HIV reactivation and replication occur, HIV protease is active within the cytosol (23), where it cleaves both viral and host substrates, leading to apoptotic cell death (24) **HIV protease induced death is dependent upon protease cleaving procaspase 8 to generate the fragment Casp8p41(25-27), which contains an α-helical BH3-like domain that binds and activates the pro-apoptotic Bcl-2 family member Bak, triggering mitochondrial outer**

membrane permeabilization (MOMP) and apoptosis (28, 29) (Figure 1).

HIV is currently incurable due to the presence of HIV integrated into the genome of resting cells, predominantly central memory CD4 T cells (T_{CM}), which constitute the major reservoir for HIV in patients on suppressive ART (30). In the context of Casp8p41 biology, there are two possible reasons why reactivating cells might not die: Either Casp8p41 is not generated, or the pro-apoptotic effects of Casp8p41 are antagonized by intrinsic apoptosis resistance mechanisms. Since T_{CM} express detectable levels of Caspase 8 (Figure 2A), we analyzed whether T_{CM} from HIV-infected persons generate Casp8p41 *in vivo* (Figure 2B). **Flow cytometry readily detected Casp8p41 in T_{CM}, suggesting that the failure of T_{CM} to die when HIV reactivates is not due to a failure to generate Casp8p41, but instead perhaps due to antagonism of Casp8p41-mediated killing.**

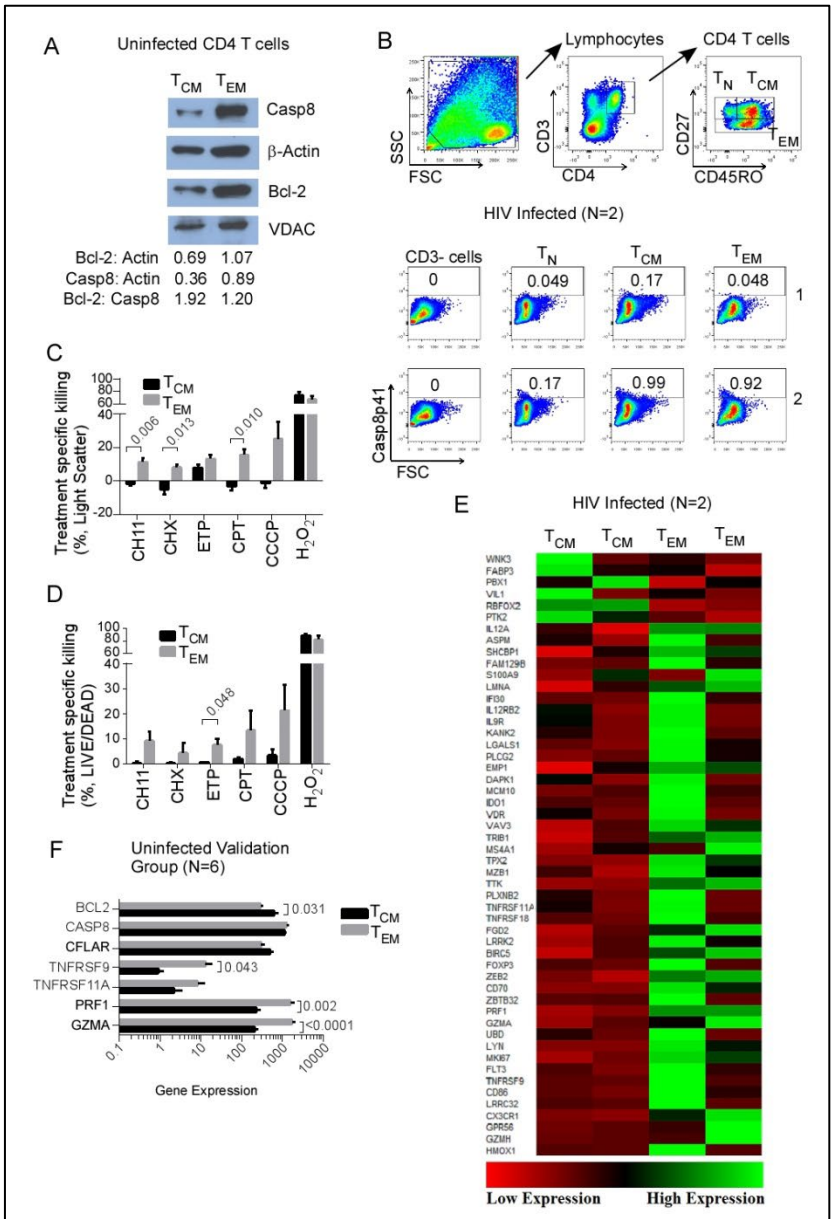


Figure 2. Despite expressing Casp8p41 in the context of HIV infection, Central memory CD4 T cells are intrinsically resistant to cell death due to a increase in Bcl-2 expression relative to procaspase 8.

T_{CM} cells are long lived and proliferate in response to antigenic re-stimulation. By contrast, effector memory CD4 T cells (T_{EM}), which are a lesser HIV reservoir (31), are found in areas of inflammation and have a shorter half-life than T_{CM} (32). The longer life span of T_{CM} prompted us to speculate that these cells might be intrinsically resistant to death stimuli. To assess this possibility, we treated primary PBMCs from 3 uninfected donors with a variety of pro-apoptotic stimuli and analyzed cell death in T_{CM} and T_{EM}. T_{CM} were significantly less susceptible than T_{EM} to both Fas ligation and various triggers of the mitochondrial apoptotic pathway (Figure 2C,D). Not only did T_{CM} die less in response to the

Fas-agonist antibody CH11 compared to T_{EM} (absolute difference -19.0 ± 3.7%, P=0.006), but T_{CM} also died less in response to chemotherapy agents such as etoposide and camptothecin, suggesting a global death resistance. **These findings extend prior results showing that phosphorylation mediated inactivation of Foxo3a results in the resistance of T_{CM} to spontaneous, Fas ligation-induced and TCR-ligation induced cell death (33).**

To understand the mechanism underlying apoptosis resistance of T_{CM} compared to T_{EM}, we performed RNAseq using cells from 2 HIV-infected donors with long term suppressed HIV infection. In comparison to T_{EM}, T_{CM} up-regulated 6 proliferation genes and down-regulated 46 cell death genes by at least 2-fold ($P=6.5x10^{-6}$ for enrichment within the death and proliferation gene sets, Figure 2E). These results were validated by querying previously published (34), publicly available gene expression data (GSE61697, Gene Expression Omnibus), comparing expression of 4 genes differentially expressed in our dataset (TNFRSF9, TNFRSF11A, PRF1 and GZMA, Figure 2F). **Therefore T_{CM} upregulate antiapoptotic genes, and down regulate proapoptotic genes consistent with prior reports that during the development of memory T cells upregulate anti-apoptotic molecules such as Bcl2, Bcl-x_L and c-IAPs (35).**

Since Bcl-2 antagonizes Bak activation in lymphoid cells (36), we assessed the relative expression of Bcl-2 and Procaspase 8 protein in T_{CM} versus T_{EM} and observed that the Bcl-2:Procaspase 8 ratio is 60% higher in T_{CM} compared to T_{EM} (Figure 2A), suggesting that T_{CM} might resist the pro-death effects of Casp8p41 by virtue of elevated Bcl-2 expression. **This relative increase in the Bcl-2:Procaspase 8 ratio in T_{CM} cells was further supported by gene expression data showing increased BCL2 gene expression in T_{CM} compared to T_{EM} (P=0.031, Figure 2F).**

Whether Bcl-2 alters the ability of Casp8p41 to kill cells is unknown. Accordingly, we compared the responses of parental Jurkat T cells to those of Jurkat cells stably overexpressing Bcl-2 (Jurkat-Bcl-2) to transfection with GFP-Casp8p41 or empty vector. Over 24 hours, significantly more parental Jurkat T cells expressing Casp8p41 became apoptotic as measured by TUNEL positivity (Figure 3A), compared to Jurkat-Bcl-2 cells expressing Casp8p41 (Mean AUC 1500 vs 870 [95% CI of difference -790, -480; P =0.004]) despite similar degrees of Casp8p41 expression (Figure 3B). **Thus, cells overexpressing Bcl-2 die less in response to Casp8p41, indicating that Bcl-2 antagonizes Casp8p41 induced killing and suggesting that Bcl-2 overexpression might also alter the outcome of HIV infection.**

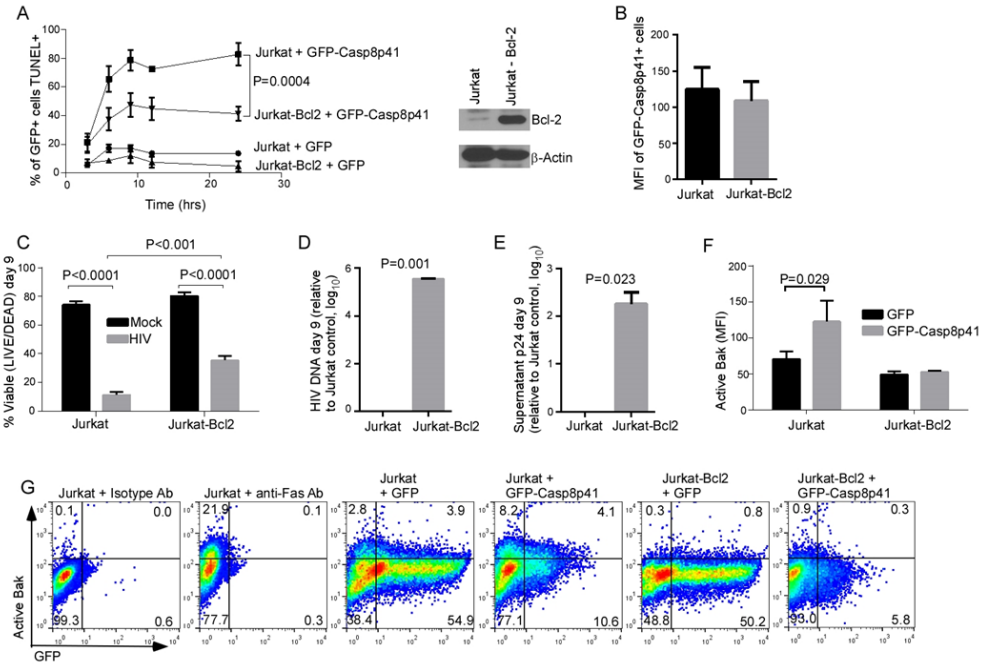


Figure 3. Bcl-2 overexpression inhibits Casp8p41-induced Bak-activation and apoptosis, and results in increased survival, HIV replication and cell associated HIV DNA in the context of acute *in vitro* infection.

To experimentally test this hypothesis, Jurkat and Jurkat-Bcl-2 cells were infected with HIV IIIb and analyzed over time for cell viability, HIV p24 production, and cell associated HIV DNA content. Bcl-2 overexpression was accompanied by increased cell survival following HIV infection (mean 36% vs 11% viability at day 9 post infection, P<0.001, Figure 3C), increased cell associated HIV DNA (5.5 log increase, P=0.001, Figure 3D), and increased HIV p24 production in culture supernatants (2.5 log increase, P=0.023, Figure 3E). **Consequently Bcl-2 reduces HIV induced cell death and increases the number of HIV-infected cells (reflected by increased HIV DNA), thereby allowing more cells to produce progeny virions (reflected by increased p24).**

To assess whether Bcl-2 overexpression impacts Casp8p41-mediated Bak activation, Jurkat or Jurkat-Bcl-2 cells were transfected with GFP or GFP-Casp8p41 and we used conformational specific antibodies which recognize activated Bak, but not inactive Bak, to assess the state of Bak activation in the GFP^+ cells. As expected, GFP-$Casp8p41^+$ parental Jurkat cells contained more activated Bak than parental cells expressing GFP alone (MFI difference 52+/-16, P=0.029; Figure 3F,G). Also and consistent with our hypothesis, GFP-Casp8p41+ Jurkat-Bcl-2 cells did not exhibit increased Bak activation compared to GFP alone (MFI difference 3.5+/-16, P=0.834), indicating that Bcl-2 prevents Bak activation in response to Casp8p41 expression. Furthermore, the overall number of activated Bak positive cells was lower in Jurkat-Bcl-2 cells compared to Jurkat cells (P=0.045). **Thus, Bcl-2, which is increased in T_{CM} relative to other T cell subsets, inhibits Casp8p41-induced Bak activation.**

We next investigated how Bcl-2 antagonizes Casp8p41 killing. Bcl-2 family members are classified into three groups based on their structure and function(37): the pro-apoptotic proteins Bax and Bak, which contain BH1, BH2, and BH3 domains, permeabilize the outer mitochondrial

membrane when activated; the anti-apoptotic proteins, e.g., Bcl-2, Bcl-x_L and Mcl-1, which contain BH1, BH2, BH3 and BH4 domains, bind and neutralize activated Bax and Bak as well as other proteins that contain BH3 domains; and the pro-apoptotic BH3-only proteins, e.g., Bim, Bid and Bad, which contain only a BH3 domain, serve as sensors of various cellular stresses and trigger apoptosis by i) directly activating Bax and Bak or ii) displacing these proteins from anti-apoptotic neutralizers.

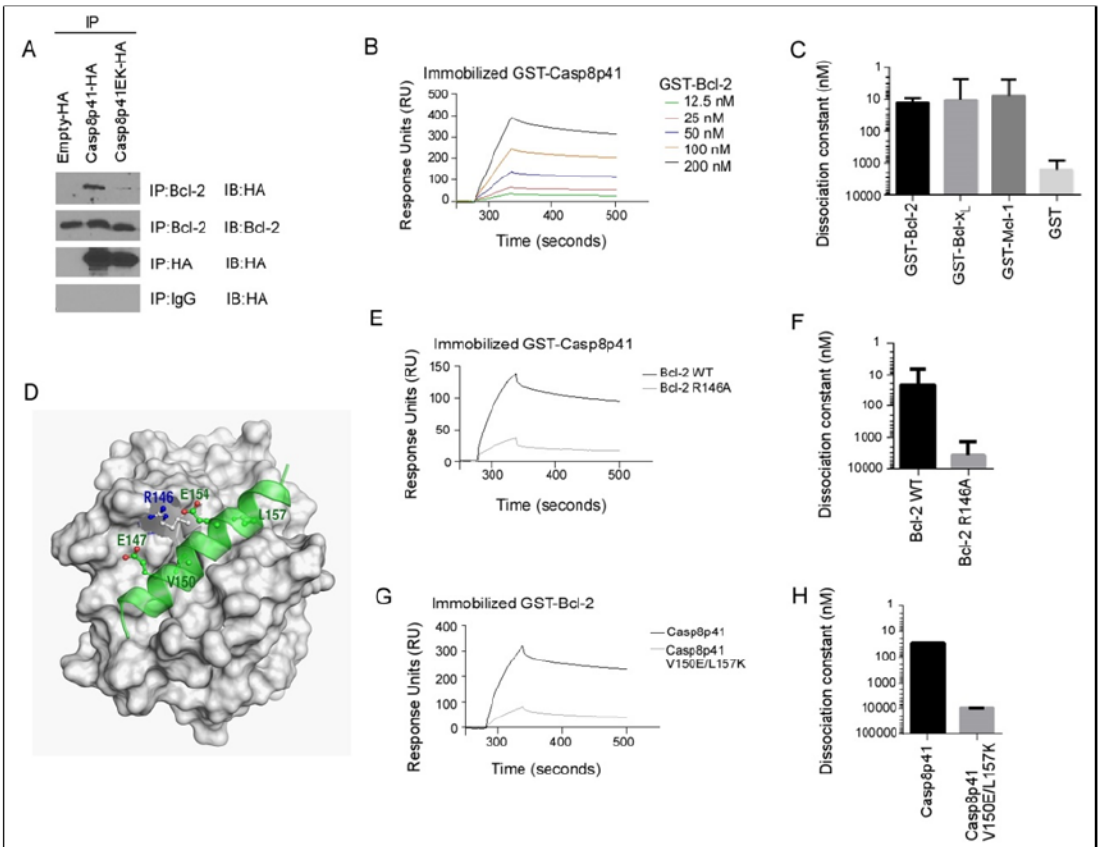


Figure 4. Casp8p41 binds Bcl-2 through interactions between a BH3-like domain unmasked by HIV protease cleavage of procaspase 8 and the BH3-binding groove of Bcl-2.

Because the α-helical BH3-like domain of Casp8p41 directly activates Bak by binding to the Bak BH3 binding groove (29), we investigated whether Casp8p41 also binds anti-apoptotic Bcl-2, Bcl-x_L and Mcl-1 through their BH3 binding grooves (38, 39). In initial experiments, lysates from 293 T cells transfected with empty vector, HA-Casp8p41, or HA-Casp8p41 V150E/L157K (Casp8p41-EK, a variant with decreased affinity for Bak due to disruption of critical binding interactions (29)) were immunoprecipitated for Bcl-2 and probed for associated HA tagged proteins. Immunoprecipitation of Bcl-2 demonstrated interaction with HA Casp8p41 (Figure 4A) that was reduced by the V150E/L157K substitutions. Surface plasmon resonance (SPR), a widely used technique to assess the affinity of protein:protein interactions, was used for more detailed study of Casp8p41 interactions with Bcl-2, Bcl-x_L, and Mcl-1, the anti-apoptotic Bcl-2 family members expressed in T cells. These experiments indicated equilibrium dissociation constants (K_ds) of 13 ± 4 nM for Casp8p41 binding to Bcl-2, 11 ± 8 nM to Bcl-x_L, and 8 nM ± 6 nM to Mcl-1 (Figure 4B and C), which are similar to affinities of the same proteins for BH3-only proteins such as Bim and Puma (8). We also confirmed that the peptide corresponding to the α□-helical BH3-like domain of Casp8p41 also bound Bcl-2, providing

evidence that the same Casp8p41 domain is responsible for binding both Bak and Bcl-2 (data not shown).

Using multiple low-mass molecular dynamic simulations (40, 41), we constructed a three-dimensional model of Casp8p41 bound in the BH3 binding groove of Bcl-2 (Figure 4D). This model predicts that Arg146 of Bcl-2 is critical for binding electrostatically with Glu147 and Glu154 of Casp8p41. Mutation of Bcl-2 Arg146 to Ala (42) decreased the affinity of Bcl-2 for the Casp8p41 activator peptide (Supplementary Figure 3) or full length Casp8p41 250-fold (Figure 3E and F), confirming that Casp8p41 is binding to the BH3 binding groove of Bcl-2. The model also suggests that Val150 and Leu157 of Casp8p41 contribute to binding in two hydrophobic regions of the Bcl-2 BH3 binding groove that are similar to the two hydrophobic holes of the Bak BH3 binding groove (43). Consistent with this prediction, the affinity of Casp8p41 for Bcl-2 was reduced over 300-fold by introduction of the Val150Glu/ Leu157Lys mutations in Casp8p41 (Figure 4G and H). **Thus, Casp8p41 favors cell death both by de-repressing Bak (by inhibiting Bcl-2, Bcl-x_L and Mcl-1) as well as by directly activating Bak (29).**

We propose a unifying model wherein activated HIV infected CD4 cells replicate virus, and HIV protease cleaves the abundantly available procaspase 8 to generate Casp8p41 which is minimally antagonized by the less prevalent Bcl-2; thus cell death ensues. Conversely HIV reactivation in T_{CM}, where less procaspase 8 is present, results in less Casp8p41 being produced, and what little Casp8p41 is produced is effectively antagonized by the abundant Bcl-2. However, the fate of Casp8p41 after it has been bound and inhibited by Bcl-2 remains unknown.

Within a cell, unneeded, misfolded, damaged and potentially toxic proteins are sometimes generated, in which case they are removed through the ubiquitin–proteasome pathway. This involves the reversible conjugation of the protein with ubiquitin chains, which target the protein to the proteasome for degradation. As proteasomal degradation has been shown to occur following neutralization of other pro-apoptotic proteins (e.g. when the pro-apoptotic NOXA is inhibited by the anti-apoptotic protein Mcl1 (44), the complex is degraded by the proteasome), we postulated that Casp8p41 bound and neutralized by Bcl2 might be ubiquitinated and degraded by the proteasome.

First, we sought to confirm that Casp8p41 is indeed ubiquitinated, by expressing HA-tagged Casp8p41 in 293T T cells. We did not detect ubiquitinated proteins in the Anti-HA pulldowns from control-transfected cells. By contrast anti HA pulldown from HA-Casp8p41-expressing cells revealed an abundance of ubiquitinated proteins, migrating at high molecular weights, corresponding to polyubiquitinated HA-Casp8p41 (Figure 5A).

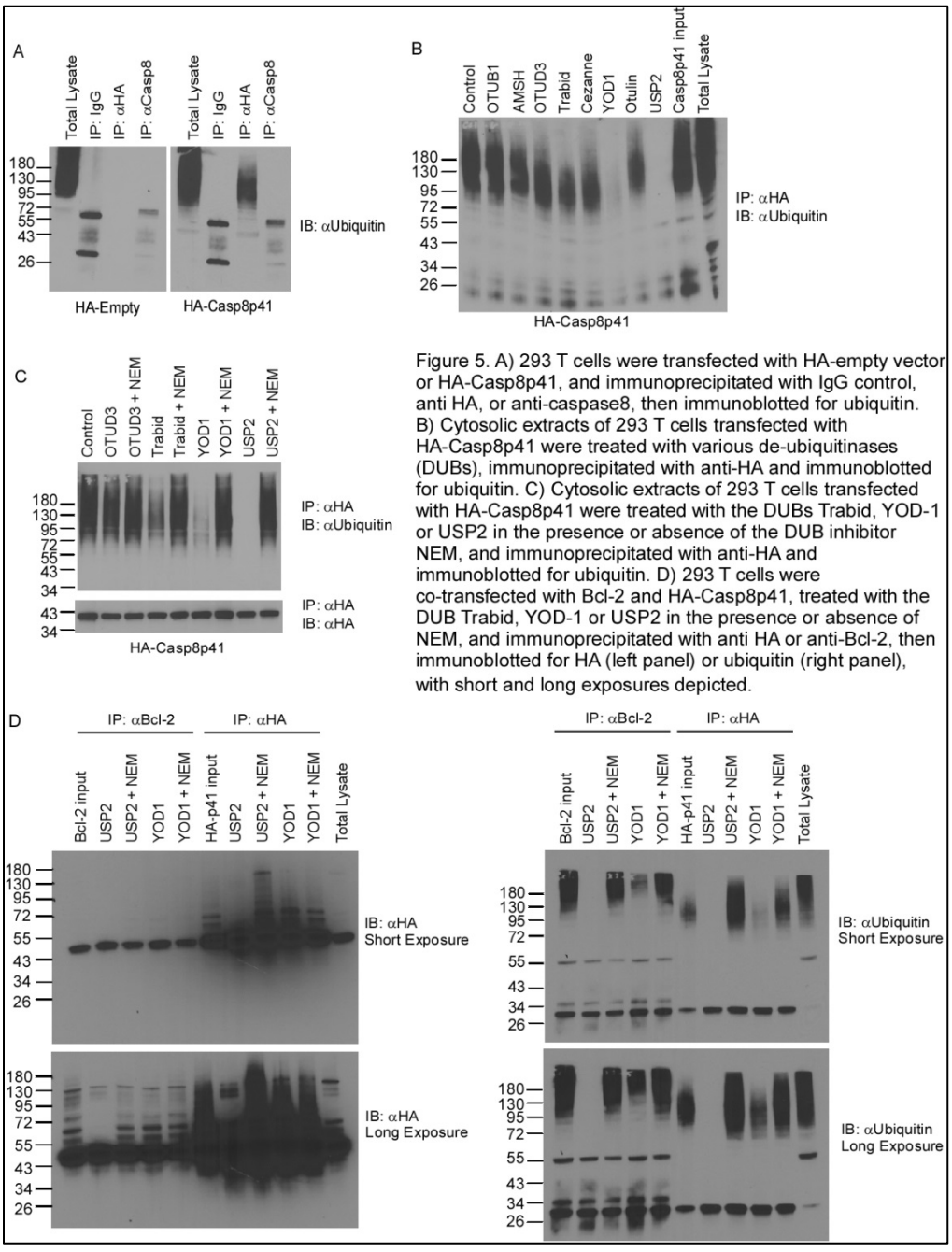


Figure 5. A) 293 T cells were transfected with HA-empty vector or HA-Casp8p41, and immunoprecipitated with IgG control, anti HA, or anti-caspase8, then immunoblotted for ubiquitin. B) Cytosolic extracts of 293 T cells transfected with HA-Casp8p41 were treated with various de-ubiquitinases (DUBs), immunoprecipitated with anti-HA and immunoblotted for ubiquitin. C) Cytosolic extracts of 293 T cells transfected with HA-Casp8p41 were treated with the DUBs Trabid, YOD-1 or USP2 in the presence or absence of the DUB inhibitor NEM, and immunoprecipitated with anti-HA and immunoblotted for ubiquitin. D) 293 T cells were co-transfected with Bcl-2 and HA-Casp8p41, treated with the DUB Trabid, YOD-1 or USP2 in the presence or absence of NEM, and immunoprecipitated with anti HA or anti-Bcl-2, then immunoblotted for HA (left panel) or ubiquitin (right panel), with short and long exposures depicted.

Covalent modification of proteins with ubiquitin can drive a number of outcomes ranging from degradative (in which the protein is tagged by ubiquitin chains at their Lys48 linkages resulting is proteasome degradation) to non-degradative (in which the protein is tagged by ubiquitin chains at their Lys63 linkages which results in modified protein:protein interactions and alteration of signal transduction pathways) (45). (46-50). To assess whether ubiquitinated Casp8p41 is targeted for degradation, we reversed this post-translational modification using specific de-ubiqitinating enzymes (DUBs), which distinguish between Lys48 and Lys63 ubiquitin linkages (51). The ubiquitination of HA-Casp8p41 was completely reversed by the Lys48-specific DUB USP2 and partially reversed by another Lys48-specific DUB YOD-1, but not other DUB's (52, 53), (Figure 5B), and this effect was abrogated by inhibiting DUB activity with the de-ubiquitinase inhibitor N-ethylmaleimide (NEM, Figure 5C) (54). This indicates that Casp8p41 is ubiquitinated in a manner that is consistent with it being targeted to the proteasome for degradation.

To confirm that Casp8p41 complexed with Bcl2 is targeted for ubiquitination and degradation, we co-expressed HA-Casp8p41 and Bcl2 in 293T cells, and performed reciprocal immunoprecipitation (IP). IP of Bcl2 and blotting for HA confirmed the association of Bcl2 with HA-Casp8p41 (Fig 5D, left panel) as a band at the expected size of ~43kDa. However, a longer exposure revealed additional HA immunoreactive bands that were detected in the IP of Bcl2 that were effectively absent in cells treated with the DUB USP2, and weakened relative to the control in cells treated with YOD1, suggesting that these bands were ubiquitinated forms of Casp8p41 in complex with Bcl2. The effects of YOD1 and USP2 were reversed by treatment with NEM, as expected. These results were confirmed by ubiquitin blotting of the immunoprecipitates (Fig 5D, right panel). Both Bcl2 and HA IP brought down ubiquitinated proteins, which completely disappeared following treatment with the DUB USP2 and partially disappeared with YOD1, and the de-ubiquitination was reversed by NEM. These data suggest that the Casp8p41–Bcl2 complex is ubiquitinated and targeted to the proteasome for degradation.

Proteasome inhibition increases Casp8p41 expression levels.

Altering the activity of the proteasome impacts a variety of biologic activities, and this characteristic has been successfully exploited with the clinical development of proteasome inhibitors. For example multiple myeloma is a disease in which plasma cells produce too much immunoglobulin protein, and blocking the ubiquitin mediated degradation of these immunoglobulin proteins using proteasome inhibitors results in the intracellular accumulation of immunoglobulin, and cell death through activation of the unfolded protein response.(55).

Based on our data above, we predict that treatment of Casp8p41 expressing cells with proteasome inhibitors will increase expression levels of Casp8p41, and since Casp8p41 is only physiologically expressed in HIV infected cells, that increase should facilitate killing of the HIV infected cells. We therefore tested whether the currently used (and approved) proteasome

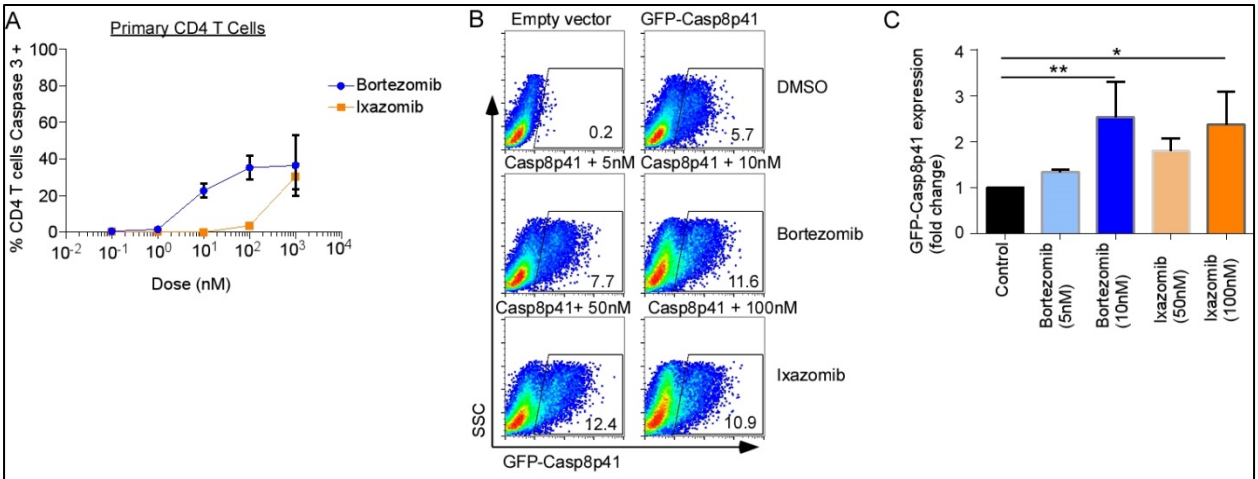


Figure 6. Proteosome inhibition increases Casp8p41 levels at drug concentrations that exhibit minimal toxicity. A) Uninfected primary CD4+ T cells were treated with bortezomib or ixazomib at the indicated concentrations for 48 hours, and cell death by apoptosis was assessed by intracellular active caspase 3 staining. Depicted is the mean (SD) of two experiments. B,C) Jurkat CD4+ T cells were transfected with empty vector or GFP-Casp8p41 then treated with control (DMSO), bortezomib or ixazomib, and GFP expression analyzed 6 hours later. C) Mean (SD) data from three independent experiments as depicted in (B). . * P<0.05; ** P<0.01.

inhibitor bortezomib (which is widely used clinically for the treatment of hematologic malignancies) and ixazomib (which is an orally bioavailable proteasome inhibitor recently approved by FDA for the treatment of refractory multiple myeloma) would also block the degradation of Casp8p41 following ectopic expression. We first performed dose-ranging toxicity

studies of these agents on primary CD4+ T cells from healthy donors. As ixazomib is administered as a citrate ester prodrug (ixazomib citrate) that is hydrolyzed under physiological conditions to its biologically active form, ixazomib, in vivo, we treated cells with ixazomib (MLN2238; hereafter referred to as ixazomib). In these experiments, toxicity as determined by active Caspase 3 staining was negligible at doses below 10nM for Bortezomib, and toxicity of Ixazomib was negligible at doses below 100nM (Figure 6A). Consequently, we used bortezomib at 5 and 10 nM and Ixazomib at 50 and 100nM for the remaining experiments. These doses also closely reflect peak plasma levels seen in patients (56, 57).

We next treated Jurkat CD4+ T cells with either bortezomib or ixazomib overnight, transfected them with GFP-tagged Casp8p41, and analyzed GFP expression 6 hours later. Consistent with our expectations, pretreatment of cells with either bortezomib or with ixazomib significantly increased the proportion of cells expressing GFP-tagged Casp8p41 – 10nM bortezomib resulted in a 2.5-fold increase in Casp8p41 relative to control cells ($p=0.009$), and 100nM ixazomib resulted in a 2.4-fold increase ($p=0.045$; Figure 6B,C).

Proteasome inhibitors independently decrease HDAC, activate NF-κB, and induce HIV reactivation.

In treating J-Lat-10.6 cells with bortezomib or ixazomib we observed enhanced expression of GFP by J-Lat-10.6 cells over time, suggesting that these agents increase HIV replication (Figure 7A), consistent with a prior report suggesting that bortezomib activates latent provirus yet paradoxically decrease HIV replication(58). Since HIV reactivation is necessary for production of Casp8p41, and is also a preferred approach to effecting HIV cure strategies, this finding is of great potential relevance, and therefore we further assessed that possibility. Treatment of J Lat 10.6 cells with ixazomib/bortezomib was accompanied by an increase in the levels of HIV p55 gag protein (Figure 7B), which was detectable 48 hours after treatment. Next we sought to determine the mechanism underlying this reactivation.

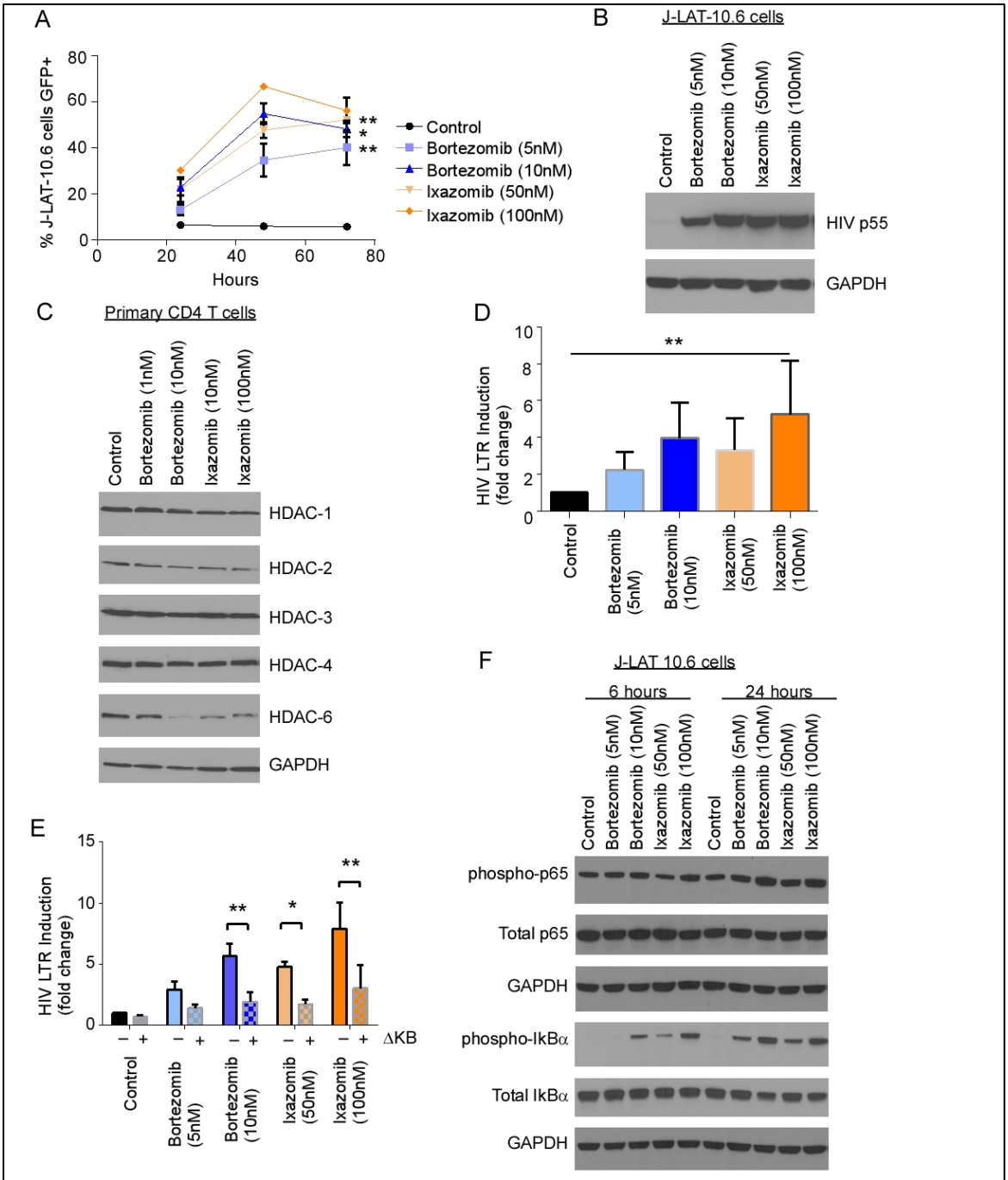


Figure 7. A) J-Lat 10.6 cells treated as shown were assessed for GFP expression. B) J-Lat 10.6 cells treated as in (A) were assessed for HIV p55 Gag. C) Uninfected primary CD4+ T cells treated as shown for 48 hours were assessed for expression of HDAC isoforms. D) Jurkat cells transfected with HIV-LTR firefly luciferase were treated as shown for 48 hours, and assessed for luciferase activity, normalized to Renilla. E) Jurkat cells transfected with HIV-LTR luciferase +/- deletions in the NF-KB-binding site were treated as shown and assessed for luciferase activity. F) J-Lat 10.6 cells were treated as indicated, and expression of NF-kB p65, Ser536 phospho-NF-kB, IkBalpa, and Ser32 phospho-IkBalpha assessed. * P<0.05; ** P<0.01.

Histone deacetylases are critical in inducing and maintaining HIV latency, and HDAC inhibitors are potent inducers of HIV reactivation (59) (60), (61) (62) . Since Bortezomib functions as a de facto HDAC inhibitor by transcriptional repression of class 1 HDAC

expression in myeloma cells (63) (64), we assessed whether treatment of primary CD4 T cells with bortezomib or Ixazomib also altered expression of different HDAC. Reproducibly, over 48 hours of treatment, HDAC 2 and HDAC 6 were reduced by treatment with either Bortezomib or Ixazomib (Figure 7C). Thus proteasome inhibitor mediated downregulation of these HDAC might contribute to enhanced HIV replication.

Bortezomib also directly activates the canonical NF-κB axis in multiple myeloma cells (65), raising the possibility that bortezomib and Ixazomib do similarly in other cell types including CD4 T cells. We therefore evaluated whether bortezomib or ixazomib affects the NFKB mediated HIV LTR activation in CD4+ T cells. Jurkat CD4+ T cells were transfected with luciferase reporter constructs in which either the HIV LTR was linked to luciferase (HIV-luc) or the HIV LTR missing the NF-κB-binding sites was linked to luciferase (HIVdeltaκB-Luc), as previously described (66). Treatment with bortezomib or ixazomib resulted in a dose-dependent increase in luciferase expression (normalized to Renilla in cells containing the HIV-Luc reporter construct), demonstrating a direct effect on HIV LTR-mediated replication in cells containing HIV-luc (Figure 7D). However, as expected, this effect was abrogated in the cells containing HIVdeltaκB-Luc reporter, indicating that proteasome activation of the HIV LTR is mediated through NF-κB (Figure 7E). Internally consistent with these findings the negative repressor of NFKB, IκB, was inactivated (through Ser32 phosphorylation(67).) and the p65 subunit of NF-κB was activated (through Ser536 phosphorylation) within 6 hours of treating J-Lat-10.6 cells with bortezomib or ixazomib, (Figure 7F)

Proteasome inhibitors preferentially kill HIV-infected T cells

As we have established that bortezomib and ixazomib independently induce HIV reactivation – one of the key requirements for the prime shock and kill model of HIV eradication (68) – we next assessed whether these agents also favor the death of the HIV-positive cells. We treated J-Lat-10.6 cells or parental Jurkat CD4+ T cells separately with vehicle control, bortezomib or ixazomib, and measured caspase 3 expression over time by flow cytometry. Both bortezomib and ixazomib increased caspase 3 expression in J-Lat-10.6 cells in a dose- and time-dependent manner compared with control-treated J-Lat-10.6 cells (Figure 8A and B) without significant toxicity to uninfected Jurkats at the same doses.

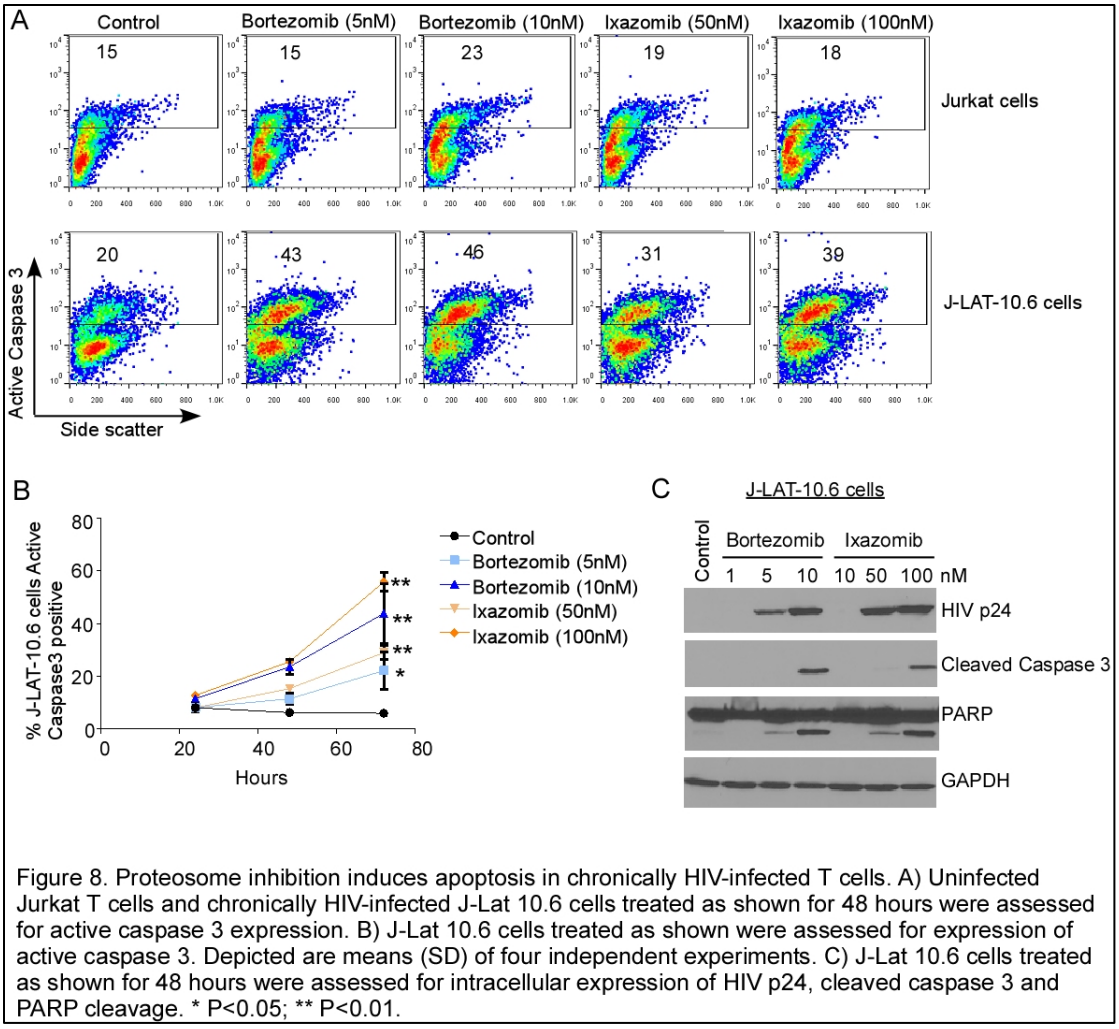


Figure 8. Proteosome inhibition induces apoptosis in chronically HIV-infected T cells. A) Uninfected Jurkat T cells and chronically HIV-infected J-Lat 10.6 cells treated as shown for 48 hours were assessed for active caspase 3 expression. B) J-Lat 10.6 cells treated as shown were assessed for expression of active caspase 3. Depicted are means (SD) of four independent experiments. C) J-Lat 10.6 cells treated as shown for 48 hours were assessed for intracellular expression of HIV p24, cleaved caspase 3 and PARP cleavage. * P<0.05; ** P<0.01.

Our model posits that HIV reactivation induced by bortezomib or ixazomib initiates a mitochondrial death pathway ultimately causing activation of caspase 3, cleavage of structural and regulatory proteins including PARP and cell death. Therefore, we performed western blot analyses of treated J-Lat-10.6 cells to assess HIV reactivation (as measured by p24 expression), and cleavage of both caspase 3 and PARP (Figure 8C). Consistent with our previous data (Figure 7A and B), treatment with bortezomib or ixazomib resulted in reactivation of HIV as evidenced by intracellular p24 expression in a dose dependent manner (Figure 8C, top panel). Consistent with the flow cytometry data (Figure 8A), treatment with bortezomib or ixazomib resulted in expression of cleaved Caspase 3 (Figure 8C, second panel), with associated cleavage of PARP (Figure 8C, third panel), indicated the cells were undergoing apoptosis.

Any therapy which kills HIV infected cells will need to kill infected cells preferentially over uninfected cells. Our data indicate that proteasome inhibitors kill HIV-infected cells by promoting HIV reactivation and prolonging the half-life of Casp8p41 (which is only present in HIV-infected cells) and so should preferentially target HIV-infected cells. Thus, we assessed whether cell death occurs preferentially in latently infected J-Lat-10.6 cells over uninfected Jurkat T cells. After confirming that both cell lines have a similar propensity to undergo apoptosis (Figure 9A and 9B), we performed clonotypic assays to assess which cells survive

following coincubation of the cells with bortezomib or ixazomib. To distinguish the phenotypically indistinguishable cell types, we first labelled chronically HIV-infected J-Lat cells with the lipophilic dye CellTracker Orange (CTO), and mixed these cells 50:50 with uninfected, unlabeled Jurkat T cells. These cell mixtures were then treated with vehicle control, bortezomib or ixazomib. Conceptually, if treatments cause non-specific killing, then the proportion of labelled CTO+ J-Lat cells should remain at ~50 percent over time, whereas if killing is selective for one cell type, then the proportion of labelled CTO+ J-Lat cells should change accordingly. Strikingly, at all doses tested, bortezomib and ixazomib resulted in a decrease in the proportion of CTO-positive J-Lat-10.6 cells (Figure 9C), indicating that the HIV-infected cells are selectively killed. Indeed, by 48 hours after treatment only approximately 25% of remaining live cells were chronically HIV-infected CTO+ J-Lat cells.

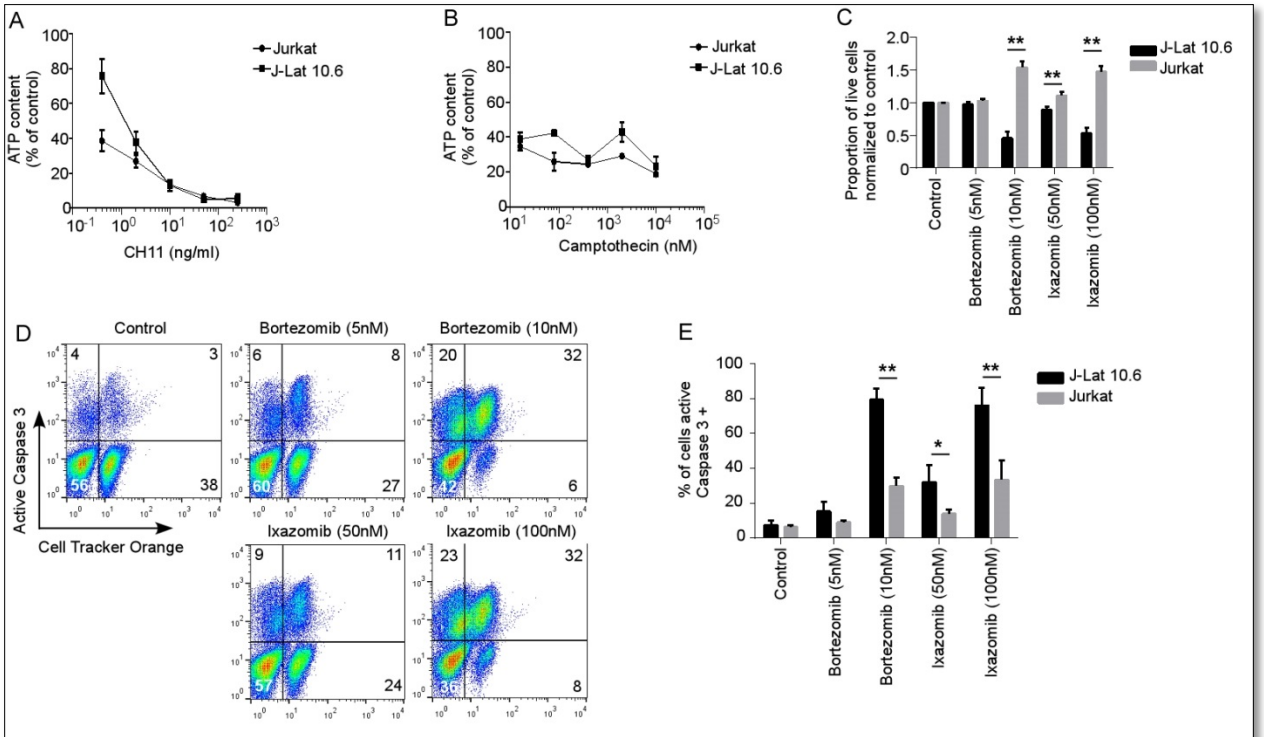


Figure 9. Proteasome inhibition preferentially kills HIV-infected as compared to uninfected T cells. A) Jurkat T cells (circles) and J-Lat 10.6 cells (squares) treated with proapoptotic anti-Fas antibody clone CH11 were assessed for cell survival by intracellular ATP content normalized to untreated control. (B) Jurkat T cells (circles) and J-Lat 10.6 cells (squares) were treated with proapoptotic chemotherapeutic agent Camptothecin and assessed for cell survival C) J-Lat 10.6 cells were labeled with the lipophilic dye Cell Tracker Orange (CTO) to distinguish them from unlabeled Jurkat T cells, and the two cell types were mixed at a 1:1 ratio. The HIV entry inhibitor T20 was added to the mixture of cells to prevent spreading infection, and then cells were treated as shown. Cells were analyzed for active caspase 3 and for expression of the CTO label. Depicted is the proportion of live (caspase 3-negative) cells normalized to control treated cells, by cell type. D) Mixed Jurkat and J-Lat 10.6 cells treated as in (C) were assessed for caspase 3 expression. E) Figure depicts the means (SD) of three independent experiments as in (D).* P<0.05; ** P<0.01.

Likewise, in the presence of 10nM bortezomib, 79% of J-Lat-10.6 and 30% of Jurkat cells were stained for caspase 3 ($P<0.0001$), while in the presence of 100nM ixazomib 76% of J-Lat-10.6 and 33% of Jurkat cells were positive for caspase 3 ($P=0.001$) (Figure 9D and 9E). Taken together, in this in vitro system, HIV-infected cells die preferentially in response to bortezomib or ixazomib treatment.

Casp8p41 is expressed in central memory T cells which reactivate HIV, is increased by proteasome inhibitors, resulting in killing of the reactivating cells.

Taken together our cumulative results indicate that proteasome inhibitors reactivate HIV in vitro, and preferentially induce the death of HIV infected cells over uninfected cells. Therefore we next assessed the effects of proteasome inhibitors in cells from HIV infected patients, treated ex vivo with proteasome inhibitors. In these cells however, it is not possible to identify which cells are latently infected and which ones are not. Therefore, it is impossible to compare rates of cell death in latently HIV-infected cells with death in cells which are not latently infected. Instead, it is only possible to make this comparison by: (i) assessing death in cells which begin to express HIV specific markers such as p24 after reactivation treatment (which would suggest that they are latently infected); or (ii) assess the amount of cell-associated HIV DNA pre versus post treatment as these values normally do not change over a several day treatment window (69)).

First, we looked for Casp8p41 expression in latently infected cells that had been induced to reactivate HIV. Using primary CD4+ T cells from two ART-suppressed, HIV-infected patients, we induced reactivation using αCD3/αCD28, and assessed for intracellular Casp8p41, using our previously validated neo-epitope-specific Casp8p41 antibody (70, 71). Consistent with our previous findings, reactivated cells contained Casp8p41 at detectable levels, and the frequency of Casp8p41 positivity was reflective of the relative frequency of HIV latency in each cell subset (Figure 10A); for instance, up to 1% of sorted resting memory CD4 T cells have detectable levels of HIV Gag DNA (72), and up to 2% of central memory CD4 T cells are positive for p24, even in the setting of ART (73).

Next, we wanted to test whether the reactivating cells were in fact the cells that died in response to treatment. We treated cells from two ART-suppressed HIV-infected patients with bortezomib or ixazomib in the presence of anti-retroviral drugs to prevent spreading infection, and analyzed cells for the co-expression of caspase 3 (as a marker of apoptosis) and p24 (as a marker of reactivation) (Figure 10B and 10C). In cells that stained positive for p24, low and high dose bortezomib increased the proportion of caspase 3-positive cells from 17% (control) to 24% (5 nM bortezomib) and 60% (10 nM bortezomib); however bortezomib also increased the proportion of HIV p24 positive cells that were caspase 3 positive, suggesting nonspecific toxicity, consistent with bortezomib causing leukopenia in a significant proportion of patients (74). In the case of ixazomib, treatment with 100 nM of the drug resulted in a significant increase in the proportion of caspase 3-expressing p24-positive cells (27% vs 17% in controls, $p=0.023$), while not significantly increasing the proportion of p24 negative cells that stain for active caspase 3, suggesting limited nonspecific toxicity, and consistent with the recent phase three trial data using ixazomib in myeloma patients, in which the rate of all side effects including leukopenia were not different between placebo and Ixazomib treatment arms. (Figure 10B and C).

There is considerable debate about which is the best technique available to estimate HIV reservoir size (75). Highly sensitive nucleic acid amplification techniques have been developed which can accurately detect low copy number HIV genes with a very low coefficient of variance, such a digital droplet PCR (ddPCR) (76). However, such approaches cannot distinguish replication-competent from replication-incompetent (defective) virus (77), the latter being estimated to represent ~80 % of detectable viral sequences. On the other hand, functional assays such as the quantitative viral outgrowth assay (QVOA) are specific for replication-competent virus, but they underestimate the size of the latent pool (69, 78, 79), and are infrequently positive in cells from ART-suppressed patients. Thus, we opted to estimate HIV reservoir size ex vivo using the more sensitive approach – ddPCR – with the understanding that any reduction in HIV DNA would confer at least a similar degree of reduction in replication competent virus.

In our first series of experiments, we established and validated ddPCR, as follows. Cell-associated HIV polymerase copies were measured repeatedly over 10 different runs in positive control samples containing a fixed ratio of uninfected Jurkat T cells and chronically HIV-infected 8E5 T cells, which contain one integrated HIV provirus per cell. We were able to obtain a coefficient of variance of ddPCR of 3.8% (Figure 10D), thereby enabling us to interpret changes of greater that 3.8% as being greater than the noise of the assay. Next, 37 HIV-negative samples were assayed for cell-associated HIV polymerase, establishing a detection limit of 45 HIV DNA copies per million cells (Figure 10E).

Next we assessed changes in cell-associated HIV DNA in primary CD4+ T cells from ART-suppressed HIV patients, following maximal HIV reactivation by αCD3/αCD28 in the presence or absence of bortezomib or ixazomib. First, we isolated CD4+ T cells from cryopreserved leukapheresis PBMC samples from 6 individual ART-suppressed HIV patients, and pre-treated with bortezomib or ixazomib for 24 hours prior to viral reactivation with αCD3/CD28 stimulation. Total cell-associated HIV DNA was measured by ddPCR 72 hours after reactivation. Treatment with bortezomib followed by reactivation did not reduce HIV DNA in the leukapheresis samples (Figure 10F left panel), whereas ixazomib followed by viral reactivation resulted in a geometric mean 31% reduction (P=0.06) in HIV DNA when compared with reactivation alone (Figure 10F right panel). We next treated freshly obtained primary CD4 T cells from 12 ART suppressed patients with ixazomib or vehicle control prior to reactivation with αCD3/28 as above. Cells from 8 of 12 patients treated with ixazomib had a decrease in cell associated HIV DNA of ≥10% compared to control, and were considered treatment responders. In these responders (N=8), ixazomib reduced cell associated HIV DNA by a geometric mean of 44% (P=0.002) compared to control treated cells (Figure 10G). We next focused on whether treatment of cells from an additional 11 HIV-infected, ART-suppressed patients with ixazomib alone would be sufficient to both reactivate HIV and prime the cells towards an apoptosis-prone phenotype, so as to result in a reduction in cell-associated HIV DNA content when used as monotherapy, in the setting of continued ART. Treatment of CD4+ T cells with ixazomib (100nM) alone for 96 hours without additional reactivation stimuli reduced cell associated HIV DNA by a geometric mean 37% compared to vehicle treated cells (P=0.02, data not shown).

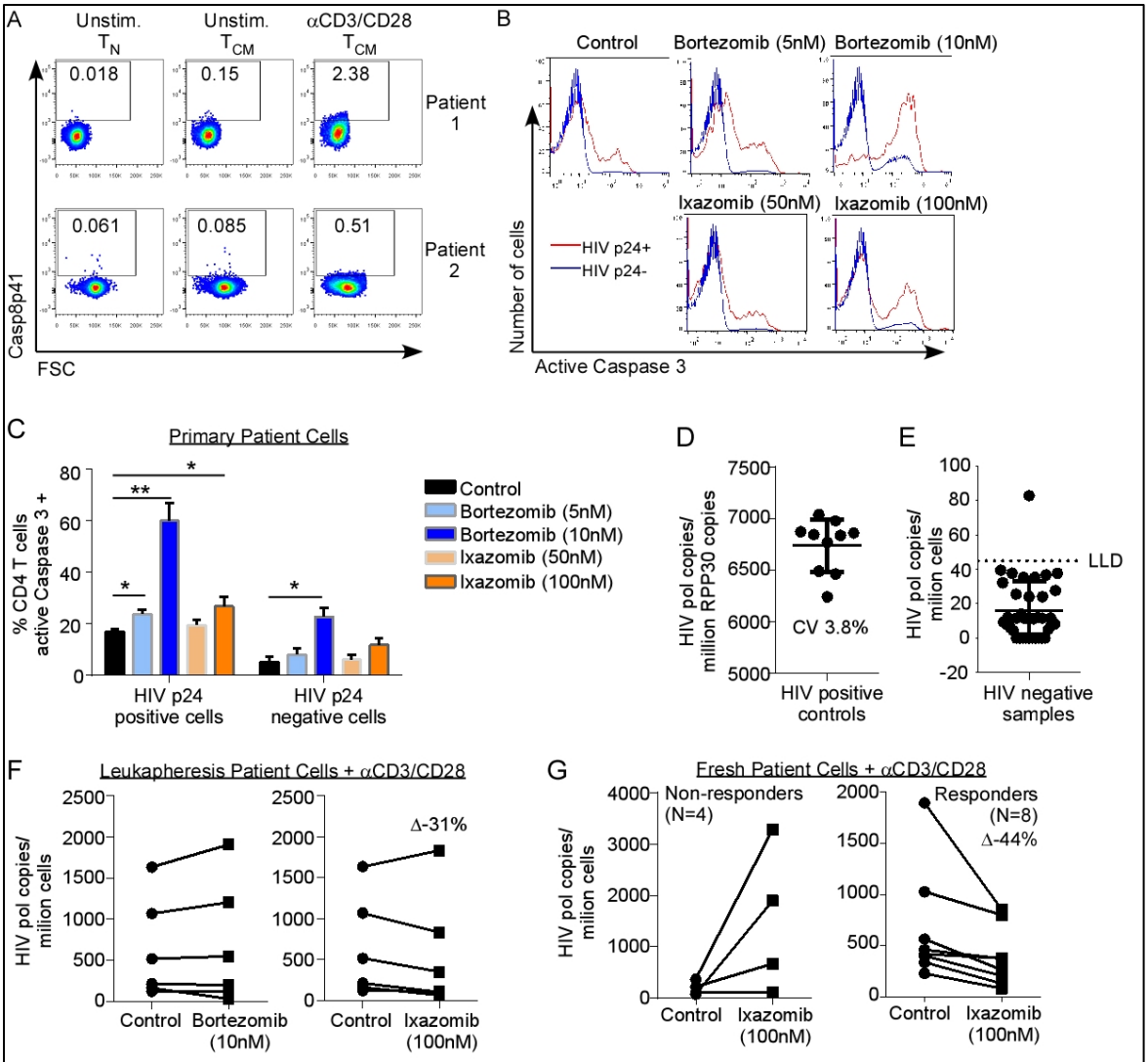
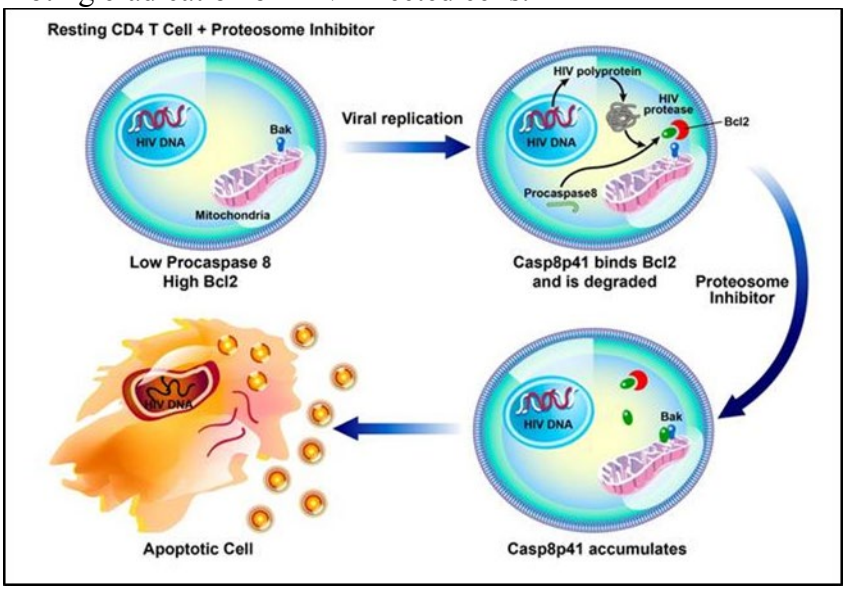


Figure 10. A) Intracellular Casp8p41 expression was assessed in central memory CD4+ T cells +/- reactivation with αCD3/CD28 stimulation. B) Primary CD4+ T cells from HIV-infected, ART-suppressed patients treated as shown for 48 hours were assessed for intracellular expression of HIV p24 and active Caspase 3. C) Mean (SD) of 3 independent experiments as in (B). D) Intrasample variability of ddPCR was assessed and coefficient of variance calculated by measurement of HIV pol DNA copies per million RPP30 (host gene) copies in 10 serial measurements of a 1:100 dilution of chronically infected 8E5 T cells mixed with uninfected Jurkat cells. E) The lower limit of detection of the ddPCR assay for HIV DNA was assessed by measurement of 37 HIV negative samples. The cut-off for positivity was determined to be 45 HIV polymerase copies/million cells. (F) CD4+ T cells obtained from frozen leukapheresis samples of HIV infected ART suppressed patients were treated as shown, with ART (T20, EFV, RAL) to prevent spreading infection. 72 hours after reactivation cells were harvested, DNA extracted and copies of HIV DNA per million cells determined by ddPCR as in D & E. (G) CD4+ T cells obtained from freshly collected samples of HIV-infected, ART-suppressed patients were treated as shown, with ART to prevent spreading infection. 72 hours after reactivation copies of HIV DNA per million cells was determined by ddPCR.

Summary of Hypothesis

Current ART effectively reduces HIV replication (measured by HIV RNA levels) to undetectable levels within several months of starting therapy. Thereafter HIV persists primarily as integrated HIV DNA within central memory CD4 T cells. The half-life of these latently HIV infected CD4 T cells is ~44 months(80), meaning that ~70 years of ARV suppression would be required for eradication of all latently infected cells. To date, no therapy has been identified that reduces HIV DNA levels from latently infected cells. For the reasons outlined above, and supported by the accompanying preliminary data, we propose that using proteasome inhibitors will stabilize Casp8p41 in cells which reactivate HIV, thereby causing apoptosis of those cells, and promoting eradication of HIV infected cells.



Choice of Bortezomib versus Ixazomib

Dr Anthony Fauci, Director of the National Institute of Allergy and Infectious Diseases (NIAID) is a long time champion of HIV cure. Due to the global nature of HIV, and the fact that persons infected with HIV do not always have access to tertiary medical care, he espouses that any curative regimen must be effective, simple, safe, and scalable (81) . Thus concerning the choice between bortezomib and ixazomib, both of which show activity in vitro for HIV eradication, it would be preferable from the standpoint of simplicity and scalability to use ixazomib as it is oral, and has permissible storage condition excursion range is 8°C to 40°C for a maximum period of 30 days Moreover since HIV infection and older HIV treatments are commonly associated with peripheral neuropathy (PN), many HIV infected patients are either predisposed to PN, or have ongoing PN, thus as ixazomib is associated with less peripheral neuropathy than bortezomib, it will be preferable to use ixazomib. Ixazomib is also associated with better overall volume of distribution given its faster dissociation and greater tissue penetration. This would be of great relevance in terms of its ability to get into lymph nodes, which are critical sites of HIV persistence and replication. Finally the use of oral ixazomib would be more acceptable compared to IV bortezomib, from a patient perspective as well. Finally the superior toxicity profile of ixazomib compared to bortezomib (82) would be favorable for this patient population.

1.2 Investigational Agent

Ixazomib, which has been formulated for both intravenous (IV) and oral (PO) administration, is a small molecule proteasome inhibitor. The proteasome is a large protein complex that degrades ubiquitinated proteins via the ubiquitin-proteasome pathway (UPP), which is responsible for the degradation of the majority of intracellular proteins. Due to the accumulation of many different proteasome substrates, proteasome inhibition affects multiple signaling cascades within cells, resulting in downstream effects including antitumor activity, promotion of apoptosis, and antiangiogenic and antiproliferative activities. These consequences of proteasome inhibition are of particular importance in plasma cells and multiple myeloma cells, which produce high levels of secreted Ig proteins. Ixazomib is a small molecule proteasome inhibitor.

Ixazomib citrate is the citrate ester of the biologically active form, ixazomib (MLN2238), a modified dipeptide boronic acid with structural differences from bortezomib. In water or aqueous systems, ixazomib citrate rapidly hydrolyzes to ixazomib (MLN2238), therefore all doses and concentrations are expressed as ixazomib (MLN2238). Nonclinical studies were conducted with a solution of either ixazomib (MLN2238) or ixazomib (MLN2238) in equilibrium with ixazomib citrate. Similar to bortezomib, ixazomib (MLN2238) potently, reversibly, and selectively inhibits the 20S proteasome. However in contrast to bortezomib, it has a shorter dissociation half-life (t1/2) that may contribute to increased tissue distribution. Bortezomib has a slowly reversible dissociation rate from the red blood cell proteasome, while ixazomib (MLN2238) demonstrates a more rapidly reversible dissociation rate from the blood but sustained effects on bone marrow and tumor proteasomes suggesting better tissue distribution. The pharmacologic implications of this difference in binding kinetics and tissue distribution may in turn result in differences in safety and efficacy profiles in a broader range of tumors. In xenograft-bearing mice, the more rapid dissociation rate correlates with an increased ratio of tumor proteasome inhibition to blood proteasome inhibition, and ixazomib shows greater antitumor activity in several xenograft models, both solid tumor and bortezomib-resistant xenografts, than bortezomib.

In Vitro Pharmacology: MLN2238 preferentially binds the β5 site of the 20S proteasome; at higher concentrations, it also inhibits the activity of the β1 and β2 sites. MLN2238 inhibits ß5 site 20S proteasome activity in vitro, with a half-maximal inhibitory concentration (IC50) of 3.4 nM. Potency is reduced roughly 10-fold versus ß1 (IC50 31 nM) and 1,000-fold versus ß2 (IC50 =3500 nM). MLN2238 was also tested for inhibition against a panel of 103 kinases, 18 receptors (neurotransmitter, ion channel, brain and gut receptors), and 9 serine proteases. In all cases, the IC50 values were > 10 μM. MLN2238 and bortezomib have different β5 proteasome dissociation half-lives (t1/2), reflecting differences in their on-off binding kinetics (the β5 proteasome dissociation t1/2 for MLN2238 and bortezomib are 18 and 110 minutes, respectively). Based on these favorable characteristics, ixazomib is anticipated to be effective against multiple myeloma. (ixazomib Investigator's Brochure (IB).
Proteasome inhibition results in the accumulation of poly-ubiquitinated substrates within the cell and leads to cell cycle disruption, with concomitant activation of apoptotic pathways and cell death. Consistent with inhibition of ß5 20S activity, MLN2238 demonstrated potent activity against cultured MDA-MB 231 human breast cancer cells in the WST cell viability assay. In nonclinical models MLN2238 has activity against both solid tumor and bortezomib-resistant xenografts

In Vivo Pharmacology: To determine the activity of MLN2238 in vivo, pharmacodynamic studies were performed in immunocompromised mice bearing either CWR22 human prostate or WSU-DLCL2 (human diffuse large B-cell lymphoma [DLBCL]) tumors. Pharmacodynamic responses in xenograft tumors were analyzed by assessing 20S proteasome inhibition and by evaluating levels of accumulated protein markers such as deoxyribonucleic acid (DNA) damage-inducible protein 34 (GADD34) and activating transcription factor-3 (ATF-3) as well as measuring growth arrest. Increased expression of GADD34 and ATF-3 is indicative of a downstream biological response to proteasome inhibition. After a single dose of MLN2238, a clear dose response was observed in CWR22 xenografts as seen in both tumor 20S proteasome inhibition and in changes in GADD34 and ATF-3 expression. In WSU-DLCL2 xenografts, greater tumor proteasome inhibition was observed with MLN2238 compared to bortezomib and resulted in increased expression of GADD34 and ATF-3.

MLN2238 efficacy experiments demonstrated strong antitumor activity in 4 xenograft models: CWR22 (a human prostate cancer cell line) and 3 human lymphoma cell lines (WSU-DLCL2, OCI-Ly7-7D1-luc, and PHTX-22L). In the case of the CWR22 xenograft model, significant antitumor activity was seen with both IV and PO dosing, demonstrating that this molecule has antitumor activity when administered via different dosing routes. In all 3 lymphoma lines, MLN2238 demonstrated stronger antitumor activity than did bortezomib.

In summary, MLN2238, similar to bortezomib, is a dipeptide boronic acid proteasome inhibitor that potently, reversibly, and selectively inhibits the proteasome. There are several features, such as sustained pharmacodynamic effects and activity in a bortezomib-refractory lymphoma xenograft model, that suggest that it may have activity that extends beyond that seen with bortezomib.

Nonclinical Pharmacokinetics and Pharmacodynamics: The pharmacokinetic (PK) properties of MLN2238 were studied in severe combined immunodeficient (SCID) mice bearing human CWR22 tumor xenografts, Sprague-Dawley rats, beagle dogs, and cynomolgus monkeys. Because of the extensive red blood cell (RBC) partitioning of MLN2238, both blood and plasma PK parameters were determined in these studies. MLN2238 had a very low blood clearance (CLb) and a moderate blood volume of distribution at steady-state (Vss,b) after IV administration. The concentration-versus-time curve of MLN2238 displayed a distinct bi-exponential profile with a steep initial distribution phase and a long terminal t1/2 (> 24 hr) in all species tested. MLN2238 had higher plasma clearance (CLp) and a larger plasma volume of distribution at steady-state (Vss,p) than in blood, largely because of the extensive RBC partitioning.

The PK properties of MLN2238 after oral administration were studied in rats and dogs. The plasma oral bioavailability (F) was 41% in rats and nearly 100% in dogs. A clinical prototype formulation of the ixazomib capsule demonstrated that MLN2238 had excellent oral F and an excellent absorption profile in dogs. In addition, interindividual variability, as measured by %CV, in Cmax and AUC0-24hr after oral administration was low to moderate, similar to that after IV administration. The terminal t1/2 after oral administration was also similar to that after IV administration. Comparison of the PK profiles after IV or PO administration in the dog is reported in further detail in the IB.

MLN2238 is predicted to have very low CLb (0.0045 L/hr/kg) and a moderate Vss,b (0.79 L/kg) with a long terminal t1/2 (> 24 hours) in humans. The human efficacious IV dose of MLN2238 is predicted to be 2.0 mg/m2 (0.054 mg/kg) twice weekly.

The human efficacious oral dose is predicted to be between 2 and 5 mg/m2 twice weekly, based on a predicted oral F of between 41% (as seen in rats) and 100% (as seen in dogs). The efficacious dose projection for once weekly oral would be higher than twice weekly oral (data not provided).

Metabolism appears to be a major route of elimination for MLN2238 and urinary excretion of the parent drug was negligible (< 5% of dose). In vitro in liver microsomes, the metabolism of MLN2238 was high in mice and low to moderate in all other species studied. Metabolism by multiple CYP enzymes and non-CYP proteins is expected to be the major clearance mechanism for ixazomib (MLN2238). At clinically relevant ixazomib concentrations, in vitro studies using human cDNA-expressed cytochrome P450 isozymes showed that no specific CYP isozyme predominantly contributes to ixazomib metabolism. At higher than clinical concentrations, ixazomib was metabolized by multiple CYP isoforms with estimated relative contributions of 3A4 (42%), 1A2 (26%), 2B6 (16%), 2C8 (6%), 2D6 (5%), 2C19 (5%) and 2C9 (<1%).

Ixazomib (MLN2238) is neither a reversible nor a time-dependent inhibitor of CYPs 1A2, 2B6, 2C8, 2C9, 2C19, 2D6, or 3A4/5. Ixazomib did not induce CYP1A2, CYP2B6, and CYP3A4/5 activity or corresponding immunoreactive protein levels. Ixazomib is not expected to produce drug-drug interactions via CYP inhibition or induction.

In a Caco-2 cell assay, MLN2238 showed medium permeability with a B-to-A/A-to-B permeability ratio of 2.9. MLN2238 may be a low-affinity substrate of para-glycoprotein (P-gp), breast cancer resistance protein (BCRP), and multidrug resistance protein 2 (MRP2) efflux pump transporters. MLN2238 is not an inhibitor of P-gp, BCRP, and MRP2 (IC50 > 100 μM). Consequently, the potential for MLN2238 to cause DDIs with substrates or inhibitors of P-gp, BCRP, and MRP2 is low.

Safety Pharmacology: In exploratory safety pharmacology studies, MLN2238 was a weak inhibitor of the cloned cardiac potassium (K+) human ether à-go-go related gene (hERG) channel, with an IC50 of 59.6 μM, which exceeds, by approximately 200-fold, the plasma Cmax (111 ng/mL [0.3 μM]) predicted to occur in humans at the optimally efficacious dose after IV administration.

In the GLP-compliant, 1-cycle, repeat-dose, PO toxicology study in beagle dogs, an increase in QTc was seen in male dogs at non-tolerated doses, and a potential increase in QTc was seen in male dogs at tolerated doses. However, increased QTc was not seen in female dogs at any dose, despite the fact that female dogs had plasma Cmax values similar to those of male dogs. Additionally, in a GLP-compliant, 2-cycle, repeat-dose, IV toxicology study in beagle dogs, no increase in QTc was seen in either male or female dogs at any dose, even though dogs in the IV study had higher MLN2238 plasma Cmax values than did the male dogs in the PO study. These data suggest that MLN2238 has a low potential for prolonging the QT interval in vivo.

1.3 Preclinical Data

Toxicology: All studies discussed in this section were conducted with a solution of either MLN2238 or MLN2238 in equilibrium with ixazomib citrate. Because ixazomib citrate was shown to dissociate immediately to MLN2238 upon exposure to plasma in vitro and therefore could not be detected in plasma samples in vitro all doses, concentrations, and PK parameters noted, here and in the IB, are expressed as the boronic acid, MLN2238.

The toxicology studies of MLN2238 were studied in SCID mice bearing human CWR22 tumor xenografts, Sprague-Dawley rats, beagle dogs, and cynomolgus monkeys. Details of these studies are included in the IB.

In Vitro Toxicology: MLN2238 was not mutagenic in a Good Laboratory Practice (GLP)-compliant bacterial reverse mutation assay (Ames assay).

In Vivo Toxicology: Details of the in vivo toxicology IV dosing and oral dosing studies are provided in the IB. To summarize, the toxicologic effects seen in the IV and PO studies are qualitatively similar to what was previously observed in rodents dosed with bortezomib, for which ixazomib is the next-generation molecule. MLN2238 did not cause significant toxicities that have not been previously observed after dosing with bortezomib. Therefore, on the basis of the similarity in the toxicity profile in rats between MLN2238 and bortezomib, MLN2238 is not known to present any additional safety risks beyond those that occur after treatment with bortezomib. In addition, there were no significant findings at tolerated exposures in dogs observed after PO administration that were not seen after IV administration, and similar exposures were tolerated regardless of the route of administration.

The potential risks identified from nonclinical studies in dogs and rats include:

- GI toxicity that could result in nausea, vomiting, diarrhea, dehydration, electrolyte imbalance, bleeding, bowel obstruction including ileus and intussusception, and sepsis.
- Reduced blood counts manifest as thrombocytopenia, neutropenia, and anemia. Reticulocytopenia was described in animals and may be associated with anemia. Reductions in blood counts may predispose to an increased susceptibility to infection, bleeding, and anemia.
- Peripheral nerve ganglia effects that may be associated with peripheral neuropathy that includes pain, burning sensation, and numbness. Autonomic and motor neuropathy may be observed, as both have been reported for bortezomib.
- Lymphoid cell depletion that may be associated with increased risk of infection, including re-activation of herpes zoster.
- Acute phase response that may result in fever and metabolic changes.

All of the effects seen in the GLP-compliant PO toxicology studies in both dogs and rats at tolerated doses were reversible/reversing and can be monitored in the clinic with routine clinical observations (GI disturbances and infections secondary to lymphoid compromise), clinical pathology assessments (inhibition of erythropoiesis, thrombocytopenia, and inflammatory leukogram), and neurologic assessment, as are commonly done for patients treated with bortezomib. The neurologic lesions in these studies are similar to what has been described

after treatment with bortezomib and are believed to be the cause of the peripheral neuropathy observed in patients treated with bortezomib.

Further details are presented in the current version of the ixazomib IB.

1.4 Clinical Data to Date

Clinical Experience: To date, there is no published experience with ixazomib in HIV positive persons.

Ixazomib has been evaluated as an oral single agent in phase 1 studies that have included patients with advanced solid tumors, lymphoma, relapse/refractory MM (RRMM), and relapsed or refractory light-chain (AL) amyloidosis and demonstrated early signs of activity. Ongoing studies continue to investigate both single-agent ixazomib and ixazomib in combination with standard treatments. Based on encouraging preliminary data observed in patients with MM requiring systemic treatment, 2 phase 3 trials in newly diagnosed MM (NDMM) (C16014) and RRMM (C16010) patient populations are currently evaluating ixazomib in combination with Revlimid and Dexamethasone (RevDex) versus placebo/RevDex. Both trials are combining ixazomib at a weekly dose of 4.0 mg on Days 1, 8, and 15 in a 28-day cycle to a standard dose of lenalidomide with a weekly dexamethasone dose of 40 mg. In addition, clinical pharmacology studies have evaluated drug-drug interactions with ketoconazole, clarithromycin, and rifampin, as well as the effect of food, renal impairment, and hepatic impairment on the PK of ixazomib. Studies evaluating the safety and pharmacokinetic (PK) of ixazomib alone (in Japanese patients) and in combination with lenalidomide and dexamethasone in Asian adult patients (including Japanese patients) with a diagnosis of RRMM are ongoing.

As of 27 March 2013, preliminary clinical data is available for a total of 653 patients across 13 studies. The emerging safety profile indicates that ixazomib is generally well tolerated. The adverse events (AEs) are consistent with the class-based effects of proteasome inhibition and are similar to what has been previously reported with VELCADE though the severity of some, for example peripheral neuropathy, is less. While some of these potential toxicities may be severe, they can be managed by clinical monitoring and standard medical intervention, or, as needed, dose modification or discontinuation.

Fatigue was the most common AE reported among 384 patients treated in the oral (PO) studies (47%). Other common AEs reported in the pooled intravenous (IV) and PO safety populations include nausea, thrombocytopenia, diarrhea, and vomiting. Rash is also a commonly reported treatment-emergent event; however, there is some variety in its characterization and causality resulting in different preferred terms to describe it. A high-level term outline of rash events includes rashes, eruptions and exanthems NEC; pruritus NEC; erythemas; papulosquamous conditions; and exfoliative conditions. The dose escalation phases of most trials reported in the IB have now completed enrollment, and gastrointestinal (GI) symptoms were the common dose-limiting toxicities (DLTs) when the use of prophylactic anti-emetics was not permitted per protocol. In the expansion cohorts or phase 2 cohorts (as per each study), the incidence and severity of GI symptoms was mitigated by the use of the lower maximum tolerated dose (MTD)/recommended phase 2 dose (RP2D) (as per each study) and standard clinical usage of anti-emetics and/or antidiarrheal medications as deemed appropriate. Prophylactic use of anti-

emetics has not been required as with other agents but (as outlined in Section 6.7) has been used according to standard practice and are effective.

The most frequent (at least 20%) treatment-emergent adverse events (TEAEs) reported with the PO formulation pooled from single-agent studies (n = 201) irrespective of causality to ixazomib, include nausea (53%), fatigue (51%), diarrhea (44%), thrombocytopenia (34%), vomiting (38%), decreased appetite (32%), fever (21%), and anemia (21%). The most frequent (at least 20%) TEAEs reported with the PO formulation pooled from combination trials (irrespective of the combination) (n = 173), irrespective of causality to ixazomib, include diarrhea (47%), fatigue (44%), nausea (38%), peripheral edema (35%), constipation (33%), insomnia (29%), thrombocytopenia (28%), anemia (26%), vomiting (26%), neutropenia (25%), back pain (24%), pyrexia (23%), peripheral edema (21%, each), fever (20%), cough (20%), hypokalemia (20%), neutropenia (20%), and upper respiratory tract infection (20%). Overall rash of all grades is reported in approximately 50% of patients and is more common when ixazomib is given in combination with lenalidomide where rash is an overlapping toxicity.

Additional detailed and more recent information regarding the clinical experience of ixazomib may be found in the IB, including information on the IV formulation.

1.5 Dose Rationale and Risk/Benefits

Safety is our first concern, therefore we have chosen a dose escalation study, starting at a low dose with regular and frequent DSMB safety review. The lowest starting dose in this study is 1 mg weekly, which is expected to achieve <30% 20S proteasome inhibition based on previously published pharmacodynamics data in advanced non-hematologic malignancies (Invest New Drugs. 2015; 33(3): 652–663). The maximum possible dose of ixazomib to be administered in this trial is similar to the doses SK Kumar et al. have previously used in a Phase I study of weekly ixazomib for relapsed/refractory multiple myeloma (60). However, during that study, dosing was BSA based, and the maximum tolerated dose was 2.97 mg/m2 (translating to a fixed dose of 5.5 mg). The estimated half-life of ixazomib was 3.6 to 11.3 days, supporting weekly dosing, which is the dosing schedule studied in Phase 2 and 3 trials, and ultimately approved by the FDA for treatment of multiple myeloma.

Furthermore, the doses of ixazomib to be administered in this trial are expected to correlate with the concentrations used in the preliminary data noted above. Maximum biologic effects were noted in the in vitro and ex vivo experiments above with a single treatment of 100 nM ixazomib, which corresponds to a peak in vitro concentration of 36.1 ng/ml. In Study C16009, Arm 3, adult patients with a diagnosis of an advanced non-hematologic malignancy or lymphoma were treated with a standard 4 mg oral dose of ixazomib, and geometic mean Cmax in the fasted state was 77 ng/ml with a 57% CV. Therefore, the proposed dose range for this study (from 1 to 4 mg) is expected to result in plasma Cmax values that approximate the doses used in the in vitro studies. Cmin and Cmax levels before and after the second dose of ixazomib on Day+15 will be measured.

Potential Risks of ixazomib: Because ixazomib has not been previously studied in HIV infected patients or otherwise healthy individuals, it is unclear what side effects low doses of ixazomib

will have in otherwise healthy HIV infected patients. In the absence of healthy volunteer data, the best indication of likely side effects is best informed from the phase three trial that has recently been reported (Moreau et al. NEJM 374;17:1621-1634) . In that phase three trial refractory multiple myeloma patients were randomly assigned to receive Dexamethasone and Lenalidomide along with either placebo , or Ixazomib 4 mg once weekly on days 1,8, and 15 of a four week cycle. The incidence of side effects was no different between study arms, and therefore additional toxicities (including peripheral neuropathy) directly attributable to ixazomib were concluded to be limited (see below for details). Based on observations from nonclinical toxicology studies of ixazomib and the clinical observations from the ongoing and completed clinical studies, the following potential safety risks of ixazomib are noted in the ixazomib IB: Anemia, neutropenia, thrombocytopenia, fatigue, nausea, vomiting, constipation, diarrhea, peripheral edema, pyrexia, decreased appetite, dizziness, dysgeusia, peripheral neuropathy, peripheral sensory neuropathy, insomnia, progressive multifocal leukoencephalopathy and rash (erythematous, generalized, macular, macular-papular, and papular) with or without pruritus have been listed in the ixazomib IB as adverse drug reactions reported with ixazomib use. It is also possible that ixazomib will have toxicities that were not previously observed in or predicted from such sources. Patients will be monitored closely for anticipated and unanticipated toxicities.

While the above listed adverse drug reactions have been reported in association with ixazomib use in previous clinical trials, definitive attribution of causation of these adverse events to ixazomib is difficult. First, participants in these trials often have underlying conditions that predispose to the development of these adverse events – i.e. up to 20% of multiple myeloma patients have peripheral neuropathy at the time of diagnosis prior to any treatment (Handb Clin Neurol. 2013;115:443-59). Second, many trial participants have received prior therapies, or receive concomitant therapies during the trail, that have overlapping toxicities. Therefore, careful examination of toxicities observed in a phase 3 trial when ixazomib is compared to placebo, in addition to an optimized background regimen is instructive..

The TOURMALINE-MM1 trial was a phase 3, double blind, placebo-controlled, randomized trial comparing ixazomib 4 mg plus lenalidomide-dexamethasone to placebo plus lenalidomide-dexamethasone in patients with relapsed and/or refractory multiple myeloma (Moreau et al. NEJM 374;17:1621-1634). Importantly, 70% of participants had prior treatment with bortezomib, which has a known association with the development of peripheral neuropathy, and other adverse events, and as noted above approximately 20% of patients with multiple myeloma have peripheral neuropathy at the time of diagnosis, prior to any treatments. Notably, the rates of serious adverse events were similar in the placebo and in the ixazomib groups (47% in the ixazomib group versus 49% in the placebo group). The incidence of any peripheral neuropathy was 29% in the ixazomib group and 24% in the placebo group ($P>0.05$); the prevalence of peripheral neuropathy at baseline prior to treatment was not reported. 2% of participants developed grade 3 peripheral neuropathy in both groups, and no participants developed grade 4 peripheral neuropathy. These data suggest that the attributable risk of peripheral neuropathy (or any other toxicity) due to ixazomib in multiple myeloma patients is low. Furthermore, previous studies have demonstrated that the peripheral neuropathy associated with bortezomib use is reversible in the majority of patients (Br J Haematol. 2009

Mar;144(6):895-903.). Preliminary clinical data with ixazomib suggest that associated peripheral neuropathy is mild and reversible (see current IB page 153).

Ixazomib has principally been studied in combinations and in patients with multiple myeloma. There are two ongoing studies evaluating ixazomib monotherapy in patients with diseases other than myeloma:

- Phase 2, open-label, dose escalation trial of ixazomib in adult patients with non-Hodgkin lymphoma. The dose and schedule of ixazomib (monotherapy) during the dose-escalation phase was either 4 mg, 5.3 mg or 7 mg administered on Days 1, 8 and 15 of a 28-day cycle (ClinicalTrials.gov Identifier: NCT01939899).
- Phase 1b, randomized placebo controlled trial of ixazomib in patients with ISN / RPS Class III or IV Lupus Nephritis (ClinicalTrials.gov Identifier: NCT02176486).

These studies will be informative as to the association of peripheral neuropathy (or lack thereof) with ixazomib monotherapy in patients with diseases other than multiple myeloma.

Additional details on the potential risks of ixazomib may be found in the current IB.

Potential Benefits of ixazomib: It is likely that individual participants in this phase I/IIa study will derive no direct clinical benefit from treatment with ixazomib, as specified in the informed consent form.

The hypothesis we are exploring based on in vitro and ex vivo data is that therapy with ixazomib decreases cell-associated HIV DNA by 1) inducing HIV reactivation, 2) increasing expression of pro-apoptotic Casp8p41 in HIV-infected cells, and 3) inducing cell death specifically in HIV-infected cells while sparing HIV-uninfected cells. If these positive effects are recapitulated when treating patients with HIV on antiretroviral therapy with ixazomib, this would represent the first clinical intervention found to decrease the size of the HIV reservoir, which is the main barrier to HIV cure.

There is an urgent need for an HIV cure. While patients with HIV on antiretroviral therapy live longer and have reduced rates of opportunistic infections and malignancies compared to untreated HIV patients, data suggest that life expectancy for patients with treated HIV in the current treatment era are still nearly 14 years shorter than age and sex-matched uninfected controls (Marcus et al. J Acquir Immune Defic Syndr. 2016 Mar 29), indicating persistent increased mortality even in treated patients. Chronic, treated HIV infection is associated with abnormal chronic inflammation, cardiovascular disease, metabolic syndrome, bone disease, neurocognitive defects and age-related non-AIDS malignancies. Furthermore, current HIV treatments require lifelong therapy with antiretroviral medications, which have substantial associated burdens of costs and adverse drug effects themselves.

Therefore, establishment of a maximal tolerated dose, and obtaining preliminary efficacy data at reducing HIV reservoir size, will inform future therapeutic study designs with the intent of establishing whether ixazomib has beneficial effects in patients with HIV infection.

2 Study Objectives

Primary Objective

To determine the safety and tolerability of ixazomib in HIV infected patients who are on a stable regimen of ART that suppresses HIV replication (HIV RNA to undetectable levels using clinical assays).

Secondary Objectives

To determine the effect of ixazomib on:
1) Change in cell associated HIV DNA in CD4 T cell subsets from Week -1 to Week 24;
2) Change in culturable HIV from Week -1 to Week 24;
3) Change in absolute CD4 T cell, CD8 T cell count and percentage as well as CD4/CD8 ratio from Week -1 to 24

3 Study Design

3.1 General Design

This study is an open label, phase I/IIa pilot trial of the safety and tolerability of ixazomib, in the treatment of HIV. Subjects will be screened at outpatient clinic visit appointments and interested qualified subjects will be offered and consented for participation in this trial. All study visits except the screening visit will be conducted at the Mayo Clinic. The screening visit may be completed at the Mayo Clinic, Hennepin County Medical Center or any other site that has been approved by the regulatory sponsor. Once consent has been obtained, baseline values will be established and subjects will begin treatment and follow-up for the next 6 months. A final visit for evaluation and collection of lab samples will be conducted at the end of the study.

3.2 Primary Study Endpoints

The primary endpoint to be analyzed in the study is the safety and tolerability of ixazomib in HIV positive patients on antiretroviral therapy.

(See pages 39 and 49 for a list of adverse events to be assessed).

3.3 Secondary Study Endpoints

Secondary endpoints will include change from baseline in measures of HIV infection (cell associated HIV DNA and culturable HIV), and immune parameters (CD4 and CD8 T cell count, as well as CD4/CD8 Ratio).

4 Subject Selection Enrollment and Withdrawal

4.1 Inclusion Criteria

- The following laboratory values obtained <=14 days prior to registration.
    - ANC ≥ LLN (lower limit of normal) and ≤ULN (upper limit of normal), Hgb ≥ 12.0 g/dL and ≤ULN, PLT ≥ LLN and ≤ULN

  - Total bilirubin ≤ULN and the direct bilirubin must be ≤ ULN; AST <1.5 x ULN and ALT <1.5 x ULN
  - Creatinine <2.0 x ULN and an estimated creatinine clearance > 60 ml/min (patients on dolutegravir based regimens will have cystatin C measured for a more accurate estimation of creatinine clearance)
- HIV infection with suppressed viral replication on at least 3 active drug ART for at least 6 months
  - Suppressed viral replication is defined by plasma HIV viral load <20copies/mL.
  - Patient must have HIV viral load <20 copies/ml on two occasions at least 3 months apart.
  - In the opinion of the treating physician, patients must have available other regimens likely to suppress HIV should their current regimen fail.
- Male or female patients age >=18 years
- A plasma HIV RNA viral load demonstrating a measure of <20 copies/mL within 30 days prior to study initiation.
- CD4 count ≥500 cells/mm^3 within 30 days prior to study enrollment
- Females must have a negative urine pregnancy test prior to receiving the 1st dose of ixazomib and be postmenopausal for at least 1 year before the screen visit, or surgically sterile,
- Male patients, even if surgically sterilized (ie, status post-vasectomy), must agree to one of the following:
  - Agree to practice effective barrier contraception AND a second method of contraception for female partners of childbearing potential during the entire study treatment period and through *90 days* after the last dose of ixazomib,
  - OR
  - Agree to practice true abstinence when this is in line with the preferred and usual lifestyle of the subject. (Periodic abstinence (eg, calendar, ovulation, symptothermal, post-ovulation methods] and withdrawal are not acceptable methods of contraception.)
  - AND
  - Agree to forego sperm donation for the same period as above.

4.2 Exclusion Criteria

- The following laboratory values obtained <=14 days prior to registration.
  - ANC < LLN or >ULN, Hgb < 12.0 g/dL or >ULN, PLT < LLN or >ULN
  - Total bilirubin >ULN or the direct bilirubin is > ULN; AST >1.5 x ULN or AST >1.5 x ULN
  - Creatinine >=2.0 x ULN or an estimated creatinine clearance <=60mL/min
- Diagnosed and treated for a malignancy within 5 years before randomization, or previously diagnosed with a malignancy and have any evidence of residual disease.

Patients with nonmelanoma skin cancer or carcinoma in situ of any type are not excluded if they have undergone complete resection

- Any infection except HIV (excluding benign conditions that is unlikely to be affected or modulated by treatment with ixazomib, e.g. stye or furuncle), or treatment with anti-infective agents within 14 days of enrollment.
- Pregnant women
- Women of childbearing potential and Nursing women
- Men who are unwilling to use a condom (even if they have undergone a prior vasectomy) while having intercourse, while taking the drug and for 90 days after stopping ixazomib.
- Any history of peripheral neuropathy, or peripheral neuropathy detected during the screening period. Screening for peripheral neuropathy will be performed using the the European Organization for Research and Treatment of Cancer QLQ-CIPN20 Questionnaire (see section 6 and Appendix II).
- Major surgery within 14 days before study registration
- Systemic treatment with strong CYP3A inducers (rifampin, rifapentine, rifabutin,carbamazepine, phenytoin, phenobarbital), or use of St. John's wort.
- Evidence of current uncontrolled cardiovascular conditions, including serious cardiac arrhythmias, congestive heart failure, angina, or myocardial infarction within the past 6 months. Note: Prior to study entry, any ECG abnormality at screening must be documented by the investigator as not medically relevant IF the ECG abnormality is judged NOT to be clinically relevant.
- QTc > 450 milliseconds (msec) for men and >470 milliseconds for women (83) on a 12 lead ECG obtained during the Screening period. Note: If a machine reading is above this value, the ECG should be reviewed by a qualified cardiologist.
- Known hepatitis B DNA positive status and/or HBsAg positive and/or HBeAg positive, or active hepatitis C replication (HCV RNA positive) or currently on hepatitis C treatment.
- Known history of cirrhosis or active liver inflammation, including "fatty liver" or non-alcohol steatohepatitis (NASH).
- Any serious medical or psychiatric illness that could, in the investigator's opinion, potentially interfere with the completion of treatment according to this protocol. This includes a recent history of substance abuse with less than 6 months of sobriety.
- Known allergy to any of the study medications, their analogues or excipients in the various formulations.
- Any other recent or concurrent medical condition that, in the Investigator's opinion, would impose any risk to the patient
- Known GI disease or GI procedure that could interfere with the oral absorption or tolerance of ixazomib including difficulty swallowing.
- Participation in other clinical trials, including those with other investigational agents not included in this trial, within 30 days of the start of this trial and throughout the duration of this trial.

4.3 Subject Recruitment, Enrollment and Screening

A maximum total of 17 subjects will be recruited from patients from the Mayo Clinic HIV Clinic and the Hennepin County Medical Center (HCMC). Potential study subjects will be identified and screened for inclusion and exclusion criteria by a member of the study team. Furthermore, other treating clinicians, including residents, fellows and staff physicians, in the Mayo Clinic HIV clinic and HCMC continuity clinic may refer potential study subjects to a study team member. Evaluation and documentation of inclusion and exclusion criteria will be obtained and recorded by an investigator prior to enrollment. Informed consent will be obtained by an MD investigator. Patients will be remunerated for participation in the study (see section 12.3).

4.4 Early Withdrawal of Subjects

4.4.1 When and How to Withdraw Subjects

Permanently discontinue ixazomib in individual subjects if any of the following occur:

- Death of any subject
- Anaphylactic reaction in any subject
- A life-threatening adverse event in any subject
- Any Grade 3 AE (except if there is clear evidence that it is not study drug related – i.e. a plausible alternative reason).
- Any Grade 4 AE
- A decrease in CD4 count below 350 cells/mm^3, confirmed on repeat testing within 2 weeks, and associated with a relative drop in CD4 T cell percentage by >25% from baseline values, in the absence of an alternative explanation
- Confirmed virologic rebound (HIV-1 RNA >500 copies/ml confirmed by a second assay done within 14 days of the initial one). If the HIV-1 viral load is >20 copies/mL and <500 copies/mL, the HIV-1 viral load will be rechecked every 2 weeks until it becomes <20 copies/mL or >500 copies/mL.
- Development of ≥ Grade 1 peripheral neuropathy with or without pain

An independent Data Safety and Monitoring Board will review the case. The committee and principal investigators will determine whether there is an association between the event and the ixazomib. The DSMB will make a recommendation accordingly as to whether the study should proceed, or if a modification in trial design is required, or if a replacement subject should be enrolled at the same dose as the withdrawn subject.

Reasons for early withdrawal of individual subjects may include:

- Subjects refuse to return for any remaining study visits.
- Subjects discontinue the ixazomib at any time during the study.
- Safety reasons, either at the discretion of the Investigator or at the subject's request.
- Protocol violations at the discretion of the principal investigators.
- Initiation of concomitant therapy that could interfere with the results of the study or ixazomib
- Subject's decision to withdraw, at any time.

All subjects are free to withdraw from participating in this study at any time and for whatever reason, specified or unspecified, and without prejudice.

4.4.2 Data Collection and Follow-up for Withdrawn Subjects

All premature discontinuations and their causes must be carefully documented by the investigators. Subjects not completing the entire study should be fully evaluated when possible. If subjects choose to withdraw before completing the study, they should return to complete the following safety and limited efficacy assessments:

- Patient tolerability and side effects questionnaire.
- Vital signs.
- A complete physical exam.
- A review of recent clinical laboratory results, including a complete blood count, serum electrolytes, creatinine, fasting glucose, liver function tests, CD4 T cell count and HIV viral load.

If subjects who prematurely withdraw from the study do not return for this limited evaluation visit, then the relevant and available clinical data will be abstracted from the patient's medical record at the appropriate time based upon the study schedule.

Patients who withdraw from the study for virologic failure or a decrease in CD4 T cell counts will be followed until these values return to their baseline values.

5 Study Drug

5.1 Description

Background

Ixazomib is a second-generation small molecule inhibitor of the 20S proteasome that is under development for the treatment of non-hematologic malignancies, lymphoma, and multiple myeloma. Ixazomib (MLN2238) refers to the biologically active, boronic acid form of the drug substance, ixazomib citrate. The transition to MLN2238 occurs in any aqueous system.

Formulation

The ixazomib capsule drug product formulation consists of drug substance, microcrystalline cellulose, talc, and magnesium stearate. Ixazomib capsules are individually packaged in cold form foil-foil blisters with a paper backing for child resistance.

The ixazomib drug product is provided in strengths of 4.0-, 3.0-, and 2.0-, and 0.5- mg capsules as the active boronic acid. The different dose strengths are differentiated by both capsule size and color as described below:

Dose Strength	Capsule Size	Capsule Color
4.0 mg	Size 4	Ivory
3.0 mg	Size 3	Light gray
2.3 mg	Size 2	Light pink
2.0 mg	Size 2	Swedish orange
0.5 mg	Size 3	Dark green
0.2 mg	Size 4	White opaque

For additional details, please see the ixazomib IB.

5.2 Treatment Regimen

Patients will be treated in sequential groups after the safety evaluation of each group by the DSMB. Visits 1 and 2 do not have a time window, and can occur without DSMB review as patients will not receive drug at those visits. The following groups will be studied in this protocol:

Group 1: 3 patients with HIV infection, who meet inclusion criteria, will receive ixazomib 1 mg on days 1, 8 and 15 for 28 days. After 28 days, the DSMB will meet to decide if enrollment of the next cohort should proceed. After 84 days (28 days x 3 cycles), the DSMB will meet and decide if patients will receive either (i) ixazomib once weekly for 12 weeks or (ii) ixazomib 1 mg on days 1, 8 and 15 for an additional three 28 days cycles. Visits 3-15 will have a window of ± 3 days.

Group 2: 3 patients with HIV infection, who meet inclusion criteria, will receive ixazomib 2 mg on days 1, 8 and 15 for 28 days. After 28 days, the DSMB will meet to decide if enrollment of the next cohort should proceed. After 84 days (28 days x 3 cycles), the DSMB will meet and decide if patients will receive either (i) ixazomib once weekly for 12 weeks or (ii) ixazomib 2 mg

on days 1, 8 and 15 for an additional three 28 days cycles. Visits 3-15 will have a window of ± 3 days.

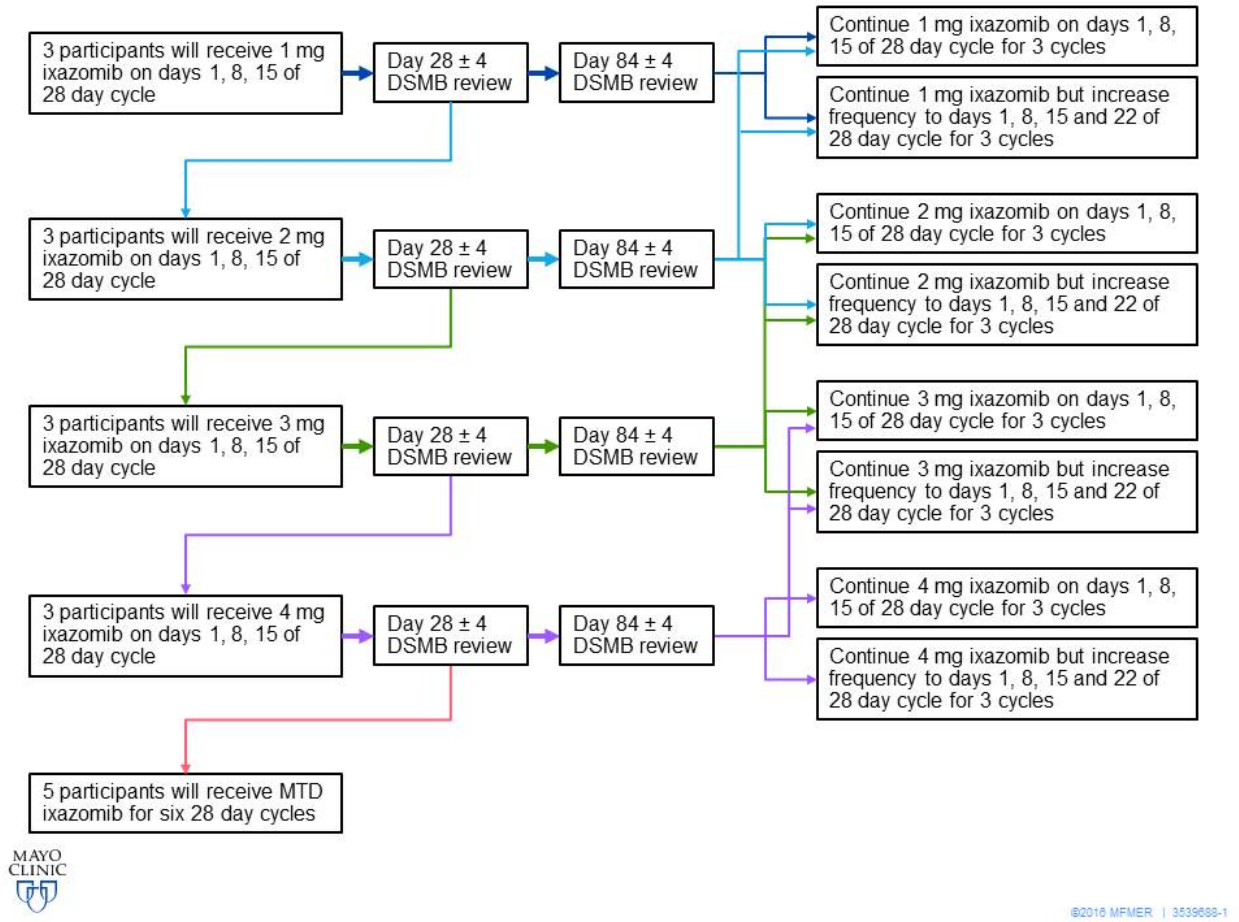
Group 3: 3 patients with HIV infection, who meet inclusion criteria, will receive ixazomib 3 mg on days 1, 8 and 15 for 28 days. After 28 days, the DSMB will meet to decide if enrollment of the next cohort should proceed. After 84 days (28 days x 3 cycles), the DSMB will meet and decide if patients will receive either (i) ixazomib once weekly for 12 weeks or (ii) ixazomib 3 mg on days 1, 8 and 15 for an additional three 28 days cycles. Visits 3-15 will have a window of ± 3 days.

Group 4: 3 patients with HIV infection, who meet inclusion criteria, will receive ixazomib 4 mg on days 1, 8 and 15 for 28 days. After 28 days, the DSMB will meet to decide if enrollment of the next cohort should proceed. After 84 days (28 days x 3 cycles), the DSMB will meet and decide if patients will receive either (i) ixazomib once weekly for 12 weeks or (ii) ixazomib 4 mg on days 1, 8 and 15 for an additional three 28 days cycles. Visits 3-15 will have a window of ± 3 days.

Group 5: 5 patients with HIV infection, who meet inclusion criteria will receive the maximum tolerated dose of ixazomib as determined by DSMB recommendation. Visits 3-15 will have a window of ± 3 days.

All participants will continue their current ART regimen.

The DSMB will review each dose group and the following recommendations can be made after each review:



5.3 Method for Assigning Subjects to Treatment Groups

Each group (or dose level) will enroll and after 4 weeks of follow up of 3 patients treated with ixazomib at a particular dose will be reviewed for safety by DSMB before initiating enrollment into the next group (or dose level). All patients in the current cohort must have completed 4 weeks and the cohort's data reviewed by the by the Safety Monitoring Committee before patients in the next cohort can receive drug. Patients from the next cohort may complete the first two visits prior to DSMB review of earlier cohorts.

Dose limiting toxicities are defined in Section 6.17 Table 2. After all the subjects in each dosing group has completed Cycle 1 of ixazomib, the DSMB may recommend:

(i) Proceed to next dose level, or

(ii) Terminate trial, or

(iii) Enroll a replacement subject for a withdrawn subject, or

(iv) Add 5 more patients at previous dose group (maximum tolerated dose). The maximum tolerated dose is that level where no more than 0 out of 3 patients experience dose limiting toxicity.

If no DLT are observed in 3 subjects, the next dosing level will be initiated.

After DSMB review of the 4 mg dosing level, if no DLT are observed, the DSMB may recommend:

(i) Terminating the trial
(ii) Enroll 5 more patients at the maximum tolerated dose.

The NNRTIs etravirine and efavirenz are moderate CYP3A enzyme inducers and have potential to decrease systemic exposure to ixazomib. That possibility will be explored by measuring trough ixazomib levels in all patients and comparing ixazomib concentrations in patients on different ART regimens. The DSMB will have to consider the number of patients treated with NNRTIs in a particular group as the safety profile of a group may be underestimated if the majority of patients are treated with NNRTIs as part of their ARV regimen.

Additional data review by the DSMB will occur after Day 84 for each dosing cohort. At this time, the DSMB may recommend:

(i) Proceed to uninterrupted weekly dosing on days 1, 8, 15 and 22 of a 28 day cycle, or
(ii) Continue previous dosing on days 1, 8, and 15 of a 28 day cycle, or
(iii) Enroll a replacement subject for a withdrawn subject, or
(iv) Terminate dosing.
This determination will be made on an individual participant basis, and not a dosing cohort basis, and will be made based on the presence or absence of adverse drug effects that do not meet the definition of dose related toxicities.

5.4 Preparation and Administration of Study Drug

Preparation and storage

Ixazomib capsules, individually packaged in foil blister strips with child resistant paper backing (see below), will be stored unopened at 2-8ºC (36-46ºF) Of note, Ixazomib can be stored up to 40ºC (104ºF) for up to 30 days, and up to 50º (122ºF) for up to 3 days). Study medication will be stored and dispensed by the Research Pharmacy at Mayo Clinic. Ixazomib that is dispensed to the patient for take-home dosing should remain in the blister packaging until the point of use. The investigative site will be providing the medication to the patient in units that comprise the correct dose configurations, which are prepackaged by the manufacturer. Capsules should remain in the blisters until the point of use. Comprehensive instructions will be provided to the patient in order to ensure compliance with dosing procedures. Patients will be instructed to store the medication in the refrigerator until the time of use.

Open Wallet View

Front Wallet View

Back Wallet View

Administration

All protocol-specific criteria for administration of ixazomib must be met and documented before drug administration. Ixazomib will be administered or dispensed only to eligible patients under the supervision of the investigator or identified subinvestigator(s). Patients should be monitored for toxicity, as necessary, and doses of ixazomib should be modified as needed to accommodate patient tolerance to treatment; this may include symptomatic treatment, dose interruptions, and adjustments of ixazomib dose. Capsules of ixazomib will also be referred to as study drug. Study drug will be supplied by Millennium as capsules of 0.5mg, 2.0mg, and 3.0mg, and 4.0mg ixazomib. Patients will be instructed to swallow ixazomib capsules whole, with water, and not to break, chew, or open the capsules. Ixazomib should be taken on an empty stomach (no food or drink) at least 1 hour before or 2 hours after a meal. Each capsule should be swallowed separately with a sip of water. A total of approximately 8 ounces (240 mL) of water should be taken with the capsules.

Missed doses can be taken as soon as the patient remembers if the next scheduled dose is 72 hours or more away. A double dose should not be taken to make up for a missed dose. If the patient vomits after taking a dose, the patient should not repeat the dose but should resume dosing at the time of the next scheduled dose.

5.5 Subject Compliance Monitoring

Reconciliation will occur accordingly when the patient returns for their next cycle of therapy. Any extremes in temperature should be reported as an excursion and will be managed on a case by case basis. Returned unused capsules should be discarded in a proper biohazard container.

5.6 Prior and Concomitant Therapy

See section 4.2 Exclusion criteria above.

5.7 Packaging

See section 5.4 above.

5.8 Receiving, Storage, Dispensing and Return

5.8.1 Receipt of Drug Supplies

Drug procurement: Investigational product will be supplied free of charge to trial participants by Takeda Pharmaceuticals, Inc.

5.8.2 Storage

See section 5.4 above.

5.8.3 Inventory/Dispensing/Labeling of Study Drug

See section 5.4. regarding storage of drug

A drug inventory/accountability log will be utilized to track study drug inventory/accountability. Upon shipment receipt, invoices will be verified and the inventory updated upon receipt. The inventory/accountability log will note name, dose, lot number, expiration, and quantity. A dispensing record will be tracked to note drug name, strength, quantity, lot number, drug expiration, patient name, date of dispensing.

Upon dispensing, a designated Research Pharmacist will label the medication with a label containing the following (Patient name, drug name/dose, dispense date, PI's name, address, telephone #, IRB #, directions for use, expiration date) and an auxiliary label noting "Caution-new drug limited by law to investigational use." The labeling and packaging will be double checked by an additional study team member prior to release to the patient. Counseling and drug information will be provided to the patient upon study drug dispensing.

Drug specific patient education/counseling

- Ixazomib capsules must be administered intact and should not be opened or manipulated in any way. Additionally, ixazomib capsules should remain in the blister packs until they are ready to be taken.

- Ixazomib should be taken on an empty stomach, at least 1 hour before or no sooner than 2 hours after a meal (as described in section 5.4 above).

- Thrombocytopenia has been observed with ixazomib. Patients will be instructed to report any unusual bruising or bleeding to the study team.

- GI side effects have been observed in patients treated with ixazomib (nausea, diarrhea, vomiting). If this should occur, patients should seek medical advice to ensure compliance with HIV ARV medications and effective management of the adverse event.

- Patients will be instructed to report any rash to study team.

- Patients will be instructed not to start any new medications or supplements without checking with the study team first. Our investigator team will assess patient's concomitant medications, including over the counter and supplements. Ixazomib is metabolized through both CYP enzymes and non-CYP proteins, and drug to drug interactions exist with strong inducers of CYP3A.

- The NNRTIs etravirine and efavirenz are moderate CYP3A enzyme inducers and have potential to decrease systemic exposure to ixazomib. That possibility will be explored by measuring trough ixazomib levels in all patients and comparing ixazomib concentrations in patients on different ART regimens.

5.8.3 Return or Destruction of Study Drug

At the completion of the study, there will be a final reconciliation of drug shipped, drug dispensed, drug returns, and drug remaining. This reconciliation will be logged on the drug reconciliation form, signed and dated. Any discrepancies noted will be documented and investigated, prior to destruction of unused study drug. Drug destroyed on site will be documented in the study files.

6 Study Procedures

Study related procedures include leukapheresis performed at baseline and at treatment completion..

Leukapheresis

Fenwal Amicus (Fenwal Inc., Lake Zurich IL, USA) apheresis systems (Version 3.1) will be used for mononuclear cell (MNC) collections. Access will be obtained using peripheral veins. Each subject will be evaluated after enrollment but prior to collection by the nursing staff of the Therapeutic Apheresis Treatment Unit to ensure that they have adequate venous access for the collection procedure. Venous access will consist of a 16 gauge steel needle placed into a vein in an antecubital fossa of one arm as a draw. The return will consist of an IV placed in the opposite arm. All MNC collections will have an endpoint of four hours processing time. Anticoagulant will be acid-citrate-dextrose solution-A (ACD-A) (Baxter Healthcare Corp., Deerfield, IL, USA) for subjects with a contraindication to heparin or a mixture of ACD-A, normal saline, and heparin for subjects without a contraindication to heparin. The heparin/ACD-A anticoagulant is preferred due to the lower incidence of citrate toxicity because of the lower citrate dose administered during the procedure. The lower dose of heparin administered also minimizes the length of systemic anticoagulation.The MNC offset will be 1.5 ml. The RBC offset start at 5.0 ml and will be adjusted during the procedure. The operator will observe the first cycle MNC transfer to determine if the RBCs pass completely through the right cassette. If the RBCs did not reach the top of the cassette, the operator will increase the RBC offsets to the next level for each succeeding cycle until the RBCs reached the top of the right cassette. Once the RBCs reach the top of the right cassette, no further adjustment will be made. If the subject's pre-procedure PLT count is ≥300 x 109/L, then the RBC offset will started at 6.0 ml. Only PBMCs will be harvested by this procedure. Nearly all blood is returned to the patient with only a small amount of blood lost in the process and in the tubing. PBMCs harvested by apheresis will be stored and used in

associated laboratory assays in the Badley HIV Immunology Laboratory BSL-3 facility on Guggenheim 5th floor in accordance with all appropriate Biosafety requirements.

Screening for peripheral neuropathy
Screening for peripheral neuropathy will occur at baseline (for study exclusion), and at each study visit (for safety monitoring), by administration of the European Organization for Research and Treatment of Cancer QLQ-CIPN20 Questionnaire. The 20-item CIPN-specific questionnaire which includes three scales assessing sensory (9 items), motor (8 items), and autonomic (3 items) symptoms and functioning with each item formatted the same as items of the EORTC QLQ-C30. It was developed to be used in conjunction with the EORTC QLQ-C30 following the EORTC guidelines for module development (15911236). The EORTC QLQ-CIPN20 has been tested in cancer patients receiving a variety of chemotherapies and has been shown to have internal consistency reliability based on Cronbach's alpha coefficients of 0.82, 0.73, and 0.76 for the three scales, respectively (PMID 23543373). The questions that comprise the QLQ-CIPN20 are listed in Appendix II. Any affirmative answer to questions in the QLQ-CIPN20 will trigger a further clinical assessment, and grading of suspected peripheral neuropathy according to the (CTCAE) Version 4.0 scale (Appendix I). Further clinical assessment of suspected peripheral neuropathy will be referred to a qualified physician in the area of drug-induced peripheral neuropathy.

The following toxicities will be considered to be dose limiting toxicities:

- Any ≥Grade 4 toxicity
- Any Grade 3 AE (except if there is clear evidence that it is not ixazomib related – i.e. a plausible alternative reason).
- Any ≥Grade 1 peripheral neuropathy (PN)

Specific management of toxicities in individual participants is discussed below.

Study related biology assays for the secondary outcomes include:

- CD4 T cell count, CD8 T cell count and CD4/CD8 ratio – by routine clinical flow cytometry (Mayo Medical Labs).
- Plasma HIV RNA viral load – by routine clinical RT-qPCR (Mayo Medical Labs).
- HIV Proviral DNA – by clinical qPCR (Mayo Medical Labs)
- Cell associated HIV DNA by digital droplet PCR – Peripheral blood CD4 T cells will be isolated from baseline and post-treatment leukapheresis samples by magnetic bead negative selection. Cell associated HIV DNA will be quantified by digital droplet PCR using RainDance™ ddPCR system (Badley HIV Laboratory).
- Culturable HIV – replication competent latent viral reservoir will be estimated using a quantitative viral outgrowth assay, considered the gold standard measurement, on peripheral blood CD4 T cells from baseline and post-treatment leukapheresis samples (Badley HIV Laboratory).

6.1 Visit 1 (Screening and Enrollment)

- Screen for study eligibility based on inclusion and exclusion criteria.
- Obtained informed consent.
- Obtain vital signs, including weight.
- Document medical history.
- Document concomitant medications and non-drug treatments (including prescription and over-the-counter medications, nutritional supplements and herbal remedies, taken within 30 days of screening)
- Perform complete physical exam.
- Review and record results of routine clinical monitoring labs, including CBC, electrolytes, glucose, creatinine, LFTs, CD4 T cell count and percentage, CD8 T cell count and percentage and CD4/CD8 ratio, HIV RNA viral load, HIV proviral DNA, urine pregnancy test and ECG. Obtain an additional four (4) heparin tubes of whole blood for stored PBMC and plasma.

When screening clinical laboratory test results are received, they must be reviewed by the Principal Investigator or designee physician for clinically significant abnormalities. If no clinically significant abnormalities or exclusions are found, the subject should be scheduled to attend Visit 2. Visits 1 and 2 may occur on the same date, but not before visit 3.

6.2 Visit 2

- Leukapheresis performed at Clinical Apheresis Unit, Charlton 8th floor.

6.3 Visit 3 (Study Entry, Time 0 weeks)

- Obtain vital signs, including weight.
- Perform brief physical exam.
- Monitor for changes to concomitant drug and non-drug treatments
- Obtain CBC and an additional four (4) heparin tubes of whole blood for stored PBMC and plasma.
- QLQ-CIPN20 Questionnaire
- Prescribe study medication.

6.3.5 Visit 3.5 (Optional post-dose blood draw)

- Obtain four (4) heparin tubes of whole blood for stored PBMC and plasma.

6.4 Visit 4 (Treatment phase, Time 1 week ±3 days)

- Obtain vital signs, including weight.
- Perform brief physical exam.
- Monitor for changes to concomitant drug and non-drug treatments
- Obtain CBC and confirm lack of hematologic toxicity prior to administering day 8 dose.
- QLQ-CIPN20 Questionnaire
- Monitor and record adverse events
- Prescribe study medication.

6.5 Visit 5 (Treatment Phase, Time 2 weeks ±3 days)

- Obtain vital signs, including weight.
- Perform brief physical exam.
- Monitor for changes to concomitant drug and non-drug treatments.
- Obtain CBC and confirm lack of hematologic toxicity prior to administering day 15 dose.
- Obtain electrolytes, glucose, creatinine, LFTs.
- QLQ-CIPN20 Questionnaire
- Monitor and record adverse events
- Prescribe study medication

6.6 Visit 6 (Treatment phase, Time 3 weeks ±3 days)

- Obtain vital signs, including weight.
- Perform brief physical exam.
- Monitor for changes to concomitant drug and non-drug treatments
- Obtain CBC, CD4 and CD8 count, and confirm lack of hematologic toxicity.
- QLQ-CIPN20 Questionnaire
- Monitor and record adverse events
- Prescribe study medication.

6.7 Visit 7 (Treatment Phase, Time 4 weeks ±3 days)

- Obtain vital signs, including weight.
- Monitor for changes to concomitant drug and non-drug treatments.
- Monitor and record adverse events.
- Perform brief physical exam.
- Obtain CD4 and CD8 count, HIV RNA viral load, HIV proviral DNA, and an additional four (4) heparin tubes of whole blood for stored PBMC and plasma.
- Obtain electrolytes, glucose, creatinine, LFTs
- Obtain CBC and confirm lack of hematologic toxicity prior to starting next 28 day cycle.
- QLQ-CIPN20 Questionnaire
- Prescribe study medication.

6.8 Visit 8 (Treatment Phase, Time 6 weeks ±3 days)

- Obtain vital signs, including weight.
- Monitor for changes to concomitant drug and non-drug treatments.
- Monitor and record adverse events.
- Perform brief physical exam
- Obtain CBC, electrolytes, glucose, creatinine, LFTs
- Prescribe study medication.QLQ-CIPN20 Questionnaire
- Draw ixazomib trough level (pre dose sample for drug level determination) and 1 hour post-dose level.

6.9 Visit 9 (Treatment Phase, Time 8 weeks ±3 days)

- Obtain vital signs, including weight.
- Monitor for changes to concomitant drug and non-drug treatments.
- Monitor and record adverse events.
- Perform brief physical exam.
- Obtain CBC, CD4 and CD8 count, HIV RNA viral load, HIV proviral DNA and an additional four (4) heparins tube of whole blood for stored PBMC and plasma.
- Obtain electrolytes, glucose, creatinine, LFTs
- QLQ-CIPN20 Questionnaire
- Prescribe study medication.

6.10 Visit 10 (Treatment Phase, Time 12 weeks ±3 days)

- Obtain vital signs, including weight.
- Monitor for changes to concomitant drug and non-drug treatments.
- Monitor and record adverse events.
- Perform brief physical exam.
- Obtain CBC, CD4 and CD8 count, HIV RNA viral load, HIV proviral DNA and an additional four (4) heparin tubes of whole blood for stored PBMC and plasma.
- Obtain electrolytes, glucose, creatinine, LFTs
- QLQ-CIPN20 Questionnaire
- Prescribe study medication.

6.11 Visit 11 (Treatment Phase, Time 16 weeks ±3 days)

- Obtain vital signs, including weight.
- Monitor for changes to concomitant drug and non-drug treatments.
- Monitor and record adverse events.
- Perform brief physical exam.
- Obtain CBC, CD4 and CD8 count, HIV RNA viral load, HIV proviral DNA and an additional four (4) heparin tubes of whole blood for stored PBMC and plasma.
- Obtain electrolytes, glucose, creatinine, LFTs
- QLQ-CIPN20 Questionnaire
- Prescribe study medication.

6.12 Visit 12 (Treatment Phase, Time 20 Weeks ±3 days)

- Obtain vital signs, including weight.
- Monitor for changes to concomitant drug and non-drug treatments.
- Monitor and record adverse events.
- Perform brief physical exam.

- Obtain CBC, CD4 and CD8 count, HIV RNA viral load, HIV proviral DNA and an additional four (4) heparin tubes of whole blood for stored PBMC and plasma.
- Obtain electrolytes, glucose, creatinine, LFTs
- QLQ-CIPN20 Questionnaire
- Prescribe study medication.

6.13 Visit 13 (Leukapheresis, Time 22 weeks ±3 days)

- Leukapheresis performed at Clinical Apheresis Unit, Charlton 8th floor.
- Monitor and record adverse events.
- Obtain CBC.
- Draw ixazomib trough level (pre dose sample for drug level determination) and 1 hour post-dose level.

6.14 Visit 14 (Treatment Completion, Time 24 weeks ±3 days)

- Obtain vital signs, including weight.
- Monitor for changes to concomitant drug and non-drug treatments.
- Monitor and record adverse events.
- Perform brief physical exam.
- Obtain CBC, CD4 count, HIV RNA viral load, HIV proviral DNA and an additional four (4) heparin tubes of whole blood for stored PBMC and plasma.
- Obtain electrolytes, glucose, creatinine, LFTs
- QLQ-CIPN20 Questionnaire
- Obtain ECG.

6.15 Visit 15 (Final Study Visit, Time 28 weeks ±3 days)

- Obtain vital signs, including weight.
- Monitor for changes to concomitant drug and non-drug treatments.
- Monitor and record adverse events.
- Perform brief physical exam.
- Obtain CBC, CD4 count, HIV RNA viral load, HIV proviral DNA
- Obtain electrolytes, glucose, creatinine, LFTsQLQ-CIPN20 Questionnaire

6.16 Table 1. Table of Study Activities

Study Activity	V 1	V 2	V 3[c]	V 3.5	V 4	V 5	V 6	V 7	V 8	V 9	V 10	V 11	V 12	V 13	V 14	V 15
Eligibility Screening	X															
Peripheral Neuropathy self-reported questionnaire	X		X		X	X	X	X	X	X	X	X	X		X	X
Informed consent	X															
Medical History	X		X		X	X	X	X	X	X	X	X	X		X	X
Concurrent meds	X															
Complete physical exam	X															
Brief physical exam			X		X	X	X	X	X	X	X	X	X		X	X
Vital Signs	X		X		X	X	X	X	X	X	X	X	X		X	X
CD4/CD8 T cell counts	X						X	X		X	X	X	X		X	X
HIV RNA Viral load	X							X		X	X	X	X		X	X
HIV Proviral DNA	X							X		X	X	X	X		X	X
CBC w/diff, platelet [a]	X		X		X	X	X	X	X	X	X	X	X	X	X	X
Heparin tubes for PBMC and plasma storage	X		X	X				X		X	X	X	X		X	
Serum chemistry [b]	X					X		X	X	X	X	X	X		X	X
Pre- and post-dose ixazomib level									X					X		
Adverse event evaluation					X	X	X	X	X	X	X	X	X		X	X
Urine Pregnancy test	X															
Leukapheresis		X												X		

a – Increased blood draw frequency may be required in the presence of hematologic toxicity. This will occur prior to each dose of ixazomib. In addition, increased blood draw frequency may be required if ixazomib is dosed weekly uninterrupted in Cycles 4-6.
b - Potassium, Creatinine, Alkaline phosphatase, ALT, AST, Total bilirubin, Direct bilirubin, Sodium, BUN, glucose, Chloride, Bicarbonate.
c- Visits 1 and 2 can occur on the same date, as long as they occur before visit 3.

6.17 Table 2: Instructions for treatment delays and dose reductions prior to ixazomib administration

CTCAE System/Organ/ Class (SOC)	**ADVERSE EVENT**	**AGENT**	**ACTION****
BASED ON INTERVAL ADVERSE EVENT			

CTCAE System/Organ/ Class (SOC)	ADVERSE EVENT	AGENT	ACTION**
Investigations	Grade 2 thrombocytopenia (PLT count <75,000 - 50,000/mm^3) and/or neutropenia <1500 - 1000/mm^3	Ixazomib	Ixazomib dose should be omitted until the PLT and/or ANC has recovered to baseline values (counts >lower limit of normal). Ixazomib may be reinitiated at the same dose if the recovery has occurred in less than 7days. If the abnormality lasts ≥7 days or recurs, then the dose of ixazomib should be reduced by one dose level. If a patient is already at the lowest dose level, ixazomib should be permanently discontinued. Patients should have CBC checked weekly until resolution of abnormality.
	≥Grade 3 thrombocytopenia and/or neutropenia		Permanently discontinue ixazomib
	≥Grade 2 increase in AST, ALT or total bilirubin	Ixazomib	Ixazomib dose should be omitted until the abnormal LFT has recovered to baseline values. Ixazomib may be reinitiated at the same dose if the recovery has occurred in less than 7days. If the abnormality lasts ≥7 days or recurs, then the dose of ixazomib should be reduced by one dose level. If a patient is already at the lowest dose level, ixazomib should be permanently discontinued. Patients should have LFTs checked weekly until resolution of abnormality.
Skin and subcutaneous tissue disorders	Any Grade ≤2 Rash	Ixazomib	Omit ixazomib until rash resolves. Restart at same dose. If the rash recurs, reduce dose by one dose level. If a patient is already at the lowest ixazomib dose, ixazomib should be permanently discontinued.
	Any rash, ≥Grade 3	Ixazomib	Ixazomib should be permanently discontinued.

CTCAE System/Organ/ Class (SOC)	ADVERSE EVENT	AGENT	ACTION**
Nervous System Disorders	Grade 1 peripheral neuropathy	Ixazomib	Ixazomib should be permanently discontinued.
Other	Any Grade 3 AE (except if there is clear evidence that it is not ixazomib related – i.e. a plausible alternative reason).	Ixazomib	Permanently discontinue ixazomib.
	Grade 4 AE	Ixazomib	Permanently discontinue ixazomib
	Plasma HIV RNA >500copies/ml on 2 consecutive occasions. (except if there is clear evidence that it is not ixazomib related – i.e. a plausible alternative reason – for example missed ART doses).	Ixazomib	Permanently discontinue ixazomib. Perform HIV genotypic resistance testing.

Use the NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.0* unless otherwise specified for all Adverse Events.

6.18 Excluded Concomitant Medications and Procedures

The following medications and procedures are prohibited during the study.

Systemic treatment with any of the following metabolizing enzyme inducers should be avoided, unless there is no appropriate alternative medication for the patient's use:

- Strong CYP3A inducers: rifampin, rifapentine, rifabutin, carbamazepine, phenytoin, and phenobarbital

- Excluded foods and dietary supplements include St. John's wort.

6.19 Permitted Concomitant Medications and Procedures

The following medications and procedures are permitted during the study:

- Antiemetics, including 5-HT3 serotonin receptor antagonists, may be used at the discretion of the investigator.
- Loperamide or other antidiarrheal should be used for symptomatic diarrhea at discretion of the investigator. The dose and regimen will be according to institutional guidelines. fluid replacement should be given to prevent volume depletion.
- Antiviral therapy (in addition to the patient's antiretroviral regimen) such as acyclovir may be administered if medically appropriate.
- Supportive measures consistent with optimal patient care may be given throughout the study.

6.20 Precautions and Restrictions

Fluid deficit should be corrected before initiation of treatment and during ixazomib treatmentNonsteroidal anti-inflammatory drugs (NSAIDs) should be avoided with impaired renal function given reported NSAID-induced renal failure in patients with decreased renal function.

The NNRTIs etravirine and efavirenz are moderate CYP3A4 enzyme inducers and have potential to decrease systemic exposure to ixazomib. That possibility will be explored by measuring ixazomib levels in all patients and comparing ixazomib concentrations in patients on different ART regimens.

Pregnancy

It is not known what effects ixazomib has on human pregnancy or development of the embryo or fetus. Therefore, female patients participating in this study should avoid becoming pregnant, and male patients should avoid impregnating a female partner. Male patients should use effective methods of contraception through defined periods during and after study treatment as specified below.

Female patients must meet 1 of the following:

Postmenopausal for at least 1 year before the screening visit, or surgically sterile.

Regardless of above criteria, females must have a negative urine pregnancy test prior to receiving the 1st dose of ixazomib.

Male patients, even if surgically sterilized (ie, status postvasectomy) must agree to 1 of the following:

- Practice effective barrier contraception AND a second method of contraception for female partners of childbearing potential during the entire study treatment period and through 90 days after the last dose of ixazomib, or

- Agree to practice true abstinence, when this is in line with the preferred and usual lifestyle of the subject. (Periodic abstinence [eg, calendar, ovulation, symptothermal, postovulation methods for the female partner] and withdrawal are not acceptable methods of contraception.)

- AND

- Agree to forego sperm donation for the same period as above.

6.21 Management of Clinical Events

Adverse drug reactions such as thrombocytopenia, diarrhea, fatigue, nausea, vomiting, and rash have been associated with ixazomib treatment. Management guidelines regarding these events are outlined below. Further details of management of ixazomib AEs are described in the ixazomib IB.

Prophylaxis Against Risk of Reactivation of Herpes Infection

Patients may be at an increased risk of infection including reactivation of herpes zoster and herpes simplex viruses. Antiviral therapy such as acyclovir, valacyclovir, or other antivirals may be initiated as clinically indicated.

Nausea and/or Vomiting

Standard anti-emetics including 5-hydroxytryptamine 3 serotonin receptor antagonists are recommended for emesis if it occurs once treatment is initiated; prophylactic anti-emetics may also be considered at the physician's discretion. Fluid deficit should be corrected before the patient takes ixazomib.

Diarrhea

Prophylactic antidiarrheals will not be used in this protocol. However, diarrhea should be managed according to clinical practice, including the administration of antidiarrheals once infectious causes are excluded. Fluid intake should be maintained to avoid dehydration. Fluid deficit should be corrected before the patient takes ixazomib.

Erythematous Rash With or Without Pruritus

As with bortezomib, rash with or without pruritus has been reported with ixazomib, primarily at the higher doses tested and when given with agents where rash is an overlapping toxicity. The rash may range from limited erythematous areas, macular and/or small papular bumps that may or may not be pruritic over a few areas of the body, to a more generalized eruption that is predominately on the trunk or extremities. Rash has been most commonly characterized as maculopapular or macular. To date, when it does occur, rash is most commonly reported within the first 3 cycles of therapy. The rash is often transient, self-limiting, and is typically Grade 1 to 2 in severity.

Symptomatic measures such as antihistamines or corticosteroids (oral or topical) have been successfully used to manage rash and have been used prophylactically in subsequent cycles. The use of a topical, IV, or oral steroid (eg, prednisone ≤ 10 mg per day or equivalent) is permitted. Management of a Grade 3 rash may require intravenous antihistamines or corticosteroids. Administration of ixazomib (and/or other causative agent if given in combination) should be modified per protocol and re-initiated at a reduced level from where rash was noted (also, per protocol).

In line with clinical practice, dermatology consult and biopsy of Grade 3 or higher rash or any SAE involving rash is recommended. Prophylactic measures should also be considered if a patient has previously developed a rash (eg, using a thick, alcohol-free emollient cream on dry areas of the body or oral or topical antihistamines). The rare risks of Stevens-Johnson syndrome, TEN, DRESS syndrome and pemphigus vulgaris have been reported in oncology studies when ixazomib (or placebo) has been given in a multi-drug regimen, with concomitant medications known to cause rash, and/or in the setting of confounding treatment emergent adverse events. These severe, potentially life-threatening, or deadly conditions may involve rash with skin peeling and mouth sores and should be clinically managed according to standard medical practice. Study medication should be discontinued in the event of severe, potentially life-threatening rash. Punch biopsies for histopathological analysis are encouraged at the discretion of the investigator.

Thrombocytopenia and anemia

Blood counts should be monitored regularly as outlined in the protocol with additional testing obtained according to standard clinical practice. Thrombocytopenia has been seen in patients with cancer and patients that were treated with combination anti-cancer therapy which included ixazomib, and may be severe but has been manageable with platelet transfusions according to standard clinical practice. Ixazomib administration should be modified as noted as

per dose modification recommendations in the protocol when thrombocytopenia occurs (See Page 39 above). Therapy can be reinitiated at a reduced level upon recovery of platelet counts. A rare risk is thrombotic thrombocytopenic purpura (TTP), a rare blood disorder where blood clots form in small blood vessels throughout the body characterized by thrombocytopenia, petechiae, fever, or possibly more serious signs and symptoms. TTP should be managed symptomatically according to standard medical practice. Red blood cell transfusions should be administered for the treatment of anemia according to standard clinical practice.

Neutropenia

Blood counts should be monitored regularly as outlined in the protocol with additional testing obtained according to standard clinical practice. Neutropenia may be severe but has been manageable. Ixazomib administration should be modified as noted as per dose modification recommendations in the protocol when neutropenia occurs (see Page 39 above). Therapy can be reinitiated at a reduced level upon recovery of ANCs.

Fluid Deficit

Dehydration should be avoided since ixazomib may cause vomiting, diarrhea, and dehydration. Acute renal failure has been reported in patients treated with ixazomib, commonly in the setting of the previously noted gastrointestinal toxicities and dehydration.

Fluid deficit should be corrected before initiation of ixazomib and as needed during treatment to avoid dehydration.

Hypotension

Symptomatic hypotension and orthostatic hypotension with or without syncope have been reported with ixazomib. Blood pressure should be closely monitored while the patient is on study treatment and fluid deficit should be corrected as needed, especially in the setting of concomitant symptoms such as nausea, vomiting, diarrhea, or anorexia. Patients taking medications and/or diuretics to manage their blood pressure (for either hypo- or hypertension) should be managed according to standard clinical practice, including considerations for dose adjustments of their concomitant medications during the course of the trial. Fluid deficit should be corrected before initiation of ixazomib and as needed during treatment to avoid dehydration.

Posterior Reversible Encephalopathy Syndrome

One case of posterior reversible encephalopathy syndrome, which ultimately resolved, has been reported with ixazomib. This condition is characterized by headache, seizures and visual loss, as well as abrupt increase in blood pressure. Diagnosis may be confirmed by

magnetic resonance imaging (MRI). If the syndrome is diagnosed or suspected, symptom-directed treatment should be maintained until the condition is reversed by control of hypertension or other instigating factors.

Transverse Myelitis

Transverse myelitis has also been reported with ixazomib. It is not known if ixazomib causes transverse myelitis; however, because it happened to a patient receiving ixazomib, the possibility that ixazomib may have contributed to transverse myelitis cannot be excluded.

7 Statistical Plan

7.1 Sample Size Determination

For this Phase I/II single-center study, the sample size is primarily determined by the estimated number of patients that can be successfully recruited during the study time period.

7.2 Statistical Methods

Descriptive Statistics

Univariate descriptive statistics and frequency distributions will be calculated, as appropriate for all variables. Baseline values for demographic, clinical, and outcome variables will be tabulated for the treatment groups. These analyses will help identify potential confounding variables to be used as covariates in sensitivity analyses. Putative prognostic variables that will be investigated through these descriptive analyses include variables such as age, sex, race/ethnicity, and duration of HIV infection, baseline CD4 T cell count and HIV viral load, mode of transmission, duration of ART, and duration of viral suppression.

Multiplicity

No *a priori* correction to the type I error rate is warranted for this study.

Primary Analysis

Ixazomib will be safe and well tolerated in HIV positive persons on suppressive ART.

Description statistics will be used to describe the rates of adverse effects by CTC grade of ixazomib among the dosing groups.

Secondary Analysis 1:

Ixazomib will reduce HIV DNA and replication competent viral reservoir size and improve CD4 T cell counts in HIV positive patients on suppressive ART.

To test this hypothesis, a mixed model will be estimated for each outcome separately. This model will consider linear trajectories and more flexible distribution of outcomes over time using each assessment point as a factor (i.e., a "repeated measures ANOVA formulation"). Given the limitations of the sample size, random effects for only subject (random intercept) models will be considered. These models will examine the treatment by time interaction and the linear trajectory model will be expected to have greater, but relatively poor, power to detect differences in outcomes such as viral load over time. An alpha of 0.1 or lower will be considered sufficiently significant to serve as preliminary evidence of a biologic effect to warrant further studies.

In the event the models are not able to be estimated from the data available, a planned post-hoc analysis will compare changes in secondary endpoints between participants on integrase inhibitor-based ART and non-nucleoside reverse transcriptase-based ART.

Interim Analysis

There is no interim analysis in this protocol, each cohort/dose level will be reviewed by the DSMB and decide upon escalation to the next dose level. This review will be conducted when all treated patients/cohort have received 4 weeks of treatment, or earlier if a DLT has occurred. Thereafter DSMB will review each cohort monthly.

7.3 Subject Population(s) for Analysis

The per-protocol analysis set will be of participants followed according to the study schedule. This subset of the entire study sample will be of particular interest to the mechanistic studies since all data will be available. The results of this potentially non-representative subset will be compared to the results obtained using the Safety evaluable Analysis Set as a sensitivity analysis. Should the results disagree qualitatively, the ITT results will be considered the less-biased results; however, a careful examination of putative causes for the differences will be fully investigated to inform a Phase II/III confirmatory study.

The Safety Evaluable Analysis Set will consist of all participants that have at least one dose of study medication. This analysis set will serve as the basis for the primary analysis noted above and adverse event reporting of treatment emergent and/or worsening events.

8 Safety and Adverse Events

8.1 Definitions

Unanticipated Problems Involving Risk to Subjects or Others (UPIRTSO)

Any unanticipated problem or adverse event that meets the following three criteria:

- Serious: Serious problems or events that results in significant harm, (which may be physical, psychological, financial, social, economic, or legal) or increased risk for the subject or others (including individuals who are not research subjects). These include: (1)

death; (2) life threatening adverse experience; (3) hospitalization - inpatient, new, or prolonged; (4) disability/incapacity - persistent or significant; (5) birth defect/anomaly; (6) breach of confidentiality and (7) other problems, events, or new information (i.e. publications, DSMB reports, interim findings, product labeling change) that in the opinion of the local investigator may adversely affect the rights, safety, or welfare of the subjects or others, or substantially compromise the research data, **AND**

- Unanticipated: (i.e. unexpected) problems or events are those that are not already described as potential risks in the protocol, consent document, not listed in the Investigator's Brochure, or not part of an underlying disease. A problem or event is "unanticipated" when it was unforeseeable at the time of its occurrence. A problem or event is "unanticipated" when it occurs at an increased frequency or at an increased severity than expected, AND
- Related: A problem or event is "related" if it is possibly related to the research procedures.

Adverse Event

An untoward or undesirable experience associated with the use of a medical product (i.e. drug, device, biologic) in a patient or research subject. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal (investigational) product whether or not it is related to the medicinal product. This includes any newly occurring event, or a previous condition that has increased in severity or frequency since the administration of ixazomib.

An abnormal laboratory value will not be assessed as an AE unless that value leads to discontinuation or delay in treatment, dose modification, therapeutic intervention, or is considered by the investigator to be a clinically significant change from baseline.

Serious Adverse Event

Adverse events are classified as serious or non-serious. Serious problems/events can be well defined and include;

- death
- life threatening adverse experience
- hospitalization
- inpatient, new, or prolonged; disability/incapacity
- persistent or significant birth defect/anomaly

and/or per protocol may be problems/events that in the opinion of the sponsor-investigator may have adversely affected the rights, safety, or welfare of the subjects or others, or substantially compromised the research data.

All adverse events that do not meet any of the criteria for serious, should be regarded as **non-serious adverse events**.

Adverse Event Reporting Period

For this study, the study treatment follow-up period is defined as 30 days following the last administration of study treatment.

Preexisting Condition

A preexisting condition is one that is present at the start of the study. A preexisting condition should be recorded as an adverse event if the frequency, intensity, or the character of the condition worsens during the study period.

General Physical Examination Findings

At screening, any clinically significant abnormality should be recorded as a preexisting condition. At the end of the study, any new clinically significant findings/abnormalities that meet the definition of an adverse event must also be recorded and documented as an adverse event.

Post-study Adverse Event

All unresolved adverse events should be followed by the sponsor-investigator until the events are resolved, the subject is lost to follow-up, or the adverse event is otherwise explained. At the last scheduled visit, the sponsor-investigator should instruct each subject to report, to the sponsor-investigator, any subsequent event(s) that the subject, or the subject's personal physician, believes might reasonably be related to participation in this study.

Table 3. Adverse Events to be Graded at Each Evaluation

SYSTEM ORGAN CLASS	Adverse event/Symptoms	Baseline	Each evaluation
Investigations	Creatinine increased	X	X
	Neutrophil count decreased	X	X
	Platelet count decreased	X	X
General disorders and administration site conditions	Edema limbs	X	X
	Fatigue	X	X
Gastrointestinal Disorders	Nausea	X	X
	Vomiting	X	X
	# of Stools	X	
	Diarrhea		X
	Constipation		X
Infections and infestations	Sepsis	X	X
Blood and lymphatic system disorders	Febrile neutropenia	X	X
Skin and subcutaneous tissue disorders	Rash, maculopapular	X	X
Nervous system	Peripheral sensory neuropathy	X	X

disorders	Peripheral motor neuropathy	X	X
	Cognitive disturbance	X	X

Hospitalization, Prolonged Hospitalization or Surgery

Any adverse event that results in hospitalization or prolonged hospitalization should be documented and reported as a serious adverse event unless specifically instructed otherwise in this protocol. Any condition responsible for surgery should be documented as an adverse event if the condition meets the criteria for an adverse event.

Neither the condition, hospitalization, prolonged hospitalization, nor surgery are reported as an adverse event in the following circumstances:

- Hospitalization or prolonged hospitalization for diagnostic or elective surgical procedures for a preexisting condition. Surgery should **not** be reported as an outcome of an adverse event if the purpose of the surgery was elective or diagnostic and the outcome was uneventful.
- Hospitalization or prolonged hospitalization required to allow efficacy measurement for the study.
- Hospitalization or prolonged hospitalization for therapy of the target disease of the study, unless it is a worsening or increase in frequency of hospital admissions as judged by the clinical investigator.

8.2 Recording of Adverse Events

At each contact with the subject, the study team must seek information on adverse events by specific questioning and, as appropriate, by examination. AEs may be spontaneously reported by the patient and/or in response to an open question from study personnel or revealed by observation, physical examination, or other diagnostic procedures. Any clinically relevant deterioration in laboratory assessments or other clinical finding is considered an AE. When possible, signs and symptoms indicating a common underlying pathology should be noted as one comprehensive event. For serious AEs, the investigator must determine both the intensity of the event and the relationship of the event to ixazomib administration.

Information on all adverse events should be recorded immediately in the source document, and also in the appropriate adverse event section of the case report form (CRF). All clearly related signs, symptoms, and abnormal diagnostic, laboratory or procedure results should recorded in the source document.

All adverse events occurring during the study period must be recorded. The clinical course of each event should be followed until resolution, stabilization, or until it has been ultimately determined that the study treatment or participation is not the probable cause. Serious adverse events that are still ongoing at the end of the study period must be followed up, to determine the final outcome. Any serious adverse event that occurs after the study period and is considered to be at least possibly related to the study treatment or study participation should be recorded and reported immediately.

8.3 Reporting of Serious Adverse Events and Unanticipated Problems

When an adverse event has been identified, the study team will take appropriated action necessary to protect the study participant and then complete the Study Adverse Event Worksheet and log. The sponsor-investigator will evaluate the event and determine the necessary follow-up and reporting required.

8.3.1 Sponsor-Investigator reporting: notifying the Mayo IRB

The sponsor-investigator will report to the Mayo IRB any UPIRTSOs and Non-UPIRTSOs according to the Mayo IRB Policy and Procedures. According to Mayo IRB Policy any serious adverse event (SAE) which the Principal Investigator has determined to be a UPIRTSO must be reported to the Mayo IRB as soon as possible but no later than 5 working days after the investigator first learns of the problem/event.

Information collected on the adverse event worksheet (and entered in the research database) will include:

- Subject's name:
- Medical record number:
- Disease/histology (if applicable):
- The date the adverse event occurred:
- Description of the adverse event:
- Relationship of the adverse event to the research (drug, procedure, or intervention):
- If the adverse event was expected:
- The severity of the adverse event:
- If any intervention was necessary:
- Resolution: (was the incident resolved spontaneously, or after discontinuing treatment)
- Date of Resolution:

The investigator will review all adverse event reports to determine if specific reports need to be made to the IRB and FDA. The sponsor-investigator will sign and date the adverse event report when it is reviewed. For this protocol, only directly related SAEs/UPIRTSOs will be reported to the IRB.

8.3.2 Sponsor-Investigator reporting: Notifying the FDA

The sponsor-investigator will report to the FDA all unexpected, serious suspected adverse reactions according to the required IND Safety Reporting timelines, formats and requirements.

Unexpected fatal or life threatening suspected adverse reactions where there is evidence to suggest a causal relationship between the ixazomib and the adverse event, will be reported as a serious suspected adverse reaction. This will be reported to the FDA on FDA Form 3500A, no later than 7 calendar days after the sponsor-investigator's initial receipt of the information about the event.

Other unexpected serious suspected adverse reactions where there is evidence to suggest a causal relationship between the ixazomib and the adverse event, will be reported as a serious suspected adverse reaction. This will be reported to the FDA on FDA Form 3500A, no later than 15 calendar days after the sponsor-investigator's initial receipt of the information about the event.

Any clinically important increase in the rate of serious suspected adverse reactions over those listed in the protocol or product insert will be reported as a serious suspected adverse reaction. This will be reported to the FDA on FDA Form 3500A no later than 15 calendar days after the sponsor-investigator's initial receipt of the information about the event.

Findings from other studies in human or animals that suggest a significant risk in humans exposed to the drug will be reported. This will be reported to the FDA on FDA Form 3500A, no later than 15 calendar days after the sponsor-investigators initial receipt of the information about the event.

AEs which are serious must be reported to Millennium Pharmacovigilance (or designee) from the first dose of ixazomib through 30 days after administration of the last dose of ixazomib. Any SAE that occurs at any time after completion of ixazomib treatment or after the designated follow-up period that the sponsor-investigator and/or sub-investigator considers to be related to ixazomib must be reported to Millennium Pharmacovigilance (or designee). In addition, new primary malignancies that occur during the follow-up periods must be reported, regardless of causality to ixazomib, for a minimum of three years after the last dose of ixazomib, starting from the first dose of ixazomib. All new cases of primary malignancy must be reported to Millennium Pharmacovigilance (or designee).

Planned hospital admissions or surgical procedures for an illness or disease that existed before the patient was enrolled in the trial are not to be considered AEs unless the condition deteriorated in an unexpected manner during the trial (e.g., surgery was performed earlier or later than planned). All SAEs should be monitored until they are resolved or are clearly determined to be due to a patient's stable or chronic condition or intercurrent illness(es).

Since this is an investigator-initiated study, the principal investigator is responsible for reporting serious adverse events (SAEs) to any regulatory agency and to the Mayo Clinic's IRB.

Regardless of expectedness or causality, all SAEs (including serious pretreatment events) must also be reported in English to Millennium Pharmacovigilance (or designee):

Fatal and Life Threatening SAEs within 24 hours of the sponsor-investigator's observation or awareness of the event

All other serious (non-fatal/non life threatening) events within 4 calendar days of the sponsor-investigator's observation or awareness of the event

See below for contact information for the reporting of SAEs to Millennium Pharmacovigilance.

The sponsor-investigator must fax or email the SAE Form per the timelines above. A sample of an SAE Form will be provided.

The SAE report must include at minimum:

- **Event term(s)**
- **Serious criteria**
- **Intensity of the event(s):** Sponsor-investigator's or sub-investigator's determination. Intensity for each SAE, including any lab abnormalities, will be determined by using the NCI CTCAE version specified in the protocol, as a guideline, whenever possible. The criteria are available online at http://ctep.cancer.gov/reporting/ctc.html.
- **Causality of the event(s):** Sponsor-investigator's or sub-investigator's determination of the relationship of the event(s) to ixazomib administration.

Follow-up information on the SAE may be requested by Millennium.

Intensity for each SAE, including any lab abnormalities, will be determined by using the NCI CTCAE version used at your institution, as a guideline, whenever possible. The criteria are available online at http://ctep.cancer.gov/reporting/ctc.html.

In the event that this is a multisite study, the sponsor-investigator is responsible to ensure that the SAE reports are sent to Millennium Pharmacovigilance (or designee) from all sites participating in the study. Sub-investigators must report all SAEs to the sponsor-investigator so that the sponsor-investigator can meet his/her foregoing reporting obligations to the required regulatory agencies and to Millennium Pharmacovigilance, unless otherwise agreed between the sponsor-investigator and sub-investigator(s).

Relationship to ixazomib for each SAE will be determined by the investigator or sub-investigator by responding yes or no to the question: Is there a reasonable possibility that the AE is associated with the ixazomib?

Sponsor-investigator must also provide Millennium Pharmacovigilance with a copy of all communications with applicable regulatory authorities related to ixazomib as soon as possible but no later than 4 calendar days of such communication.

SAE and Pregnancy Reporting Contact Information

US & Canada
Fax Number: 1-800-963-6290
Email: TakedaOncoCases@cognizant.com

Rest of World
Fax #: 1 202 315 3560

E-mail: TakedaOncoCases@cognizant.comSuggested Reporting Form:

- SAE Report Form (provided by Millennium)
- US FDA MedWatch 3500A: http://www.fda.gov/Safety/MedWatch/HowToReport/DownloadForms/default.htm
- Any other form deemed appropriate by the sponsor-investigator

8.4 Procedures for Reporting Drug Exposure During Pregnancy and Birth Events

If a woman becomes pregnant or suspects that she is pregnant while participating in this study or within 90 days after the last dose of ixazomib, she must inform the investigator immediately and permanently discontinue ixazomib . The sponsor-investigator must immediately fax a completed Pregnancy Form to the Millennium Department of Pharmacovigilance or designee (see Section 8.2). The pregnancy must be followed for the final pregnancy outcome.

If a female partner of a male patient becomes pregnant during the male patient's participation in this study, the sponsor-investigator must also immediately fax a completed Pregnancy Form to the Millennium Department of Pharmacovigilance or designee (see Section 8.2). Every effort should be made to follow the pregnancy for the final pregnancy outcome.

Suggested Pregnancy Reporting Form:

- Pregnancy Report Form (provided by Millennium)

9 ADMINISTRATIVE REQUIREMENTS

9.1 Product Complaints

A product complaint is a verbal, written, or electronic expression that implies dissatisfaction regarding the identity, strength, purity, quality, or stability of a drug product. Individuals who identify a potential product complaint situation should immediately contact Millennium (see below) and report the event. Whenever possible, the associated product should be maintained in accordance with the label instructions pending further guidance from a Millennium Quality representative.

For Product Complaints,

- Phone: 1-844-N1-POINT (1-844-617-6468)
- E-mail: GlobalOncologyMedinfo@takeda.com
- FAX: 1-800-881-6092
- Hours: Mon-Fri, 9 a.m. – 7 p.m. ET (US)

Product complaints in and of themselves are not AEs. If a product complaint results in an SAE, an SAE form should be completed and sent to Millennium Pharmacovigilance

9.2 Unmasking/Unblinding Procedures

Not applicable as this is an open-label study.

9.3 Stopping Rules

If any of the following occur:

- Death of any subject
- Anaphylactic reaction in any subject
- A life-threatening adverse event in any subject
- Any Grade 3 AE (except if there is clear evidence that it is not study drug related – i.e. a plausible alternative reason).
- Any Grade 4 AE
- A decrease in CD4 count below 350 cells/mm3
- Confirmed virologic rebound (HIV-1 RNA >500 copies/ml confirmed by a second assay done within 14 days of the initial one). If the HIV-1 viral load is >20 copies/mL and <500 copies/mL, the HIV-1 viral load will be rechecked every 2 weeks until it becomes <20 copies/mL or >500 copies/mL.

An independent Data Safety and Monitoring Committee will review the case. The committee and principal investigators will determine whether there is an association between the event and the ixazomib and make a recommendation accordingly as to whether the study should proceed or if a modification in trial design is required. If this should occur, the principal investigators will report this action to the IRB and the FDA within the timelines noted in Sections 8.3.1 and 8.3.2.

9.4 Medical Monitoring

It is the responsibility of the Principal Investigator to oversee the safety of the study at all study sites. This safety monitoring will include careful assessment and appropriate reporting of adverse events as noted above, as well as the construction and implementation of a site data and safety-monitoring plan (see section 10 "Study Monitoring, Auditing, and Inspecting"). Medical monitoring will include a regular assessment of the number and type of serious adverse events.

9.4.1 Internal Data and Safety Monitoring Board

An Internal Data and Safety Monitoring Board (DSMB) will be established based on the FDA Guidance on Establishment and Operation of Clinical Trial Data Monitoring Committees, (http://www.fda.gov/RegulatoryInformation/Guidances/ucm127069.htm). The DSMB will have a charter describing its function, standard operating procedures, responsibilities, and an analysis plan for pre-planned safety analyses. The DSMB will consist of an HIV physician (Zelalem Temesgen, Division of Infectious Diseases, Rochester MN), a clinician with expertise in clinical use of ixazomib (Vincent Rajkumar, Division of Hematology, Rochester MN), and a general infectious disease physician (Rana Chakraborty, Division of Infectious Diseases, Rochester MN). The DSMB will review the clinical and laboratory safety data after each group of patients have completed the first month of therapy and a decision will be made as to whether to proceed with enrolling the subsequent group. Subsequent review of the safety data will be done on a regular basis, at a minimum every quarter.

10 Data Handling and Record Keeping

10.1 Confidentiality

Information about study subjects will be kept confidential and managed according to the requirements of the Health Insurance Portability and Accountability Act of 1996 (HIPAA). Those regulations require a signed subject authorization informing the subject of the following:

- What protected health information (PHI) will be collected from subjects in this study
- Who will have access to that information and why
- Who will use or disclose that information
- The rights of a research subject to revoke their authorization for use of their PHI.

In the event that a subject revokes authorization to collect or use PHI, the investigator, by regulation, retains the ability to use all information collected prior to the revocation of subject authorization. For subjects that have revoked authorization to collect or use PHI, attempts should be made to obtain permission to collect at least vital status (long term survival status that the subject is alive) at the end of their scheduled study period.

10.2 Source Documents

Source data is all information, original records of clinical findings, observations, or other activities in a clinical trial necessary for the reconstruction and evaluation of the trial. Source data are contained in source documents. Examples of these original documents, and data records include: hospital records, clinical and office charts, laboratory notes, memoranda, subjects' diaries or evaluation checklists, pharmacy dispensing records, recorded data from automated instruments, copies or transcriptions certified after verification as being accurate and complete,

microfiches, photographic negatives, microfilm or magnetic media, x-rays, subject files, and records kept at the pharmacy, at the laboratories, and at medico-technical departments involved in the clinical trial.

10.3 **Data Management**

The investigator is responsible to ensure the accuracy, completeness, legibility, and timeliness of the data reported. All source documents should be completed in a neat, legible manner to ensure accurate interpretation of data. Black ink is recommended to ensure clarity of reproduced copies. When making changes or corrections, cross out the original entry with a single line, and initial and date the change. DO NOT ERASE, OVERWRITE, OR USE CORRECTION FLUID OR TAPE ON THE ORIGINAL. Data reported in the eCRF derived from source documents should be consistent with the source documents or the discrepancies should be explained.

10.4 **Data Processing**

Qualified study staff at each site will perform primary data collection from source-document reviews. This study will use eCRFs developed through a validated, Medidata RAVE, the information technology endorsed by Mayo Clinic's Clinical Trial Management System (CTMS).

10.5 Records Retention

The sponsor-investigator will maintain records and essential documents related to the conduct of the study. These will include subject case histories and regulatory documents. Subject-specific data and Case Report Forms will receive a study-specific unique code for each subject. These materials, and the subject identification code list, will be stored in the principal investigator's office in a locked cabinet so as to protect the subjects' confidentiality. Subject names or other directly identifiable information will not appear on any reports, publications, or other disclosures of clinical study outcomes.

The sponsor-investigator will retain the specified records and reports for:

1. Up to 2 years after the marketing application is approved for the drug; or, if a marketing application is not submitted or approved for the drug, until 2 years after shipment and delivery of the drug for investigational use is discontinued and the FDA has been so notified. OR
2. As outlined in the Mayo Clinic Research Policy Manual –"Access to and Retention of Research Data Policy" http://mayocontent.mayo.edu/research-policy/MSS_669717, whichever is longer.

11 Study Monitoring, Auditing, and Inspecting

11.1 Study Monitoring Plan

This study will be monitored on a routine basis during the conduct of the trial. The Mayo Clinic Office of Research Regulatory Support will provide clinical monitoring for the trial as a service for the sponsor-investigator. Clinical trial monitoring requires review of the study data generated throughout the duration of the study to ensure the validity and integrity of the data along with the protection of human research subjects. This will assist sponsor-investigators in complying with Food and Drug Administration regulations.

The investigator will allocate adequate time for such monitoring activities. The Investigator will also ensure that the monitor or other compliance or quality assurance reviewer is given access to all the study-related documents and study related facilities (e.g. pharmacy, diagnostic laboratory, etc.), and has adequate space to conduct the monitoring visit.

11.2 Auditing and Inspecting

The investigator will permit study-related monitoring, audits, and inspections by the IRB, the sponsor, and government regulatory agencies, of all study related documents (e.g. source documents, regulatory documents, data collection instruments, study data etc.). The investigator will ensure the capability for inspections of applicable study-related facilities (e.g. pharmacy, diagnostic laboratory, etc.).

Participation as an investigator in this study implies acceptance of potential inspection by government regulatory authorities and applicable compliance offices.

12 Ethical Considerations

This study is to be conducted according to United States government regulations and Institutional research policies and procedures.

This protocol and any amendments will be submitted to a properly constituted local Institutional Review Board (IRB), in agreement with local legal prescriptions, for formal approval of the study. The decision of the IRB concerning the conduct of the study will be made in writing to the sponsor-investigator before commencement of this study.

All subjects for this study will be provided a consent form describing this study and providing sufficient information for subjects to make an informed decision about their participation in this study. This consent form will be submitted with the protocol for review and approval by the IRB for the study. The formal consent of a subject, using the Approved IRB consent form, must be obtained before that subject undergoes any study procedure. The consent form must be signed by the subject or the subject's legally authorized representative, and the individual obtaining the informed consent.

13 Study Finances

13.1 Funding Source

Funding for portions of this study and ixazomib will be provided from Takeda. This funding will be used for study personnel costs, patient care costs including leukapheresis, and use of the Clinical Research and Trials Unit, study drug storage and administration, and measurements of secondary outcomes (CD4 T cells counts, HIV RNA viral loads, HIV proviral DNA, QVOA, safety and monitoring labs).

13.2 Conflict of Interest

Any study team member who has a conflict of interest with this study (patent ownership, royalties, or financial gain greater than the minimum allowable by their institution, etc.) must have the conflict reviewed by a properly constituted Conflict of Interest Committee with a Committee-sanctioned conflict management plan that has been reviewed and approved by the study sponsor-investigator prior to participation in this study.

13.3 Subject Stipends or Payments

Participants will be remunerated for participation based on the following schedule:

- Leukapheresis $150 each, for a possible total of $300.
- Research Blood draws $25 each, for a possible total of $225.
- $50 for each study visit (not leukapheresis)
- Total potential compensation $1,175.
- Patients referred from HCMC will be reimbursed for travel costs.
- Participants will also receive parking passes for each study visit.

14 Publication Plan

The principal investigators hold the primary responsibility for publication of the results of the study. This study will be registered with ClinicalTrials.gov (https://register.clinicaltrials.gov/) prior to subject recruitment and enrollment. In addition, results of the study will be posted to ClinicalTrials.gov within 12 months of final data collection for the primary outcome.

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16 Appendix I

Peripheral Neuropathy Scale
Common Terminology Criteria for Adverse Events (CTCAE) Version 4.0
Published: May 28, 2009 (v4.03: June 14, 2010)
U.S.DEPARTMENT OF HEALTH AND HUMAN SERVICES
National Institutes of Health
National Cancer Institute

Adverse Event	Grade 1	Grade 2	Grade 3	Grade 4	Grade 5
Peripheral neuropathy	Asymptomatic; clinical or diagnostic observations only; intervention not indicated	Moderate symptoms; limiting instrumental ADL	Severe symptoms; limiting self care ADL; assistive device indicated	Life-threatening consequences; urgent intervention indicated	Death
Questions	Sample answers for each toxicity grade				
Do you have problems tying your shoe laces, buttoning your shirts, fastening buckles or pulling up zippers?	"No, I might feel some tingling in my hands, but I have no problems tying laces, buttoning shirts, fastening buckles or pulling up zippers"	"It is a bit harder than before, but I can still tie laces, button shirts, fasten buckles or pull up zippers"	"I have severe difficulties tying shoe laces, buttoning shirts, fastening buckles or pulling up zippers" or "I cannot tie laces, button shirts, fasten buckles or pull up zippers anymore"	"I haven't been able to tie laces, button shirts, fasten buckles or pull up zippers for weeks"	
Do you have problems writing?	"No, I might feel some tingling in my hands, but I have no problems writing"	"It is a bit harder than before, but I can still write"	"I have severe difficulties writing" or "I cannot write anymore"	"I haven't been able to write for weeks"	
Do you have problems	"No, I might feel some	"It is a bit harder than	"I have severe difficulties	"I haven't been able to put on	

putting on your jewelry or your watch?	tingling in my hands, but I have no problems putting on my jewelry or my watch"	before, but I can still put on my jewelry or my watch"	putting on my jewelry or my watch" or "I cannot put on my jewelry or my watch anymore"	my jewelry or my watch for weeks"	
Do your have problems walking?	"No, I might feel some tingling in my feet, but I have no problems walking"	"It is a bit harder than before, but I can still walk"	"I have severe difficulties walking" or "I cannot walk anymore"	"I haven't been able to walk for weeks	

Appendix II

European Organization for Research and Treatment of Cancer QLQ-CIPN20 Questionnaire.
Lavoie Smith EM, Barton DL, Qin R, Steen PD, Aaronson NK, Loprinzi CL. Qual Life Res. 2013 Dec;22(10):2787-99. doi: 10.1007/s11136-013-0379-8. Epub 2013 Mar 30.

1.Do you have tingling fingers or hands?

2.Do you have tingling toes or feet?

3.Do you have numbness in your fingers or hands?

4.Do you have numbness in your toes or feet?

5.Do you have shooting or burning pain in your fingers or hands?

6.Do you have shooting or burning pain in your toes or feet?

7.Do you have cramps in your hands?

8.Do you have cramps in your feet?

9.Do you have problems standing or walking because of difficulty feeling the ground under your feet?

10.Do you have difficulty distinguishing between hot and cold water?

11.Do you have a problem holding a pen, which made writing difficult?

12.Do you have difficulty manipulating small objects with your fingers (for example, fastening small buttons)?

13.Do you have difficulty opening a jar or bottle because of weakness in your hands?

14.Do you have difficulty walking because your feet dropped downwards?

15.Do you have difficulty climbing stairs or getting up out of a chair because of weakness in your legs?

16.Are you dizzy when standing up from a sitting or lying position?

17.Do you have blurred vision?

18.Do you have difficulty hearing?

Please answer the following question only if you drive a car

19.Do you have difficulty using the pedals?

Please answer the following question only if you are a man

20.Do you have difficulty getting or maintaining an erection?