EProst # 20150567

A Phase II Trial of Belinostat as Consolidation Therapy with Zidovudine for Adult T-Cell Leukemia-Lymphoma (ATLL)

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Regulatory Sponsor	Juan Carlos Ramos, MD [redacted]
Funding Source(s)	Sylvester Comprehensive Cancer Center
Investigational Agent(s)	Acrotech Bioharma: Belinostat (Beleodaq®)
Other Agent(s)	Commercial Supplies: Zidovudine (AZT or Retrovir®) IFN-alfa-2b (Intron A®) PEG-IFN-alfa-2b (Pegintron®)
IND Status	IND #: Exempt

	Spectrum IND #:070789

Participating Sites/Institutions – if multicenter study coordinated by UM/SCCC	PI's name	Site's role
		Choose an item.
		Choose an item.
		Choose an item.

INVESTIGATOR AGREEMENT

I confirm that I have read this protocol, I understand it, and I will work according to this protocol and to the ethical principles stated in the latest version of the Declaration of Helsinki, the applicable ICH guidelines for good clinical practices, and the applicable federal, state, and local laws, rules, and regulations relating to the conduct of the protocol.

I have read and understand the information in the Investigators' Brochure (and/or other such pertinent safety information) regarding the risks and potential benefits.

I agree to inform all those who assist/collaborate with me in the conduct of this study of their responsibilities and obligations.

Once the protocol has been reviewed and approved by the Institutional Review Board (IRB) I understand that any change(s) made during the course of the study must also (first) be approved by the IRB prior to implementation, except when such modification is made to remove any immediate hazard(s) to the subject(s).

I certify that I and the study staff responsible have received the requisite training to conduct this research protocol.

I agree to maintain adequate and accurate records in accordance with the University of Miami policies, federal, state and local laws and regulations.

I agree to maintain the confidentiality of all information received and/or developed in connection with this protocol.

eProst Number:	
Protocol Version Number: 9.0	Protocol Version Date: 4 December, 2025

Signature of Investigator:	Date:
Name of Investigator (printed):	Institution:

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ABBREVIATIONS & DEFINITIONS

Term	Abbreviation	Definition
Failure-free Survival	FFS	The length of time from the start of treatment for a disease until documented disease progression, relapse after response or death from any cause.
Overall Survival	OS	The length of time from either the date of diagnosis or the start of treatment for a disease, that patients diagnosed with the disease are still alive.
Progression-Free Survival	PFS	The length of time during and after the treatment of a disease that a patient lives with the disease but it does not get worse.
Time to Progression	TTP	The length of time from the date of the start of treatment for a disease until the disease starts to get worse or spread to other parts of the body.

Reference: National Cancer Institute (NCI) Dictionary of Cancer Termshttp://www.cancer.gov/dictionary

PROTOCOL SYNOPSIS

<table>
<tr><td>Protocol Title</td><td>A phase II trial of belinostat as consolidation therapy with zidovudine for adult T-cell leukemia-lymphoma (ATLL).</td></tr>
<tr><td>Targeted Patient Population</td><td>• Presence of residual ATLL based on morphology, histology, flow cytometry or morphology, or T-cell clonality by gene rearrangement studies in peripheral blood (at the time of enrollment)

AND

• Treatment with prior chemotherapy or other antineoplastic agents ≥ 2 weeks prior to enrollment with the exception of dose-reduced vincristine/and or cyclophosphamide, or high dose steroids, administered for cytoreductive purpose. (Note: Continuation of zidovudine and interferon therapy is allowed)</td></tr>
<tr><td>Study Design</td><td>This is an open-label, single arm, phase II study using belinostat/AZT in combination as consolidation therapy followed by standard zidovudine (AZT)-based maintenance therapy with optional interferon-alfa-2b (IFN-alfa-2b) or pegylated interferon-alfa-2b (PEG-IFN-alfa-2b) during the study. For subjects receiving interferon therapy at baseline, the corresponding commercially available interferon, for example IFN-alfa-2b at 5 million IU daily or PEG-IFN-alfa-2b at 1.5 μg/kg once weekly (rounded up to the closest commercially available individual vial/syringe preparation), or newer forms of pegylated interferon that become available at the recommended package insert doses, may be continued. Subjects will receive 8 cycles of belinostat treatment combined with AZT (± IFN-alfa or PEG-IFN-alfa). Thereafter subjects may receive additional AZT (± IFN-alfa) for 6 more months, depending on response assessments.</td></tr>
<tr><td>Treatment Schema (see also Section 8.6.1 for Concurrent Medications/ Measures)</td><td>
<table>
<tr><th colspan="6">Regimen Description</th></tr>
<tr><th>Agent</th><th>Premedication; Precautions</th><th>Dose</th><th>Route</th><th>Schedule</th><th>Cycle Length</th></tr>
<tr><td>Belinostat[A]</td><td>see Appendix E</td><td>1,000 mg/m^2(or 750 mg/m^2[B])</td><td>IV 30 min</td><td>Days 1-5 x
8 cycles</td><td>21 days</td></tr>
</table>
</td></tr>
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<table>
<tr><td></td><td>
<table>
<tr><td>Zidovudine (AZT)[A]</td><td>see Appendix I</td><td>300mg tablet</td><td>PO</td><td>Three times daily, or continue dose given prior to enrollment</td><td rowspan="3"></td></tr>
<tr><td>(OPTIONAL) IFN-alfa-2b or</td><td>(for subjects already receiving IFN)</td><td>5 million IU</td><td>SQ</td><td>Once daily</td></tr>
<tr><td>PEG-IFN-alfa-2b</td><td>(for subjects already receiving PEG-IFN)</td><td>1.5 µg/kg (rounded up to the closest commercially available dosing preparation), or dose recommended by package insert for pegylated interferon substitutes that become commercially available</td><td>SQ</td><td>Once weekly</td></tr>
</table>
[A] Zidovudine must be administered (for patients who are AZT-experienced, as well as for patients who are AZT-naïve) at least 24-hours prior to the first dose (i.e. 24-hours before C1D1) of belinostat

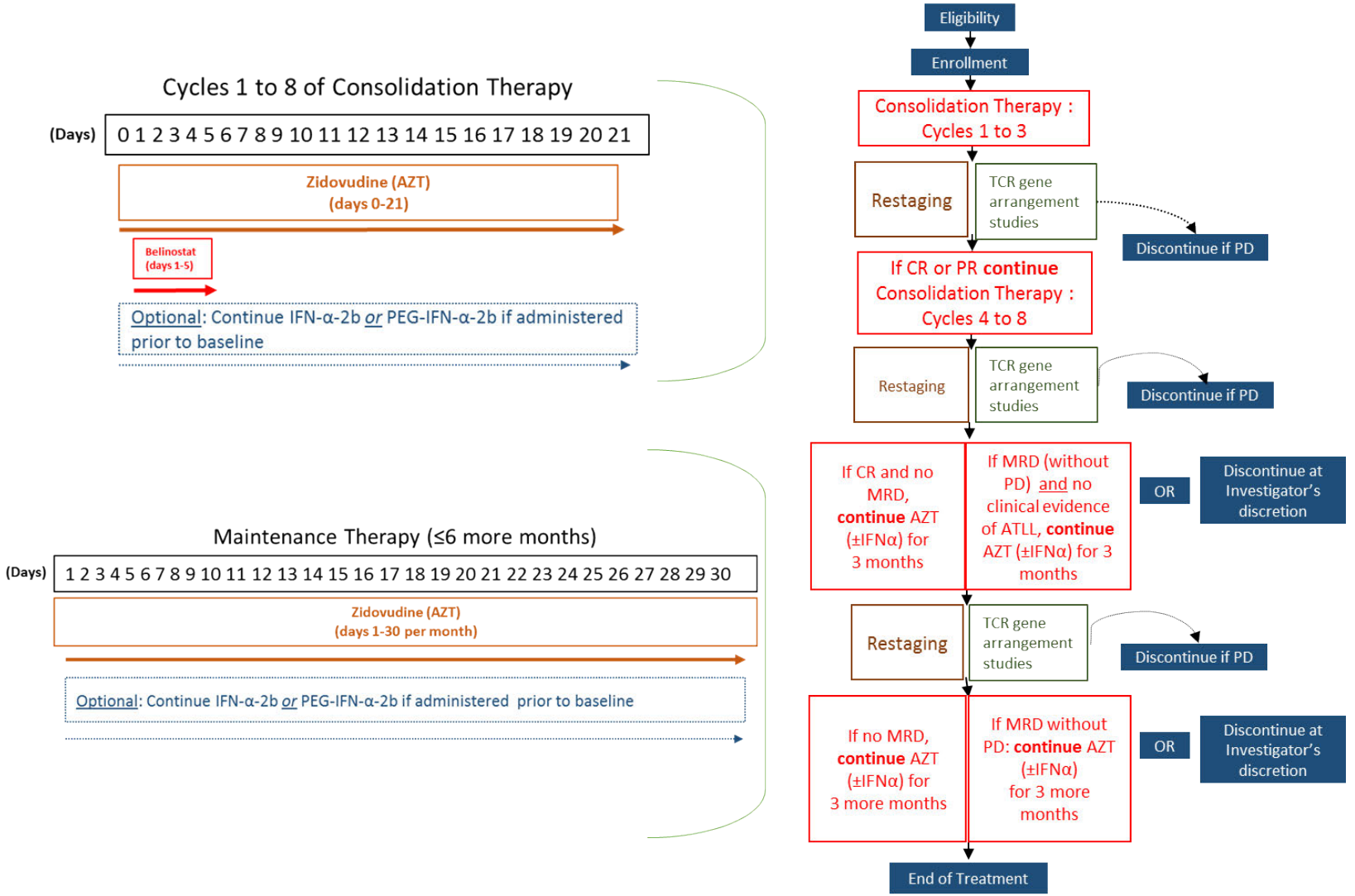
[B]As per FDA-approved Package Insert: In patients known to be homozygous for the UGT1A1*28 allele, the starting belinostat dose must be 750mg/m^2
</td></tr>
<tr><td>Duration of Treatment</td><td>Approximately six months of belinostat/AZT (+/-IFN-alfa-2b) with an additional six months of maintenance AZT (+/-IFN-alfa-2b) for at least 1 year of treatment on-study. After this, subjects who enter a complete molecular response (CMR) may stop maintenance AZT (+/-IFN-alfa-2b) or continue standard maintenance AZT +/- IFN-alfa-2b at the discretion of the treating physician. Those with molecular evidence of residual disease may continue standard maintenance AZT +/- IFN-alfa-2b at the discretion of the treating physician, or be removed from the study at the discretion of the treating physician.</td></tr>
</table>

Follow-up Required Post-Treatment	All subjects will be followed at approximately 30-days (+/-7 days) after the last dose of study treatment. Subjects who complete one year [i.e. 8 cycles of belinostat/AZT plus 6 months of AZT (±IFN-alfa-2b) maintenance] of study treatment will be followed every 3 months (+/-2 weeks) for a at least one year (up to month 24), with response assessment at months 18 and 24 (+/- 1 month). Survival data will be collected up to 5 years in all subjects. Those who stop therapy under protocol early and remain on the study will continue to be followed every 3 months (+/-2 weeks) for a at least one year, and for failure-free survival for at least 12 months or until they receive a new different treatment, whichever occurs first; they may continue all protocol study-related assessments until disease progression or until initiation of new different treatment after being removed from the study .
Objectives	Primary Objectives: • To determine the complete molecular response (CMR) rate after adding belinostat as consolidation therapy for ATLL during AZT-based maintenance treatment. • To determine the safety of adding belinostat to AZT-based regimen as consolidation therapy for ATLL. Secondary Objectives: • To evaluate the clinical response rates, minimum 1-year failure-free and overall survivals • To investigate whether belinostat disrupts HTLV-1 latency load *in vivo* • To determine whether belinostat provokes an immune or cytotoxic T-cell response *in vivo* • To determine the impact of belinostat/AZT (+/- IFNα) on HTLV-1 proviral load, a measure of HTLV-1 infected reservoirs, and clonal abundance *in vivo*. Exploratory Objectives: • To study the molecular effects of belinostat on ATLL cells *in vivo*, including on histone acetylation, expression of HTLV-1 genes, including Tax and HTLV-1 basic zipper factor (HBZ), and cellular genes and proteins that can be affected. To bank available baseline ATLL specimens in order to perform genomic studies including next generation RNA and exome sequencing, or future technologies that become available, which may help identify putative biomarkers that can predict treatment response and disease outcome.
Expected Number of Subjects	A minimum of 10 (as long as primary end point is reached) or maximum of 20

Expected Number of Centers	Sylvester Comprehensive Cancer Center *Note: SCCC is inclusive of the constituent satellite sites.*
Expected Duration of the Protocol	Expected time to complete accrual is 5 years. Expected time to study completion is approximately 6 years from date of first enrollment.
Inclusion Criteria	1. Histologically or cytologically documented adult T-cell leukemia/lymphoma (ATLL) with the following characteristics (for definition of ATLL subtypes see Appendix H): • Any stage of disease • Aggressive types (except smoldering or chronic type with favorable features, i.e. normal LDH) • Documented presence of ATLL cells in peripheral blood by either morphology, histology, flow cytometry or gene rearrangement studies Received chemotherapy or antineoplastic drug treatment ≥ 2 weeks prior to enrollment with the exception of dose-reduced vincristine/and or cyclophosphamide, or high dose steroids, administered for cytoreductive purpose. (Note: Continuation of zidovudine and interferon therapy is allowed.) 2. Presence of residual ATLL in peripheral blood either by morphology, histology, flow cytometry or gene rearrangement studies (T-cell clonality) during screening prior to enrollment. 3. Documented HTLV-1 infection: Documentation may be serologic assay (ELISA) confirmed by Western blot or polymerase chain reaction (PCR). 4. Measurable or evaluable clinical or molecular disease, which may only be detected by flow cytometry or T-cell clonality (gene rearrangement studies). 5. 18 years of age or older. 6. Karnofsky performance status (KPS) ≥ 50% or ECOG performance status ≤ 3 (See Appendix C) 7. Patients must have adequate end organ and bone marrow function as defined below: • Absolute neutrophil count (ANC)≥ 1,000 cells/mm^3[Exception: Unless cytopenias are secondary to ATLL] • Platelets (PLT) ≥ 50,000 cells/mm^3 [Exception: Unless cytopenias are secondary to ATLL] • Adequate hepatic function: transaminase ≤ 2.5 x the institutional upper limit of normal, total bilirubin ≤ 1.5 x institutional upper limit of normal, [Exception: Unless secondary to hepatic infiltration with lymphoma. If the elevated bilirubin is felt to be secondary to *indinavir* or *atazavinir* therapy (or anti-HIV medications), patients will be

	allowed to enroll.] Creatinine clearance (CrCl) ≥ 40 mL/min, unless secondary to renal involvement by lymphoma. 8. Patients who are human immunodeficiency virus positive (HIV+) are also eligible. 9. Females of childbearing potential (CBP) must have a negative serum pregnancy test within one week of enrollment. Women should avoid pregnancy while receiving study treatment. Males and females must agree to use adequate methods of birth control during participation in this trial and for 3 months after completing therapy. 10. Patients receiving erythropoietin or G-CSF from baseline are eligible. 11. Ability to understand and willingness to sign a written informed consent document.
Exclusion Criteria	1. Patients with chronic leukemia with favorable features (normal LDH level), or smoldering type ATLL (for definition of ATLL subtypes see Appendix H). 2. Patients receiving any other investigational agents within 14 days prior to initiation of study therapy. (Exception: Patients actively receiving IFN-alfa-2b, PEG-IFN-alfa-2b, or similar commercially available substitutes are permitted). 3. Uncontrolled inter-current illness including, but not limited to, ongoing or active infection, symptomatic congestive heart failure (CHF), unstable angina pectoris, cardiac arrhythmia, or psychiatric illness/social situations that are likely in the judgment of the Investigator(s) to interfere or limit compliance with study requirements/treatment. 4. Pregnant or breast-feeding women. 5. Known hypersensitivity to histone deacetylases (HDACs), zidovudine, belinostat or any other component(s) of the formulation(s). 6. Acute hepatitis or decompensated liver disease, unless due to lymphoma. Chronic hepatitis will be required to be on prophylactic treatment during the study if provided liver function test meet criteria listed above without evidence of cirrhosis induced by viral hepatitis, to be eligible. 7. Concurrent active malignancies, with the exception of *in situ* carcinoma of the cervix, non-metastatic, non-melanomatous skin cancer, or Kaposi's sarcoma not requiring systemic chemotherapy. 8. Known NYHA Class 3 or 4 heart disease as per Appendix D. 9. Known history of cardiomyopathy with ejection fraction < 45% (or lower limit of institutional normal value) or uncontrolled arrhythmia. 10. Psychological, familial, sociological or geographical conditions likely in the judgment of the Investigator(s) to interfere or limit compliance with study requirements/treatment.

PROTOCOL SCHEMA



Abbreviations: CR=Complete Response; MRD=Minimal Residual Disease; PR=Partial Response; PD=Progressive Disease

1.0 BACKGROUND

1.1 Study Disease

Adult T-cell leukemia-lymphoma (ATLL) was first described as a distinct clinical entity in 1979 and its association with the human T-cell leukemia virus type 1 (HTLV-1) was reported shortly thereafter (1). ATLL can present in multiple forms and is generally sub-classified into four subtypes (2). Lymphoma and acute ATLL are the two most aggressive variants where patients usually present with a high tumor burden and hypercalcemia. The chronic and smoldering forms of ATLL have a more indolent course, although they often progress to the more malignant forms of the disease (3).

Therapies available for ATLL, particularly for acute and lymphoma subtypes have demonstrated low clinical benefit. A large published retrospective series, which included over 800 Japanese patients with ATLL, show that the median survival times for acute and lymphomatous types were 6.2 and 10.2 months respectively regardless of treatment (2). With some of the most intensive chemotherapy regimens, complete response rates of ~35% or more have been reported (4, 5). Allogeneic bone marrow transplantation, including reduced-intensity regimens, has been successful in a number of ATLL patients although severe immunodeficiency due to both the underlying disease and preparatory regimens poses a serious risk (6, 7). Even after stem cell transplant, the majority of patients succumb to their disease.

Newly developed investigational therapies include the use of monoclonal antibodies targeting T-cell receptor molecules, such as Tac (CD25) and CCR4 (8, 9). However, a benefit of adding these drugs in combination with chemotherapy has yet to be proven in randomized studies. Such agents are also expensive and not widely available in regions where HTLV-1 is endemic, such as the Caribbean and South America.

1.2 HTLV-1 Endemic Regions and Affected Populations

HTLV-1 affects about 10-20 million people worldwide and is endemic in southwest Japan, sub-Saharan Africa, the Caribbean, and parts of South America, particularly Brazil and Peru (11). Miami in Florida (USA), due to its close proximity to the Caribbean, has a large population of immigrants from HTLV-1 endemic areas. African-American patients are also frequently diagnosed with ATLL at our institution (10). The University of Miami (UM) and affiliated hospitals represent outstanding resources for the study of HTLV-1 related diseases (12). ATLL patients are also frequently encountered in New York City (13).

1.3 Treatment of ATLL

1.3.1 *Chemotherapy*

ATLL is considered generally chemotherapy resistant, and the long-term outcome of patients with this disease is dismal. Clinical trials performed in the U.S. using standard aggressive chemotherapy have only yielded modest results. A phase II trial of 15 patients with aggressive ATLL treated with CHOP (cyclophosphamide, doxorubicin, vincristine and prednisone) in combination with daclizumab (a humanized anti-CD25 monoclonal antibody) resulted in OS of 10 months (14). Another phase II trial using EPOCH (etoposide, prednisone, vincristine, cyclophosphamide, and doxorubicin) followed by AZT

plus interferon-alfa-2b (IFNα) as maintenance showed that a significant number of subjects progressed early during their treatment, and all patients eventually relapsed (15). At UM, we have recently completed the largest retrospective study of ATLL cases in the U.S. history to this date (12), and we have not observed any cures in any patients treated with conventional chemotherapy at our institution.

In Japan, a randomized phase III study of 188 patients demonstrated a trend to overall survival advantage favoring the ranimustine-containing chemotherapy regimen VCAP-AMP-VECP over intensive CHOP (16). The complete response (CR) rate was higher in the VCAP-AMP-VECP arm as compared to biweekly CHOP (40% v 25%, respectively; p=0.020). Progression-free survival (PFS) at 1 year was 28% in the VCAP-AMP-VECP arm compared with 16% in the CHOP arm (p= 0.100, two-sided p = 0.200). Overall survival (OS) at 3 years was 24% in the VCAP-AMP-VECP arm and 13% in the CHOP arm (p= 0.085, two-sided P = 0.169). However, the VCAP-AMP-VECP was significantly more toxic resulting in universal neutropenia and 3 treatment-related deaths.

1.3.2 *Zidovudine (AZT) Plus Interferon (IFNα) Therapy*

Several phase II studies carried out outside of Japan, including at our center, have demonstrated that AZT/IFNα as induction followed by maintenance therapy can be efficacious as first line treatment in patients with leukemia-type ATLL (17-21). High response rates were noted in chemotherapy naïve with some patients achieving very prolonged responses. A recent meta-analysis suggests AZT/IFNα therapy may be superior to conventional chemotherapy for acute and chronic leukemic type ATLL in terms of overall survival (22). AZT/IFNα can have a major impact in extending survival in a subset of ATLL patients. At UM, we have observed that patients with aggressive ATLL who achieved a CR after AZT/IFNα therapy (n=16) had a significantly longer progression-free survival (PFS), as compared to those achieving a CR after conventional chemotherapy (n=35) (PFS= 48 months vs. 11 months respectively, p=0.003) (12). However, CR rate using AZT/IFNα was 24% in acute type ATLL and not significantly effective in lymphomatous type ATLL. The results of an international meta-analysis on AZT/IFNα as first-line therapy which were presented at the 13th International Conference on Human Retrovirology: HTLV and at the 49th Annual Meeting of the American Society of Hematology, showed strong promise as an option for the treatment of leukemic types of ATLL (23). AZT/IFNα is now considered an acceptable first-line therapy option for ATLL per the latest National Comprehensive Cancer Network (NCCN) recommendation guidelines.

In summary, while chemotherapy or AZT-IFNα can be effective in some patients with ATLL, response rates are suboptimal, and patients usually relapse and succumb to their disease. Therefore, new therapeutic approaches and strategies are urgently needed to treat ATLL.

1.4 **HDAC Inhibitors in ATLL**

HDAC inhibitors are broadly active agents in cancer treatment. Currently, three HDAC inhibitors (vorinostat/SAHA, romidepsin, and belinostat) are FDA-approved and clinically available for the treatment of relapsed cutaneous or peripheral T-cell lymphoma (PTCL).

The mechanism of action of HDAC inhibitors in activating the HTLV-1 promoter has been elucidated. Virtually all ATLL cells contain clonally integrated HTLV-1 provirus. The HTLV-1 promoter is regulated by histone deacetylases; histone acetylation results in HTLV-1 promoter activation (24). The HTLV-1 promoter is trans-activated by the HTLV-1 protein Tax through binding of CREB and p300/CBP at the 5'LTR (25). Tax binding to p300/CBP promotes its histone acetyl transferase (HAT) activity resulting in chromatin unwinding and transcription of the viral genome (26). The p300/CBP-mediated HAT activity is countered by HDAC1, and by the HTLV-1 basic leucine zipper factor (HBZ), which binds CREB and p300/CBP thus displacing Tax (24, 25). The HBZ gene is transcribed from the 3' end of the proviral genome, and unlike Tax, it is the only viral gene consistently expressed in ATLL (25). Overall, the effect of HDAC inhibitors is to promote the activation of the HTLV-1 provirus and expression of the viral genome.

1.5 Clinical Experience with HDAC Inhibitors in ATLL

The dual anti-neoplastic and HTLV-1 transcriptional activating role of HDAC inhibitors can be exploited in the treatment of ATLL. Selective induction of HTLV might allow the immune response under antiretroviral treatment (AZT) to clear HTLV-1 infection (27). A similar approach using the old generation HDAC inhibitor (HDI) valproic acid (VPA) in combination with AZT has been utilized in a primate (baboon) model infected with simian T-cell leukemia virus I (STLV-1), which is highly homologous and related to HTLV-1, to eradicate virus infected cells (28).

1.5.1 *Prospective Study using an HDAC Inhibitor in ATLL: University of Miami*

We have recently conducted a pilot trial for aggressive ATLL incorporating the old generation HDAC inhibitor valproic acid (VPA) during AZT/IFNα maintenance therapy entitled "Prospective Study of the Molecular Characteristics of Sensitive and Resistant Disease in Patients with HTLV-1 Associated Adult T Cell Leukemia Treated with Zidovudine (AZT) Plus Interferon alpha-2b". This study was designed to add VPA to AZT/IFN-α maintenance therapy after successful induction. We hypothesized that through HDAC inhibition, VPA would reactivate HTLV-I thus leading to an immune response against HTLV-1 and infected cells, thereby effectively eradicating blood circulating ATLL clones that normally persist after AZT/IFN-α (27). The interim results of this study were updated at the 2013 International Conference on HTLV-1 and Related Viruses conference (Montreal, Canada) (12), and the 2014 HTLV-1 European Research Network Conference (Rome, Italy). Results of this study have been reported at *ClinicalTrials.gov* (study identifier: NCT01964755). Thirteen subjects were accrued on this study (10 acute types, and 3 lymphomatous types with circulating ATLL cells). Of 12 subjects with evaluable responses, 3 patients received VPA that was added at Day 60 during maintenance therapy using AZT and pegylated-IFNα; VPA was given for a maximum of 6 months. ACR was achieved in 4 patients, 2 of which received had achieved a partial response to AZT/IFN-α prior to starting VPA. One patient relapsed shortly after beginning VPA, while the addition of VPA resulted in clinical complete remission and decrease of HTLV-1 proviral load over time in two ATLL subjects, and a molecular remission in one subject (ATLL-84) based on disappearance of signal indicative of T-cell receptor gene rearrangement on serial multiplex PCR studies at months 6, 9, and 12 (Figure 1). This patient continued to be in

clinical remission for 4 years. In past experience we had not observed such effect in long-term survivors treated with AZT/IFNα therapy alone (21).

Overall these data support the innovative notion that targeting ATLL with HDAC inhibitors under anti-retroviral therapy (i.e. AZT) treatment may eliminate ATLL clones and HTLV-1 infected reservoirs *in vivo*.

Figure 1

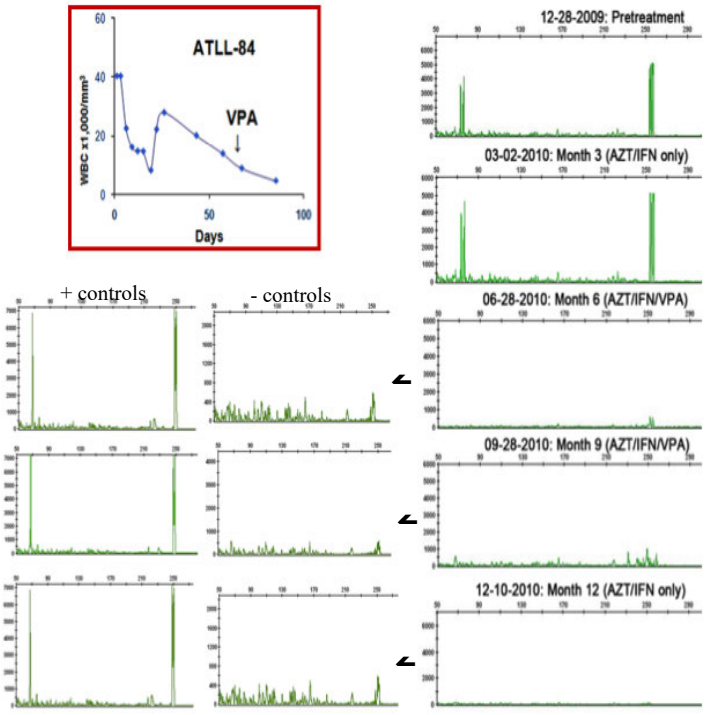


Figure 1. Hematologic and molecular response of ATLL patient to AZT/IFN-α therapy with valproic acid (VPA). Shows WBC plot over time and combined multiplex PCR analyses for acute ATLL patient (ATLL-84) during AZT/IFN-α therapy. Multiplex PCR studies were performed utilizing two sets of reaction primers including those specific to the $V_{1\text{-}8,}$ V_9 and consensus J_1 and J_2 regions of β-chain TCR genes (peaks on the right) or alternate J_1 and J_2 regions (peaks on the left) at the indicated dates (month/year). VPA was started on Day 60 (indicated by arrow on WBC plot). Positive and negative control multiplex PCR experiments using a monoclonal T-cell line and polyclonal T-cells as indicated were performed on the same dates of the experiments indicated by the corresponding arrows (🡐)

1.6 Investigational Agent: Belinostat

Belinostat is a pan-HDAC inhibitor that recently obtained accelerated approval in the U.S. for the treatment of relapsed/refractory PTCL based on efficacy and duration of response (29). The largest safety analysis of belinostat to date was conducted in the BELIEF trial (30). In this study, 120 evaluable patients with relapsed or refractory PTCL with platelets ≥ 50,000/μL who had received a median of 2 prior therapies were treated with belinostat intravenously at 1000 mg/m^2 on days 1-5 of a 3-week cycle schedule for a median of 2 cycles. The overall response rate was 26% (CR = 10%; PR = 16%). The median time to response was 5.6 weeks, the median duration of response was 8.3 months, and the longest duration of response was 29.4 months. The most common grade ¾ adverse events were thrombocytopenia and neutropenia (13%), anemia (10%), dyspnea (6%), pneumonia (6%), and fatigue (5%). The authors concluded that belinostat was well tolerated in patients with

platelet counts less than 100,000/μL. Progressive disease was observed in 64% of patients, which was the major cause for drug discontinuation.

Belinostat is also active in other hematologic malignancies and in solid tumors. As of May, 2015, a total of 34 clinical trials using belinostat alone or in combination with other chemotherapies were listed in *ClinicalTrials.gov*. At University of Miami, one patient with refractory ATLL was treated with intravenous belinostat at 1000 mg/m^2 on days 1-5 with daily oral AZT at a maximum dose of 600 mg twice daily. After four 3-week cycles, this patient tolerated treatment well and had an objective response in skin disease (unpublished data). The patient experienced no side effects to belinostat/AZT and no hematologic toxicity was observed. Based on this experience, belinostat appeared to be clinically safe in combination with the antiviral drug zidovudine.

1.7 Preclinical Efficacy of Belinostat in ATLL

ATLL is challenging to study in part due to the lack of suitable pre-clinical models. A well-known *in vitro* phenomenon is that ATLL cells express Tax immediately upon culture. Tax protein exerts wide cellular effects driving malignant growth in T-cells resulting in genetic instability and transformation through the interaction with pleotropic transcription factors such as NF-κB (31-33). However, Tax is not significantly expressed in primary ATLL tumors, which often harbor a defective provirus (33). Unfortunately, most published work describing the biology of ATLL and drug therapeutic pre-clinical studies involve Tax+ cell lines and animal models (34).

We tested the *in vitro* effects of belinostat on ATLL cells using a patient-derived low-passage cell line (JEA) established from patient ATLL-84 in our laboratory. We found that belinostat caused H3 subunit acetylation in JEA cells (low Tax-expressing) inducing the expression of high levels of Tax, resulting in cell death using concentrations within the nanoMolar range that are well below those achievable in patients in a dose-dependent manner (Figure 2 A-C). Further, belinostat blocked the expression of the HTLV-1 basic zip factor (HBZ), which is transcribed from the 3' end of the proviral genome, and therefore not subject to 5'LTR promoter regulation (Figure 3). Unlike Tax, HBZ the only viral gene consistently expressed in ATLL, and it is thought to play a major pathogenic role in ATLL, and to drive the propagation of latently infected reservoirs in patients (25). The belinostat-induced apoptosis was augmented by increasing doses of AZT that can be achieved in patients after oral administration (Figure 4). In JEA cells, the combination of IFNα 2b, AZT, and belinostast separately, resulted in higher cell death (Figure 5A). However, an interesting observation we have made is that IFNα may diminish the killing effects of bellinostat in long-established, interferon-resistant ATLL-derived cell lines and freshly isolated leukemic cells (ATLL-151 shown in Figure 5B). Based on these data and concepts, we proposed the use of belinostat with AZT for the treatment of ATLL after

disease cytoreduction using chemotherapy or AZT-IFNα, and to omit IFNα in those patients who have not received or benefited from this drug.

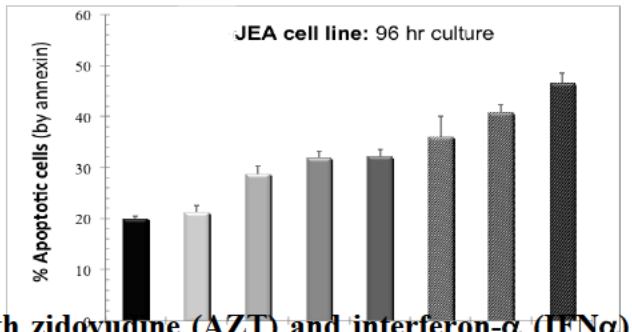


Figure 4. Zidovudine augments apopotosis in combination with belinostat in ATLL cells. Shows percent apoptosis (measured by annexin V) of JEA cells treated once with fixed dose of belinostat (BEL) and increasing concentration of zidovudine (AZT) at the indicated doses, and their combinations, after 5 days of culture. Control cells were treated with DMSO.

Figure 5. The combination of belinostat with zidovudine (AZT) and interferon-α (IFNα) augments apoptosis in ATLL cells, while IFNα may dir

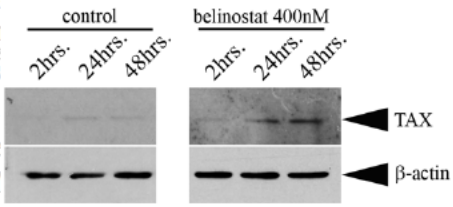
Figure 2. A. **B.**

belinostat + AZT-IFNα-resistant ATLL cells. A-B. Shows

annexin V) of JEA cell line a ic cell

once with fixed doses of AZ nd the

doses after 5 days of culture. C vith DI



C.

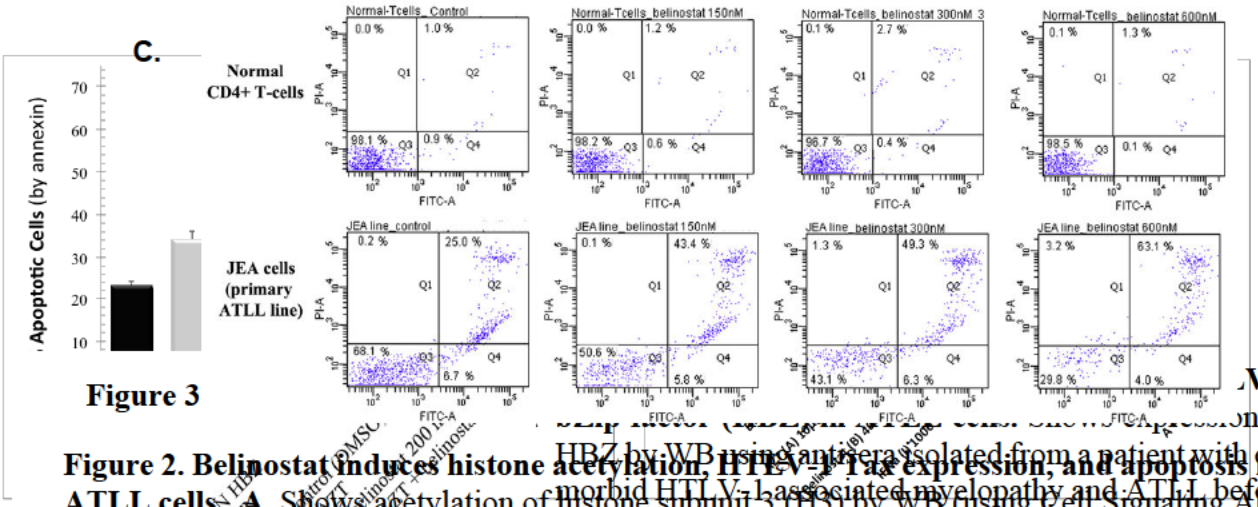


Figure 3

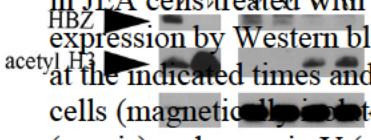


Figure 2. Belinostat induces histone acetylation, HTLV-1 Tax expression, and apoptosis in ATLL cells. A. Shows acetylation of histone subunit 3 (H3) by WB (using Cell Signaling Ab) in JEA cells treated with increasing belinostat doses for 24 hrs. **B.** Shows HTLV-1 Tax protein expression by Western blot (WB) (using Santa Cruz Ab) in JEA cells before and after belinostat at the indicated times and doses. **C.** Shows histograms from FACS analysis of normal CD4+ T-cells (magnetic bead isolated by negative selection) and JEA cells stained with propidium iodide (y-axis) and annexin V (x-axis) using increasing belinostat doses.

V-1

HBZ by WB using antisera isolated from a patient with co-morbid HTLV-1 associated myelopathy and ATLL before and after 48 hours at the indicated treatment and doses. 293TN cells transduced with HBZ-encoding vector were used as positive control. Acetylation of H3 upon belinostat treatment is also shown.

1.8 Study Rationale

ATLL is an aggressive malignancy caused by HTLV-1. ATLL cannot be cured by conventional chemotherapy thus urging the development of new therapeutic strategies. HDAC inhibitors are broadly active anti-neoplastic agents that can be exploited for the treatment of ATLL as it has been demonstrated that pharmacologic inhibition of HDACs

promotes acetylation of nucleosomes and chromatin unwinding at the HTLV-1 5' LTR, which results in transcription of the viral genome. Belinostat, a potent pan HDAC inhibitor, causes H3 subunit acetylation and induces HTLV-1 Tax expression in cultured ATLL cells resulting in dose-dependent apoptosis (Figure 2). Further, belinostat blocks HBZ, a key viral protein that is essential for propagation of HTLV-1 infected clones and increases apoptosis in the presence of AZT (Figures 3, 4, and 5).

We propose to use belinostat in combination with AZT as consolidation therapy for the treatment of ATLL. Patients will receive standard maintenance AZT-based therapy (35). Patients who are receiving interferon-alfa 2b (IFN-α) or other similar forms of IFN-alfa available at baseline and demonstrate to have derived clinical benefit may continue the drug. As our pre-clinical data suggest that IFN-α decrease the killing effect of HDAC inhibitors in some cell lines and primary fresh ATLL isolates from patient samples (Figure 5), patients who have not received IFN-α pre-study will receive AZT only along with belinostat.

Patients' blood samples will be assessed for minimal residual disease (MRD) by T-cell clonality studies (multiplex PCR) and HTLV-1 proviral loads before, during, and after belinostat treatment using assays available at our collaborating laboratories.

Table 1: UM laboratories participating in correlative studies

Investigator/ Collaborator Laboratory	**Correlative Studie(s)**
Hilda B. Ye, , PhD	Immunologic and cytotoxic T-cell assays
Anne Van den Broeke, PhD	Clonal abundance studies
Jennifer Chapman, MD	Immunophenotyping studies on ATLL tumors, and TCR Gene Rearrangement(s) via multiplex PCR
Juan Carlos Ramos, MD	HTLV-1 proviral loads and reactivation, molecular evaluations of belinostat in ATLL

Correlative studies will include measuring HTLV-1 reactivation in peripheral blood T-cells, assessing the cytotoxic T-cell response *in vivo*, and investigating the molecular effects of belinostat in ATLL cells *in vivo*. The clinicians involved in this study have a track record in treating ATLL and well-established clinics where patients will be followed closely. In this way, alternative therapies can be quickly offered to non-responders (i.e. intensive chemotherapy, targeted therapies, or allogeneic bone marrow transplant when feasible). The study will be initiated at the University of Miami first and may be expanded to committed domestic sites in New York City and international sites contingent upon available funding in the future. An average of 8-15 new ATLL cases are encountered at the University of Miami (based on trend over last 5 years). Similar number

of ATLL cases are encountered at Memorial West Hospital (Pembroke Pines, FL) and in New York City Hospitals (i.e. Weil Cornell Medical College-affiliated Columbia Presbyterian Hospital, and Albert Einstein/Montefiore Medical Center). With the participation of any of these collaborating sites in the future, we expect to exceed accrual goals under strict eligibility criteria.

1.9 Correlative Studies

1.9.1 Eradication of ATLL clones and HTLV-1 infected reservoirs

We hypothesize that adding belinostat under anti-retroviral control will result in eradication of ATLL and HTLV-1 infected reservoirs. To test this hypothesis, patients will be assessed for minimal residual disease (MRD) at specified times during treatment (after cycles 3 and 8), and at the end of months 9, 12 using multiplex PCR for T-cell receptor gene rearrangements as used previously (36). Matching DNA samples will be preserved for measuring HTLV-1 proviral loads using single molecule droplet-based digital PCR before belinostat, during treatment (Day 15, after cycles 3 and 8), and at the end of months 9 and 12.

1.9.2 Evaluation of HTLV-1 reactivation and cytotoxic T-cell immune response *in vivo*

We hypothesize that through HDAC inhibition belinostat will reactivate HTLV-1 provirus in ATLL cells and infected T-cell reservoirs in subjects. The HTLV-1 protein Tax is highly immunogenic. Therefore, HTLV-1 reactivation should provoke a potent cytotoxic T-cell response against ATLL cells and other virus harboring cell reservoirs. To confirm this biological effect *in vivo*, CD4+ T-cell enriched peripheral blood mononuclear cells (PBMC's) will be isolated before and within 48-72 hours after belinostat treatment initiation. Expression of HTLV-1 genes (Tax, gag, Pol) will be determined by single molecule droplet based digital RT-PCR. Cytotoxic T-cell lymphocyte (CTL) assays using isolated CD4+ T-cell enriched PBMC's and HTLV-1 specific generated peptides will be performed before belinostat, at Day 8, and at the end of cycle 1 (Day 21). CTL studies will be performed at Dr. Barber's laboratory (37-41).

1.9.3 Molecular effects of belinostat in ATLL

We hypothesize that belinostat will exert its anti-neoplastic effects in ATLL cells through a variety of molecular mechanisms including the activation of silenced key cellular genes (i.e. cell cycle regulators and tumor suppressors), and suppression of HBZ, which is the only HTLV-1 protein that is consistently expressed in all ATLL tumors; this protein is thought to play a major role in HTLV-1 latency and propagation of immortalized clones. To investigate the molecular effects of belinostat we will isolate CD4+ T-cell from enriched PBMCs before and shortly after belinostat treatment initiation (48-72 hours). We will determine histone acetylation, HBZ and Tax mRNA and protein levels, and expression of various cellular genes affected by viral proteins or epigenetic effects of belinostat. These studies will be performed at UM Genomics Core facilities, and at Ramos and Barber laboratories.

1.9.4 Immunohistochemistry Studies on ATLL tumors

Immunohistochemistry studies for ATLL associated markers will be performed in collaboration with the hematopathology lab (Dr. Jennifer Chapman). ATLL important markers include, but are not limited to: CD25, CD30, and MUM-1. While the sample number of this clinical trial may not be powered to detect events significantly associated with disease subtype, phenotype, malignant

course, and treatment resistance, these studies will provide us with preliminary data to conduct further exploratory studies in future.

2.0 OBJECTIVES

2.1 Primary Objectives

- To determine the complete molecular response (CMR) rate after adding belinostat as consolidation therapy for ATLL during AZT-based maintenance treatment.
- To determine the safety of adding belinostat to AZT-based regimen as consolidation therapy for ATLL.

Note: Adverse event data regarding the use of AZT (+/- IFNα or PEG-IFNα) and belinostat as consolidation treatment will be collected in this study (as per NCI CTCAE version 4.03). Strict dose modification guidelines have been established for this protocol (Section 10.0).

2.2 Secondary Objectives

- To evaluate the clinical response rates, and at minimum 1-year failure-free survival (FFS) and overall survival (OS)
- To investigate whether belinostat disrupts HTLV-1 latency *in vivo*
- To determine whether belinostat provokes an immune or cytotoxic T-cell response load *in vivo*
- To determine the impact of belinostat/AZT (+/- IFNα) on HTLV-1 proviral load as a measure of HTLV-1 infected reservoirs, and clonal abundance *in vivo*.

2.3 Exploratory Objectives

- To study the molecular mechanisms of belinostat on ATLL cells *in vivo*, including but not limited to determining its effects on histone acetylation, expression of HTLV-1 genes including Tax and HTLV-1 basic zipper factor (HBZ), and expression of various cellular genes affected by viral proteins or epigenetic effects of belinostat.
- To bank available baseline ATLL specimens in order to perform genomic studies including next generation RNA and exome sequencing to identify putative biomarkers that can predict treatment response and disease outcome.

3.0 ENDPOINTS

3.1 Primary endpoints

Complete Molecular Response (CMR): The proportion of subjects achieving CMR will be reported. Complete Molecular Response (CMR) is defined as the disappearance of malignant clone(s), as proven by negative T-cell receptor gene rearrangement studies of peripheral blood DNA, and bone marrow if previously involved (see Section 14.0).

In addition the proportion of subjects with minimal residual disease (MRD) will also be reported. Minimal Residual Disease (MRD) is defined as the presence of malignant clone(s) as determined by negative T-cell receptor gene rearrangement studies of peripheral blood DNA.

Molecular response will be evaluated based upon T-cell clonality studies to be conducted at the end of cycles 3 and 8 while subjects are on or off belinostat, and at the end of months 9 and 12 while subjects are receiving no treatment, interferon-alfa, or standard AZT-based maintenance treatment (after belinostat completion).

Safety: Treatment-related adverse events (AEs) including serious adverse events (SAEs). AEs will be assessed by and assigned severity and treatment attribution using the National Cancer Institute Common Terminology Criteria for Adverse Events (NCICTCAE), Version 4.03.

3.1.1 Evaluable for Molecular Response:

- Eligible subjects who receive **at least three (3) doses** of belinostat (with AZT), have evaluable disease at baseline and at least one post-baseline molecular disease assessment.

3.1.2 Evaluable for Safety:

- Eligible subjects who receive **at least one dose** of belinostat and AZT study treatment.

3.2 Secondary endpoints

Clinical Response: Clinical response per Section 14.0 will be evaluated during treatment by scheduled assessments. The overall best clinical response rate is defined as the proportion of subjects with clinical complete response (CR) or partial response (PR).

3.2.1 Evaluable for Clinical Response:

- Eligible subjects, who receive **at least one dose** of belinostat and AZT study treatment, have evaluable disease at baseline and at least one post-baseline clinical disease assessment.

1-year Failure-Free Survival (FFS): Subjects will be evaluated during treatment or by follow-up assessments post-treatment. 1-year FFS is defined as the time from study treatment initiation until documented disease progression, relapse after response or death (by any cause, in the absence of progression). In the failure-free subjects, FFS will be censored at the last documented date of failure-free status. In patients who transfer out to to another institution of clinic, FFS data will be collected from existing medical records obtained from outside and communication with any treating physician.

1-year Overall Survival (OS): Follow-up for OS post initiation of treatment will occur every 3 months during year 1 under the proposed study. Survival data from existing medical records or telephone contact every 6 months for up to 5 years from treatment initiation or until death or withdrawal of consent from the study for the purpose of reporting trial result updates in the future. OS is defined as the elapsed time from study treatment initiation to death or date of censoring. Subjects alive or those lost to follow-up will be censored at the last date known to be alive.

3.2.2 Evaluable for FFS and OS:

- Eligible subjects who receive **at least one dose** of belinostat and AZT study treatment.

3.3 Exploratory endpoints

Serial blood sampling at specified time points to describe the molecular and epigenetic actions of belinostat in combination with AZT.

3.3.1 Evaluable for Molecular, Biomarker& Genomic Studies:

Eligible subjects who receive **at least one dose** of belinostat and AZT study treatment and provide at least one sample of blood for molecular studies, and for banking left over material for future biomarker and genomic studies in the future.

4.0 SUBJECT RECRUITMENT & SCREENING

Sites must have this protocol approved by their Institutional IRB before they may screen and enroll participants in the study.

Potential subjects will be recruited from investigators' clinical practices. The identification of subjects must protect the subjects' privacy. Privacy refers to persons and their interest in controlling the access of others to themselves.

Both men and women of all races and ethnic groups are eligible for this trial. Once a potential participant has been identified and the current version of Informed Consent Form has been signed by the potential participant or legally authorized representative (LAR), site Principal Investigator or Sub-Investigator must contact Dr. Juan Carlos Ramos to discuss the case. Relevant source documents including subject medical history and physical exam, previous and concomitant therapies/medications, admission or discharge notes, diagnostic reports, pathologic confirmation of diagnosis, and relevant subject-specific written communication, as applicable will be submitted to University of Miami Study Project Manager for Coordinating Center PI review. Confirmation of enrollment must be obtained prior to subject enrollment.

5.0 PATIENT SELECTION

5.1 Inclusion Criteria

5.1.1 Histologically or cytologically documented adult T-cell leukemia/lymphoma (ATLL) with the following characteristics (for definition of ATLL subtypes see Appendix H):

- Any stage of disease,
- Aggressive types (for definition of ATLL subtypes see Appendix H),
- Documented presence of ATLL cells in peripheral blood by either morphology, histology, flow cytometry or gene rearrangement studies

5.1.2 One of the following:

Initiated AZT/IFNα therapy prior or at the time of enrollment.

OR

Received chemotherapy or other antineoplastic drug therapy ≥ 2 weeks prior to enrollment with the exception of dose-reduced vincristine/and or

cyclophosphamide, or high dose steroids, administered for cytoreductive purpose. (Note: Continuation of zidovudine and interferon therapy is allowed.)

5.1.3 Presence of ATLL based on morphology, histology, flow cytometry, or T-cell clonality in peripheral blood during screening period prior enrollment

5.1.4 Documented HTLV-1 infection: Documentation may be serologic assay (ELISA) confirmed by Western blot or polymerase chain reaction (PCR).

5.1.5 Measurable or evaluable disease, including presence of ATLL by immunophenotyping from either histology or flow cytometry studies, or molecular disease as evidence by T-cell clonality detected by gene rearrangement studies.

5.1.6 18 years of age or older.

5.1.7 Karnofsky performance status (KPS) ≥ 50% or ECOG performance status ≤ 3 (See Appendix C)

5.1.8 Patients must have adequate end organ and bone marrow function as defined below:

- Absolute neutrophil count (ANC)≥ 1,000 cells/mm3 [Exception: Unless cytopenias are secondary to ATLL]
- Platelets (PLT) ≥ 50,000 cells/mm3 [Exception: Unless cytopenias are secondary to ATLL]
- Adequate hepatic function:
  - transaminase ≤ 2.5 the institutional upper limit of normal (ULN),
  - total bilirubin ≤ 1.5 x the institutional upper limit of normal (ULN), [Exception: Unless secondary to hepatic infiltration with lymphoma. If the elevated bilirubin is felt to be secondary to *indinavir* or *atazavinir* therapy (or anti-HIV medications), patients will be allowed to enroll.]
- Creatinine clearance (CrCl) ≥ 40 mL/min, [Exception: Unless secondary to renal involvement by lymphoma is suspected]

5.1.9 Patients who are human immunodeficiency virus positive (HIV+) are also eligible.

5.1.10 Females of childbearing potential (CBP) must have a negative serum pregnancy test within one week of enrollment. Women should avoid pregnancy while receiving study treatment. Males and females must agree to use adequate birth control during participation in this trial and for 3 months after completing therapy.

5.1.11 Patients receiving erythropoietin or G-CSF from baseline are eligible.

5.2 Exclusion Criteria

5.2.1 Patients with progressive disease (after previous chemotherapy or AZT/IFNα) at the time of enrollment.

5.2.2 Patients with chronic leukemia with favorable features, or smoldering type ATLL (for definition of ATLL subtypes see Appendix H).

5.2.3 Patients receiving any other investigational agents within 14 days prior to initiation of study therapy. (Exception: Patients actively receiving IFN-alfa-2b, PEG-IFN-alfa-2b, or similar forms of IFN-alfa are permitted).

5.2.4 Uncontrolled inter-current illness including, but not limited to, ongoing or active infection, symptomatic congestive heart failure (CHF), unstable angina pectoris, cardiac arrhythmia, or psychiatric illness/social situations that are likely in the judgment of the Investigator(s) to interfere or limit compliance with study requirements/treatment.

5.2.5 Pregnant or breast-feeding women.

5.2.6 Known hypersensitivity to histone deacetylases (HDACs), zidovudine, belinostat or any component of the formulation(s).

5.2.7 Acute hepatitis or decompensated liver disease unless due to lymphoma. Chronic hepatitis will be required to be on prophylactic treatment during the study if provided liver function test meet criteria listed above without evidence of cirrhosis to be eligible.

5.2.8 Concurrent active malignancies, with the exception of *in situ* carcinoma of the cervix, non-metastatic, non-melanomatous skin cancer, or Kaposi's sarcoma not requiring systemic chemotherapy.

5.2.9 Known NYHA Class 3 or 4 heart disease as per Appendix D.

5.2.10 Known ejection fraction < 45% or institutional limit of normal range

5.2.11 Psychological, familial, sociological or geographical conditions likely in the judgment of the Investigator(s) to interfere or limit compliance with study requirements/treatment.

6.0 **Enrollment Procedures**

Participating sites must first receive written permission from the Coordinating Center to enroll a participant.

The following documents must be provided to the Coordinating Center and the Protocol Chair for registration approval (i.e., enrollment of a consented subject):

1. **De-Identified**, signed consent document(s) and HIPAA
2. Completed Registration Cover Sheet
3. Signed/dated eligibility checklist
4. All **De-Identified** eligibility source documents (e.g., lab reports, scans, test, etc.)
5. Desired timeline for the registration / proposed start date for treatment

Please refer to study specific manual of Procedures for further details.

6.1 **Cancellation Guidelines**

If a patient does not receive protocol therapy, the patient may withdraw. Contact the CRS Representative, or e-mail the information including the reasons for withdrawal within 10-business days.

6.2 **Emergency Registration**

If an emergency registration takes place after business hours, the items listed above must be submitted by the next business day.

7.0 **STUDY DESIGN**

This is an open-label, single arm, phase II study of zidovudine (AZT) and belinostat, as consolidation therapy with *optional* interferon-alfa-2b (IFN-alfa-2b), interferon-alfa-2a, pegylated interferon-alfa-2b (PEG-IFN-alfa-2b), peginterferon-alfa-2a or generic preparations of IFN-alfa that become commercially available to replace existing agents or the one approved by patient's insurance. For subjects receiving interferon therapy at baseline, the corresponding interferon may be continued. Subjects will receive up to 8 cycles of belinostat combined with AZT (as consolidation) followed by AZT-based maintenance therapy (± IFN-alfa) as tolerated for at least 6 more months.

Subjects will be restaged at the end of belinostat Cycle 3 (-7 days) or end of Month 3, belinostat Cycle 8 or end of month 6 (-14 days), end of Month 9 (≤14 days) and end of Month 12 (≤14 days). Subjects who have progression of disease at any of these time points will be taken off study treatment. See Sections 12.0 and 14.0 for details.

At the end of Cycle 3, subjects who achieve a *molecular* complete response or maintain a *clinical* stable response may continue on the study.

At the end of Cycle 8 (i.e. completion of belinostat) or end of Month 6, subjects who achieve or maintain a complete molecular response (CMR) with no evidence of minimal residual disease (MRD) may continue to receive AZT (±IFN-alfa) at the discretion of the investigator. Subjects with MRD with no clinical evidence of disease progression nor clinical evidence of ATLL may also continue to receive AZT (± IFN-alfa) *OR* be removed from treatment at the discretion of the Investigator.

At the end of Month 9, subjects who maintain/achieve CR with no evidence of MRD may continue to receive AZT (± IFN-alfa) at the discretion of the investigator for 3 more months, up to Month 12. Those with MRD (without clinical evidence of disease progression or clinical evidence of ATLL) may also continue to receive AZT (± IFN-alfa) for 3 more months to Month 12 *OR* be removed from the study at the discretion of the Investigator.

At least 10 subjects are expected to be enrolled at Sylvester Comprehensive Cancer Center (SCCC), University of Miami affiliated hospitals, including Jackson Memorial Hospital, and any future collaborating sites. Expected time to complete total accrual is approximately 5 years. Expected time to study completion is 6 years (12 months after the last enrollment) from the time of first enrollment.

Correlative evaluations will also be performed at specified visits. Please refer to Section 13.0 CORRELATIVE STUDIES and Appendices F & G for specific details.

8.0 **TREATMENT PLAN**

During belinostat administration, a cycle of treatment will consist of 3 weeks or 21 days (±3 days). Thereafter, AZT (+/- IFN-alfa) may be continued monthly up to the end of Month 12. Trial treatment should be administered after all procedures/assessments have been completed as detailed in Section 12.0. See also Section 8.6.1 for Concurrent Medications and Prophylactic Measures.

8.1 Zidovudine (AZT)

Zidovudine shall be administered in the outpatient setting as 300 mg tablets orally (PO), three times daily (TID) or continue the dose patient was receiving prior to enrollment along with belinostat for 21 days on cycles 1 to 8, followed by maintenance therapy (+/- IFN-alfa) up to the end of Month 12. For patients who are AZT-experienced, as well as for patients who are AZT-naïve, zidovudine must be administered at least 24-hours prior to the first dose (i.e. 24-hours before C1D1) of belinostat. AZT may be administered for a minimum of 12 months from the beginning of the study. Because AZT is a standard maintenance drug for ATLL, starting the drug before enrolment should not be considered a deviation from the protocol whatsoever.

For additional information on zidovudine including mechanism of action, drug metabolism, pharmacokinetics & toxicology, known side effects, composition, and storage recommendations, (see Section 9.2).

8.2 Belinostat

Belinostat will be administered as 1,000 mg/m^2 IV infusion over 30 minutes on Days 1-5 every 21 days (**Exception as per FDA-approved Package Insert**: In patients known to be homozygous for the UGT1A1*28 allele, the starting belinostat dose must be 750mg/m^2) for up to 8 cycles. (UGT1A1 activity is reduced in individuals with genetic polymorphisms that lead to reduced enzyme activity such as the UGT1A1*28 polymorphism. Approximately 20% of the black population, 10% of the white population, and 2% of the Asian population are homozygous for the UGT1A1*28 allele. Because belinostat is primarily (80-90%) metabolized by UGT1A1, the clearance of belinostat could be decreased in patients with reduced UGT1A1 activity (e.g., patients with UGT1A1*28 allele). Testing for the UGT1A1*28 allele is not required for eligibility on this study.

Every effort to target infusion timing to be as close to days 1 to 5, and infusion times 30 minutes as possible, should be made. However, given the variability of outpatient scheduling conflicts (including weekend and holidays), infusion pumps, a window of +3 days for scheduled drug administration, and -5 minutes and +10 minutes infusion times is permitted (i.e. 30 minute infusion time -5 min/+10min).

For additional information on belinostat including mechanism of action, drug metabolism, pharmacokinetics & toxicology, known side effects, composition, and storage recommendations, (see Section 9.1).

8.3 Interferon alfa-2b (IFN-α-2b) *or* pegylated interferon alfa-2b (PEG-IFN-α-2b) *or* other forms of IFN-alfa that become commercially available to replace existing preparations *or* those preferred by patient's insurance.

Optional: For subjects receiving interferon therapy at baseline, continue Interferon alfa-2b 5 million IU daily *or* pegylated interferon alfa-2b 1.5 µg/kg based on (PegIntron recommended dosing, rounded up to the closest dose to existing vial/syringe dose preparations) once weekly, subcutaneously (SQ) for up to 12 months. Subjects should be advised to only use the brand and type of interferon that their doctor prescribes. They should not use another brand of interferon or switch between interferon alfa-2b and peg

interferon alfa-2b in vials and/or injection pens *without* talking to their doctor(s) first. In case interferon alfa-2b *or* pegylated interferon alfa-2b are unavailable or not covered by the subject's medical insurance, other commercially available forms of IFN-alfa may be administered at doses recommended by package insert only after approval by the protocol Chair, and administration doses for such form of interferon may be modified (dose-reduced) at the protocol Chair's discretion based on previous treatment tolerance.

For additional information on IFN-alfa-2b, PEG-IFN-alfa-2b/-2a including mechanism of action, drug metabolism, pharmacokinetics & toxicology, known side effects, composition, and storage recommendations, (see Section 9.3 and 9.4, respectively).

8.4 **Lymphodepletion therapy**: A one-time administration of standard low dose cyclophosphamide (up to 375 mg/m^2) will be permitted during cycle 1 after administration of Day 5 (+ 7 days) belinostat to treat any increase in absolute lymphocyte count, which may occur transiently during cycle 1 after belinostat from re-activation of HTLV-1 in ATLL or T-lymphocytes based on early trial observations.

8.5 Treatment Schema

Table 2: Treatment Schema

Regimen Description					
Agent	**Premedication; Precautions (see also Section 8.6.1 Concurrent Medications/ Prophylactic Measures)**	**Dose**	**Route**	**Schedule**	**Cycle Length** for belinostat
Belinostat[A]	(see Appendix E)	1,000 mg/m^2 or 750 mg/m^{2B}	IV 30 min	Days 1-5 x 8 cycles	21 days (3 weeks)
Zidovudine (AZT)[A]	(see Appendix I)	300mg tablet	PO	3 times a day (or continue previous dose prior to enrollment) x 12 months	
(OPTIONAL) IFN-alfa-2b ***or***	(for subjects already receiving IFN)	5 million IU	SQ	Daily x 12 months	
(OPTIONAL) IFN-alfa-2a ***or***	(for subjects already receiving IFN)	5 million IU	SQ	Daily x 12 months	
(OPTIONAL) PEG-IFN-alfa-2a	(for subjects already receiving PEG-IFN)	Package insert dose	SQ	Once weekly x 12 months	
(OPTIONAL) PEG-IFN-alfa-2b	(for subjects already receiving PEG-IFN)	1.5 µg/kg	SQ	Once weekly x 12 months	
(OPTIONAL) Lymphodepleting therapy	(for any increase in absolute lymphocyte count)	Cyclo-phosphamide 375 mg/m^2	IV 60 mins	Once Cycle 1 only (after Day 5 belinostat)	1 day

[A]Zidovudine must be administered (for patients who are AZT-experienced, as well as for patients who are AZT-naïve) at least 24-hours prior to the first dose (i.e. 24-hours before C1D1) of belinostat

[B]As per FDA-approved Package Insert: In patients known to be homozygous for the UGT1A1*28 allele, the starting belinostat dose must be 750mg/m^2.

8.6 Treatment Dispensation, Compliance and Accountability

8.6.1 Belinostat (Beleodaq®)

Eligible subjects shall be treated with the investigational supply of belinostat, as provided by *Acrotech Biopharma, LLC*. Clinical supplies may not be used for any purpose other than that stated in the protocol.

Clinical supplies must be stored in a secure, limited-access location under the storage conditions specified.

Receipt and dispensing of investigational product must be recorded by authorized person(s) at the trial site. The Investigator is responsible for maintaining accurate records of the clinical supplies received from *Acrotech Biopharma, LLC*, the amount dispensed to subjects and the amount remaining at the conclusion of the trial.

Upon completion or termination of the study, all unused and/or partially used investigational product will be destroyed at the site per institutional policy. It is the Investigator's responsibility to arrange for the disposal of all empty containers, provided that procedures for proper disposal have been established according to applicable federal, state, local and institutional guidelines and procedures, and provided that appropriate records of disposal are kept.

8.6.2 Zidovudine (AZT, Retrovir®)

(During cycles 1-8 of belinostat administration). Eligible subjects will be prescribed the appropriate number of zidovudine capsules required for 21 days of dosing, plus an additional 3-day supply to account for visit scheduling. The subject will be requested to maintain a medication diary of each dose of zidovudine (AZT). The date, time and dose should be recorded at each dosing time point to confirm that each dose of zidovudine was taken for each day. See Appendix I for the dosing diary.

If a dose of zidovudine AZT is missed due to subject error/oversight, then s/he should take the zidovudine AZT dose within 6 hours of the missed dose. If more than 6 hours have elapsed, that missed dose should be omitted. The subject should resume treatment at the next scheduled dosing time point and the reason for missed dose reported on the dosing diary. **NOTE: Although patients will be requested to maintain a medication diary, missed doses of AZT due to error/oversight will not be deemed significant since AZT is an acceptable NCCN standard guideline for first-line and maintenance treatment of ATLL.**

If a dose of zidovudine AZT is held due to an AE, SAE or at the Investigator's discretion, then s/he should resume treatment at the Investigator's discretion taking into account all of the dose modification/discontinuation guidelines provided in Section 10.0 Treatment/Dose Modifications.

8.6.3 Interferon Alfa-2b (INTRON® A) *OR* Peg interferon Alfa-2b (PEGINTRON®) OR other commercially available forms of Interferon Alfa-2b (INTRON® A) *OR* Peg interferon Alfa-2b (PEGINTRON® in the case these brands become unavailable.

For subjects receiving interferon therapy at baseline, continue during belinostat/AZT consolidation and for up to 6 more months thereafter. The commercial supply of INTRON® A or PEGINTRON® will be used (Subjects should not switch between interferon alfa-2b, peg-interferon alfa-2b, or other commercially available forms of interferon alfa that replace these agents in vials and/or injection pens *without* talking to their clinical trial's doctor(s) first).

8.7 Supportive Care Guidelines

8.7.1 Concurrent Medications/ Prophylactic Measures

Central Nervous System (CNS) Prophylaxis: Subjects who have not previously received meningeal prophylaxis with intrathecal chemotherapy may receive prophylaxis at the discretion of the investigator or treating physician. It is recommended that lumbar puncture and prophylactic intrathecal chemotherapy is postponed until absolute lymphocyte blood count is less than 3.5 X $10^3/\mu l$

Antiretroviral therapy (ART): Subjects who are HIV positive may receive antiretroviral therapy at the discretion of the investigator. Zidovudine (AZT) as part of ART regimen is prohibited. Antiretroviral regimens may be modified before or during the course of protocol treatment as necessitated by the occurrence of drug-induced toxicities or based upon measurement of viral load.

Prevention of tumor lysis syndrome: Subjects with evidence of high tumor burden (bulky disease, bone marrow involvement, LDH > twice the upper limit of normal) should receive allopurinol 300 mg daily starting 24 hours prior to the initiation of chemotherapy followed for at least the first seven treatment days. Appropriate IV hydration should be maintained and serum electrolytes, BUN, phosphorous, creatinine, calcium and uric acid should be monitored closely. Allopurinol dosing may be adjusted at the discretion of the treating physician.

Antibiotics may be administered as clinically indicated, at the discretion of the treating Investigator.

Topical, anti-infectives and/or antifungal agents are permitted.

Growth factors may be used as clinically indicated, at the discretion of the treating Investigator.

Pneumocystis Carinii Prophylaxis: All subjects should receive prophylaxis with co-trimoxozole, dapsone, atovaquone, or inhaled pentamidine as per recommended, standard of care guidelines.

8.8 Duration of Treatment

While on-study subjects will receive a maximum of 8 cycles (about 6 months) of belinostat consolidation therapy with AZT (±IFN-alfa). Thereafter, depending upon restaging assessments and clinical/molecular evaluations, subjects may receive an additional 6 months of AZT-based maintenance therapy (±IFN-alfa).

8.9 Duration of Follow-Up

All subjects will be followed at approximately ≤30 days (+/- 7 days) after the last dose of belinostat for toxicity.

Subjects who achieve/maintain CR and have completed one year of therapy (i.e. 8 cycles of belinostat/AZT by about 6 months, with AZT-based maintenance therapy for an additional 6 months), will be followed every 3 months (±14 days) for clinical assessment

(Complete PE, CBC, CMP, and LDH), with response assessment (CT, BMX and skeletal X-ray survey if indicated) every 6 months for 1 year.

Thereafter, these subjects will continue to be followed every 6 months (± one month) during Years 3 to 5 at a minimum for survival via a telephone call.

Subjects who are off treatment after early discontinuation not due to disease progression may remain on the study and will be followed for clinical assessments mentioned above and for overall and failure free-survival for at least 12 months or until they receive new treatment, at which time they will be censored.

Subjects who discontinue treatment for disease progression will come off treatment and will be followed for survival every 6 months (±one month) for up to 5 years.

Subjects who withdraw consent will come off study

9.0 TREATMENT/ DOSE MODIFICATIONS

9.1 Dose Modification Guidelines

9.1.1 Hematologic Toxicity (grade 4 neutropenia or thrombocytopenia unless present at baseline due to lymphomatous bone marrow involvement)

For new Grade 4 thrombocytopenia, hold all drugs until the toxicity grade returns to ≤Grade 2. Once the toxicity grade returns to ≤ Grade 2, belinostat should be administered at 75% the original dose (i.e. 750 mg/m^2) on the next cycle. Subjects may receive AZT and IFNα (if applicable) at the original dose(s). In the case of known bone marrow involvement by lymphoma, subjects may continue to receive AZT and/or IFNα (standard maintenance treatment for ATLL to prevent disease progression) at reduced doses at the discretion of the investigator regardless of AE grade level if not recovered. AZT and/or IFNα may be also held at any time at the discretion of the investigator.

For new Grade 4 neutropenia (based on absolute neutrophil count or ANC), hold all drugs until the toxicity grade returns to ≤ Grade 3. Once the toxicity grade returns to ≤ Grade 3, belinostat should be administered at 75% the original dose (i.e. 750 mg/m^2). Subjects may receive AZT and IFNα or PEG-IFN-alfa2b (if applicable) at the original dose(s). In the case of known bone marrow involvement by lymphoma, subjects may continue to receive AZT and/or IFNα (standard maintenance treatment for ATLL to prevent disease progression) at reduced doses at the discretion of the investigator regardless of AE grade level if not recovered. AZT and/or IFNα may be also held at any time at the discretion of the investigator.

If Grade 4 thrombocytopenia or Grade 4 neutropenia recur after the above dose adjustments, then all drugs will be held until the toxicity grade returns to ≤ Grade 2 platelets and/or ≤ Grade 3 ANC, or to baseline in patients with lymphomatous bone marrow involvement at initial presentation before treatment. Once the toxicity grade returns to these limits, belinostat should administered at 75% the previous dose on the next cycle (second dose reduction). AZT should be reduced to 300 mg twice daily or lower at the discretion of the investigator. If applicable, IFNα-2b should be reduced to 5MU three times week and PEG-IFN-alfa-2b should be restarted at 50% of the previous dose. Other commercially available substitutes of interferon alfa that replace these agents should be re-started at 50% of the previous dose. In the case of known bone marrow involvement by lymphoma, subjects may continue to receive AZT and/or IFNα (standard maintenance treatment

for ATLL to prevent disease progression) at reduced doses at the discretion of the investigator regardless of AE grade level if not recovered. AZT and/or IFNα may be also held at any time at the discretion of the investigator.

If Grade 4 thrombocytopenia or Grade 4 neutropenia recur, then all drugs will be held until the toxicity grade returns to ≤ Grade 2 platelets and/or ≤ Grade 3 ANC, or to baseline in patients with lymphomatous bone marrow involvement at initial presentation before treatment. Discontinue belinostat in subjects who have recurrent ANC less than 0.5 x 10^9/L and/or recurrent platelet count nadirs less than 25 x 10^9/L after two dosage reductions. Once the toxicity grade returns to ≤ Grade 3, subjects may re-start AZT, and IFNα or PEG-IFN-alfa2b if applicable, at the previous doses.

In subject with lymphomatous bone marrow involvement who present with grade 4 neutropenia or thrombocytopenia grade 4 prior to next treatment, study drugs will be held at the discretion of the investigator and if continued reasons should be documented in the medical record.

If any of these drug-induced toxicities persist for more than 28 days at any time or recur after belinostat was discontinued, then all drugs will be permanently stopped, and the subject will be removed from the study. Exception: In the case of known bone marrow involvement by lymphoma, subjects may continue to receive AZT and/or IFNα (standard maintenance treatment for ATLL to prevent disease progression) at reduced doses at the discretion of the investigator regardless of AE grade level and continue on the study. AZT and/or IFNα may be also held at any time at the discretion of the investigator during this time.

G-CSF or GM-CSF may be used for neutropenia > Grade 3, at the discretion of the investigator.

Platelet and blood transfusions, and recombinant erythropoietin (rEPO), may be given at any time at the discretion of the investigator.

9.1.2 Non-Hematological Toxicity: Hepatotoxicity

For Grade 2 or greater elevations in AST (SGOT) and/or ALT (SGPT), or elevation of total bilirubin ≥ 1.5 institutional upper limit of normal, all study medications will be held. Once the toxicity grade returns to ≤ Grade 1 and total bilirubin returns to < 1.5 institutional upper limit of normal within 14 days, belinostat should be administered at 75% the original dose (i.e. 750 mg/m^2). AZT should be reduced to 300 mg twice daily. When applicable, IFNα2b should be reduced to 5MU three times weekly and PEG-IFN-alfa2b or equivalent drug form restarted at 50% of the last dose.

If Grade 2 or greater elevations in AST (SGOT) and/or ALT (SGPT), or elevation of total bilirubin ≥ 1.5 institutional upper limit of normal persist for 14 days, then belinostat will be discontinued and the subject will be removed from the study.

If Grade 2 or greater elevations in AST (SGOT) and/or ALT (SGPT) and/or elevation of total bilirubin ≥ 1.5 institutional upper limit of normal recur then all drugs will be held. Once the toxicity grade returns to ≤ Grade 1 and total bilirubin returns to < 1.5 institutional upper limit of normal within 14 days, belinostat should be administered at 75% the previous dose. AZT should be re-started at 300 mg twice daily. Whenever applicable, IFN-alfa2b orPEG-IFN-alfa2b or equivalent drug form should be held and discontinued.

If Grade 2 or greater elevations in AST (SGOT) and/or ALT (SGPT) and/or elevation of total bilirubin ≥ 1.5 institutional upper limit of normal recur then all drugs will be discontinued and the subject will be removed from the study.

9.1.3 Non-Hematological Toxicity: Gastrointestinal Toxicity

Belinostat should be withheld if ≥ Grade 3 drug-related nausea, vomiting, diarrhea, or dehydration. After resolution to Grade 1 or less, belinostat may be re-started 75% the previous dose.

If ≥ Grade 3 toxicity recurs at the reduced dose the drug will then be discontinued permanently.

Subjects may continue to receive AZT and IFNα2b/PEG-IFN-alfa2b (or equivalent drug form) at the discretion of the Investigator.

9.1.4 Pregnancy

Belinostat will be stopped for subjects who become pregnant. If this drug is used during pregnancy, or if the subject becomes pregnant while taking this drug, the subject should be apprised of potential hazard to the fetus. See also Appendix A.

AZT and IFN-alfa2b/ PEG-IFN-alfa2b or equivalent drug form may be continued at the discretion of the Investigator(s).

Males and females must agree to use adequate birth control during participation in this trial and for 3 months after completing therapy.

9.1.5 Grade ≥3 Fatigue, Anorexia and/or Mood Alteration

When applicable, IFN-α should be held first in subjects receiving this drug until the toxicity grade returns to ≤ Grade 2 or baseline. IFN-α should be reduced to 5MU three times weekly and PEG-IFN-alfa2b or equivalent drug form restarted at 50% of the last dose. In subject(s) not receiving IFN-α2b/PEG-IFN-alfa2b or equivalent drug form, belinostat should be withheld until toxicity is Grade 1 or less. Belinostat may then be re-started at 75% the previous dose. If ≥ Grade 3 toxicity recurs belinostat should be withheld until toxicity is Grade 1 or less. Belinostat may be restarted 75% the previous dose. If ≥ Grade 3 toxicity recurs at the reduced dose (second dose reduction) the drug will then be discontinued permanently, and the subject will be removed from the study.

If any of these toxicities persists for more than 14 days, then all study medications will be permanently discontinued.

10.0 TREATMENT DISCONTINUATION

Treatment may be discontinued for any of the following reasons:

- The subject demonstrates progression of disease (Exception: Subjects may remain on the study if in the opinion of the Investigator, he/she is deriving clinical benefit from study treatment)
- The subject withdraws consent from the study
- The subject has not received study treatment for >35 days due to an adverse event/toxicity (See Section 10.0). Exception: Subjects may remain on the study if in the opinion of the Investigator, he/she is deriving clinical benefit from being in the protocol). **Patients who have been previously removed from the study after treatment discontinuation and who have not received other therapies or progressed may re-enter the study after**

re-consent for the purpose of capturing progression-free data and continue disease assessments and monitor any unresolved toxicities as per protocol, as long as they do not progress or receive any new treatment. Those receiving AZT and/or IFNα as standard maintenance will be also allowed to re-enter the study.

- The subject experiences an adverse event that in the opinion of the Investigator makes continued study treatment an unacceptable risk
- The subject becomes pregnant (also see Appendix A: Expedited Adverse Event Reporting Requirements)
- The subject requires continuous treatment with a prohibited concomitant drug(s) for which no safe alternatives can be substituted
- The subject is significantly noncompliant with the requirements of the protocol

Should discontinuation of study therapy occur, all efforts should be made to execute/ report End-of-Treatment and Follow-up Evaluations as completely as possible and to determine/ document the reason for discontinuation (unless the patient withdraws consent for follow-up).

If a subject wishes to withdraw consent from the study, the PI must be notified. The information regarding withdrawal (i.e. subject identifiers and date of withdrawal) should be documented in the subject's record and updated within any other research database(s).

11.0 SCHEDULE OF CLINICAL & LABORATORY EVALUATIONS

Prior to performing any study-specific procedures or evaluations, written informed consent and authorization for the use of protected health information (HIPAA) must be obtained in accordance with all applicable policies, regulations and laws. Investigator(s) should also perform routine clinical and laboratory evaluations as necessary for standard of care treatment.

Correlative evaluations must also be performed at specified visits. Please refer to Section 13.0 CORRELATIVE STUDIES and Appendices F & G for specific details.

11.1 Pre-Treatment Evaluations (Screening)

The following must be collected/performed ≤ 21days prior to Cycle 1, day 1 of treatment with the exception of some laboratory tests as specified. Clinical and laboratory evaluations performed as part of routine standard of care do not need to be repeated if performed within the appropriate window.

- Complete medical history that includes:
  - Date of initial and/or relapsed diagnosis of lymphoma. A copy of the pathology and/or flow cytometry report must be available in the medical record.
  - Presence or absence of "B" symptoms (i.e. unexplained fevers, night sweats, involuntary weight loss > 10% of normal body weight)
  - History of other symptoms related to ATLL.
  - History of drug allergies.
  - Current medication list.
  - Previous therapy received for this lymphoma.
- Complete physical examination (PE): includes neurologic examination, careful measurement of all palpable peripheral lymph nodes and measurement of other

sites of disease present on physical examination including skin. Palpable lymph nodes or masses should be measured in at least two dimensions.

- ECOG or Karnofsky performance score (ECOG PS or KPS). See also Appendix C
- Vital signs (V/S: including heart rate (HR), blood pressure (BP), respiratory rate (RR), and oral temperature (oral temp)
- Weight
- Height
- Baseline laboratory tests (to be repeated **within 48 hours prior** to initiation of zidovudine (unless patient was already on this drug), unless otherwise indicated:
    - Blood chemistries: serum electrolytes, creatinine, total bilirubin, calcium, phosphorous, AST, ALT, alkaline phosphatase, total protein, albumin
    - Hematology: CBC, with differential, and platelet (PLT) count
    - LDH level
    - Uric acid level
    - Hepatitis profile including hepatitis B surface antibody, hepatitis B surface antigen and hepatitis C antibody (**within 6 months prior** to enrollment).
    - Serum pregnancy test if premenopausal within 14 days prior to treatment initiation and at any time during protocol therapy if pregnancy is suspected.
    - Leukemia-lymphoma standard panel (flow cytometry) with standard T-cell receptor gene rearrangement (i.e. by multiplex PCR) within 14 days prior to enrollment but not within 14 days of any previous therapy (excluding zidovudine and interferon) to determine presence of clonal disease.
- Correlative studies (see Section 13.0)
- 12-Lead ECG
- Staging Evaluation: The following studies will be done for baseline evaluation of the extent of disease within 30 days prior to on-study treatment initiation in subjects already on AZT-IFN and 14 days after any previous therapy (excluding zidovudine and interferon):
    - CT scans of the chest, abdomen, and pelvis (C/A/P) with contrast unless contraindicated, in which case nuclear imaging methods for lymphoma staging can be used or non-contrast CTs. Optional: Skeletal X-ray survey (chest, skull, shoulder girdle, spine, and pelvic girdle and bone survey) to check for bone lytic lesions if the patient had hypercalcemia upon initial diagnosis and no visible bone lesions on CT scans.
    - Bone marrow biopsy unless there is confirmed evidence of leukemia in peripheral blood, in which case it is not needed at baseline but can be done at the discretion of the investigator; If performance of BM biopsy will delay treatment initiation (e.g., falling on a weekend or holiday), it may be delayed ±3 days or must be performed to confirm CR at the end of month 2 (i.e. at the end of cycles 3), month 6 (i.e. at the end of cycles 8) and at the end of months 9 and 12.

- Lumbar puncture with routine studies and cytology will be done at the discretion of the treating Investigator(s) if lymphomatous CNS involvement is suspected. It is discouraged in patients with blood circulating ATLL cells (i.e. active leukemia) unless otherwise warranted by symptoms.

11.2 Evaluations on Treatment

Collection of Concomitant Medications and Adverse Events (AEs) should occur throughout the study, as described. Patients will be asked to maintain a medication diary for AZT (see Section 8.5.2 and Appendix I).

11.2.1 **Cycle 1 Day 1** (assessments done one day before can be used, including baseline evaluations)

- Complete physical examination: includes neurologic examination, careful measurement of all palpable peripheral lymph nodes and measurement of other sites of disease present on physical examination including skin. Palpable lymph nodes or masses should be measured in at least two dimensions.
- ECOG or Karnofsky performance score (Appendix C)
- Vital signs (HR, BP, RR, and oral temp)
- Weight
- CBC with differential and platelet count
- Blood chemistries: serum electrolytes, creatinine, total bilirubin, calcium, phosphorous, AST, ALT, alkaline phosphatase, total protein, albumin
- LDH level

11.2.2 **Cycle 1, days 3 to 4 (72 to 96 hours after belinostat initiation)**

- CBC, with differential and platelet count
- Blood chemistries (serum electrolytes, creatinine, total bilirubin, calcium, phosphorous, AST, ALT, alkaline phosphatase, total protein, albumin), and LDH level.
- Correlative studies (see Section 13.0)

11.2.3 **Cycle 1, day 8 (±1 day)**

- CBC, with differential and platelet count
- Blood chemistries (serum electrolytes, creatinine, total bilirubin, calcium, phosphorous, AST, ALT, alkaline phosphatase, total protein, albumin), and LDH level.
- Correlative studies (see Section 13.0)

11.2.4 **Cycle 1, day 15 (±3 days)**

- CBC, with differential and platelet count (*to check nadirs*)

11.2.5 Correlative studies (see Section 13.0) **Subsequent Cycles 2 through 8, day 1 (≤ 3 days prior to day 1) (end of cycles assessments can be used and do not need to be repeated)**

- Complete physical examination: includes neurologic examination, careful measurement of all palpable peripheral lymph nodes and measurement of other sites of disease present on physical examination including skin. Palpable lymph nodes or masses should be measured in at least two dimensions.
- vital signs (HR, BP, RR, and oral temp)
- weight
- ECOG or Karnofsky performance score (Appendix C)
- CBC, with differential and platelet count
- Blood chemistries (serum electrolytes, creatinine, total and direct bilirubin, calcium, phosphorous, AST, ALT, alkaline phosphatase, total protein, albumin), and LDH level.
- Correlative studies only prior Cycle 2 (see Section 13.0)

11.2.6 **Subsequent Cycles 2 through 8, day 8 (± 3 days)**

- CBC, with differential and platelet count (*as standard safety to check nadirs*)

11.2.7 **Subsequent Cycles 2 through 8, day 15 (±3 days)**

- CBC, with differential and platelet count (*as standard safety to check nadirs*)

11.2.8 **End of Cycle 3 (≤7 days prior to cycle 4)**

- Complete Physical examination: Palpable lymph nodes on physical examination should be measured in at least two dimensions
- CBC, with differential and platelet count
- Blood chemistries (serum electrolytes, creatinine, total bilirubin, calcium, phosphorous, AST, ALT, alkaline phosphatase, total protein, albumin), and LDH level.
- Leukemia-lymphoma standard panel (flow cytometry with standard T-cell receptor gene rearrangement (i.e. by multiplex PCR). In case of persistent ATLL evidence by flow cytometry, T-cell receptor gene rearrangement (i.e. by multiplex PCR) is not required.
- CT scans of the chest, abdomen, and pelvis (C/A/P) with contrast unless contraindicated, in which case nuclear imaging methods for lymphoma staging can be used or non-contrast CTs. If non-contrast CT-PET is obtained, for instance in patients where IV contrast is contraindicated due to allergy or renal dysfunction, it is recommended that only the CT portion be used for response assessment during treatment. Optional: Skeletal X-ray Survey if positive for bone lesions at baseline.
- Correlative studies (see Section 13.0)
- Bone marrow biopsy (if presumed or involved at baseline to confirm CR)

11.2.9 **End of Cycle 8 (≤14 days prior to Day 21)**

- Complete Physical examination: palpable lymph nodes on physical examination should be measured in at least two dimensions.
- Standard laboratory tests:

  - CBC, with differential and platelet count
  - Blood chemistries (serum electrolytes, creatinine, total bilirubin, calcium, phosphorous, AST, ALT, alkaline phosphatase, total protein, albumin), and LDH level.
- Leukemia-lymphoma panel (flow cytometry) with standard T-cell receptor gene rearrangement (i.e. by multiplex PCR). In case of persistent ATLL evidence by flow cytometry, T-cell receptor gene rearrangement (i.e. by multiplex PCR) is not required.
- Correlative studies (see Section 13.0)
- Standard restaging:
- CT scans of the neck (if involved at baseline only), chest, abdomen, and pelvis. Optional: Skeletal X-ray Survey if positive for bone lesions at baseline.
- Bone marrow biopsy (if presumed or involved at baseline to confirm CR)

Note: Subjects who have clinical or molecular evidence of ATLL without evidence of disease progression may remain on study receiving AZT (or AZT/ IFNα) or may be removed from treatment protocol to receive alternative therapies at the discretion of the treating Investigator. Subjects who maintain or achieve a complete remission will continue on the study.

11.2.10 End of Month 9 (≤14 days)

- Physical examination: palpable lymph nodes on physical examination should be measured in at least two dimensions.
- Standard laboratory tests:
  - CBC, with differential and platelet count
  - Blood chemistries (serum electrolytes, creatinine, total bilirubin, calcium, phosphorous, AST, ALT, alkaline phosphatase, total protein, albumin), and LDH level.
- Leukemia-lymphoma panel (flow cytometry) with standard T-cell receptor gene rearrangement (i.e. by multiplex PCR). In case of persistent ATLL evidence by flow cytometry, T-cell receptor gene rearrangement (i.e. by multiplex PCR) is not required.
- Correlative studies (see Section 13.0)
- Standard restaging:
- CT scans of the neck (if involved at baseline only), chest, abdomen, and pelvis. Optional: Skeletal X-ray Survey if positive for bone lesions at baseline.
- Bone marrow biopsy (if presumed or involved at baseline to confirm CR)

Note: Subjects who maintain a response with clinical or molecular evidence of ATLL may remain on study receiving AZT (or AZT/ IFNα) or may be removed from the protocol to receive alternative therapies at the discretion of the treating Investigator. Subjects who maintain or achieve a complete remission will continue on the study.

11.2.11 End of Month 12 (≤14 days)

- Physical examination: palpable lymph nodes on physical examination should be measured in at least two dimensions.

- Standard laboratory tests:
  - CBC, with differential and platelet count
  - Blood chemistries (serum electrolytes, creatinine, total bilirubin, calcium, phosphorous, AST, ALT, alkaline phosphatase, total protein, albumin), and LDH level.
- Leukemia-lymphoma panel (flow cytometry) with standard T-cell receptor gene rearrangement (i.e. by multiplex PCR). In case of persistent ATLL evidence by flow cytometry, T-cell receptor gene rearrangement (i.e. by multiplex PCR) is not required.
- Correlative studies (see Section 13.0)
- Standard restaging (±4 weeks) or more frequently if clinically indicated: CT scans of the neck (if involved at baseline only), chest, abdomen, and pelvis. Optional: Skeletal X-ray Survey if positive for bone lesions at baseline.
- Bone marrow biopsy (if involved at baseline and only to confirm CR)

Note: If at the end of month 12, no minimal residual disease is detected, patients may continue AZT (or AZT/IFNα2b) at the discretion of the treating Investigator.

11.3 Off-Treatment Evaluations (Early Discontinuation not due to disease progression, End of Treatment or EOT visit)

At ≤ 30-days after the last dose of study treatment or before the initiation of new anti-cancer therapy (early discontinuation not due to disease progression), whichever occurs first, the following assessments should be performed:

- Complete physical examination (PE): includes neurologic examination, careful measurement of all palpable peripheral lymph nodes and measurement of other sites of disease present on physical examination including skin. Palpable lymph nodes or masses should be measured in at least two dimensions.
- ECOG or Karnofsky performance score (ECOG PS or KPS) See also Appendix C
- Vital signs (V/S: including heart rate (HR), blood pressure (BP), respiratory rate (RR), and oral temperature (oral temp))
- Weight
- CBC, with differential and platelet count
- Blood chemistries (serum electrolytes, creatinine, total bilirubin, calcium, phosphorous, AST, ALT, alkaline phosphatase, total protein, albumin), and LDH level.
- Leukemia-lymphoma panel (flow cytometry) in the absence of clinical disease and only to confirm complete remission

with standard T-cell receptor gene rearrangement (i.e. by multiplex PCR) in the absence of clinical disease only to confirm complete remission. In case of persistent ATLL evidence by flow cytometry, T-cell receptor gene rearrangement (i.e. by multiplex PCR) is not required.

- Correlative studies (see also Section 13.0): Patients who discontinue belinostat/AZT treatment prior to completion of study therapy for any reason (e.g.

disease progression) will have 5 yellow tops drawn for HTLV-1 proviral load. (Appendix G).

- Standard restaging (±4 weeks) or more frequently if clinically indicated: CT scans of the neck (if involved at baseline only), chest, abdomen, and pelvis
- Bone marrow biopsy (if presumed or involved at baseline to confirm CR if patient agrees)

11.4 Follow-up Evaluations

Subjects who achieve/maintain CR and have completed one year of protocol treatment (i.e. 8 cycles of belinostat/AZT followed by AZT-based maintenance therapy) and subjects who are removed from belinostat treatment early and remain on the study will be followed every 3 months (±30 days) up to the end of month 24 according to study calendar for clinical assessments:

Complete physical examination (PE): includes neurologic examination, careful measurement of all palpable peripheral lymph nodes and measurement of other sites of disease present on physical examination including skin. Palpable lymph nodes or masses should be measured in at least two dimensions.

- ECOG or Karnofsky performance score (ECOG PS or KPS) See also Appendix C,
- Vital signs (V/S: including heart rate (HR), blood pressure (BP), respiratory rate (RR), and oral temperature (oral temp)
- Weight
- CBC, with differential and platelet count

Response assessment will then be performed every 6 months for 1 year.:

- Skeletal X-ray Survey if positive for bone lesions at baseline
- CT scans of the neck, chest, abdomen, and pelvis
- Bone marrow biopsy (if involved at baseline and only to confirm CR)

Thereafter, these subjects will continue to be followed every 6 months (± one month) during year 3 to 5, for overall survival via a telephone call, at a minimum and failure-free survival from available medical records.

Subjects who are removed from the study for disease progression or due to adverse event will be followed for survival every 6 months (±one month) for 5 years.

Subjects who withdraw consent will come off study.

11.5 Calendar of Clinical and Laboratory Evaluations

	Screening	Cycle 1					Cycles 2, 3, 4, 5, 6, 7, and 8			End of Cycle 3	End of Cycle 8, 9M and 12M	End of Treatment	Follow up
		Day -1	Day 1	Days 3-4	Day 8 ±2 days	Day 15 ±2 days	Day 1 (≤3 days)	Day 8 (±2 days)	Day 15 ±2 days	Day 21 (≤7 days)		EDT (≤35 days)	±30 days
Informed Consent	X												
Eligibility	X												
Complete Medical History	X												
Complete PE	X		X				X[F]			X	X	X	X[I]
ECOG PS or KPS	X		X				X[F]					X	
V/S (HR, BP, RR, and oral temp)	X		X				X[F]					X	
Height	X												
Weight	X		X				X[F]					X	
CBC w/ diff, PLT	X[A]		X	X	X	X	X[F]	X	X	X	X	X	X[I]
CMP	X		X	X	X		X[F]			X	X	X	X[I]
LDH	X		X	X	X		X[F]			X	X	X	
Uric acid	X												
Hepatitis profile	X												
Serum Pregnancy test[E]	X												
Leukemia/Lymphoma standard panel (by Flow Cytometry)	X									X	X	X	
Standard T-cell receptor gene rearrangement (by multiplex PCR)										X	X	X	
12 Lead ECG	X												
Skeletal Survey	X										X[C]	X[C]	X[C, I]
CT (neck, C/A/P) with contrast unless contrindicated)	X										X	X	X[I]
Bone Marrow Biopsy[G]	X									X	X	X	X[I]
Lumbar Puncture with routine studies and cytology[H]	X												
Zidovudine (AZT); sec 8.5.2		X[D]	X										
Belinostat			X (Starting at Cycle 1 Day 1 to Cycle 8 Day 21)										
Optional: IFN-α-2b or PEG-IFN-α2b	X	X											
Correlative studies (see Sec 13.0 and Appenices F & G for details)	X			X	X	X	X			X	X	X	
Concomitant Medications		X											
Adverse Events			X										XJ
Survival (Can be done via telephone call)													X[I]

[A] The baseline laboratory tests: CBC with differentials and PLT, CMP (electrolytes, creatinine, total bilirubin, calcium, phosphorus, AST, ALT, alk phos, total protein, albumin), LDH, and uric acid are to be repeated within 48-hours prior to the initiation of zidovudine (AZT) (Day 1) unless subject was already on this drug prior to enrollment).

[B] Baseline hepatitis profile including hepatitis B surface antibody, hepatitis B surface antigen, and hepatitis C antibody are to be done within 6 months prior to study enrollment.

[C] Skeletal X-ray survey (skull, chest, pelvic and shoulder girdle, spine and bone survey) is optional at baseline and thereafter and ONLY if hypercalcemia is present at baseline/ pretreatment and if no bone lesions were identified by CT scan.

[D] Zidovudine (AZT) to be administered at least 24-hours prior to the first dose of belinostat (for patients who are AZT-experienced, as well as for patients who are AZT-naïve) (i.e. 24-hours before C1D1 of belinostat).

[E]Repeat pregnancy test, serum Theophylline and thyroid stimulating hormone (TSH) after baseline/screening only, if clinically indicated.

[F]Do not need to repeat for Cycle 2, day 1 if these assessments completed within the day before (i.e. Cycle 1, day 21).

[G]If performance of bone marrow biopsy will delay treatment initiation (e.g., falling on a weekend or holiday), it may be delayed ±3 days but must be performed to confirm CR at the end of months 2, 6, 9, and 12 (i.e. at the end of cycles 3, 8, and end of months 9 and 12 during AZT maintenance treatment).

[H]Lumbar puncture with routine studies and cytology will be done at the discretion of the treating Investigator(s) if lymphomatous CNS involvement is suspected. It is discouraged in patients with blood circulating ATLL cells (i.e. active leukemia) unless otherwise warranted by symptoms.

[I] Follow up schedule for patients who achieve/maintain CR and completed 1 year of therapy: every 3 months (±one month) for clinical assessment (Complete PE, CBC, CMP, and LDH), with response assessment (CT, BMX and Skeletal scans if indicated only) every 6 months for 1 year (during Year 2). Then every 6 months (±one month) during Years 3 to 5, for overall survival (OS) via a telephone call, at minimum. Subjects who discontinue treatment for disease progression will come off treatment and will be followed for survival every 6 months (±one month) for 5 years. Adverse events will be collected starting on Day 1 of treatment and continuing until 30 days post treatment

12.0 SCHEDULE OF CORRELATIVE EVALUATIONS

Venous blood will be collected at the following time points (up to 10-ml per tube) for immunologic assays as well as molecular evaluations and analysis of ATLL and HTLV-1 clones. Please also refer to Appendices F and G for more information.

12.1 Pre-Treatment Specimen Collection (Screening)

The following must be collected/ performed **within 7 days prior** to administering Cycle 1, day 1 of treatment:

- Immune response and cytotoxic T-cell (CTL) assays: a total of 3 green cap tubes will be collected (See Appendix F).
- Molecular, TCR PVL, and clonal abundance studies: A total of 5 yellow cap tubes will be collected for processing to perform the following: (See Appendix G)
  - Measurements of baseline histone acetylation, Tax, and HBZ levels
  - HTLV-1 proviral (PVL) load (single molecule droplet-based digital PCR)

12.2 On Treatment Specimen Collection

12.2.1 Cycle 1, days 3 to 4 (72 to 96 hours after belinostat initiation)

- 5 yellow cap tubes for correlative studies: to investigate molecular mechanisms of belinostat on ATLL cells (MOL), including HTLV-1 reactivation (PVL), histone acetylation, and expression of HTLV-1 genes Tax and HBZ (see also Appendix G)

12.2.2 Cycle 1, day 8 (± 3 days)

- 3 green cap tubes for immune response and cytotoxic T-cell assays (see also Appendix F)

12.2.3 Cycle 1, day 15 (±3 days)

- 5 yellow cap tubes for HTLV-1 proviral load and clonal abundance studies (see also Appendix G)

12.2.4 Cycle 1, day 21 (± 3 days)

- 3 green cap tubes (for immune response and cytotoxic T-cell assays as also detailed in Appendix F)

12.2.5 End of Cycle 3 (≤7 days prior to day 21)

- 5 yellow cap tubes for HTLV-1 proviral load and clonal abundance studies (as in Appendix G)

12.2.6 End of Cycle 8 (≤14 days prior to day 21)

- 5 yellow cap tubes for HTLV-1 proviral load and clonal abundance studies (as in Appendix G)

12.2.7 End of Month 9 (≤14 days prior)

- 5 yellow cap tubes for HTLV-1 proviral load and clonal abundance studies (as in Appendix G)

12.2.8 End of Month 12 (≤14 days prior)

- 5 yellow cap tubes for HTLV-1 proviral load and clonal abundance studies (as in Appendix G)

12.3 Off-Treatment Specimen Collection (End of Treatment or EOT visit)

The following must be performed at the EOT visit and should occur 30-days (± 7 days) after the last dose of study treatment.

- 5 yellow cap tubes for HTLV-1 proviral load and clonal abundance studies (as in Appendix G)

12.4 Calendar of Specimen Collection for Correlative Studies

Table 3: Table of Correlative Studies

Time points	MOL (5 yellow top)*	PVL (5 yellow top)*	CTL (3 green top)
After enrollment (≤7 days prior to treatment initiation)	X (5 yellow tops (Acid Citrate Dextrose (ACD) solutions A/B additives –Trisodium citrate 22.0/13.2, citric acid 8.0/4.8 and dextrose 24.5/14.7 (in g/L) total for both assays together)		X (Sodium heparin,Lithium heparin)

Cycle 1, day 3-4(±1 day)	X		
Cycle 1, day 8 (±1)			X
Cycle 1, day 15 (±2)		X	
Cycle 1, day 21 (≤3)			X
Cycle 3, day 21 (≤7)		X	
Cycle 8, day 21 (≤14)		X	
End of Month 9, (≤14 days)		X	
End of Month 12, (≤14 days)		X	
End of Treatment*(early discontinuation not due to disease progression)*		X	

13.0 MEASUREMENT OF EFFECT

All subjects will be evaluated during (including *pre*- and *post*-dose) cycle 1, 8 (i.e. end of month 6), and at the end of months 9 and 12 by physical examination, white blood cell counts (WBC), and measurements of any initially elevated serum markers due to tumor, including calcium, lactose dehydrogenase (LDH), alkaline phosphatase, and liver enzymes.

Complete re-staging by imaging scans and evaluations for molecular response by gene rearrangement studies (i.e. minimal residual disease) will also be done at these time points.

Thereafter, follow-up for lymphoma subjects will be conducted at least every 6 months in year 2. After this period, patients will be followed as routine/standard-of-care approximately every 6 months or sooner if clinically indicated for up to 5 years from treatment initiation for the purpose of future trial results updates.

Comparison to baseline/pre-study evaluations will be made for imaging scans, bone marrow biopsy (if marrow was initially involved), and peripheral blood smear (if applicable).

13.1 Response Assessment

Response is assessed on the basis of clinical, radiologic, molecular and pathologic (i.e. bone marrow) criteria. Studies will be performed after belinostat end of cycle 3, cycles 8 (end of month 6), at the end of months 9 and 12 under this study, and then every 6 months up to year 2.

- CT scans remain the standard for evaluation of nodal disease.

- If non-contrast CT-PET is obtained, for instance in patients where IV contrast is contraindicated due to allergy or renal dysfunction, it is recommended that only the CT portion be used for response assessment during treatment.
- After the completion of treatment, the PET portion can be useful for confirming CR or relapse/progression, however positive PET findings could be seen in reactive lymph nodes, especially in patients with HTLV-1.
- Thoracic, abdominal, and pelvic scans will be performed for restaging even if those areas were not initially involved because of the unpredictable pattern of recurrence in ATLL.

- A bone marrow aspirate and biopsy should only be performed to confirm a CR if they were initially positive or if it is clinically indicated by new abnormalities in the peripheral blood counts or blood smear
- Evaluation for minimal residual disease will be done by standard flow cytometry and T-cell receptor gene rearrangement studies.

13.2 Complete Response (CR)

The designation of Complete Response (CR) requires the following:

- Complete disappearance of all detectable clinical evidence of disease and disease-related symptoms if present before therapy.
- Absolute lymphocyte count must be < 3.5 X 10^3/µl with no more than 5% of circulating typical lymphocytes cells on peripheral blood by morphology
- Normalization of those biochemical abnormalities (e.g. calcium) definitely assignable to the tumor.
- All lymph nodes and nodal masses must have regressed on CT to normal size (1.5 cm in their greatest transverse diameter for nodes > 1.5 cm before therapy). Previously involved nodes that were 1.1 to 1.5 cm in their long axis and more than 1.0 cm in their short axis before treatment must have decreased to 1.0 cm in their short axis after treatment. (Lymph nodes should be considered abnormal if the long axis is more than 1.5 cm regardless of the short axis. If a lymph node has a long axis of 1.1 to 1.5 cm, it should only be considered abnormal if its short axis is more than 1.0. Lymph nodes ≤ 1.0 x ≤ 1.0 cm will not be considered as abnormal for relapse or progressive disease).
- The spleen and/or liver, if considered to be enlarged before therapy on the basis of a physical examination or CT scan, should not be palpable on physical examination and should be considered normal size by imaging studies, and nodules related to lymphoma should disappear. However, determination of splenic involvement is not always reliable because a spleen considered normal in size may still contain lymphoma, whereas an enlarged spleen may reflect variations in anatomy, blood volume, the use of hematopoietic growth factors, or other causes other than lymphoma.
- If the bone marrow was involved by lymphoma before treatment, the infiltrate must be cleared on repeat bone marrow biopsy. The biopsy sample on which this determination is made must be adequate. If the sample is indeterminate by morphology, it should be negative by immunohistochemistry. A sample that is

negative by immunohistochemistry but that demonstrates a small population of clonal lymphocytes by flow cytometry will be considered a CR until data become available demonstrating clear difference in subject outcome.

- No new sites of disease.
- May have minimal residual disease at the molecular level.
- Stable complete response: Response must be durable for at least 1 month.
- Standard CT-PET if available and if covered by insurance may be used to confirm CR.

13.3 Partial Response (PR)

The designation of Partial Response (PR) requires the following:

- At least a 50% decrease in sum of the product of the diameters (SPD) of up to six of the largest dominant nodes or nodal masses. These nodes or masses should be selected according to all of the following: they should be clearly measurable in at least two perpendicular dimensions, if possible they should be from disparate regions of the body, and they should include mediastinal and retroperitoneal areas of disease whenever these sites are involved.
- No increase should be observed in the size of other nodes, liver, or spleen.
- Splenic and hepatic nodules must regress by ≥ 50% in their SPD or, for single nodules, in the greatest transverse diameter.
- With the exception of splenic and hepatic nodules, involvement of other organs is usually assessable and no measurable disease should be present.
- Bone marrow assessment is irrelevant for determination of a PR if positive before treatment. However, if positive, the cell type should be specified in the report, (e.g., large-cell lymphoma or small neoplastic B cells).
    - Subjects who achieve a CR by the above criteria (Section 14.2), but who have persistent morphologic bone marrow involvement will be considered partial responders.
    - In cases when the bone marrow was involved before therapy and a clinical CR was achieved, but no bone marrow assessment was performed after treatment, subjects should also be considered partial responders.
- No new sites of disease should be observed
- At least 50% decrease in absolute lymphocyte count from baseline if it continues to be > 3,500 cells/µl.
- (Stable Partial Response :) Response must be durable for at least 1 month.
- Standard CT-PET if available and if covered by insurance may be used to help determine response and to evaluate any suspicious new disease.

13.4 Stable Disease (SD)

Stable Disease is defined as less than a PR (see above) but is not progressive disease (see below).

13.5 Progressive Disease (PD, non-responders)

The designation of Progressive Disease (PD, non-responders) require the following:

- Appearance of any new lesion more than 1.5 cm in any axis during or at the end of therapy, even if other lesions are decreasing in size.
  - If CT/PET is done FDG uptake in a previously unaffected site should only be considered relapsed or progressive disease after confirmation with other modalities.
  - In subjects with no prior history of pulmonary lymphoma, new lung nodules identified by CT are mostly benign. Thus, a therapeutic decision should not be made solely on the basis of the PET without histologic confirmation.
- At least a ≥ 50% increase from nadir in the SPD of any previously involved nodes or in a single involved node, or the size of other lesions (e.g., splenic or hepatic nodules).
- To be considered progressive disease, a lymph node with a diameter of the short axis of less than 1.0 cm must increase by ≥ 50% and to a size of 1.5 x1.5 cm or more than 1.5 cm in the long axis.
- At least a 50% increase in the longest diameter of any single previously identified node more than 1 cm in its short axis.
- At least 50% increase in absolute lymphocyte count from the greater of the following: baseline absolute lymphocyte count or 3.5 X 10^3/µl (cut off normal absolute lymphocyte count in patients with HTLV-1). To meet PD criteria the 50% increase in absolute lymphocyte should be confirmed by repeat CBC on or after 3 days. **Note: A transient increase in absolute lymphocyte count during cycle 1, which may occur after belinostat (possibly due to re-activation of HTLV-1), and transient (sporadic) skin lesions that disappear during treatment will not be considered disease progression.**
- Standard CT-PET if available and if covered by insurance may be used to help determine response and to evaluate any suspicious new disease.

13.6 Recurrent Disease

Recurrent disease is defined as the appearance of tumor following documentation of a complete remission.

13.7 Time to Response

Time to response is defined as time from the first dose of chemotherapy until documentation of first *response*.

13.8 Time to progression

Time to progression is defined as time from initiation of chemotherapy to documentation of first *progression*.

14.0 ADVERSE EVENTS

The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.03 or subsequent new versions will be utilized for adverse event reporting.

14.1 Purpose

Adverse event data collection and reporting, which are required as part of every clinical trial, are done to ensure the safety of patients enrolled in the studies, as well as those who

will enroll in future studies using similar agents. Adverse events are reported in a routine manner at scheduled times during a trial. Additionally, certain adverse events must be reported in an expedited manner for timelier monitoring of patient safety and care.

14.2 Adverse Event

Adverse Event (AE): Can be any unfavorable and unintended sign (e.g., an abnormal laboratory finding), symptom, or disease temporally associated with the use of a drug, medical treatment, or procedure without judgment about causality. An adverse event can arise from any use and from any route of administration, formulation, or dose including an overdose. This includes any newly occurring event or a previous condition that has increased in severity or frequency since initiation of a drug, medical treatment, or procedure.

Abnormal Findings

In any clinical assessment, a value outside the normal or reference range (such as a clinical laboratory, vital sign, or ECG) will not be reported or assessed as an AE unless that value is considered to be of clinical significance by the investigator. A value of clinical significance is one that leads to discontinuation or delay in protocol treatment, dose modification, therapeutic intervention*, or is considered to be a clinically significant new finding or change from baseline by the investigator.

*Transfusion support administered to offset clinical symptoms of anemia or thrombocytopenia will not be considered therapeutic intervention.

Signs and Symptoms

Signs/symptoms resulting from an underlying clinical diagnosis should be documented as one comprehensive AE. If no underlying clinical diagnosis can be identified, each sign/symptom should be reported as a separate independent event. (A new or worsening event resulting from an underlying clinical diagnosis or a reaction to concurrent medications should be documented as a separate independent AE unless it is within the normal range of fluctuation for that patient.)

Grade Changes/Fluctuations

AEs will be reported at the maximum grade/severity experienced for the duration of the event. Should one particular event warrant further investigation, additional details may be collected at the discretion of the Principal Investigator.

Progression of Disease

Progression of disease, if documented in accordance to standard of care, should not be reported as an AE.

Tests and Procedures

Tests and procedures should not be reported as AEs. The underlying clinical diagnosis (or sign/symptom in the event an underlying clinical diagnosis is not known) requiring testing or a procedure, should be reported as an adverse event if it meets criteria for reporting.

14.3 Serious Adverse Events (see also Appendix A)

Serious AE (SAE) means any untoward medical occurrence that occurs at any dose:

1. **Results in death.**
2. **Is life-threatening.**
   The term "life-threatening" in the definition of "serious" refers to an event in which the patient was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe).
3. **Requires inpatient hospitalization or prolongation of present hospitalization.**
   Elective hospitalization to simplify protocol treatment/evaluations or to treat a baseline condition that did not worsen from baseline will not be considered an SAE.
4. **Results in persistent or significant disability/incapacity.**
   Disability is defined as a substantial disruption of a person's ability to conduct normal life functions.
5. **Is a congenital anomaly/birth defect**.
6. **Is a medically important event.**
   A medically important event may not be immediately life threatening or result in death or hospitalization but may jeopardize the patient or may require intervention to prevent one of the other outcomes listed in the definition above, or involves suspected transmission via a medicinal product of an infectious agent. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse; any organism, virus, or infectious particle (e.g., prion protein transmitting Transmissible Spongiform Encephalopathy), pathogenic or nonpathogenic, is considered an infectious agent.

   Clarification should be made between the terms *serious* and *severe* because they ARE NOT synonymous. The term *severe* is often used to describe the intensity (severity) of a specific event (as in mild, moderate, or severe myocardial infarction); the event itself, however, may be of relatively minor medical significance (such as a severe headache). This is NOT the same as *serious*, which is based on patient/event outcome or an action criterion described above and is usually associated with events that pose a threat to a patient's life or functioning. A severe AE does not necessarily need to be considered serious. For example, persistent nausea of several hours duration may be considered severe nausea but not an SAE. On the other hand, a stroke resulting in only a minor degree of disability may be considered mild but would be defined as an SAE based on the above noted criteria. Seriousness (not severity) serves as a guide for defining regulatory reporting obligations.

14.4 Adverse Event Collection Period

In this protocol, adverse events include only treatment-emergent adverse events. A treatment-emergent adverse event (TEAE) is defined as any event that begins or worsens after the start of protocol treatment. All baseline-emergent adverse events, any event that begins or worsens after completion of the informed consent but prior to the start of protocol treatment, should be reported as a Baseline/Comorbid Condition.

All adverse events that occur within ≤ 30 days of the last dose of study therapy will be reported and followed until resolution. Resolution is defined as a return to baseline status or the stabilization of an event with the expectation that it will remain chronic. (Exception: If a patient begins an alternative therapy that confounds accurate assessment of AEs within ≤ 30 days of the last dose of study therapy, all adverse event collection will stop and any ongoing events will be left open.)

14.5 Adverse Event Reporting Requirements

The information to be reported in AEs will be assessed by and assigned severity using the National Cancer Institute Common Terminology Criteria for Adverse Events (NCI CTCAE), Version 4.03. The NCI CTCAE provides descriptive terminology and a grading scale for each adverse event listed. A copy of the NCI CTCAE v4.03can be downloaded from the CTEP home page (http://evs.nci.nih.gov/ftp1/CTCAE/About.html).

Information to be reported in the description of each adverse event may be included, but is not limited to:

1. Clinical Diagnosis of the event as determined by NCI CTCAE, Version 4.03descriptive terminology. If no clinical diagnosis can be identified, each sign/symptom should be reported as a separate independent event.
2. Date of onset of the AE (start date).
3. Date of resolution of the AE (end date).
4. Severity of the event determined by NCI CTCAE, Version 4.03 grading scale.
5. Relationship of the AE to study therapy. Categorized as follows:

Definite	The adverse event is clearly related to the investigational agent(s)
Probable	The adverse event is likely related to the investigational agent(s)
Possible	The adverse event may be related to the investigational agent(s)
Unlikely	The adverse event is doubtfully related to the investigational agent(s)
Unrelated	The adverse event is clearly not related to the investigational agent(s)

6. Whether or not the AE is Serious or Not Serious as defined in Section 15.3 Serious Adverse Events.
7. Whether the AE is Suspected and/or Unexpected.

Suspected	Any AE for which there is a reasonable possibility that the drug caused the AE. For the purposes of expedited safety reporting, ‘reasonable

	possibility' means there is evidence to suggest a causal relationship between the drug and the AE.
Unexpected	Any AE for which the nature or severity of the event is not consistent with the applicable product information, e.g., the Investigator's Brochure or Package Insert.

8. Action taken as a result of the AE.
9. Outcome.

14.6 Expedited Adverse Event Reporting Requirements

All AEs, regardless if serious or not, will be described in the source documents, reported on the applicable AE page of the CRFs, and entered into *Velos*. However, certain adverse events must also be reported in an expedited manner for timelier monitoring of patient safety and care. Appendix A provides information about these expedited reporting requirements.

15.0 STATISTICAL CONSIDERATIONS

15.1 Overview

The primary objectives of this prospective clinical study in ATLL patients with leukemia types (chronic or acute) receiving belinostat-based treatment are the following:

1) To determine the complete molecular response (CMR) rate after adding belinostat as consolidation therapy for ATLL during AZT-based maintenance treatment.

2) To determine the safety of adding belinostat to AZT-based maintenance regimen (+/- IFNα or PEG-IFNα) as consolidation therapy for ATLL.

Secondary objectives are to determine best clinical response, failure-free survival, and overall survival. Correlative and scientific endpoints in this study include investigating whether belinostat disrupts HTLV-1 latency *in vivo*, determining whether belinostat provokes a cytotoxic T-cell response *in vivo*, measuring the overall impact of belinostat/AZT on HTLV-1 proviral load *in vivo*, and determining its effects on histone acetylation, HTLV-1 basic zipper factor (HBZ) and Tax expression.

15.2 Definitions

- A patient is considered to be on study at the time an informed consent is signed.
- Evaluable patients will be study-eligible patients who initiate the study treatment, receiving at least one (1) dose of belinostat, regardless of treatment being completed. However, those patients who will be considered **Evaluable for the primary endpoint "molecular response"** will be study-eligible patients who receive **at least three (3) doses** of belinostat (with AZT), have evaluable disease at baseline and at least one post-baseline molecular disease assessment. All evaluable patients will be assessed for treatment response, toxicity, disease progression, and survival.
- Patients are considered to be on treatment as long as they continue to receive study treatment. Patients who are off treatment will remain on study and will be followed for toxicity, response, progression, and survival.

Exclusions (not evaluable patients) from study:

- Patients who are enrolled on study but not treated (do not receive any dose of study treatment) will be excluded from all analyses. Reasons for such withdrawals, such as eligibility not confirmed or consent withdrawn, will be characterized.
- Any patient who is treated but later found to be ineligible for study (a "protocol violation") will be withdrawn from study but followed for survival and toxicity. Such patient experience will be characterized separately from that of evaluable patients.
- Failure–free survival (FFS) will be measured from the date of treatment initiation until date of documented disease progression, relapse after response, or death from any cause. For patients alive and free of relapse or progression, follow-up time will be censored at the last documented date of failure-free status.
- Overall survival (OS) will be measured from the date of initiation of study treatment until date of death from any cause. In the absence of death, the follow-up will be censored at date last known to be alive (censored observation).

15.3 Sample Size

This study plans to enroll up to a total minimum of 10 (in the case primary end point is reached) or maximum of 20 evaluable patients with the leukemic form of ATLL within a period of 5 years and follow them for a minimum of 1 year to obtain information on response to belinostat-based treatment, disease progression and overall survival. Expected time to study completion is approximately 6 years from date open to enrollment. The main objective of this study is to investigate whether adding belinostat to AZT-based consolidation therapy eradicates residual ATLL. **The study can be closed at protocol Chair's discretion after reaching the primary end point.** A study with 20 patients treated with belinostat will have 80% power to detect a complete molecular response (CMR) rate of 20.2% based on a one-sided binomial test at the 10% significance level (7.6% actual significance level), and assuming an expected complete molecular response rate without treatment of 5% (1/20). (Hintze, J. (2014). PASS 13. NCSS, LLC. Kaysville, Utah, USA. www.ncss.com.) Based on a sample size n=10, we can calculate the 90% lower-limit confidence interval from the observed complete molecular response rate (P). With n=10, two or more complete molecular responses are needed to conclude at the target 10% one sided significance level that the true rate is greater than 5%. Note that the lower limit is 5.5% if observed rate is 20%, 11.6% if observed rate is 30% (3 responses in 10), and 18.8% if observed rate is 40% (4 responses in 10 patients). A sample size of 10 produces a one-sided 90% lower-limit confidence interval with a distance from the observed sample proportion 0.4 to the lower limit that is equal to 0.212.

Confidence Intervals for One Proportion

Numeric Results for One-Sided Lower-Limit Confidence Intervals for One Proportion

Confidence Interval Formula: Exact (Clopper-Pearson)

	Sample	Target Distance	Actual Distance	**Observed**			Distance from P to

Confidence Level	Size (N)	from P to Lower Limit	from P to Lower Limit	**Proportion (P)**	**Lower Limit**	Upper Limit	Limit if P = 0.5
0.9	10		0.145	0.2	0.055	1	0.233
0.9	10		0.184	0.3	0.116	1	0.233
0.9	10		0.212	**0.4**	**0.188**	1	0.233
0.9	10		0.233	0.5	0.267	1	0.233
0.9	20		0.11	0.2	0.09	1	0.162
0.9	20		0.134	0.3	0.166	1	0.162
0.9	20		0.151	0.4	0.249	1	0.162
0.9	20		0.162	0.5	0.338	1	0.162

Tests for One Proportion

Numeric Results for Testing One Proportion using the Exact Test

Alternative Hypothesis: One-Sided (H0: P ≤ P0 vs. H1: P > P0)

Power*	**n**	**Proportion Given H0 P0**	**Proportion Given H1 P1**	**Difference P1-P0**	**Target Alpha**	**Actual Alpha*†**	**Reject H0 If R ≥**
0.80000	10	0.0500	0.2710	0.2210	0.100	0.086	2
0.80000	20	0.0500	0.2020	0.1520	0.100	0.075	3

15.4 Statistical Analyses

Analysis of primary and secondary endpoints will be based on all evaluable patients, as per Section 16.2.

Adverse events and toxicities will be tabulated by severity and attribution to treatment according to the NCI CTCAE 4.03 criteria.

Baseline descriptive statistics will be provided for study patients. These will include demographic variables (age, sex and race), and disease characteristics (tumor stage, tumor size, lymph nodes).

The complete molecular response (CMR) rate, as well as overall clinical response rate (CR+PR) and rates of the individual categories of best clinical response (CR, PR, SD, and PD) will be estimated by the percentage of patients achieving each specific response type. The precision of these estimates will be characterized by the corresponding 95%

confidence intervals using the exact binomial method. The duration of response will be summarized (median and range) for each response type.

Addressing the primary objective, we will also report the proportion of patients with minimal (molecular) residual disease present among responders at the end of months 2, 6, 9, and 12.

Addressing secondary objectives, the Kaplan-Meier method will be used in the analysis of failure-free survival (FFS) and overall survival (OS). We will report median FFS and OS times and corresponding rates at 6 and 12 months.

Addressing other secondary objectives, we will report the proportion of patients exhibiting a cytotoxic T-cell response upon treatment with belinostat *in vivo*, or specific molecular alterations from correlative studies, and correlate with response using two-sample t-test.

16.0 DATA REPORTING

Data must be submitted according to the protocol requirements for all patients registered. Patients for whom documentation is inadequate to determine eligibility will generally be deemed ineligible.

16.1 Data and Safety Monitoring

The Sylvester Comprehensive Cancer Center (SCCC) Data and Safety Monitoring Committee (DSMC) will monitor this clinical trial according to the Cancer Center's DSM Plan. In its oversight capacity, the DSMC bears responsibility for suspending or terminating this study. DSMC oversight of the conduct of this trial includes ongoing review of accrual and adverse event (AE) data, and periodic review of the combination therapy of belinostat with AZT (and Interferon-alfa2b when applicable). The guidelines appearing in the Section 17.2is offered for DSMC consideration in assessing AEs. In addition, the DSMC will review reports from all audits, site visits, or study reviews pertaining to this clinical trial and take appropriate action. The SCCC DSM Plan to which this study is subject can also be found at www.sylvester.org.

16.2 Early Stopping Rules (for Interim Monitoring of Toxicity)

In the following sections, we provide stopping guidelines for the DSMC in its review of accumulating data on toxicity over the course of this trial. The proposed guidelines were developed using Bayesian methods, which can be applied at any stage of enrollment without advance specification of the number of interim analyses to be performed, or the number of patients evaluable for toxicity at the time such assessments are made. Under the Bayesian method, we assign a prior probability (level of belief at the start of the trial) to a range of possible values for the true toxicity rate. As data on treated patients become available, each of these probability distributions is revised and the resulting posterior probability becomes the basis for recommending either early termination or continuation of the study. Underlying assumptions for the prior distributions are also presented.

16.2.1 Guidelines for interim monitoring of toxicity

If a treatment-related (possible, probable or definite) death occurs, enrollment will be suspended and continuation of the study will be reassessed by the DSMC.

Due to the nature of the disease and the patients' preexisting conditions, we expect grade 3 or 4 hematologic toxicities to occur in at least 50% of the already bone marrow-compromised leukemic patients. The hematologic toxicities we expect to occur are leukopenia (low white blood cell count), neutropenia (low percentage and absolute neutrophil count), lymphopenia (low percentage and absolute lymphocyte count), anemia (low hemoglobin and hematocrit), and thrombocytopenia (low platelets).

Unexpected grade 3 and 4 treatment–related (possible, probable, or definite) toxicities may occur in 10% or less of the patients on study.

We suggest as a guideline for possible early termination, evidence that the proportion of patients experiencing unexpected treatment-related grade 3 or 4 toxicity exceeds 15% (see Section 9.0 for expected toxicity). Specifically, we suggest as a guideline for early termination a posterior probability of 95% or higher that the rate of unexpected treatment-related grade 3 or higher toxicity exceeds 15%. The table below present's specific instances where this guideline is met, suggesting early termination due to evidence of an excessive level of unexpected toxicity.

Table 4: Early Termination Guidelines for Unexpected Toxicity

Number of patients experiencing unexpected, treatment-related grade 3 or 4 toxicity (%)	Number of patients assessed for toxicity
5	3 to 6
6	7 to 10
7	11 to 14
8	15 to 19

To illustrate the stopping guidelines, suppose that 5 evaluable patients have been assessed for toxicity and 3 of them have experienced unexpected treatment-related grade 3 or 4 toxicity (row 2 of the above table.) Under this circumstance, the observed rate of unacceptable unexpected toxicity is 60%, resulting in a posterior probability of 96.7% that the true underlying rate exceeds 15%, thereby suggesting early termination of study.

Posterior probabilities for the table above are calculated under a weak prior beta distribution with parameters $\beta 1=0.2$ and $\beta 2=1.8$, which corresponds to an expected unacceptable-toxicity rate of 10% based on very limited information, roughly equal to having studied 2 patients. This prior distribution implies also a prior chance of only 21.6% that true rate of unacceptable unexpected toxicity is 15% or greater.

16.3 Interim Review of Safety Data (Role of the Research Team)

The protocol management team, consisting of two of the investigators for this study AND the statistician, will continuously monitor study accruals, toxicities, and response to treatment.

The protocol management team may convene at their discretion to review the accumulated safety data. Accrual will continue as long as the protocol management team has no clinical concerns regarding the incidence of unexpected, treatment-related Grade 3 or 4 adverse events. Within 10 working days, after 5 patients enrolled in the trial are treated for at least one month the protocol management team will review the safety data.

Consideration must be given to amending or closing this study if the incidence of Grade 3 or higher adverse events is exceedingly high compared to the incidence reported for previous or ongoing trials.

16.4 Study Termination

The DSMC/IRB retains the right to terminate the study for any cause, suspend patient enrollment and related study materials from the study site at any time. Specific instances, which may precipitate such termination at a site, are as follows:

- Deviation from protocol requirements
- Inaccurate and/or incomplete data recording on a recurrent basis
- Unauthorized use of investigational products or administration to any subject not enrolled as part of the protocol
- Delinquent fulfillment of obligation on the part of the Investigator with regard to adverse reaction reporting, unacceptable patient enrollment or other responsibilities as outlined in this protocol.

17.0 STUDY MONITORING

This study will be monitored according to http://research.med.miami.edu/clinical-research/crors/monitoring and http://uresearch.miami.edu/regulatory-compliance-services/rcqa.

18.0 INVESTIGATOR RESPONSIBILITIES

18.1 Investigator Responsibility/Performance

The investigator will ensure that this study is conducted in accordance with all regulations governing the protection of human subjects. The investigator will ensure that all work and services described in or associated with this protocol will be conducted in accordance with the investigational plan, applicable regulations, and the highest standards of medical and clinical research practice.

18.2 Confidentiality

The investigator must ensure that each subject's anonymity will be maintained and each subject's identity will be protected from unauthorized parties. A number will be assigned to each subject upon study entry and the number and the subject's initials will be used to identify the subject for the duration of the study. The investigator will maintain all documents related to this study in strict confidence.

18.3 Informed Consent and Permission to Use Protected Health Information

It is the responsibility of the investigator to obtain written informed consent from each subject participating in this study after adequate explanation, in lay language, of the methods, objectives, anticipated benefits, and potential hazards of the study. The investigator must also explain that the subject is completely free to refuse to enter the study or to discontinue participation at any time (for any reason) and receive alternative conventional therapy as indicated. Prior to study participation, each subject will sign an IRB approved informed consent form and receive a copy of same (and information leaflet, if appropriate). For subjects not qualified or able to give legal consent, consent must be obtained from their legally authorized representative (LAR). The investigator or designee **must** explain to the subject before enrollment into the study that for evaluation of study results, the subject's protected health information obtained during the study may be shared with the study sponsor, regulatory agencies, and the IRB. It is the investigator's (or designee's) responsibility to obtain permission to use protected health information per HIPAA from each subject, or if appropriate, the subjects' parent or legal guardian.

18.4 Source Documentation and Investigator Files

The investigator must maintain adequate and accurate records to fully document the conduct of the study and to ensure that study data can be subsequently verified. These documents should be classified into two separate categories: (1) investigator study file and (2) subject clinical source documents that corroborate data collected on the CRF's. Subject clinical source documents may include hospital/clinic patient records; physician's and nurse's notes; appointment book; original laboratory, ECG, EEG, radiology, pathology, and special assessment reports; pharmacy dispensing records; subject diaries; signed informed consent forms; and consultant letters. When the CRF or any form is used as the source document, this must be clearly stated in the investigator study file. Minimally, the following be documented in source documents:

- Medical history/physical condition and diagnosis of the subject before involvement in the study sufficient to verify protocol entry criteria
- Study number, assigned subject number, and verification that written informed consent was obtained (each recorded in dated and signed notes on the day of entry into the study)
- Progress notes for each subject visit
- Documentation of treatment
- Laboratory test results
- Adverse events (action taken and resolution)
- Condition and response of subject upon completion of or early termination from the study

18.5 Recording and Processing of Data

If using hard copies of CRF's, study center personnel will complete individual CRF's in black ink. All corrections to entered data will be made by drawing a single line through the information to be corrected without obscuring it. All corrections will be initialed, dated and explained, if necessary. The use of "white-out" or obscuring correction tape will be prohibited. A CRF is required for every patient who received any amount of study treatment. The investigator will ensure that the CRF's are accurate, complete, legible and timely. Separate source records are required to support all CRF entries except those for which use of the CRF as source document is clearly allowed per note in the investigator study file.

Data must be submitted according to the protocol requirements for ALL patients registered. Patients for whom documentation is inadequate to determine eligibility will generally be deemed ineligible.

Data must be submitted according to the protocol requirements for ALL patients registered. Patients for whom documentation is inadequate to determine eligibility will generally be deemed ineligible.

A list of forms to be submitted, as well as expectation dates may be found in Appendix B.

18.6 Non-Protocol Research

No investigative procedures other than those described in this protocol will be undertaken on the enrolled subjects without the agreement of the IRB.

18.7 Ethics

The investigator agrees to conduct the study in compliance with the protocol, current good clinical practices, and all applicable (local, FDA) regulatory guidelines and standard of ethics.

UM Ethics Programs' Research Ethics Consultation Service (RECS) is a free resource for UM Researchers. See the website for further information: http://www.miami.edu/index.php/ethics/projects/recs/

18.8 Essential documents for the conduct of a clinical trial

Essential documents are those documents with individually and collectively permit evaluation of the conduct of a trial and the quality of the data produced. The following documents should be on file: 1) 1572 (for studies involving IND drugs or devices); 2) CV's and license of all Investigators; 3) IRB documentation/correspondance and 4) Documentation of IRB certification

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APPENDIX A: EXPEDITED ADVERSE EVENT (AE) REPORTING REQUIREMENTS

For all AEs that meet criteria for expedited reporting, the Principal Investigator (PI) is obligated to pursue and provide follow-up reporting information until the event has resolved or until an acceptable medical endpoint has been reached (i.e. for the duration specified in the protocol), or the patient is lost to follow-up.

The PI and all applicable research study team members should become familiar with the safety profile of the investigational agent(s) and/or intervention at the start of the study and for the duration of the research, e.g. by reviewing the Investigator's Brochure (IB) and any Safety Reports released by the Sponsor, as applicable.

A. FDA Expedited Reporting
   a. Sponsor-Investigators i.e. IND Holders, have additional reporting requirements to the FDA and other committees, and should consult the applicable regulations and agency guidelines for these requirements.
   b. Since this protocol involves the use of FDA IND agent(s), completion of the FDA MedWatch 3500A Reporting Form is required for Sponsor-Investigators. The Form can be obtained electronically at: http://www.fda.gov/downloads/AboutFDA/ReportsManualsForms/Forms/UCM048334.pdf
      i. All serious, unexpected (unanticipated) and suspected adverse events must be directly reported to the FDA within 15 calendar days of being made known to the Principal Investigator (PI).
      ii. All fatal or life-threatening AEs must be directly reported to the FDA within 7 calendar days of being made known to the PI.
   c. For more information regarding reporting to the FDA, please refer to the FDA website for REPORTING GUIDELINES: http://www.fda.gov/Safety/MedWatch/HowToReport/default.htm

B. IRB Expedited Reporting
   a. All Investigators should also be aware of local Institutional requirements for AE reporting. For more information regarding the IRB policy, please refer to the UM HSRO's Investigator Manual: http://hsro.med.miami.edu/documents/HRP-103_-_INVESTIGATOR_MANUAL_4.11.2014.docx and the UM HSRO SOP on New Information (HRP-024) https://eprost.med.miami.edu/eProst/Doc/0/HLJ5OTJVQEH419E0I6QPT3B199/HRP-024%20-%20SOP%20-%20New%20Information.docx
   b. All AEs that are serious, unanticipated and probably related will be reported to the IRB within ten (10) working days of being made known to the PI.
   c. Events that are more frequent than anticipated or more severe than expected must be reported to the IRB within ten (10) working days of being made known to the PI.
   d. All unanticipated deaths must be reported to the IRB within 24 hours of being made known to the PI.

APPENDIX B: PERFORMANCE STATUS SCALES

PERFORMANCE STATUS CRITERIA					
ECOG (Zubrod)		**Karnofsky**		**Lansky**	
Score	Description	Score	Description	Score	Description
0	Fully active, able to carry on all pre-disease performance without restriction.	100	Normal, no complaints, no evidence of disease.	100	Fully active, normal.
		90	Able to carry on normal activity, minor signs or symptoms of disease.	90	Minor restrictions in physically strenuous activity.
1	Restricted in physically strenuous activity but ambulatory and able to carry out work of a light or sedentary nature, e.g., light housework, office work.	80	Normal activity with effort, some signs or symptoms of disease.	80	Active, but tires more quickly.
		70	Cares for self, unable to carry on normal activity or do active work.	70	Both greater restriction of, and less time spent in, play activity.
2	Ambulatory and capable of all selfcare but unable to carry out any work activities. Up and about more than 50% of waking hours.	60	Requires occasional assistance, but is able to care for most of his/her needs.	60	Up and around, but minimal active play; keeps busy with quieter activities.
		50	Requires considerable assistance and frequent medical care.	50	Gets dressed, but lies around much of the day; no active play; able to participate in all quiet play and activities.
3	Capable of only limited selfcare, confined to bed or chair more than 50% of waking hours.	40	Disabled, requires special care and assistance.	40	Mostly in bed, participates in quiet activities.
		30	Severely disabled, hospitalization indicated. Death not imminent.	30	In bed, needs assistance even for quiet play.
4	Completely disabled. Cannot carry on any selfcare. Totally confined to a bed or chair.	20	Very sick, hospitalization indicated. Death not imminent.	20	Often sleeping, play entirely limited to very passive activities.
		10	Moribund, fatal processes progressing rapidly.	10	No play, does not get out of bed.
5	Dead	0	Dead	0	Dead

As published in Am J Clin Oncol: Oken MM, Creech RH, Tormey DC, Horton J, Davis TE, McFadden ET, Carbone PP. Toxicity and Response Criteria of the Eastern Cooperative Oncology Group. Am J Clin Oncol 1982;5:649-655. The Eastern Cooperative Oncology Group, Robert Comis, MD, Group Chair.

APPENDIX C: NYHA CLASSIFICATION OF HEART DISEASE

New York Heart Association (NYHA) classification of heart disease

NYHA Class	Symptoms
I	No symptoms and no limitation in ordinary physical activity, e.g. shortness of breath when walking, climbing stairs etc.
II	Mild symptoms (mild shortness of breath and/or angina) and slight limitation during ordinary activity.
III	Marked limitation in activity due to symptoms, even during less-than-ordinary activity, e.g. walking short distances (20–100 m). Comfortable only at rest.
IV	Severe limitations. Experiences symptoms even while **at rest**. Mostly bedbound patients.

APPENDIX D: INFORMATION ON POSSIBLE DRUG INTERACTIONS

Information on Possible Interactions with Other Agents for Patients and Their Caregivers and Non-Study Healthcare Team*

[Note to Investigators: This **suggested** appendix consists of an "information sheet" to be handed to the patient at the time of enrollment. Use or modify the text as appropriate for the study agent, so that the patient is aware of the risks and can communicate with their regular prescriber(s) and pharmacist. A convenient wallet-sized information card is also included for the patient to clip out and retain at all times. **This document must be IRB-reviewed and approved prior to patient distribution**.]

The patient _____ is enrolled on a clinical trial using the experimental agent belinostat**(Beleodaq®)**. This form is addressed to the patient, but includes important information for others who care for this patient.

Belinostat **(Beleodaq®)** interacts with some drugs. Because of this, it is very important to tell your study doctors about all of your medicine before you start this study. It is also very important to tell them if you stop taking any regular medicine, or if you start taking a new medicine while you take part in this study. When you talk about your medicine with your study doctor, include medicine you buy without a prescription at the drug store (over-the-counter remedy), or herbal supplements such as St. John's wort.

Many health care prescribers can write prescriptions. You must also tell your other prescribers (doctors, physicians' assistants or nurse practitioners) that you are taking part in a clinical trial. **Bring this paper with you and keep the attached information card in your wallet**. These are the things that you and they need to know:

- Belinostat (Beleodaq®) must be used very carefully with other medicines such as carvedilol, levothyroxine, paracetamol, and raloxifene. Combined use can lead to harmful side effects and/or reduce the effectiveness of those medications.
- You and healthcare providers who prescribe drugs for you must be careful about adding or removing any drug in this category.
- Before you start the study, your study doctor will work with your regular prescriber to switch any medicines that are considered harmful.
- Your prescribers should consult a medical reference to see if any medicine they want to prescribe is on a list of drugs to avoid.
- Please be very careful! Over-the-counter drugs have a brand name on the label—it's usually big and catches your eye. They also have a generic name—it's usually small and located above or below the brand name, and printed in the ingredient list. Find the generic name and determine, with the pharmacist's help, whether there could be an adverse interaction.

Other medicines can be a problem with your study drug(s).

- You should check with your doctor or pharmacist whenever you need to use an over-the-counter medicine or herbal supplement.
- Your regular prescriber should check a medical reference or call your study doctor before prescribing any new medicine for you. Your study doctor's name is

_____ and he or she can be contacted at

_____.

APPENDIX E: IMMUNOLOGIC ASSAYS

Venous blood will be collected in 3 green tubes (Sodium heparin or Lithium heparin) (10 ml capacity) from patients diagnosed with leukemia-type (chronic or acute) ATLL at baseline prior to the initiation of belinostat, and after belinostat at the end of week 1 cycle 1 (Day 8), and end of cycle 1 (Day 21). These tubes will be collected in addition to any blood required for standard diagnostic laboratory tests processed by the hospital (i.e. cell counts, serologies, chemistries, and flow cytometry studies). Collected blood specimens will be processed on the same day by the local lab. Peripheral blood mononuclear cell (PMBC) cells will be freshly isolated from peripheral blood by centrifugation using standard Lymphoprep (ficol) procedure. At any future collaborating sites, the extracted cells will be cryopreserved in liquid nitrogen for transport to Dr. Ramos' s laboratory (University of Miami-Sylvester Comprehensive Cancer Center) at below shipping address. All assays described below have been validated in cryopreserved samples in prior clinical studies.

Immunologic Assays: In order to capture data defining the quantity, maturation/differentiation status and overall function of the recovering immune system, both phenotypic and functional flow cytometry panels will be employed to assess total numbers of mononuclear cell subsets, as well as global immune function, in addition to T cell responses directed at HTLV-1. Immunophenotyping: A modified version of the panels utilized to study immune reconstitution of cord blood transplant recipients will be employed (37). In multiple panels, the immunophenotyping will include staining for CD4, CD8, CD45RA, CD27 (to define naïve/memory CD4+ and CD8+T subsets); CD25, CD127 and intracellular Foxp3 staining (to define Treg, which may be altered by HDI therapy), CD14 (monocytes), CD16 and CD56 (NK cells), and CD19 (B cells). Studies of HTLV-1-specific and global T cell function antigen-specific T cell responses (including specific for HTLV-1, will be assessed using CFC, to assess polyfunctional T cell responses critical for protective immunity (e.g., secreting combinations of cytokines shown to be relevant for control of persistent retroviruses) (39-41). To assess the hypothesis that HTLV-1 specific CD8+ T cells will be augmented by this clinical strategy, antigen-specific responses by stimulating PBMCs will be assessed using the following antigens: 1) HTLV-1 lysates, 2) Purified HTLV-1 Tax protein; 3) super antigen staphylococcus enterotoxin B (SEB); 4) for CMV-seropositive donors (estimated to be >80% of HTLV-1+ patients), CMV pp65 and IE1 peptide mixtures. Using published methods (40-42), PBMCs will be stimulated for 6 hr and stained for CD4, CD8, CD27, CD45RA, and intracellular cytokines IL-2, IFN-α, TNF-α, and MIP1-b as previously described. FACS data will be acquired on a 4-laser, 15-color LSRII (Becton Dickinson) and will be analyzed using FlowJo software.

Shipping address:

Attn: Dr. Ramos Laboratory

University of Miami, Miller School of Medicine

Department of Hematology/Oncology (M877)

1501 NW 10thAvenue

Papanicolau (PAP) Building, Room 531

Miami, FL 33136

Tel: (305) 243-4605

APPENDIX F: MOLECULAR EVALUATION/ ANALYSIS OF ATLL AND HTLV-1+CLONES

Venous blood will be collected in 5 yellow top tubes (Acid Citrate Dextrose (ACD) solutions A/B additives –Trisodium citrate 22.0/13.2, citric acid 8.0/4.8 and dextrose 24.5/14.7 (in g/L)) (10 ml capacity) from patients diagnosed with leukemia-type (chronic or acute) ATLL prior to the initiation of AZT and belinostat. These tubes will be collected in addition to any blood required for standard diagnostic laboratory tests processed by the hospital (i.e. cell counts, serologies, chemistries, flow cytometry, and gene rearrangement studies) at baseline, 48-72 hours after belinostat treatment (i.e. Day 3 or 4), (cycle 1) Day 15, and end of cycles 3 (i.e. Day 21 of cycle 3 or prior cycle 4), 8 (i.e. Day 21 of cycle 8), at the end of month 9 &12, and at the end of treatment visit. Collected blood specimens will be processed on the same day by the local lab. Peripheral blood mononuclear cell (PMBC) cells will be freshly isolated from peripheral blood by centrifugation using standard Lymphoprep (ficol) procedure. At collaborating sites, the extracted cells will be cryopreserved in liquid nitrogen for transport to Dr. Ramos' laboratory (University of Miami-Sylvester Comprehensive Cancer Center) at the shipping address below. After thawing, a portion of cells will be subjected to magnetic CD4-enrichement by negative selection using commercially available kits. These cells will serve as source for protein, genomic and proviral DNA, and RNA, after standard extraction procedures.

Clonality and clonal abundance studies: Subjects will be assessed for minimal residual disease at baseline, and at specified times during treatment (after cycles 3 and 8), at the end of months 9, 12. Multiplex PCR designed to detect T-cell receptor gene rearrangements will be performed by immunopathology lab using extracted DNA from PBMC's (36). Samples will be analyzed at baseline, at the above time points during and post belinostat therapy, and when relapses occur off treatment. Where available, DNA from lymphomatous tissue or body fluids will also be analyzed.

Measurement of HTLV-1 proviral loads and reactivation: HTLV-1 proviral loads will be measured using extracted DNA from PBMC's cells before belinostat, during treatment (Day 15, after cycles 3 and 8), at the end of months 9 and 12 by single molecule droplet based digital PCR at UM Oncogenomics core facility.

Measurement of HTLV-1 reactivation: CD4+ enriched PBMC's cells will be assayed at baseline, and 48-72 hours after belinostat treatment for HTLV-1 Tax expression using HTLV-1 at UM Oncogenomics core facility by single molecule droplet-based digital RT-PCR using extracted RNA at UM Oncogenomics core facility.

Molecular effects of belinostat: To study the molecular mechanisms of belinostat on ATLL cells *in vivo* CD4+ enriched PBMC's cells collected at baseline, and 48-72 hours after belinostat treatment. Extracted protein will be analyzed for acetylation of histone proteins, expression of HTLV-1 proteins HBZ and Tax, and cellular genes affected by viral proteins or by epigenetic effects of belinostat by Western Blot. Extracted RNA will be analyzed for Tax and HBZ by single molecule droplet based digital PCR UM Oncogenomics core facility.

Shipping address:

Attn: Dr. Ramos Laboratory

University of Miami Miller School of Medicine

Department of Hematology/Oncology (M877)

1550 NW 10th Avenue
Papanicolau (PAP) Building, Room 531
Miami, FL 33136
Tel: (305) 243-4605

APPENDIX G: DEFINITION OF CLINICAL SUBTYPES OF ATLL

1. **Smoldering**
    a. 5% or more abnormal lymphocytes of T-cell nature in peripheral blood
    b. presence of skin lesions
    c. absence of hypercalcemia
    d. absence of visceral and lymph node involvement
    e. pulmonary lesions may be present
    f. normal LDH level
    g. if <5% circulating leukemic cells are present, there must be at least either histologically-proven skin and/or pulmonary lesions present
2. **Chronic**
    a. absolute lymphocytosis (≥4 X 10^9/L) greater
    b. elevated lactate dehydrogenase (LDH) < twice baseline or normal value
    c. lymphadenopathy and involvement of the spleen, liver, skin and lungs may be present
    d. 5% or more abnormal T lymphocytes in the peripheral blood may be present.
    e. no hypercalcemia
    f. no CNS, bone or gastrointestinal tract involvement
    g. no ascites or pleural effusion
3. **Lymphomatous**: Patients with lymphoma type should have histologically-proven lymphadenopathy and no leukemic manifestation that is defined as no lymphocytosis (<4 X 10^9/L) and 1% or less abnormal T-lymphocytes.
4. **Acute**: Remaining ATL patients who usually have leukemic manifestation and tumor lesions, but are not classified as any of the three other types –
    a. elevated lymphocyte count
    b. morphologically atypical circulating mature T-helper lymphocytes that express the CD4 antigen
    c. lymphadenopathy, hepatosplenomegaly, skin, and any other tumor lesions or organ involvement may be present
    d. elevated LDH
    e. hypercalcemia with or without lytic bone lesions (seen in approximately 50% of patients)

APPENDIX H: PATIENT DOSING DIARY

Note to Investigators: This appendix consists of a "directions sheet and diary" to be given to the patient for each cycle of belinostat/AZT (±IFN-alfa), for the planned treatment duration of Cycles 1 to 8 (i.e. about 6 months). After belinostat completion, standard AZT-based maintenance therapy (±IFN-alfa) during months 7 to 12 are dependent on subject's disease status and/or at the Investigator's discretion. These sheets MUST be approved by the Institutional Review Board (IRB) before they may be given to patients.

Diary Directions

Begin taking zidovudine (AZT) as your doctor has told you to during the study. Do not change your dose unless your doctor tells you to do so:

1. Each dose of AZT should be taken by mouth. For this study, your doctor is asking you to take 300mg of AZT three times a day, in divided doses, or continuation of same dose taken prior to enrollment at the discretion of the treating investigator physician, or a specified dose made after any adjustments made during the study.

2. You should not eat 2 hours before and 1 hour after each dose. Do not chew, crush or break tablets. Swallow each tablet whole.

3. If a dose is missed, you may take the missed dose if it has been less than 6 hours from your scheduled dose. Do not make up missed doses if it has been more than 6 hours from your scheduled dose.

7. Taking certain drugs may interfere with the absorption of zidovudine (AZT). Please discuss with your doctor(s) before taking medications like stavudine, ribavirin, doxorubicin, or ganciclovir.

8. Please be sure to contact your Doctor, if you may have any questions.

9. Please bring the empty bottle or any leftover tablets and this dosing diary to your next clinic visit. You will be asked to complete this diary every 21-days for about 6 months.

Patient Dosing Diary Zidovudine (AZT) Dose Level: 300 mg, up to three times a day, days 1-21

Patient Initials (L, F, M): **Patient ID:** _____ **Cycle:** _____

(Please also see Diary Directions listed on the page before.) This is a diary on which you are to **record the date, time and number of tablets you take each day on the days that you are supposed to take zidovudine (AZT)**. You should take your scheduled dose of each tablet. If you forget to take a dose, please write "0", but remember to take your prescribed dose at the next regularly scheduled time.

Day	Date(s)	# of tablets	Time Taken *(Circle AM or PM)*	# of tablets	Time Taken *(Circle AM or PM)*	# of tablets	Time Taken *(Circle AM or PM)*
1			AM PM		AM PM		AM PM
2			AM PM		AM PM		AM PM
3			AM PM		AM PM		AM PM
4			AM PM		AM PM		AM PM
5			AM PM		AM PM		AM PM
6			AM PM		AM PM		AM PM
7			AM PM		AM PM		AM PM
8			AM PM		AM PM		AM PM
9			AM PM		AM PM		AM PM
10			AM PM		AM PM		AM PM
11			AM PM		AM PM		AM PM
12			AM PM		AM PM		AM PM
13			AM PM		AM PM		AM PM
14			AM PM		AM PM		AM PM

15			AM PM		AM PM		AM PM
16			AM PM		AM PM		AM PM
17			AM PM		AM PM		AM PM
18			AM PM		AM PM		AM PM
19			AM PM		AM PM		AM PM
20			AM PM		AM PM		AM PM
21			AM PM		AM PM		AM PM

Patient Signature: _____ **Date:** _____

APPENDIX I: BIOBANKING CONSIDERATIONS

Blood banking:

Patient specimens will be used to develop a specimen bank that will serve as an invaluable resource for current and future research by the investigators named on this study. Blood samples will be obtained from all consenting study patients.

Upon collection of the samples from the patients, a code system will be used to de-identify all samples, assigning a unique code to each specimen. The samples will be tied to patient-identifying information only at the time of sample collection. The patient's name will be written on the specimen collection tubes. Once the samples are de-identified, which will happen as soon as the sample tubes arrive in Dr. Ramos' lab, the patient's name will be blacked out on the original sample tubes and the original tubes will be discarded in biohazardous waste, which is subsequently sent for incineration.

The database will be the only location where patient's names will be tied to their code. The database will be kept on a password protected computer in Dr. Ramos' research lab and only Dr. Ramos and his research staff named on this protocol will have access to the database. The only information that will be stored with the actual samples in the bank is the code identifier used to identify each sample. No samples will be accepted into the bank unless the subject's signed consent form has first been submitted.

Blood obtained for research purposes (in 10 ml tubes) will be drawn at the same time as other blood samples are being collected. The research samples will be incorporated with other blood draws being done as standard of care for their treatment.

Data and Specimen Banking

Samples in the bank will be stored indefinitely, or until the patient withdraws consent for inclusion in the protocol. At that time all samples and data will be destroyed. All patient samples will be stored in Dr. Ramos' laboratory: Medical Oncology Laboratory, Sylvester Comprehensive Cancer Center, 1550 NW 10th Ave, Pap 531, Miami, FL 33136 (Pap Building). Phone number: (305) 243-4605 (lab). Access to patient identifiers, including "hidden" variables, is customizable for each user and will be limited to the investigators listed on this protocol.

Researchers wishing to gain access to either the clinical data (de-identified) or samples must first submit the appropriate IRB protocol for use and analysis of the information. Upon approval, the investigator will then be granted appropriate access to the data and/or samples as indicated by his/her respective protocol.

The only investigator who will have access to samples and data being kept in the bank is Dr. Ramos and his research staff named specifically for maintenance of the bank. The database will be the only location where patient's names and medical record numbers will be tied to their code identifiers. The only information that will be stored with the actual samples in the bank is the code identifier used to identify each sample.

Data Management

As discussed above, the data will be maintained entirely in an online platform behind the university firewall and requiring UM CaneID password identification. This protocol covers establishing the database and the sample bank and is *not* comprehensive for use of the samples.

Additional IRB approval will be necessary to use and publish any additional research performed using this data and the specimens.

Risks to Subjects

This study poses minimal risks to the subjects. They may undergo additional discomfort due to the additional blood draws however the times of draws are designed to correspond with the additional blood draws required for clinical care. Additionally, there is a small risk of the compromise of their protected health information (PHI) however Researchers are taking maximal precautions to prevent this due to the rapid coding of the blood samples and entirely web-based platform of the clinical database.

There are no additional costs to the patient associated with collecting the additional samples for this study during routine tests/procedures.

Potential Benefits to Subjects

There is no direct benefit to the patients by participating in this study. There may ultimately be significant benefit to other patients based on improved understanding of the pathogenesis and mechanisms behind the diseases.

Setting

Obtaining phlebotomy samples may be performed at UMHC, UMH or SCCC depending on the setting of the patient's procedures. Storage of the specimens and the database was summarized above.

Resources Available

Dr. Ramos is a PI with experience in the design and use of retrospective and prospective clinical databases for research material(s). Drs. Ramos' and Komanduri's laboratory also have extensive experience in the establishment of models from blood specimens.

Recruitment Methods

Prospective participants will be patients of the Investigators named in this protocol that they see in their clinic, and will be approached by the Investigators named in this protocol personally during a standard or study clinic visit. Subjects will receive no compensation for participation in the study and participation will not affect clinical care.

Local Number of Subjects

Up to 20 prospective subjects.

Confidentiality

The only investigators who will have access to samples and data being kept in the bank are Dr. Ramos and his research staff. The bank database will be the only location where patient's names will be tied to their code identifiers. The only information that will be stored with the actual samples in the bank is the code identifier used to identify each sample.

Data and specimens will be maintained in the bank indefinitely. If at some point in time, a subject decides they wish to remove their specimens and data from the bank, any remaining samples belonging to that patient will be destroyed and their data removed from the database.

Provisions to Protect the Privacy Interests of Subjects

All samples will be de-identified to protect each subjects' identity as detailed above. All PHI included in the database will be hidden and access will be limited to those requiring it for establishment and maintenance of the database. All discussions of participation in the study will be performed in private clinic rooms. Signed HIPAA Authorization will be obtained from all subjects.

Consent Process

Written informed consent for the prospective use of data and samples for purposes of this protocol will be obtained. Patients will be provided with an informed consent process during their clinic visits and/or routine care visits. For subjects not qualified or able to give legal consent, consent must be obtained from their legally authorized representative (LAR).

APPENDIX J: AGENTS (DRUG FORMULATION AND PROCUREMENT)

Belinostat

[Refer to the FDA-approved package insert for more information: http://dailymed.nlm.nih.gov/dailymed/drugInfo.cfm?setid=84b2e16e-f0d1-4757-8da8-79dfa83aab79.]

Other name(s)

PXD101, Beleodaq®

Mechanism of Action

Beleodaq is a histone deacetylase (HDAC) inhibitor. HDACs catalyze the removal of acetyl groups from the lysine residues of histones and some non-histone proteins. *In vitro*, belinostat caused the accumulation of acetylated histones and other proteins, inducing cell cycle arrest and/or apoptosis of some transformed cells. Belinostat shows preferential cytotoxicity towards tumor cells compared to normal cells. Belinostat inhibited the enzymatic activity of histone deacetylases at nanomolar concentrations (<250 nM).

Drug Metabolism, Pharmacokinetics and Toxicology

Belinostat is primarily metabolized by hepatic UGT1A1. Strong UGT1A1 inhibitors are expected to increase exposure to belinostat. Belinostat also undergoes hepatic metabolism by CYP2A6, CYP2C9, and CYP3A4 enzymes to form belinostat amide and belinostat acid. The enzymes responsible for the formation of methyl belinostat and 3-(anilinosulfonyl)-benzenecarboxylic acid, (3-ASBA) are not known.

The pharmacokinetic characteristics of belinostat were analyzed from pooled data from phase 1/2 clinical studies that used doses of belinostat ranging from 150 to 1200 mg/m. The total mean plasma clearance and elimination half-life were 1240 mL/min and 1.1 hours, respectively. The total clearance approximates average hepatic blood flow (1500 mL/min), suggesting high hepatic extraction (clearance being flow dependent).

UGT1A1 Inhibitors: Belinostat is primarily metabolized by UGT1A1. Avoid concomitant administration of Beleodaq with strong inhibitors of UGT1A1.

Warfarin: Co-administration of Beleodaq and warfarin resulted in no clinically relevant increase in plasma exposure of either R-warfarin or S-warfarin that would require a dose adjustment.

Carcinogenicity studies have not been performed with belinostat.

Belinostat was genotoxic in a bacterial reverse mutation test (Ames assay), an in vitro mouse lymphoma cell mutagenesis assay, and an in vivo rat micronucleus assay.

Beleodaq may impair male fertility. Fertility studies using belinostat were not conducted. However, belinostat effects on male reproductive organs observed during the 24-week repeat-dose dog toxicology study included reduced organ weights of the testes/epididymides that correlated with a delay in testicular maturation.

Management of Agent-Specific Adverse Events

Hematologic Toxicity

Beleodaq can cause thrombocytopenia, leukopenia (neutropenia and lymphopenia), and/or anemia; monitor blood counts weekly during treatment, and modify dosage as necessary.

Infections

Serious and sometimes fatal infections, including pneumonia and sepsis, have occurred with Beleodaq. Do not administer Beleodaq to subjects with an active infection. Subjects with a history of extensive or intensive chemotherapy may be at higher risk of life threatening infections.

Hepatotoxicity

Beleodaq can cause fatal hepatotoxicity and liver function test abnormalities. Monitor liver function tests before treatment and before the start of each cycle. Interrupt or adjust dosage until recovery, or permanently discontinue Beleodaq based on the severity of the hepatic toxicity.

Tumor Lysis Syndrome

Tumor lysis syndrome has occurred in Beleodaq-treated patients in the clinical trial of patients with relapsed or refractory PTCL. Monitor patients with advanced stage disease and/or high tumor burden and take appropriate precautions.

Gastrointestinal Toxicity

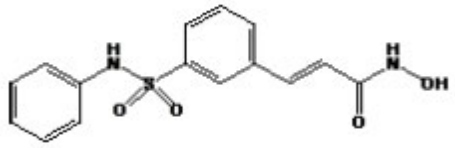
Nausea, vomiting and diarrhea occur with Beleodaq and may require the use of antiemetic and antidiarrheal medications.

Embryo-fetal Toxicity

Beleodaq can cause fetal harm when administered to a pregnant woman. Beleodaq may cause teratogenicity and/or embryo-fetal lethality because it is genotoxic and targets actively dividing cells. Women of childbearing potential should be advised to avoid pregnancy while receiving Beleodaq. If this drug is used during pregnancy, or if the patient becomes pregnant while taking this drug, the patient should be apprised of potential hazard to the fetus.

Composition

Beleodaq is a histone deacetylase inhibitor with a sulfonamide-hydroxamide structure. The chemical name of belinostat is (2E)-N-hydroxy-3-[3-(phenylsulfamoyl) phenyl]prop-2-enamide. The structural formula is as follows:



The molecular formula is $C_{15}H_{14}N_2O_4S$ and the molecular weight is 318.35 g/mol.

Storage Recommendations and Dosage Forms

For injection: 500 mg, lyophilized powder in single-use vial for reconstitution.

Beleodaq (belinostat) for injection is supplied in single vial cartons; each 30 mL clear vial contains sterile, lyophilized powder equivalent to 500 mg belinostat.

NDC 68152-108-09: Individual carton of Beleodaq 30 mL single-use vial containing 500 mg belinostat.

Store Beleodaq (belinostat) for injection at room temperature 20°C to 25°C (68°C to 77°F). Excursions are permitted between 15°C and 30°C (59°F and 86°F). Retain in original package until use. [see USP Controlled Room Temperature].

Beleodaq is a cytotoxic drug. Follow special handling and disposal procedures.

Dispensation and Accountability

See also Section 8.5.1; Belinostat will be supplied by Spectrum Pharmaceuticals, Inc.

Beleodaq® (belinostat) for injection is supplied in single vial cartons; each 30 mL clear vial contains sterile, lyophilized powder equivalent to 500 mg belinostat.

Manufacturer contact information:

CENEXI-Laboratoires Thissen S.A.,

Rue de la papyrée, 2-4-6 ,

1420 BRAINE L'ALLEUD,

BELGIUM

Drug Label Information:

Patient ID: _____ Study # XXXXXXXX
Belinostat (PXD101) For Injection 500mg/vial
Batch No: XXXXX Store at 20 - 25°C
See protocol for administration directions
Caution : New Drug -- Limited by United States law to investigational use

Sponsor : XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX

Any City, Any State XXXXX

Tel : XXX-XXX-XXXX

Zidovudine

[Refer to the FDA-approved package insert for more information: http://dailymed.nlm.nih.gov/dailymed/drugInfo.cfm?setid=6df09f15-b102-431c-adde-d7aeef6f5d84.]

Other name(s)

azidothymidine (AZT), Retrovir®

Mechanism of Action

Zidovudine is an antiviral agent. It is a synthetic nucleoside analogue. Intracellularly, zidovudine is phosphorylated to its active 5'-triphosphate metabolite, zidovudine triphosphate (ZDV-TP). The principle mode of action of ZDV-TP is inhibition of reverse transcriptase (RT) via DNA chain termination after incorporation of the nucleotide analogue. ZDV-TP is a weak inhibitor of the cellular DNA polymerases alpha and gamma and has been reported to be incorporated into the DNA of cells in culture.

Drug Metabolism, Pharmacokinetics and Toxicology

Zidovudine is primarily eliminated by hepatic metabolism. The major metabolite of zidovudine is GZDV. GZDV AUC is about 3-fold greater than the zidovudine AUC. Urinary recovery of zidovudine and GZDV accounts for 14% and 74%, respectively, of the dose following oral administration and 18% and 60%, respectively, following IV dosing. A second metabolite, 3′-amino-3′-deoxythymidine (AMT), has been identified in the plasma following single-dose IV administration of zidovudine. The AMT AUC was one-fifth of the zidovudine AUC. Pharmacokinetics of zidovudine were dose independent at oral dosing regimens ranging from 2 mg per kg every 8 hours to 10 mg per kg every 4 hours.

Following IV dosing, dose-independent kinetics was observed over the range of 1 to 5 mg per kg. The mean steady-state peak and trough concentrations of zidovudine at 2.5 mg per kg every 4 hours were 1.1 and 0.1 mcg per mL, respectively.

In adults, following oral administration, zidovudine is rapidly absorbed and extensively distributed, with peak serum concentrations occurring within 0.5 to 1.5 hours. The AUC was equivalent when zidovudine was administered as RETROVIR tablets or syrup compared with RETROVIR capsules. The pharmacokinetic properties of zidovudine in fasting adult subjects are summarized in the table below [where a): Median [range] for 50 paired samples drawn 1 to 8 hours after the last dose in subjects on chronic therapy with RETROVIR and b): Approximate range.]

Parameter	Mean ± SD (except where noted)
Oral bioavailability (%)	64 ± 10 (n = 5)
Apparent volume of distribution (L/kg)	1.6 ± 0.6 (n = 8)
Cerebrospinal fluid (CSF):plasma ratio[a]	0.6 [0.04 to 2.62] (n = 39)
Systemic clearance (L/h/kg)	1.6 ± 0.6 (n = 6)
Renal clearance (L/h/kg)	0.34 ± 0.05 (n = 9)
Elimination half-life (h)[b]	0.5 to 3 (n = 19)

Carcinogenesis

Zidovudine was administered orally at 3 dosage levels to separate groups of mice and rats (60 females and 60 males in each group). Initial single daily doses were 30, 60, and 120 mg per kg per day in mice and 80, 220, and 600 mg per kg per day in rats. The doses in mice were reduced to 20, 30, and 40 mg per kg per day after day 90 because of treatment-related anemia, whereas in rats only the high dose was reduced to 450 mg per kg per day on Day 91 and then to 300 mg per kg per day on Day 279.

In mice, 7 late-appearing (after 19 months) vaginal neoplasms (5 non-metastasizing squamous cell carcinomas, 1 squamous cell papilloma, and 1 squamous polyp) occurred in animals given the highest dose. One late-appearing squamous cell papilloma occurred in the vagina of a middle-dose animal. No vaginal tumors were found at the lowest dose.

In rats, 2 late-appearing (after 20 months), non-metastasizing vaginal squamous cell carcinomas occurred in animals given the highest dose. No vaginal tumors occurred at the low or middle dose in rats. No other drug-related tumors were observed in either sex of either species.

At doses that produced tumors in mice and rats, the estimated drug exposure (as measured by AUC) was approximately 3 times (mouse) and 24 times (rat) the estimated human exposure at the recommended therapeutic dose of 100 mg every 4 hours.

It is not known how predictive the results of rodent carcinogenicity studies may be for humans.

Two trans placental carcinogenicity studies were conducted in mice. One study administered zidovudine at doses of 20 mg per kg per day or 40 mg per kg per day from gestation Day 10 through parturition and lactation with dosing continuing in offspring for 24 months postnatally. The doses of zidovudine administered in this study produced zidovudine exposures approximately 3 times the estimated human exposure at recommended doses. After 24 months, an increase in incidence of vaginal tumors was noted with no increase in tumors in the liver or lung or any other organ in either gender. These findings are consistent with results of the standard oral carcinogenicity study in mice, as described earlier. A second study administered zidovudine at maximum tolerated doses of 12.5 mg per day or 25 mg per day (approximately 1,000 mg per kg non-pregnant body weight or approximately 450 mg per kg of term body weight) to pregnant mice from Days 12 through 18 of gestation. There was an increase in the number of tumors in the lung, liver, and female reproductive tracts in the offspring of mice receiving the higher dose level of zidovudine.

Mutagenesis

Zidovudine was mutagenic in a 5178Y/TK mouse lymphoma assay, positive in an in vitro cell transformation assay, clastogenic in a cytogenetic assay using cultured human lymphocytes, and positive in mouse and rat micronucleus tests after repeated doses. It was negative in a cytogenetic study in rats given a single dose.

Impairment of Fertility

Zidovudine, administered to male and female rats at doses up to 7 times the usual adult dose based on body surface area, had no effect on fertility judged by conception rates.

Animal Toxicology and/or Pharmacology

Oral teratology studies in the rat and in the rabbit at doses up to 500 mg per kg per day revealed no evidence of teratogenicity with zidovudine. Zidovudine treatment resulted in embryo/fetal toxicity as evidenced by an increase in the incidence of fetal resorptions in rats given 150 or 450 mg per kg per day and rabbits given 500 mg per kg per day. The doses used in the teratology studies resulted in peak zidovudine plasma concentrations (after one-half of the daily dose) in rats 66 to 226 times, and in rabbits 12 to 87 times, mean steady-state peak human plasma concentrations (after one-sixth of the daily dose) achieved with the recommended daily dose (100 mg every 4 hours). In an in vitro experiment with fertilized mouse oocytes, zidovudine exposure resulted in a dose-dependent reduction in blastocyst formation. In an additional teratology study in rats, a dose of 3,000 mg per kg per day (very near the oral median lethal dose in rats of 3,683 mg per kg) caused marked maternal toxicity and an increase in the incidence of fetal malformations. This dose resulted in peak zidovudine plasma concentrations 350 times peak human plasma concentrations. (Estimated AUC in rats at this dose level was 300 times the daily AUC in humans given 600 mg per day.) No evidence of teratogenicity was seen in this experiment at doses of 600 mg per kg per day or less.

Management of Agent-Specific Adverse Events

RETROVIR is contraindicated in patients who have had a potentially life-threatening hypersensitivity reaction (e.g., anaphylaxis, Stevens-Johnson syndrome) to any of the components of the formulations.

Hematologic Toxicity/Bone Marrow Suppression

RETROVIR should be used with caution in patients who have bone marrow compromise evidenced by granulocyte count less than 1,000 cells per mm or hemoglobin less than 9.5 g per dL. Hematologic toxicities appear to be related to pretreatment bone marrow reserve and to dose and duration of therapy.

In patients with advanced symptomatic HIV-1 disease, anemia and neutropenia were the most significant adverse events observed. In patients who experience hematologic toxicity, a reduction in hemoglobin may occur as early as 2 to 4 weeks, and neutropenia usually occurs after 6 to 8 weeks. There have been reports of pancytopenia associated with the use of RETROVIR, which was reversible in most instances after discontinuance of the drug. However, significant anemia, in many cases requiring dose adjustment, discontinuation of RETROVIR, and/or blood transfusions, has occurred during treatment with RETROVIR alone or in combination with other anti-retrovirals.

Frequent blood counts are strongly recommended to detect severe anemia or neutropenia in patients with poor bone marrow reserve, particularly in patients with advanced HIV-1 disease who are treated with RETROVIR. For HIV-1-infected individuals and patients with asymptomatic or early HIV-1 disease, periodic blood counts are recommended. If anemia or neutropenia develops, dosage interruption may be needed.

Latex

The vial stoppers for RETROVIR injection contain natural rubber latex which may cause allergic reactions in latex-sensitive individuals.

Myopathy

Myopathy and myositis with pathological changes, similar to that produced by HIV-1 disease, have been associated with prolonged use of RETROVIR.

Lactic Acidosis /Severe Hepatomegaly with Steatosis

Lactic acidosis and severe hepatomegaly with steatosis, including fatal cases, have been reported with the use of nucleoside analogues alone or in combination, including zidovudine and other anti-retrovirals. A majority of these cases have been in women. Obesity and prolonged exposure to antiretroviral nucleoside analogues may be risk factors. Particular caution should be exercised when administering RETROVIR to any patient with known risk factors for liver disease; however, cases have also been reported in patients with no known risk factors. Treatment with RETROVIR should be suspended in any patient who develops clinical or laboratory findings suggestive of lactic acidosis or pronounced hepatotoxicity (which may include hepatomegaly and steatosis even in the absence of marked transaminase elevations).

Use with Interferon- and Ribavirin-based Regimens in HIV-1/HCV Co-infected Patients

In vitro studies have shown ribavirin can reduce the phosphorylation of pyrimidine nucleoside analogues such as zidovudine. Although no evidence of a pharmacokinetic or pharmacodynamics interaction (e.g., loss of HIV-1/HCV virologic suppression) was seen when ribavirin was co-administered with zidovudine in HIV-1/HCV co-infected subjects, exacerbation of anemia due to ribavirin has been reported when zidovudine is part of the HIV regimen.

Co-administration of ribavirin and zidovudine is not advised. Consideration should be given to replacing zidovudine in established combination HIV-1/HCV therapy, especially in patients with a known history of zidovudine-induced anemia.

Hepatic decompensation (some fatal) has occurred in HIV-1/HCV co-infected patients receiving combination antiretroviral therapy for HIV-1 and interferon alfa with or without ribavirin. Patients receiving interferon alfa with or without ribavirin and zidovudine should be closely monitored for treatment-associated toxicities, especially hepatic decompensation, neutropenia, and anemia.

Discontinuation of zidovudine should be considered as medically appropriate. Dose reduction or discontinuation of interferon alfa, ribavirin, or both should also be considered if worsening clinical toxicities are observed, including hepatic decompensation (e.g., Child-Pugh greater than 6) (see the complete prescribing information for interferon and ribavirin).

Use with Other Zidovudine-containing Products

RETROVIR should not be administered with combination products that contain zidovudine as one of their components (e.g., COMBIVIR [lamivudine and zidovudine] tablets or TRIZIVIR [abacavir sulfate, lamivudine, and zidovudine] tablets).

Immune Reconstitution Syndrome

Immune reconstitution syndrome has been reported in patients treated with combination antiretroviral therapy, including RETROVIR. During the initial phase of combination antiretroviral treatment, patients whose immune systems respond may develop an inflammatory response to indolent or residual opportunistic infections (such as Mycobacterium avium infection, cytomegalovirus, Pneumocystis jirovecii pneumonia [PCP], or tuberculosis), which may necessitate further evaluation and treatment.

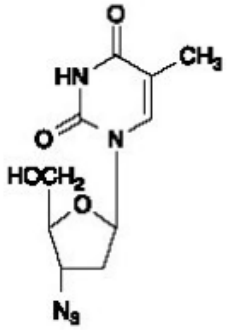
Autoimmune disorders (such as Graves' disease, polymyositis, and Guillain-Barré syndrome) have also been reported to occur in the setting of immune reconstitution; however, the time to onset is more variable, and can occur many months after initiation of treatment.

Fat Redistribution

Redistribution/accumulation of body fat, including central obesity, dorsocervical fat enlargement (buffalo hump), peripheral wasting, facial wasting, breast enlargement, and "cushingoid appearance," have been observed in patients receiving antiretroviral therapy. The mechanism and long-term consequences of these events are currently unknown. A causal relationship has not been established.

Composition

RETROVIR is the brand name for zidovudine (formerly called azido thymidine [AZT]), a pyrimidine nucleoside analogue active against HIV-1. The chemical name of zidovudine is 3′-azido-3′-deoxythymidine; it has the following structural formula:



Zidovudine is a white to beige, odorless, crystalline solid with a molecular weight of 267.24 and a solubility of 20.1 mg per mL in water at 25°C. The molecular formula is $C_{10}H_{13}N_5O_4$.

RETROVIR capsules are for oral administration. Each capsule contains 100 mg of zidovudine and the inactive ingredients corn starch, magnesium stearate, microcrystalline cellulose, and sodium starch glycolate. The 100-mg empty hard gelatin capsule, printed with edible black ink, consists of black iron oxide, dimethylpolysiloxane, gelatin, pharmaceutical shellac, soya lecithin, and titanium dioxide.

Storage Recommendations and Dosage Forms

Oral Dosing: The recommended oral dose of RETROVIR is 300 mg twice daily in combination with other antiretroviral agents.

RETROVIR 100-mg capsules are supplied as white, opaque cap and body capsules containing 100 mg zidovudine per capsule. Each capsule is printed with “Wellcome” and unicorn logo on cap and “Y9C” and “100” on body.

Bottles of 100 (NDC 49702-211-20).

Store at 15° to 25°C (59° to 77°F) and protect from moisture.

Dispensation and Accountability

Zidovudine is commercially available. See also Section 8.5.2.

Instruct subjects that if they miss a dose, they should just take their next dose at the usual time. Subjects should not double their next dose.

Interferon alfa-2b

[Refer to the FDA-approved package insert for more information: http://dailymed.nlm.nih.gov/dailymed/drugInfo.cfm?setid=30789790-8317-49f9-b97b-8c5ba17b53d2.]

Other name(s)

INTRON® A, IFN-alfa-2b

Mechanism of Action

General

The interferons are a family of naturally occurring small proteins and glycoproteins with molecular weights of approximately 15,000 to 27,600 daltons produced and secreted by cells in response to viral infections and to synthetic or biological inducers.

Preclinical Pharmacology

Interferons exert their cellular activities by binding to specific membrane receptors on the cell surface.

Once bound to the cell membrane, interferons initiate a complex sequence of intracellular events. In vitro studies demonstrated that these include the induction of certain enzymes, suppression of cell proliferation, immunomodulating activities such as enhancement of the phagocytic activity of macrophages and augmentation of the specific cytotoxicity of lymphocytes for target cells, and inhibition of virus replication in virus-infected cells.

In a study using human hepatoblastoma cell line HB 611, the in vitro antiviral activity of alpha interferon was demonstrated by its inhibition of hepatitis B virus (HBV) replication.

The correlation between these in vitro data and the clinical results is unknown. Any of these activities might contribute to interferon's therapeutic effects.

Drug Metabolism, Pharmacokinetics and Toxicology

Serum Neutralizing Antibodies

In INTRON A-treated patients tested for antibody activity in clinical trials, serum anti-interferon neutralizing antibodies were detected in 0% (0/90) of patients with hairy cell leukemia, 0.8% (2/260) of patients treated intralesionally for condylomata acuminata, and 4% (1/24) of patients with AIDS-Related Kaposi's Sarcoma. Serum neutralizing antibodies have been detected in less than 3% of patients treated with higher INTRON A doses in malignancies other than hairy cell leukemia or AIDS-Related Kaposi's Sarcoma. The clinical significance of the appearance of serum anti-interferon neutralizing activity in these indications is not known.

Serum anti-interferon neutralizing antibodies were detected in 7% (12/168) of patients either during treatment or after completing 12 to 48 weeks of treatment with 3 million IU TIW of INTRON A therapy for chronic hepatitis C and in 13% (6/48) of patients who received INTRON A therapy for chronic hepatitis B at 5 million IU QD for 4 months, and in 3% (1/33) of patients treated at 10 million IU TIW.

Serum anti-interferon neutralizing antibodies were detected in 9% (5/53) of pediatric patients who received INTRON A therapy for chronic hepatitis B at 6 million IU/m TIW. Among all chronic hepatitis B or C patients, pediatrics and adults with detectable serum neutralizing antibodies, the titers detected were low (22/24 with titers less than or equal to 1:40 and 2/24 with titers less than or equal to 1:160). The appearance of serum anti-interferon neutralizing activity did not appear to affect safety or efficacy.

Pharmacokinetics

The pharmacokinetics of INTRON A were studied in 12 healthy male volunteers following single doses of 5 million IU/m administered intramuscularly, subcutaneously, and as a 30-minute intravenous infusion in a crossover design.

The mean serum INTRON A concentrations following intramuscular and subcutaneous injections were comparable. The maximum serum concentrations obtained via these routes were approximately 18 to 116 IU/mL and occurred 3 to 12 hours after administration. The elimination half-life of INTRON A following both intramuscular and subcutaneous injections was approximately 2 to 3 hours. Serum concentrations were undetectable by 16 hours after the injections.

After intravenous administration, serum INTRON A concentrations peaked (135-273 IU/mL) by the end of the 30-minute infusion, then declined at a slightly more rapid rate than after intramuscular or subcutaneous drug administration, becoming undetectable 4 hours after the infusion. The elimination half-life was approximately 2 hours.

Urine INTRON A concentrations following a single dose (5 million IU/ml) were not detectable after any of the parenteral routes of administration. This result was expected since preliminary studies with isolated and perfused rabbit kidneys have shown that the kidney may be the main site of interferon catabolism.

There are no pharmacokinetic data available for the intralesional route of administration.

Contraindications

INTRON A is contraindicated in patients with:

Hypersensitivity to interferon alpha or any component of the product

Autoimmune hepatitis

Decompensated liver disease

INTRON A and REBETOL combination therapy is additionally contraindicated in:

Patients with hypersensitivity to ribavirin or any other component of the product

Women who are pregnant

Men whose female partners are pregnant

Patients with hemoglobinopathies (e.g., thalassemia major, sickle cell anemia)

Patients with creatinine clearance less than 50 mL/min.

(See REBETOL prescribing information for additional information.)

Management of Agent-Specific Adverse Events

General

Moderate to severe adverse experiences may require modification of the patient's dosage regimen, or in some cases termination of INTRON A therapy. Because of the fever and other "flu-like" symptoms associated with INTRON A administration, it should be used cautiously in patients with debilitating medical conditions, such as those with a history of pulmonary disease (e.g., chronic obstructive pulmonary disease) or diabetes mellitus prone to ketoacidosis. Caution should also be observed in patients with coagulation disorders (e.g., thrombophlebitis, pulmonary embolism) or severe myelosuppression.

Cardiovascular Disorders

INTRON A therapy should be used cautiously in patients with a history of cardiovascular disease.

Those patients with a history of myocardial infarction and/or previous or current arrhythmic disorder who require INTRON A therapy should be closely monitored (see PRECAUTIONS, Laboratory Tests). Cardiovascular adverse experiences, which include hypotension, arrhythmia, or tachycardia of

150 beats per minute or greater, and rarely, cardiomyopathy and myocardial infarction have been observed in some INTRON A-treated patients. Some patients with these adverse events had no history of cardiovascular disease. Transient cardiomyopathy was reported in approximately 2% of the AIDS-Related Kaposi's Sarcoma patients treated with INTRON A. Hypotension may occur during INTRON A administration, or up to 2 days post-therapy, and may require supportive therapy including fluid replacement to maintain intravascular volume.

Supraventricular arrhythmias occurred rarely and appeared to be correlated with preexisting conditions and prior therapy with cardio toxic agents. These adverse experiences were controlled by modifying the dose or discontinuing treatment, but may require specific additional therapy.

Cerebrovascular Disorders

Ischemic and hemorrhagic cerebrovascular events have been observed in patients treated with interferon alpha-based therapies, including INTRON A. Events occurred in patients with few or no reported risk factors for stroke, including patients less than 45 years of age. Because these are spontaneous reports, estimates of frequency cannot be made and a causal relationship between interferon alpha-based therapies and these events is difficult to establish.

Neuropsychiatric Disorders

DEPRESSION AND SUICIDAL BEHAVIOR INCLUDING SUICIDAL IDEATION, SUICIDAL ATTEMPTS, AND COMPLETED SUICIDES, HOMICIDAL IDEATION, AND AGGRESSIVE BEHAVIOR SOMETIMES DIRECTED TOWARDS OTHERS, HAVE BEEN REPORTED IN ASSOCIATION WITH TREATMENT WITH ALPHA INTERFERONS, INCLUDING INTRON A THERAPY. If patients develop psychiatric problems, including clinical depression, it is recommended that the patients be carefully monitored during treatment and in the 6-month follow-up period.

INTRON A should be used with caution in patients with a history of psychiatric disorders. INTRON A therapy should be discontinued for any patient developing severe psychiatric disorder during treatment.

Obtundation and coma have also been observed in some patients, usually elderly, treated at higher doses. While these effects are usually rapidly reversible upon discontinuation of therapy, full resolution of symptoms has taken up to 3 weeks in a few severe episodes. If psychiatric symptoms persist or worsen, or suicidal ideation or aggressive behavior towards others is identified, it is recommended that treatment with INTRON A be discontinued and the patient followed, with psychiatric intervention as appropriate. Narcotics, hypnotics, or sedatives may be used concurrently with caution and patients should be closely monitored until the adverse effects have resolved. Suicidal ideation or attempts occurred more frequently among pediatric patients, primarily adolescents, compared to adult patients (2.4% versus 1%) during treatment and off-therapy follow-up. Cases of encephalopathy have also been observed in some patients, usually elderly, treated with higher doses of INTRON A.

Treatment with interferons may be associated with exacerbated symptoms of psychiatric disorders in patients with co-occurring psychiatric and substance use disorders. If treatment with interferons is initiated in patients with prior history or existence of psychiatric condition or with a history of substance use disorders, treatment considerations should include the need for drug screening and periodic health evaluation, including psychiatric symptom monitoring. Early intervention for reemergence or development of neuropsychiatric symptoms and substance use is recommended.

Bone Marrow Toxicity

INTRON A therapy suppresses bone marrow function and may result in severe cytopenias including aplastic anemia. It is advised that complete blood counts (CBC) be obtained pretreatment and monitored routinely during therapy (see PRECAUTIONS, Laboratory Tests). INTRON A therapy should be discontinued in patients who develop severe decreases in neutrophil (less than 0.5×10 /L) or platelet counts (less than 25×10 /L) (see DOSAGE AND ADMINISTRATION, Guidelines for Dose Modification).

Ophthalmologic Disorders

Decrease or loss of vision, retinopathy including macular edema, retinal artery or vein thrombosis, retinal hemorrhages and cotton wool spots; optic neuritis, papilledema, and serous retinal detachment may be induced or aggravated by treatment with interferon alfa-2b or other alpha interferons. All patients should receive an eye examination at baseline. Patients with preexisting ophthalmologic disorders (e.g., diabetic or hypertensive retinopathy) should receive periodic ophthalmologic exams during interferon alpha treatment. Any patient who develops ocular symptoms should receive a prompt and complete eye examination. Interferon alfa-2b treatment should be discontinued in patients who develop new or worsening ophthalmologic disorders.

Endocrine Disorders

Infrequently, patients receiving INTRON A therapy developed thyroid abnormalities, either hypothyroid or hyperthyroid. The mechanism by which INTRON A may alter thyroid status is unknown. Patients with preexisting thyroid abnormalities whose thyroid function cannot be maintained in the normal range by medication should not be treated with INTRON A. Prior to initiation of INTRON A therapy, serum TSH should be evaluated. Patients developing symptoms consistent with possible thyroid dysfunction during the course of INTRON A therapy should have their thyroid function evaluated and appropriate treatment instituted. Therapy should be discontinued for patients developing thyroid abnormalities during treatment whose thyroid function cannot be normalized by medication.

Discontinuation of INTRON A therapy has not always reversed thyroid dysfunction occurring during treatment. Diabetes mellitus has been observed in patients treated with alpha interferons. Patients with these conditions who cannot be effectively treated by medication should not begin INTRON A therapy.

Patients who develop these conditions during treatment and cannot be controlled with medication should not continue INTRON A therapy.

Gastrointestinal Disorders

Hepatotoxicity, including fatality, has been observed in interferon alpha-treated patients, including those treated with INTRON A. Any patient developing liver function abnormalities during treatment should be monitored closely and if appropriate, treatment should be discontinued.

Pulmonary Disorders

Dyspnea, pulmonary infiltrates, pneumonia, bronchiolitis obliterans, interstitial pneumonitis, pulmonary hypertension, and sarcoidosis, some resulting in respiratory failure and/or patient deaths, may be induced or aggravated by INTRON A or other alpha interferons. Recurrence of respiratory failure has been observed with interferon re-challenge. The etiologic explanation for these pulmonary findings has yet to be established. Any patient developing fever, cough, dyspnea, or other respiratory symptoms should have a chest X-ray taken. If the chest X-ray shows pulmonary infiltrates or there is evidence of pulmonary function impairment, the patient should be closely monitored, and, if appropriate, interferon alpha treatment should be discontinued. While this has been reported more often in patients with chronic hepatitis C treated with interferon alpha, it has also been reported in patients with oncologic diseases treated with interferon alpha.

Autoimmune Disorders

Rare cases of autoimmune diseases including thrombocytopenia, vasculitis, Raynaud's phenomenon, rheumatoid arthritis, lupus erythematosus, and rhabdomyolysis have been observed in patients treated with alpha interferons, including patients treated with INTRON A. In very rare cases the event resulted in fatality. The mechanism by which these events developed and their relationship to interferon alpha therapy is not clear. Any patient developing an autoimmune disorder during treatment should be closely monitored and, if appropriate, treatment should be discontinued.

Human Albumin

The powder formulations of this product contain albumin, a derivative of human blood. Based on effective donor screening and product manufacturing processes, it carries an extremely remote risk for transmission of viral diseases. A theoretical risk for transmission of Creutzfeldt-Jakob disease (CJD) also is considered extremely remote. No cases of transmission of viral diseases or CJD have ever been identified for albumin.

AIDS-Related Kaposi's Sarcoma

INTRON A therapy should not be used for patients with rapidly progressive visceral disease (see CLINICAL PHARMACOLOGY). Also of note, there may be synergistic adverse effects between INTRON A and zidovudine. Patients receiving concomitant zidovudine have had a higher incidence of neutropenia than that expected with zidovudine alone. Careful monitoring of the WBC count is indicated in all patients who are myelosuppressed and in all patients receiving other myelosuppressive medications. The effects of INTRON A when combined with other drugs used in the treatment of AIDS-related disease are unknown.

Chronic Hepatitis C and Chronic Hepatitis B

Patients with decompensated liver disease, autoimmune hepatitis or a history of autoimmune disease, and patients who are immunosuppressed transplant recipients should not be treated with INTRON A. There are reports of worsening liver disease, including jaundice, hepatic encephalopathy, hepatic failure, and death following INTRON A therapy in such patients. Therapy should be discontinued for any patient developing signs and symptoms of liver failure.

Chronic hepatitis B patients with evidence of decreasing hepatic synthetic functions, such as decreasing albumin levels or prolongation of prothrombin time, who nevertheless meet the entry criteria to start therapy, may be at increased risk of clinical decompensation if a flare of aminotransferases occurs during INTRON A treatment. In such patients, if increases in ALT occur during INTRON A therapy for chronic hepatitis B, they should be followed carefully, including close monitoring of clinical symptomatology and liver function tests including ALT, prothrombin time, alkaline phosphatase, albumin, and bilirubin. In considering these patients for INTRON A therapy, the potential risks must be evaluated against the potential benefits of treatment.

Peripheral Neuropathy

Peripheral neuropathy has been reported when alpha interferons were given in combination with telbivudine. In one clinical trial, an increased risk and severity of peripheral neuropathy was observed with the combination use of telbivudine and pegylated interferon alfa-2a as compared to telbivudine alone. The safety and efficacy of telbivudine in combination with interferons for the treatment of chronic hepatitis B has not been demonstrated.

Use with Ribavirin (see also REBETOL prescribing information)

REBETOL may cause birth defects and/or death of the unborn child. REBETOL therapy should not be started until a report of a negative pregnancy test has been obtained immediately prior to planed initiation of therapy. Patients should use at least two forms of contraception and have monthly pregnancy tests (see CONTRAINDICATIONS and PRECAUTIONS, Information for Patients).

Combination treatment with INTRON A and REBETOL was associated with hemolytic anemia.

Hemoglobin less than 10 g/dL was observed in approximately 10% of adult and pediatric patients in clinical trials. Anemia occurred within 1 to 2 weeks of initiation of ribavirin therapy. Combination treatment with INTRON A and REBETOL should not be used in patients with creatinine clearance less than 50 mL/min. See REBETOL prescribing information for additional information.

PRECAUTIONS

General

Acute serious hypersensitivity reactions (e.g., urticaria, angioedema, bronchoconstriction, anaphylaxis) have been observed rarely in INTRON A-treated patients; if such an acute reaction develops, the drug should be discontinued immediately and appropriate medical therapy instituted. Transient rashes have occurred in some patients following injection, but have not necessitated treatment interruption.

While fever may be related to the flu-like syndrome reported commonly in patients treated with interferon, other causes of persistent fever should be ruled out.

There have been reports of interferon, including INTRON A, exacerbating preexisting psoriasis and sarcoidosis as well as development of new sarcoidosis. Therefore, INTRON A therapy should be used in these patients only if the potential benefit justifies the potential risk.

Variations in dosage, routes of administration, and adverse reactions exist among different brands of interferon. Therefore, do not use different brands of interferon in any single treatment regimen.

Triglycerides

Elevated triglyceride levels have been observed in patients treated with interferons, including INTRON A therapy. Elevated triglyceride levels should be managed as clinically appropriate. Hypertriglyceridemia may result in pancreatitis. Discontinuation of INTRON A therapy should be considered for patients with persistently elevated triglycerides (e.g., triglycerides greater than 1000 mg/dL) associated with symptoms of potential pancreatitis, such as abdominal pain, nausea, or vomiting.

Drug Interactions

Interactions between INTRON A and other drugs have not been fully evaluated. Caution should be exercised when administering INTRON A therapy in combination with other potentially myelosuppressive agents such as zidovudine. Concomitant use of alpha interferon and theophylline decreases theophylline clearance, resulting in a 100% increase in serum theophylline levels.

Carcinogenesis, Mutagenesis, Impairment of Fertility

Studies with INTRON A have not been performed to determine carcinogenicity.

Interferon may impair fertility. In studies of interferon administration in nonhuman primates, menstrual cycle abnormalities have been observed. Decreases in serum estradiol and progesterone concentrations have been reported in women treated with human leukocyte interferon. Therefore, fertile women should not receive INTRON A therapy unless they are using effective contraception during the therapy period. INTRON A therapy should be used with caution in fertile men.

Mutagenicity studies have demonstrated that INTRON A is not mutagenic.

Studies in mice (0.1, 1.0 million IU/day), rats (4, 20, 100 million IU/kg/day), and cynomolgus monkeys (1.1 million IU/kg/day; 0.25, 0.75, 2.5 million IU/kg/day) injected with INTRON A for up to 9 days, 3 months, and 1 month, respectively, have revealed no evidence of toxicity. However, in cynomolgus monkeys (4, 20, 100 million IU/kg/day) injected daily for 3 months with INTRON A, toxicity was observed at the mid and high doses and mortality was observed at the high dose.

However, due to the known species-specificity of interferon, the effects in animals are unlikely to be predictive of those in man.

INTRON A in combination with REBETOL should be used with caution in fertile men. See the REBETOL prescribing information for additional information.

Composition

INTRON A (Interferon alfa-2b) for intramuscular, subcutaneous, intralesional, or intravenous Injection is a purified sterile recombinant interferon product.

INTRON A recombinant for Injection has been classified as an alpha interferon and is a water-soluble protein with a molecular weight of 19,271 daltons produced by recombinant DNA techniques. It is obtained from the bacterial fermentation of a strain of Escherichia coli bearing a genetically engineered plasmid containing an interferon alfa-2b gene from human leukocytes. The fermentation is carried out in a defined nutrient medium containing the antibiotic tetracycline hydrochloride at a concentration of 5 to 10 mg/L; the presence of this antibiotic is not detectable in the final product. The specific activity of interferon alfa-2b, recombinant is approximately 2.6 × 10 IU/mg protein as measured by the HPLC assay.

Storage Recommendations and Dosage Forms

Storage

INTRON A Powder for Injection/Reconstitution

INTRON A Powder for Injection should be stored in the refrigerator at 2° to 8°C (36°-46°F). After reconstitution, the solution should be used immediately, but may be stored up to 24 hours at 2° to8°C (36°-46°F). Throw away any medicine left in the vial after you withdraw 1 dose.

INTRON A Solution for Injection in Vials

INTRON A Solution for Injection in vials should be stored in the refrigerator at 2° to 8°C (36°-46°F).

INTRON A Solution for Injection should not be frozen and should be kept away from heat. Throw away any unused INTRON A Solution for Injection remaining in the vial after one month.

General

IMPORTANT: INTRON A is supplied as 1) Powder for Injection/Reconstitution; 2) Solution for Injection in Vials.

Not all dosage forms and strengths are appropriate for some indications. It is important that you carefully read the instructions below for the indication you are treating to ensure you are using an appropriate dosage form and strength.

To enhance the tolerability of INTRON A, injections should be administered in the evening when possible.

To reduce the incidence of certain adverse reactions, acetaminophen may be administered at the time of injection.

The solution should be allowed to come to room temperature before using.

Powder for Injection

Vial Strength Million IU	mL Diluent	Final Concentration after Reconstitution million IU/mL*	mg INTRON A† per vial	Route of Administration
10	1	10	0.038	IM, SC, IV, IL
18	1	18	0.069	IM, SC, IV
50	1	50	0.192	IM, SC, IV

* Each mL also contains 20 mg glycine, 2.3 mg sodium phosphate dibasic, 0.55 mg sodium phosphate monobasic, and 1.0 mg human albumin.

† Based on the specific activity of approximately 2.6×10^8 IU/mg protein, as measured by HPLC assay.

Prior to administration, the INTRON A Powder for Injection is to be reconstituted with the provided Diluent for INTRON A (Sterile Water for Injection USP) (see DOSAGE AND ADMINISTRATION).

INTRON A Powder for Injection is a white to cream-colored powder.

Solution Vials for Injection

Vial Strength	Concentration*	mg INTRON A† per vial	Route of Administration
18‡ MIU multidose	3 million IU/0.5 mL	0.088	IM, SC
25§ MIU multidose	5 million IU/0.5 mL	0.123	IM, SC, IL

* Each mL contains 7.5 mg sodium chloride, 1.8 mg sodium phosphate dibasic, 1.3 mg sodium phosphate monobasic, 0.1 mg edetate disodium, 0.1 mg polysorbate 80, and 1.5 mg m-cresol as a preservative.

† Based on the specific activity of approximately 2.6×10^8 IU/mg protein as measured by HPLC assay.

‡ This is a multidose vial which contains a total of 22.8 million IU of interferon alfa-2b, recombinant per 3.8 mL in order to provide the delivery of six 0.5-mL doses, each containing 3 million IU of INTRON A (for a label strength of 18 million IU).

§ This is a multidose vial which contains a total of 32.0 million IU of interferon alfa-2b, recombinant per 3.2 mL in order to provide the delivery of five 0.5-mL doses, each containing 5 million IU of INTRON A (for a label strength of 25 million IU).

These packages do not require reconstitution prior to administration (see DOSAGE AND ADMINISTRATION). INTRON A Solution for Injection is a clear, colorless solution.

Dispensation and Accountability

INTRON A is commercially available.

Reconstitution of INTRON A Powder for Injection

Reconstitute INTRON A Powder for Injection with 1 mL of Sterile Water for Injection, USP. The Sterile Water for Injection supplied contains 5 mL and is intended for single use. Discard the unused portion. The reconstituted solution is clear and colorless to light yellow. The INTRON A powder reconstituted with Sterile Water for Injection USP is a single-use vial and does not contain a preservative. DO NOT RE-ENTER VIAL AFTER WITHDRAWING THE DOSE. DISCARD UNUSED PORTION (see DOSAGE AND ADMINISTRATION). Once the dose from the single dose vial has been withdrawn, the sterility of any remaining product can no longer be guaranteed.

Pooling of unused portions of some medications has been linked to bacterial contamination and morbidity.

• Intramuscular, Subcutaneous, or Intralesional Administration

Inject 1 mL Diluent (Sterile Water for Injection USP) for INTRON A into the INTRON A vial. Swirl gently to hasten complete dissolution of the powder. The appropriate INTRON A dose should then be withdrawn and injected intramuscularly, subcutaneously, or intralesionally (see MEDICATION GUIDE and Instructions for Us e for detailed instructions).

Please refer to the MEDICATION GUIDE and Instructions for Us e for detailed, step-by-step instructions on how to inject the INTRON A dose. After preparation and administration of the INTRON A injection, it is essential to follow the procedure for proper disposal of syringes and needles (see MEDICATION GUIDE and Ins tructions for Us e for detailed instructions).

Parenteral drug products should be inspected visually for particulate matter and discoloration prior to administration.

• Intravenous Infusion

The infusion solution should be prepared immediately prior to use. Based on the desired dose, the appropriate vial strength(s) of INTRON A should be reconstituted with the diluent provided. Inject 1 mL Diluent (Sterile Water for Injection USP) for INTRON A into the INTRON A vial. Swirl gently to hasten complete dissolution of the powder. The appropriate INTRON A dose should then be withdrawn and injected into a 100-mL bag of 0.9% Sodium Chloride Injection USP. The final concentration of INTRON A should not be less than 10 million IU/100 mL.

INTRON A Solution for Injection in Vials

INTRON A Solution for Injection is supplied in two multi-dose vials. The solutions for injection do not require reconstitution prior to administration; the solution is clear and colorless.

The appropriate dose should be withdrawn from the vial and injected intramuscularly, subcutaneously, or intralesionally.

INTRON A Solution for Injection is not recommended for intravenous administration.

Please refer to the MEDICATION GUIDE and Instructions for Us e for detailed, step-by-step instructions on how to inject the INTRON A dose. After preparation and administration of INTRON A, it is essential to follow the procedure for proper disposal of syringes and needles.

INTRON A Powder for Injection

INTRON A Powder for Injection, 10 million IU per vial and Diluent for INTRON A (Sterile Water for Injection USP) 5 mL per vial; boxes containing 1 INTRON A vial and 1 vial of INTRON A Diluent (NDC 0085-4350-01).

INTRON A Powder for Injection, 18 million IU per vial and Diluent for INTRON A (Sterile Water for Injection USP) 5 mL per vial; boxes containing 1 vial of INTRON A and 1 vial of INTRON A Diluent (NDC 0085-4351-01).

INTRON A Powder for Injection, 50 million IU per vial and Diluent for INTRON A (Sterile Water for Injection USP) 5 mL per vial; boxes containing 1 INTRON A vial and 1 vial of INTRON A Diluent (NDC 0085-4352-01).

INTRON A Solution for Injection in Vials

INTRON A Solution for Injection, 18 million IU multi-dose vials (22.8 million IU per 3.8 mL per vial); boxes containing 1 vial of INTRON A Solution for Injection (NDC 0085-1168-01).

INTRON A Solution for Injection, 25 million IU multi-dose vials (32 million IU per 3.2 mL per vial); boxes containing 1 vial of INTRON A Solution for Injection (NDC 0085-1133-01).

Peg interferon alfa-2b

[Refer to the FDA-approved package insert for more information: http://dailymed.nlm.nih.gov/dailymed/drugInfo.cfm?setid=b70816bb-913a-467f-acb8-67ef62cf8dac.]

Other name(s)

PEGINTRON®, PEG-IFN-alfa-2b

Mechanism of Action

Pegylated recombinant human interferon alfa-2b is an inducer of the innate antiviral immune response.

The biological activity of Peg Intron is derived from its interferon alfa-2b moiety. Peg interferon alfa-2b binds to and activates the human type 1 interferon receptor. Upon binding, the receptor subunits dimerize, and activate multiple intracellular signal transduction pathways. Signal transduction is initially mediated by the JAK/STAT activation, which may occur in a wide variety of cells. Interferon receptor activation also activates NFκB in many cell types. Given the diversity of cell types that respond to interferon alfa-2b, and the multiplicity of potential intracellular responses to interferon receptor activation, peg interferon alfa-2b is expected to have pleiotropic biological effects in the body.

The mechanism by which ribavirin contributes to its antiviral efficacy in the clinic is not fully understood. Ribavirin has direct antiviral activity in tissue culture against many RNA viruses. Ribavirin increases the mutation frequency in the genomes of several viruses and ribavirin triphosphate inhibits HCV polymerase in a biochemical reaction.

Antiviral Activity

The anti-HCV activity of interferon was demonstrated in cell culture using self-replicating HCV-RNA (HCV replicon cells) or HCV infection and resulted in an effective concentration (EC) value of 1 to 10 IU/mL.

The antiviral activity of ribavirin in the HCV-replicon is not well understood and has not been defined because of the cellular toxicity of ribavirin.

Resistance

HCV genotypes show wide variability in their response to pegylated recombinant human interferon/ribavirin therapy. Genetic changes associated with the variable response have not been identified.

Cross-resistance

There is no reported cross-resistance between pegylated/non-pegylated interferons and ribavirin.

Drug Metabolism, Pharmacokinetics and Toxicology

Following a single subcutaneous dose of Peg Intron, the mean absorption half-life (t ½ ka) was 4.6 hours. Maximal serum concentrations (Cmax) occur between 15 and 44 hours post dose, and are sustained for up to 48 to 72 hours. The Cmax and AUC measurements of Peg Intron increase in a dose related manner. After multiple dosing, there is an increase in bioavailability of Peg Intron. Week 48 mean trough concentrations (320 pg/mL; range 0, 2960) are approximately 3-fold higher than Week 4 mean trough concentrations (94 pg/mL; range 0, 416). The mean Peg Intron elimination half-life is approximately 40 hours (range 22-60 hours) in patients with HCV infection. The apparent clearance of Peg Intron is estimated to be approximately 22 mL/hr·kg. Renal elimination accounts for 30% of the clearance.

Pegylation of interferon alfa-2b produces a product (Peg Intron) whose clearance is lower than that of non-pegylated interferon alfa-2b. When compared to INTRON A, Peg Intron (1 mcg/kg) has approximately a 7-fold lower mean apparent clearance and a 5-fold greater mean half-life, permitting a reduced dosing frequency. At effective therapeutic doses, Peg Intron has approximately 10-fold greater Cmax and 50-fold greater AUC than interferon alfa-2b.

Renal Dysfunction

Following multiple dosing of Peg Intron (1 mcg/kg subcutaneously given every week for 4 weeks) the clearance of Peg Intron is reduced by a mean of 17% in subjects with moderate renal impairment (creatinine clearance 30-49 mL/min) and by a mean of 44% in subjects with severe renal impairment (creatinine clearance 10-29 mL/min) compared to subjects with normal renal function. Clearance was similar in subjects with severe renal impairment not on dialysis and subjects who are receiving hemodialysis. The dose of Peg Intron for monotherapy should be reduced in patients with moderate or severe renal impairment [see Dosage and Administration (2.3) and REBETOL labeling]. REBETOL should not be used in patients with creatinine clearance less than 50 mL/min [see REBETOL labeling, WARNINGS].

Gender

During the 48-week treatment period with Peg Intron, no differences in the pharmacokinetic profiles were observed between male and female subjects with chronic hepatitis C infection.

Geriatric Patients

The pharmacokinetics of geriatric subjects (65 years of age and older) treated with a single subcutaneous dose of 1 mcg/kg of Peg Intron were similar in Cmax , AUC, clearance, or elimination half-life as compared to younger subjects (28-44 years of age).

Pediatric Patients

Population pharmacokinetics for Peg Intron and REBETOL (capsules and oral solution) were evaluated in pediatric subjects with chronic hepatitis C between 3 and 17 years of age. In pediatric patients receiving Peg Intron 60 mcg/m /week subcutaneously, exposure may be approximately 50% higher than observed in adults receiving 1.5 mcg/kg/week subcutaneously. The pharmacokinetics of REBETOL (dose-normalized) in this trial were similar to those reported in a prior trial of REBETOL in combination with INTRON A in pediatric subjects and in adults.

Effect of Food on Absorption of Ribavirin

Both AUC and Cmax increased by 70% when REBETOL capsules were administered with a high-fat meal (841 kcal, 53.8 g fat, 31.6 g protein, and 57.4 g carbohydrate) in a single-dose pharmacokinetic trial.

Drug Interactions

Drugs Metabolized by Cytochrome P-450

The pharmacokinetics of representative drugs metabolized by CYP1A2 (caffeine), CYP2C8/9 (tolbutamide), CYP2D6 (dextromethorphan), CYP3A4 (midazolam), and N-acetyltransferase (dapsone) were studied in 22 subjects with chronic hepatitis C who received Peg Intron (1.5 mcg/kg) once weekly for 4 weeks. Peg Intron treatment resulted in a 28% (mean) increase in a measure of CYP2C8/9 activity.

Peg Intron treatment also resulted in a 66% (mean) increase in a measure of CYP2D6 activity; however, the effect was variable as 13 subjects had an increase, 5 subjects had a decrease, and 4 subjects had no significant change.

No significant effect was observed on the pharmacokinetics of representative drugs metabolized by CYP1A2, CYP3A4, or N-acetyltransferase. The effects of Peg Intron on CYP2C19 activity were not assessed.

Methadone

The pharmacokinetics of concomitant administration of methadone and Peg Intron were evaluated in 18 Peg Intron-naïve chronic hepatitis C subjects receiving 1.5 mcg/kg Peg Intron subcutaneously weekly.

All subjects were on stable methadone maintenance therapy receiving greater than or equal to 40 mg/day prior to initiating Peg Intron. Mean methadone AUC was approximately 16% higher after 4 weeks of Peg Intron treatment as compared to baseline. In 2 subjects, methadone AUC was approximately double after 4 weeks of Peg Intron treatment as compared to baseline.

Use with Ribavirin, Zidovudine, Lamivudine, and Stavudine

Ribavirin has been shown in vitro to inhibit phosphorylation of zidovudine, lamivudine, and stavudine.

However, in a trial with another pegylated interferon in combination with ribavirin, no pharmacokinetic (e.g., plasma concentrations or intracellular triphosphorylated active metabolite concentrations) or pharmacodynamics (e.g., loss of HIV/HCV virologic suppression) interaction was observed when ribavirin and lamivudine (n=18), stavudine (n=10), or zidovudine (n=6) were co-administered as part of a multi-drug regimen to HIV/HCV co-infected subjects.

Didanosine

Exposure to didanosine or its active metabolite (dideoxyadenosine 5'- triphosphate) is increased when didanosine is co-administered with ribavirin, which could cause or worsen clinical toxicities.

Management of Agent-Specific Adverse Events

Adults

Study 1 compared Peg Intron monotherapy with INTRON® A monotherapy. Study 2 compared combination therapy of Peg Intron/REBETOL with combination therapy with INTRON A/REBETOL. In these clinical trials, nearly all subjects experienced one or more adverse reactions. Study 3 compared a Peg Intron/weight-based REBETOL combination to a Peg Intron/flat dose REBETOL regimen. Study 4 compared two Peg Intron (1.5 mcg/kg/week and 1 mcg/kg/week) doses in combination with REBETOL and a third treatment group receiving Pegasys (180 mcg/week)/Copegus (1000-1200 mg/day).

Adverse reactions that occurred in Studies 1 and 2 at greater than 5% incidence are provided in Table A (below) by treatment group. Due to potential differences in ascertainment procedures, adverse reaction rate comparisons across trials should not be made. Table B (below) summarizes the treatment-related adverse reactions in Study 4 that occurred at a greater than or equal to 10% incidence.

Table A: Adverse Reactions Occurring in >5% of Subjects

	*Percentage of Subjects Reporting Adverse Reactions**			
	Study 1		**Study 2**	
Adverse Reactions	**PegIntron 1 mcg/kg (N=297)**	**INTRON A 3 MIU (N=303)**	**PegIntron 1.5 mcg/kg/ REBETOL (N=511)**	**INTRON A/ REBETOL (N=505)**
Application Site				
Injection Site Inflammation/Reaction	47	20	75	49
Autonomic Nervous System				
Dry Mouth	6	7	12	8
Increased Sweating	6	7	11	7
Flushing	6	3	4	3
Body as a Whole				
Fatigue/Asthenia	52	54	66	63
Headache	56	52	62	58
Rigors	23	19	48	41
Fever	22	12	46	33
Weight Loss	11	13	29	20
Right Upper Quadrant Pain	8	8	12	6
Chest Pain	6	4	8	7
Malaise	7	6	4	6
Central/Peripheral Nervous System				
Dizziness	12	10	21	17
Endocrine				
Hypothyroidism	5	3	5	4
Gastrointestinal				
Nausea	26	20	43	33
Anorexia	20	17	32	27
Diarrhea	18	16	22	17
Vomiting	7	6	14	12
Abdominal Pain	15	11	13	13

(…continued on following page…)

Dyspepsia	6	7	9	8
Constipation	1	3	5	5
Hematologic Disorders				
Neutropenia	6	2	26	14
Anemia	0	0	12	17
Leukopenia	<1	0	6	5
Thrombocytopenia	7	<1	5	2
Liver and Biliary System				
Hepatomegaly	6	5	4	4
Musculoskeletal				
Myalgia	54	53	56	50
Arthralgia	23	27	34	28
Musculoskeletal Pain	28	22	21	19
Psychiatric				
Insomnia	23	23	40	41
Depression	29	25	31	34
Anxiety/Emotional Lability/Irritability	28	34	47	47
Concentration Impaired	10	8	17	21
Agitation	2	2	8	5
Nervousness	4	3	6	6
Reproductive, Female				
Menstrual Disorder	4	3	7	6
Resistance Mechanism				
Viral Infection	11	10	12	12
Fungal Infection	<1	3	6	1
Respiratory System				
Dyspnea	4	2	26	24
Coughing	8	5	23	16
Pharyngitis	10	7	12	13
Rhinitis	2	2	8	6
Sinusitis	7	7	6	5
Skin and Appendages				
Alopecia	22	22	36	32
Pruritus	12	8	29	28
Rash	6	7	24	23
Skin Dry	11	9	24	23
Special Senses, Other				
Taste Perversion	<1	2	9	4
Vision Disorders				
Vision Blurred	2	3	5	6
Conjunctivitis	4	2	4	5

* Subjects reporting one or more adverse reactions. A subject may have reported

Table B: Treatment-related Adverse Reactions (≥10% incidence) by Descending Frequency

Adverse Reactions	*Percentage of Subjects Reporting Treatment-Related Adverse Reactions* Study 4		
	PegIntron 1.5 mcg/kg with REBETOL (N=1019)	PegIntron 1 mcg/kg with REBETOL (N=1016)	Pegasys 180 mcg with Copegus (N=1035)
Fatigue	67	68	64
Headache	50	47	41
Nausea	40	35	34
Chills	39	36	23
Insomnia	38	37	41
Anemia	35	30	34
Pyrexia	35	32	21
Injection Site Reactions	34	35	23
Anorexia	29	25	21
Rash	29	25	34
Myalgia	27	26	22
Neutropenia	26	19	31
Irritability	25	25	25
Depression	25	19	20
Alopecia	23	20	17
Dyspnea	21	20	22
Arthralgia	21	22	22
Pruritus	18	15	19
Influenza-like Illness	16	15	15
Dizziness	16	14	13
Diarrhea	15	16	14
Cough	15	16	17
Weight Decreased	13	10	10
Vomiting	12	10	9
Unspecified Pain	12	13	9
Dry Skin	11	11	12
Anxiety	11	11	10
Abdominal Pain	10	10	10
Leukopenia	9	7	10

The adverse reaction profile in Study 3, which compared Peg Intron/weight-based REBETOL combination to a Peg Intron/flat-dose REBETOL regimen, revealed an increased rate of anemia with weight-based dosing (29% vs. 19% for weight-based vs. flat-dose regimens, respectively). However, the majority of cases of anemia were mild and responded to dose reductions.

The incidence of serious adverse reactions was comparable in all trials. In the Peg Intron monotherapy trial (Study 1) the incidence of serious adverse reactions was similar (about 12%) in all treatment groups. In Study 2, the incidence of serious adverse reactions was 17% in the Peg Intron/REBETOL groups compared to 14% in the INTRON A/REBETOL group. In Study 3, there was a similar incidence of serious adverse reactions reported for the weight-based REBETOL group (12%) and for the flat dose REBETOL regimen.

In many but not all cases, adverse reactions resolved after dose reduction or discontinuation of therapy.

Some subjects experienced ongoing or new serious adverse reactions during the 6-month follow-up period.

There have been 31 subject deaths that occurred during treatment or during follow-up in these clinical trials. In Study 1, there was 1 suicide in a subject receiving Peg Intron monotherapy and 2 deaths among subjects receiving INTRON A monotherapy (1 murder/suicide and 1 sudden death). In Study 2, there was 1 suicide in a subject receiving Peg Intron/REBETOL combination therapy, and 1 subject death in the INTRON A/REBETOL group (motor vehicle accident). In Study 3, there were 14 deaths, 2 of which were probable suicides, and 1 was an unexplained death in a person with a relevant medical history of depression. In Study 4, there were 12 deaths, 6 of which occurred in subjects receiving Peg Intron/REBETOL combination therapy; 5 in the Peg Intron 1.5 mcg/REBETOL arm (N=1019) and 1 in the Peg Intron 1 mcg/REBETOL arm (n=1016); and 6 of which occurred in subjects receiving Pegasys/Copegus (N=1035). There were 3 suicides that occurred during the off-treatment follow-up period in subjects who received Peg Intron (1.5 mcg/kg)/REBETOL combination therapy.

In Studies 1 and 2, 10% to 14% of subjects receiving Peg Intron, alone or in combination with REBETOL, discontinued therapy compared with 6% treated with INTRON A alone and 13% treated with INTRON A in combination with REBETOL. Similarly in Study 3, 15% of subjects receiving Peg Intron in combination with weight-based REBETOL and 14% of subjects receiving Peg Intron and flat-dose REBETOL discontinued therapy due to an adverse reaction. The most common reasons for discontinuation of therapy were related to known interferon effects of psychiatric, systemic (e.g., fatigue, headache), or gastrointestinal adverse reactions. In Study 4, 13% of subjects in the Peg Intron

1.5 mcg/REBETOL arm, 10% in the Peg Intron 1 mcg/REBETOL arm, and 13% in the Pegasys 180 mcg/Copegus arm discontinued therapy due to adverse events. In Study 2, dose reductions due to adverse reactions occurred in 42% of subjects receiving Peg Intron (1.5 mcg/kg)/REBETOL and in 34% of those receiving INTRON A/REBETOL. The majority of subjects (57%) weighing 60 kg or less receiving Peg Intron (1.5 mcg/kg)/REBETOL required dose reduction. Reduction of interferon was dose-related (Peg Intron 1.5 mcg/kg more than Peg Intron 0.5 mcg/kg or INTRON A), 40%, 27%, 28%, respectively. Dose reduction for REBETOL was similar across all three groups, 33% to 35%. The most common reasons for dose modifications were neutropenia (18%) or anemia (9%). Other common reasons included depression, fatigue, nausea, and thrombocytopenia. In Study 3, dose modifications due to adverse reactions occurred more frequently with weight-based dosing (WBD) compared to flat dosing (29% and 23%, respectively). In Study 4, 16% of subjects had a dose reduction of Peg Intron to 1 mcg/kg in combination with REBETOL, with an additional 4% requiring the second dose reduction of Peg Intron to 0.5 mcg/kg due to adverse events, compared to 15% of subjects in the Pegasys/Copegus arm, who required a dose reduction to 135 mcg/week with Pegasys, with an additional 7% requiring a second dose reduction to 90 mcg/week with Pegasys.

In the Peg Intron/REBETOL combination trials the most common adverse reactions were psychiatric, which occurred among 77% of subjects in Study 2 and 68% to 69% of subjects in Study 3. These psychiatric adverse reactions included most commonly depression, irritability, and insomnia, each reported by approximately 30% to 40% of subjects in all treatment groups. Suicidal behavior (ideation, attempts, and suicides) occurred in 2% of all subjects during treatment or during follow-up after treatment cessation [see Warnings and Precautions (5.2)]. In Study 4, psychiatric adverse reactions occurred in 58% of subjects in the Peg Intron 1.5 mcg/REBETOL

arm, 55% of subjects in the Peg Intron 1 mcg/REBETOL arm, and 57% of subjects in the Pegasys 180 mcg/Copegus arm.

Peg Intron induced fatigue or headache in approximately two-thirds of subjects, with fever or rigors in approximately half of the subjects. The severity of some of these systemic symptoms (e.g., fever and headache) tended to decrease as treatment continued. In Studies 1 and 2, application site inflammation and reaction (e.g., bruise, itchiness, and irritation) occurred at approximately twice the incidence with Peg Intron therapies (in up to 75% of subjects) compared with INTRON A. However, injection-site pain was infrequent (2-3%) in all groups. In Study 3, there was a 23% to 24% incidence overall for injection site reactions or inflammation.

In Study 2, many subjects continued to experience adverse reactions several months after discontinuation of therapy. By the end of the 6-month follow-up period, the incidence of ongoing adverse reactions by body class in the Peg Intron 1.5/REBETOL group was 33% (psychiatric), 20% (musculoskeletal), and 10% (for endocrine and for GI). In approximately 10% to 15% of subjects, weight loss, fatigue, and headache had not resolved.

Individual serious adverse reactions in Study 2 occurred at a frequency less than or equal to 1% and included suicide attempt, suicidal ideation, severe depression; psychosis, aggressive reaction, relapse of drug addiction/overdose; nerve palsy (facial, oculomotor); cardiomyopathy, myocardial infarction, angina, pericardial effusion, retinal ischemia, retinal artery or vein thrombosis, blindness, decreased visual acuity, optic neuritis, transient ischemic attack, supraventricular arrhythmias, loss of consciousness; neutropenia, infection (sepsis, pneumonia, abscess, cellulitis); emphysema, bronchiolitis obliterans, pleural effusion, gastroenteritis, pancreatitis, gout, hyperglycemia, hyperthyroidism and hypothyroidism, autoimmune thrombocytopenia with or without purpura, rheumatoid arthritis, interstitial nephritis, lupus-like syndrome, sarcoidosis, aggravated psoriasis; urticaria, injection-site necrosis, vasculitis, and photo toxicity.

Subjects receiving Peg Intron/REBETOL as re-treatment after failing a previous interferon combination regimen reported adverse reactions similar to those previously associated with this regimen during clinical trials of treatment-naïve subjects.

Laboratory Values

Adults

Changes in selected laboratory values during treatment with Peg Intron alone or in combination with REBETOL treatment are described below. Decreases in hemoglobin, neutrophils, and platelets may require dos e reduction or permanent dis continuation from therapy [see Dosage and Administration (2.3) and Warnings and Precautions (5.1, 5.7)].

Hemoglobin

Hemoglobin levels decreased to less than 11 g/dL in about 30% of subjects in Study 2. In Study 3, 47% of subjects receiving WBD REBETOL and 33% on flat-dose REBETOL had decreases in hemoglobin levels less than 11 g/dL. Reductions in hemoglobin to less than 9 g/dL occurred more frequently in subjects receiving WBD compared to flat dosing (4% and 2%, respectively). In Study 2, dose modification was required in 9% and 13% of subjects in the Peg Intron/REBETOL and INTRON A/REBETOL groups. In Study 4, subjects receiving Peg Intron (1.5 mcg/kg)/REBETOL had decreases in hemoglobin levels to between 8.5 to less than 10 g/dL (28%) and to less than 8.5 g/dL (3%), whereas in subjects receiving Pegasys 180 mcg/Copegus these decreases occurred in

26% and 4% of subjects, respectively. Hemoglobin levels became stable by treatment Weeks 4 to 6 on average. The typical pattern observed was a decrease in hemoglobin levels by treatment Week 4 followed by stabilization and a plateau, which was maintained to the end of treatment. In the PegIntronmonotherapy trial, hemoglobin decreases were generally mild and dose modifications were rarely necessary [see Dosage and Administration (2.3)].

Neutrophils

Decreases in neutrophil counts were observed in a majority of subjects treated with Peg Intron alone (70%) or as combination therapy with REBETOL in Study 2 (85%) and INTRONA/REBETOL (60%). Severe potentially life-threatening neutropenia (less than 0.5 × 10 /L) occurred in

1% of subjects treated with Peg Intron monotherapy, 2% of subjects treated with INTRONA/REBETOL, and in approximately 4% of subjects treated with Peg Intron/REBETOL in Study 2. Two percent of subjects receiving Peg Intron monotherapy and 18% of subjects receiving Peg Intron/REBETOL in Study 2 required modification of interferon dosage. Few subjects (less than1%) required permanent discontinuation of treatment. Neutrophil counts generally returned to pretreatment levels 4 weeks after cessation of therapy [see Dosage and Administration (2.3)].

Platelets

Platelet counts decreased to less than 100,000/mm in approximately 20% of subjects treated with Peg Intron alone or with REBETOL and in 6% of subjects treated with INTRON A/REBETOL. Severe decreases in platelet counts (less than 50,000/mm) occur in less than 4% of subjects. Patients may require discontinuation or dose modification as a result of platelet decreases [see Dosage and Administration (2.3)]. In Study 2, 1% or 3% of subjects required dose modification of INTRON A or Peg Intron, respectively. Platelet counts generally returned to pretreatment levels 4 weeks after the cessation of therapy.

Triglycerides

Elevated triglyceride levels have been observed in patients treated with interferon alphas, including Peg Intron [see Warnings and Precautions (5.17)].

Thyroid Function

Development of TSH abnormalities, with or without clinical manifestations, is associated with interferon therapies. In Study 2, clinically apparent thyroid disorders occurred among subjects treated with either INTRON A or Peg Intron (with or without REBETOL) at a similar incidence (5% for hypothyroidism and 3% for hyperthyroidism). Subjects developed new-onset TSH abnormalities while on treatment and during the follow-up period. At the end of the follow-up period,7% of subjects still had abnormal TSH values [see Warnings and Precautions (5.4)].

Bilirubin and Uric Acid. In Study 2, 10% to 14% of subjects developed hyperbilirubinemia and 33% to38% developed hyperuricemia in association with hemolysis. Six subjects developed mild to moderate gout.

Immunogenicity

As with all therapeutic proteins, there is potential for immunogenicity. Approximately 2% of subjects receiving Peg Intron (32/1759) or INTRON A (11/728) with or without REBETOL developed low-titer(less than or equal to 160) neutralizing antibodies to Peg Intron or INTRON A. The clinical and pathological significance of the appearance of serum-neutralizing antibodies is unknown. The incidence of antibody formation is highly dependent on the sensitivity and specificity of the assay. Additionally, the observed incidence of antibody (including neutralizing antibody) positivity in an assay may be influenced by several factors, including assay methodology, sample handling, timing of sample collection, concomitant medications, and underlying disease. For these reasons, comparison of the incidence of antibodies to Peg Intron with the incidence of antibodies to other products may be misleading.

Carcinogenesis and Mutagenesis

Peg Intron has not been tested for its carcinogenic potential. Neither Peg Intron nor its components, interferon or methoxypolyethylene glycol, caused damage to DNA when tested in the standard battery of mutagenesis assays, in the presence and absence of metabolic activation.

Use with Ribavirin: See ribavirin labeling for additional warnings relevant to Peg Intron therapy in combination with ribavirin.

Impairment of Fertility

Peg Intron may impair human fertility. Irregular menstrual cycles were observed in female cynomolgus monkeys given subcutaneous injections of 4239 mcg/m Peg Intron alone every other day for 1 month (approximately 345 times the recommended weekly human dose based upon body surface area). These effects included transiently decreased serum levels of estradiol and progesterone, suggestive of anovulation. Normal menstrual cycles and serum hormone levels resumed in these animals 2 to 3 months following cessation of Peg Intron treatment. Every other day dosing with 262 mcg/m (approximately 21 times the weekly human dose) had no effects on cycle duration or reproductive hormone status. The effects of Peg Intron on male fertility have not been studied.

Composition

Peg Intron, peg interferon alfa-2b, is a covalent conjugate of recombinant alfa-2b interferon with monomethoxy polyethylene glycol (PEG). The average molecular weight of the PEG portion of the molecule is 12,000 daltons. The average molecular weight of the Peg Intron molecule is approximately 31,000 daltons. The specific activity of peg interferon alfa-2b is approximately 0.7 × 108 IU/mg protein.

Interferon alfa-2b is a water-soluble protein with a molecular weight of 19,271 daltons produced by recombinant DNA techniques. It is obtained from the bacterial fermentation of a strain of Escherichia coli bearing a genetically engineered plasmid containing an interferon gene from human leukocytes.

Storage Recommendations and Dosage Forms

Peg Intron is supplied in both vials and the REDIPEN single-use pre-filled pen for subcutaneous use.

Vials

Each vial contains either 74 mcg, 118.4 mcg, 177.6 mcg, or 222 mcg of Peg Intron as a white to off-white tablet-like solid that is whole/in pieces or as a loose powder, and 1.11 mg dibasic sodium phosphate anhydrous, 1.11 mg monobasic sodium phosphate dehydrate, 59.2 mg sucrose, and 0.074 mg polysorbate 80. Following reconstitution with 0.7 mL of the supplied Sterile Water for Injection USP, each vial contains Peg Intron at strengths of either 50 mcg per 0.5 mL, 80 mcg per 0.5 mL, 120 mcg per 0.5 mL, or 150 mcg per 0.5 mL.

REDIPEN single-use pre-filled pen

REDIPEN pre-filled pen is a dual-chamber glass cartridge containing lyophilized Peg Intron as a white to off-white tablet or powder that is whole or in pieces in the sterile active chamber and a second chamber containing Sterile Water for Injection USP. Each Peg Intron REDIPEN pre-filled pen contains either 67.5 mcg, 108 mcg, 162 mcg, or 202.5 mcg of Peg Intron, and 1.013 mg dibasic sodium phosphate anhydrous, 1.013 mg monobasic sodium phosphate dehydrate, 54 mg sucrose, and 0.0675 mg polysorbate 80. Each cartridge is reconstituted to allow for the administration of up to 0.5 mL of solution. Following reconstitution, each REDIPEN pre-filled pen contains Peg Intron at strengths of either 50 mcg per 0.5 mL, 80 mcg per 0.5 mL, 120 mcg per 0.5 mL, or 150 mcg per 0.5 mL for a single use. Because a small volume of reconstituted solution is lost during preparation of Peg Intron, each REDIPEN pre-filled pen contains an excess amount of Peg Intron powder and diluent to ensure delivery of the labeled dose.

Storage

Peg Intron REDIPEN single-use pre-filled pen

Peg Intron REDIPEN pre-filled pen should be stored at 2-8°C (36-46°F).

After reconstitution, the solution should be used immediately, but may be stored up to 24 hours at 2-8°C (36-46°F). The reconstituted solution contains no preservative, and is clear and colorless. **DO NOT FREEZE. Keep away from heat.**

Peg Intron Vials

Peg Intron should be stored at 25°C (77°F); excursions permitted to 15-30°C (59-86°F) [see USP Controlled Room Temperature]. After reconstitution with supplied diluent, the solution should be used immediately but may be stored up to 24 hours at 2-8°C (36-46°F). The reconstituted solution contains no preservative, and is clear and colorless. **DO NOT FREEZE. Keep away from heat.**

Dispensation and Accountability

Peg intron is commercially available.

Patients should be advised to read the FDA-approved patient labeling (Medication Guide and Instructions for Use). A patient should self-inject Peg Intron only if it has been determined that it is appropriate, the patient agrees to medical follow-up as necessary, and training in proper injection technique has been given to him/her.(For patent information: www.merck.com/product/patent/home.html.)

Instructions for Use

Patients receiving Peg Intron should be directed in its appropriate preparation, handling, measurement, and injection, and referred to the Instructions for Use for Peg Intron Powder for Solution and Peg Intron REDIPEN Single-use Pre-filled pen.

Patients should be instructed that the Sterile Water for Injection vial supplied with Peg Intron Powder for Solution contains an excess amount of diluent (5 mL) and only 0.7 mL should be withdrawn to reconstitute Peg Intron Powder for Solution. The vial of Sterile Water for Injection is intended for single use only. Discard the unused portion of the sterile water. Do not save or reuse.

Patients should be directed to store Peg Intron before mixing as follows:

Peg Intron REDIPEN single-use pre-filled pens: store in the refrigerator between 36-46°F (2-8°C)

Peg Intron Powder for Solution: store at room temperature between 59-86°F (15-30°C)

Patients should be instructed on the importance of site selection for self-administering the injection, as well as the importance on rotating the injection sites.

Disposal Instructions

Patients should be thoroughly instructed in the importance of proper disposal. After preparation and administration of Peg Intron for Injection, patients should be advised to use a puncture-resistant container for the disposal of used syringes, needles, and the REDIPEN pre-filled pen. The full container should be disposed of in accordance with state and local laws. Patients should also be cautioned against reusing or sharing needles, syringes, or the REDIPEN pre-filled pen.

CYCLOPHOSPHAMIDE:

Cyclophosphamide is an alkylating agent and is cell cycle nonspecific. It causes cross-linking of DNA and is the most active single agent in the treatment of non-Hodgkin's lymphoma. Side effects of cyclophosphamide include nausea, vomiting, myelosuppression and alopecia. Sterility and testicular atrophy are common in men and amenorrhea is seen in women. Hemorrhagic cystitis is caused by metabolites of cyclophosphamide excreted through the urine. Bladder irritation can be reduced by adequate hydration. Please refer to the approved package insert for complete prescribing and toxicity information.