# Targeting Exportin-1 to Inhibit HTLV-1 Infection

by

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Human T-cell Leukemia Virus Type-1 (HTLV-1) is a complex human retrovirus that infects around 20 million people globally. Transmission of this retrovirus occurs by sexual intercourse, contaminated blood, and from mother to child by breastfeeding. While HTLV-1 is asymptomatic in most of the infected hosts, around 5% will develop an HTLV-1 associated disease such as Adult T-cell Leukemia/Lymphoma (ATLL) and HTLV-1 Associated Myelopathy/Tropical Spastic Paraparesis (HAM/TSP). ATLL is a malignant and aggressive form of cancer that occurs in $CD4^+$ T-cells. HAM/TSP is a neurological disorder that is believed to be caused by the infiltration of HTLV-1 infected cells into the central nervous system leading to axon demyelination. There are no effective treatments or cures for HTLV-1-associated diseases, and there is no

prophylactic vaccine. Furthermore, there is no anti-retroviral treatment that has yet been successful to inhibit HTLV-1 infection in clinical studies.

We focused our research on the protein exportin-1 (XPO-1). XPO-1 mediates the nuclear export of messenger RNA (mRNA) for the purpose of cell survival, proliferation, and metastasis. Interestingly, XPO-1 has been shown to facilitate the nuclear export of certain HTLV-1 mRNA necessary for virion formation, such as *Gag/Pol* and *Env* mRNA. Recently, some compounds known as Selective Inhibitor of Nuclear Export (SINE) have been designed to bind to a cysteine residue of XPO-1 to prevent the binding of nuclear cargo. We used one of these SINE molecules, KPT-185, to determine its efficiency in inhibiting HTLV-1 infection. Our results revealed that treatment of HTLV-1 cells derived from ATLL patients treated with KPT-185 did not lead to cell death. However, KPT-185 was successful in inhibiting the export of the *Gag/Pol* mRNA leading to reduced levels of Gag protein in the cells. In addition, we also find that KPT-185 reduced the level of the envelope protein that coat HTLV-1 virions. In correlation with these observations, we found less virions to be released and a significant reduction of HTLV-1 capability to infect other $CD4^+$ T-cells. Therefore, our observation that KPT-185 inhibits HTLV-1 infection indicate that this compound could be an effective form of prevention of HTLV-1 spread and the development of associated diseases.

# Targeting Exportin-1 to Inhibit HTLV-1 Infection

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### *1.1 Human T-cell Leukemia Virus Type-1 (HTLV-1)*

Human T-cell Leukemia Virus Type-1 (HTLV-1) is a complex human virus classified as a Deltaretrovirus of the Retrovirus family (Lairmore et al., 2012). Being a Retrovirus, HTLV-1 is a negative sense RNA virus that packages a reverse transcriptase in the viral particle to reverse transcribe its RNA to DNA. The viral particle also contains an integrase to integrate the HTLV-1 genome into the host cell DNA. Conservative estimates put the number of infected individuals to be around 20 million worldwide with endemic regions being in Japan, Africa, South America, and the Caribbean (Gessain & Cassar, 2012). Though HTLV-1 can infect a variety of cells; the virus has the greatest reproductive viability in $CD4^+$ T-cells. Unlike other viruses, HTLV-1 infection does not lead to the manifestations of symptoms in the host. The lack of symptoms in the HTLV-1 hosts means the virus can easily be spread from host to host. HTLV-1 is spread vertically from mother to child through breastfeeding and horizontally through sexual contact and blood transfusions. Furthermore, the lack of symptoms in HTLV-1 makes it difficult to diagnose. Most of those who are infected will go decades or even their entire lives without diagnosis and around 5% of those infected will develop an HTLV-1 associated disease. There are a variety of diseases that are caused by HTLV-1 but, the most common are Adult T-cell Leukemia/Lymphoma (ATLL) and HTLV-1 Associated Myelopathy/Tropical Spastic Paraparesis (HAM/TSP); however, infective dermatitis and other inflammatory diseases can occur. ATLL is an aggressive form of leukemia/lymphoma with no effective treatment or cure. HAM/TSP is a neurological disorder resulting from the demyelination of axons in the central nervous system. The

gaps in knowledge about HTLV-1 and the diseases associated with it drive research into further understanding the disease and how to treat it. Our research has been focused in treating HTLV-1 infected cells with a compound to inhibit the export of the viral mRNA to reduce the rate of infection.

### *1.2 HTLV-1 Discovery*

In 1977, researchers in Japan discovered and characterized an aggressive and malignant form of $CD4^+$ T-cell cancer in patients and named the diseases Adult T-cell Leukemia/Lymphoma (ATLL) (Matsuoka, 2005). In 1980, antibodies for a virus were discovered in the serum of samples taken from a lymphoma patient in the United States, the virus was named Human T-cell Leukemia Virus (HTLV) (Poiesz et al., 1980). In 1982, a virologic agent of ATLL was discovered and subsequently named Adult T-cell Leukemia Virus (ATLV) (Yoshida et al., 1982). Sometime after the characterization of ATLV, it was demonstrated that ATLV and HTLV were the same virus after analysis of the viral genomes and antigens, the virus was renamed HTLV-1 (Watanabe et al., 1984).

### *1.3 HTLV-1 Classification and Related Viruses*

HTLV-1 is a negative sense RNA virus characterized as a Deltaretrovirus based upon its nucleotide and amino acid sequence. HTLV-1 is also in the classification because it carries a reverse transcriptase to convert its RNA to DNA and an integrase for inserting the viral genome into the host genome. The HTLV group also contains the leukemia viruses HTLV-2, HTLV-3, and HTLV-4. Though there is some genetic overlap between the four HTLV viruses, the pathology for HTLV-2, 3, and 4 has not been clearly established.

HTLV-2 was discovered in patients with Hairy Cell Leukemia (HCL) (Rosenblatt, 1988). Unlike HTLV-1, HTLV-2 primarily infects $CD8^+$ T-cells and extensive studies have shown that the virus is not oncogenic despite it being discovered in HCL patients. However, in rare cases, patients have developed neurological symptoms similar to those with HAM/TSP (Martinez et al., 2019). HTLV-3 and HTLV-4 are localized in certain regions in Africa and have yet to be shown to be associated with any diseases (Mahieux & Gessain, 2009). The genome of HTLV viruses also strongly resemble that of the Simian T-cell Leukemia Viruses (STLV); a group of viruses known to infect African monkeys. The similarities between the two groups of viruses suggests a common evolutionary ancestor prior to a species jump.

### *1.4 HTLV-1 Transmission*

The primary pathway of HTLV-1 transmission is through a mother breastfeeding her child (Goncalves et al., 2010). The longer a child is breastfed the greater the chance the virus will be transmitted to him/her. The association between breastfeeding and HTLV-1 transmission has been established through studies comparing infection rates among breastfed and formula fed infants. Formula feeding infants presents a way of reducing vertical transmission; however, this is not feasible in some of the endemic areas such as Africa. This is due to a variety of reasons such as; beliefs held about proper infant feeding, influence from an older generation, low socio-economic status, and lack of proper health promotion (Ogbo et al., 2017).

Other forms of transmission include horizontal forms of transmission. A common form occurs through blood containing infected lymphocytes which has high rate of infection. Since 1988 blood donations in the United States have been tested for HTLV-1

and HTLV-2 under the recommendation of the Food and Drug Administration (FDA). The World Health Organization (WHO) recommends that the blood donations in endemic areas be tested as well. Transmission of HTLV-1 is also a concern among intravenous drug use and sexual transmission but, the latter is less efficient.

### *1.5 HTLV-1 Life Cycle*

The transmission of HTLV-1 is exclusively performed through cell-to-cell contact. Free viral particles have been shown to be ineffective at infecting other cells. This makes HTLV-1 different than other retroviruses such as Human Immunodeficiency Virus (HIV) which can spread through cell-to-cell contact and free-floating viral particles. The HTLV-1 viral particles are retained on surface of the infected cell by interactions with the cell surface protein tetherin (Ilinskaya et al., 2013). Tetherin is part of the viral biofilm, a specialized extracellular matrix, that is also composed of a different components such as collagen, agrin, galectin-3, CD43, and CD45 (Gross & Thoma-Kress, 2016). When an infected cell makes contact with a non-infected cell it forms a viral synapse through connections between cell surface proteins ICAM-1 and LFA-1 (Tibbetts et al., 2000). The formation of the viral synapse is mediated by the viral proteins Tax and HBZ promoting the expression of ICAM-1 (Fazio et al., 2019; Gross et al., 2016). The viral particles are released from the surface of the infected cells and will attach to receptors on the surface of the non-infected cell; GLUT-1, Neuropilin-1, and Heparan Sulfate Proteoglycans. GLUT-1 is the primary protein responsible for the binding of the viral particle to the cell through interactions with the viral envelope glycoproteins (Figure 1). Neuropilin-1 acts as a secondary binding protein for the viral particle (Ghez et al., 2006).

Heparan Sulfate Proteoglycan plays a role of an accessory protein to amplify the uptake of the viral particle (Jones et al., 2011).

Once the viral particle enters the cell, the viral envelope is removed. Following the removal of the envelope, the viral RNA is reverse transcribed with the reverse transcriptase carried in the viral particle. The HTLV-1 genome is then integrated into the hosts genome through the action of the integrase that the virus carries (Figure 1). The HTLV-1 genome is bookended by two long terminal repeats (LTR) on the 5’ and 3’ ends (Polakowski & Lemasson, 2010). The LTRs are divided into three subdivisions; U5, R, and U3. The LTRs are not transcribed; however, they contain features and structures important for transcription.

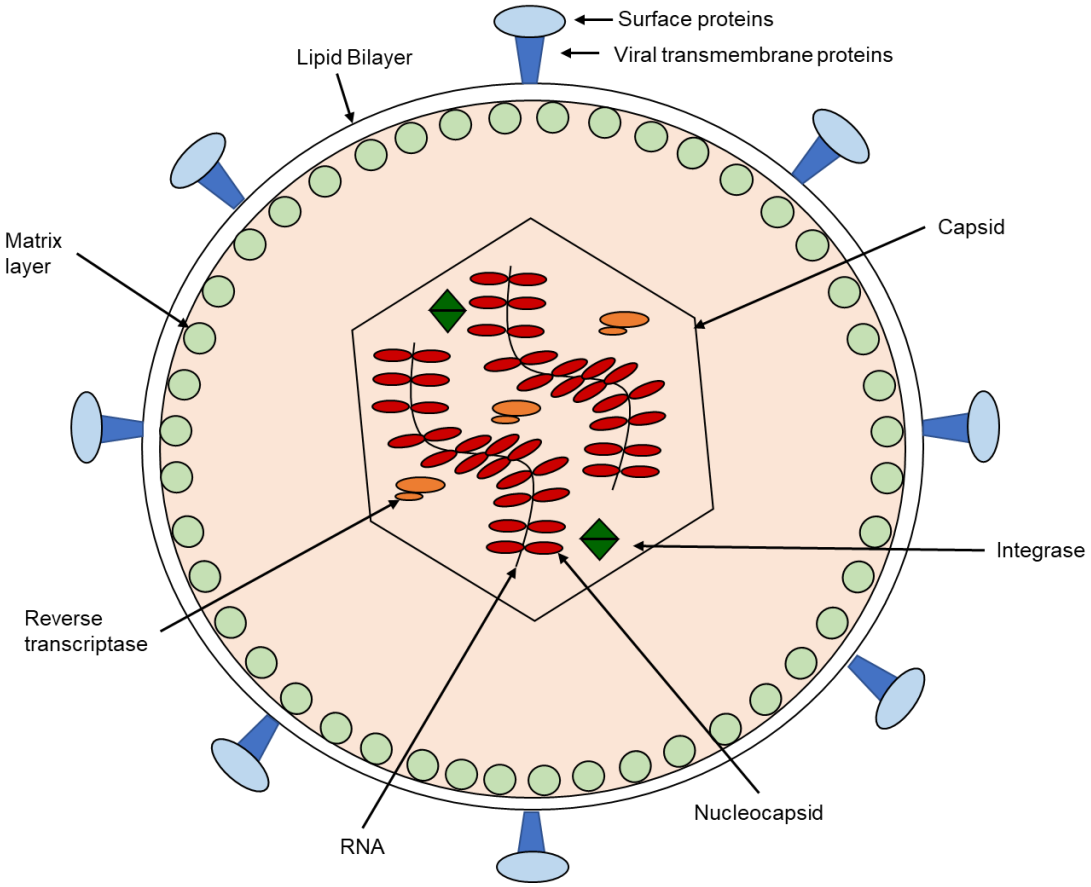


**Figure 1 - HTLV-1 viral particle structure.** The HTLV-1 viral particle is surrounded by a lipid bilayer that contains surface proteins that interact with host cell membrane receptors GLUT-1, Neuropilin-1, and Heparan Sulfate Proteoglycan. The virus also carries its own reverse transcriptase to convert the viral RNA into DNA and an integrase to integrate the viral DNA into the host cell DNA.

HTLV-1 integrates into the host cell genome at active regions of the chromatin such as transcription start sites or CpG islands (Polakowski & Lemasson, 2010). Infected, untransformed cells will typically contain only once copy of the provirus. After integration, HTLV-1 initiates mRNA transcription through the recruitment of host cell RNA polymerase II in a process called basal transcription. Basal transcription is required to produce the viral protein Tax which plays a role in mediating strong HTLV-1

transcription. Basal transcription involves the recruitment of different host cell factors; AP-1, Sp1, Sp3, SRF, and ELK-1 (Polakowski & Lemasson, 2010).

AP-1 stimulates basal transcription through interactions with the viral cyclic AMP response element binding protein (CREB) responsive element (CRE) located in the U3 region of the 5' LTR. The viral CRE contains a similar sequence to the original CRE except it is flanked by GC rich sequences. Sp1 and Sp3 are regulators of basal transcription, this regulation is achieved though interactions with GC-box and GT-box elements that represent high- and low-affinity binding sites respectively. SRF and ELK-1 are cofactors that act as regulators for basal transcription through interactions with the viral serum response elements (vSRE).

Once an adequate level of Tax is produced, Tax-mediated transcription can occur. The first step in the process is the formation of a transcription complex comprised of Tax, CREB, and p300/CBP (Figure 2). This transcription complex binds to the viral CRE in the 5' LTR and recruits the general transcription machinery such as RNA Polymerase II. The RNA Polymerase II then progresses in the sense direction until it reaches the poly A tail in the R region of the 3' LTR that terminates transcription. This transcription process produces unspliced (*Gag/Pol*), singly-spliced (*Env*), and doubly-spliced (*Tax/Rex*) mRNAs. Most of the HTLV-1 is transcribed in the 5' to 3' direction with the exception of the mRNA for the HTLV-1 basic leucine zipper factor (*Hbz)*. *Hbz* is a singly-spliced mRNA that is transcribed in the antisense direction. The transcription of *Hbz* is initiated by Sp1 that binds to Sp1 binding sites located in the 3' LTR (Yoshida et al., 2008). After production of the HBZ protein it will form a complex with the host factor JunD to amplify *Hbz* transcription (Gazon et al., 2012). HBZ also acts as a regulator of

Tax transcription through forming a complex with p300/CBP (Clerc et al., 2008) and CREB (Lemasson et al., 2007).

HTLV-1 mRNAs are also regulated by features located within the LTR. One feature is *cis*-acting response sequences (CRS) which plays a role in mRNA export regulation (Nakano & Watanabe, 2012). The HTLV-1 genome contains two CRS sites located in the R/U5 regions of the 5' and 3' LTR (Figure 2). The individual CRS perform individual functions despite being the same kind of structural feature. The 5' CRS has been shown to play a role in the retention of unspliced mRNA such as *Gag/Pol*. The 3' CRS has been shown to retain all HTLV-1 transcripts. The CRS act as nuclear retention signals that induce destabilization and insufficient nuclear export of mRNA. The exact proteins that interact with the CRS, whether they are viral or cellular, have yet to be defined.

The export of certain mRNA is mediated by an RNA secondary structure known as the Rex Responsive Element (RxRE) (Nakano & Watanabe, 2012). The RxRE is transcribed from the R region of the 3' LTR. The RxRE assists in mediating nuclear-cytoplasmic export of HTLV-1 mRNA by forming complexes with export proteins and the HTLV-1 protein Rex. All HTLV-1 mRNA are transcribed with the RxRE except for *Hbz* due to the splice in the mRNA occurring before the R region of the 3' LTR (Figure 2).

The viral mRNA are exported to the cytoplasm for translation into viral proteins necessary for viral formation and RNA packaging. The viral mRNA and proteins migrate to the host cells plasma membrane and form the viral particles through budding. As the

viral particle forms, it is retained in the viral biofilm (Gross & Thoma-Kress, 2016). The infected cell then progresses to infect other cells once cell-to-cell contact occurs.

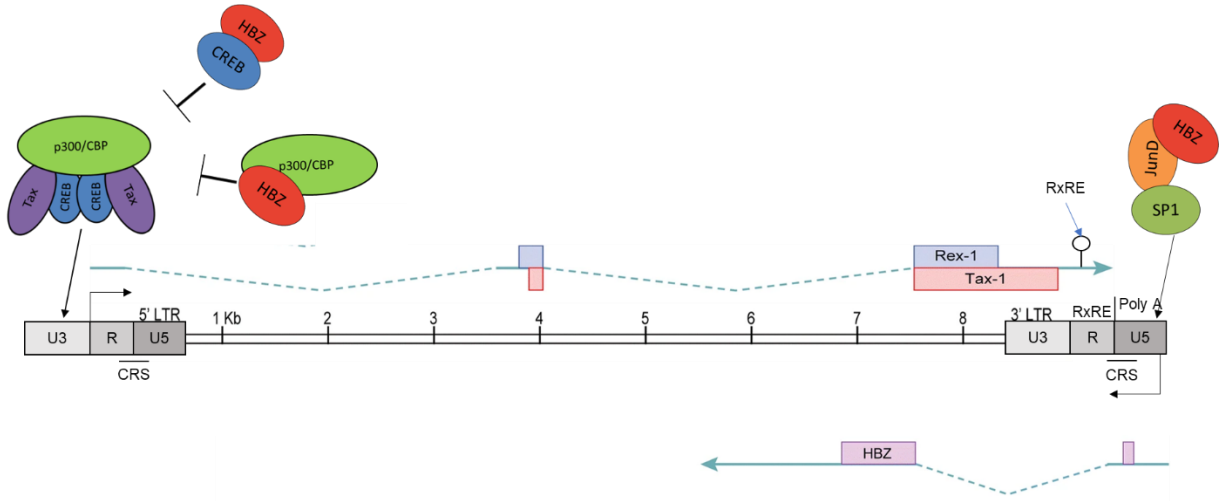


**Figure 2 - Model of HTLV-1 viral transcription**. The HTLV genome is bookended by two LTRs that each contain important features for transcription. The R/U5 of the 5' LTR and 3' LTR contain the CRS for nuclear retention of mRNA. The R region of the 3' LTR contains the RxRE which mediates the nuclear export of mRNA through interactions with the HTLV-1 protein Rex. The transcription process begins with basal transcription through the recruitment of host cell factors to produce the viral protein Tax. A transcription complex is then formed by Tax interacting with p300/CBP and CREB. The complex binds to the vCRE in the U3 region of the 5' LTR and the RNA Polymerase II progresses across the genome for transcription of viral mRNA in the sense direction. All HTLV-1 mRNA are transcribed this way with the exception of *Hbz*. HBZ is transcribed from and regulated by SP1 binding in the U5 region of the 3' LTR and transcribed in the antisense direction. Once the HBZ protein is produced, it forms a complex with JunD to amplify its own transcription and with p300/CBP and CREB to regulate Tax-mediated transcription. Modified from (Martinez et al., 2019).

### *1.6 HTLV-1 mRNA and Associated Proteins*

HTLV-1 mRNA can be divided into structural or functional categories and whether the mRNA is singly-, doubly, or unspliced (Figure 3). The unspliced and singly-spliced mRNA encode for multiple proteins essential for HTLV-1 viral particle structure.

While other mRNA and their associated proteins perform multiple functions pertaining to viral persistence and disease progression.

*Gag/Pol* mRNA is responsible for the encoding of several structural proteins including the matrix, capsid, and nucleocapsid, which make up the core of the viral particle (Nam et al., 1993). *Pol* translation is responsible for the production of the HTLV-1 reverse transcriptase and integrase which is responsible for RNA reverse transcription and DNA integration into the host cell genome respectively (Mitchell et al., 2006). *Pol* also encodes the viral protease that cleaves Gag precursor into matrix, capsid and nucleocapsid. Another structural protein, *Env*, is responsible for the production of envelope proteins and encodes for two subunits; the surface unit (gp46) and the transmembrane unit (gp21) (Tezuka et al., 2018).

*Tax/Rex* mRNA is responsible for encoding the proteins Tax and Rex. Tax is primarily retained in the nucleus, but has been shown to occasionally shuttle between the nucleus and cytoplasm (Moles et al., 2019). As previously stated, Tax is responsible for mediating strong HTLV-1 transcription by forming a transcription complex with p300/CBP and CREB and binding to vCRE in the U3 region of the 5' LTR (Polakowski & Lemasson, 2010). Tax also alters the regulation of host cell gene expression particularly transcription factor genes and genes involved in apoptosis (Azran et al., 2004). Tax also deactivates the tumor suppressor protein p53 by sequestering p300/CBP for transcription (Van Orden et al., 1999) and stimulating NF-κB activation (Pise-Masison et al., 2000b). Tax is also important for promoting T-cell proliferation and transformation. Once cell transformation occurs, Tax is no longer required for cell maintenance and it

has been shown that many ATLL cells have lower, and even stop expression of the Tax protein. Rex is a protein that plays an important role in mediating the nuclear export of structural mRNA by binding to the RxRE (Princler et al., 2003).

The *Hbz* mRNA and the HBZ protein have a variety of roles in affecting the host cell and HTLV-1 function (Zhao, 2016). Two functions of HBZ, as previously mentioned, include amplification of its own mRNA transcription through forming a complex with JunD and regulating Tax-mediated transcription through forming a complex with p300/CBP and CREB (Clerc et al., 2008; Lemasson et al., 2007). HBZ also promotes T-cell proliferation by inhibiting certain function of C/EBP$\alpha$ and ATF3 by the formation of heterodimers (Zhao, 2016). C/EBP$\alpha$ has been shown to act as an important negative regulator for cell proliferation in cancer cells. The HBZ inhibition of ATF3 attenuates certain negative effects of ATF3, such as the activation of p53, allowing ATLL cells to escape ATF-3/p53 mediated apoptosis and proliferate. HBZ also upregulates and activates cell factors to achieve T-cell proliferation. The HBZ protein upregulates expression of miroRNA-17 and mircoRNA-21 that, with HBZ, downregulates OBFC2A leading to cell proliferation and genomic instability. HBZ protein expression activates that expression of neurotrophin BDNF promoting BDNF/TrkB signaling loop, promoting cell survival (Polakowski et al., 2014). The interaction between JunD and HBZ also activates the human telomerase reverse transcriptase (Kuhlmann et al., 2007). In addition, HBZ upregulates Wnt5a (Ma et al., 2013), E2F1, and surviving (Mitobe et al., 2015) to promote proliferation. HBZ also suppresses apoptosis in the cell by down regulating the NF-$\kappa$B pathway and deactivating p53 (Wright et al., 2016). Cell-mediated immunity is downregulated by the suppression of IFN-$\gamma$. Lastly, HBZ induces T-cell

lymphoma and inflammation by binding Foxp2 and NFAT which leads to increased IFN-γ (Satou et al., 2011).

The other mRNA; *p12*, *p13*, and *p30*, have been shown to play important roles in establishing viral infection and allowing it to persist (Bai & Nicot, 2012). The protein p12 has been determined to play an important role in the establishing of viral infection due to studies involving knockouts of *p12* failed to infect other cells. The role of p13 has been shown to be in viral pathogenesis. Lastly, p30 has been shown to have a variety of roles in the cell pertaining to cell survival, viral latency, and evidence suggests that this protein plays a role in mediating the export of *Tax/Rex* mRNA.

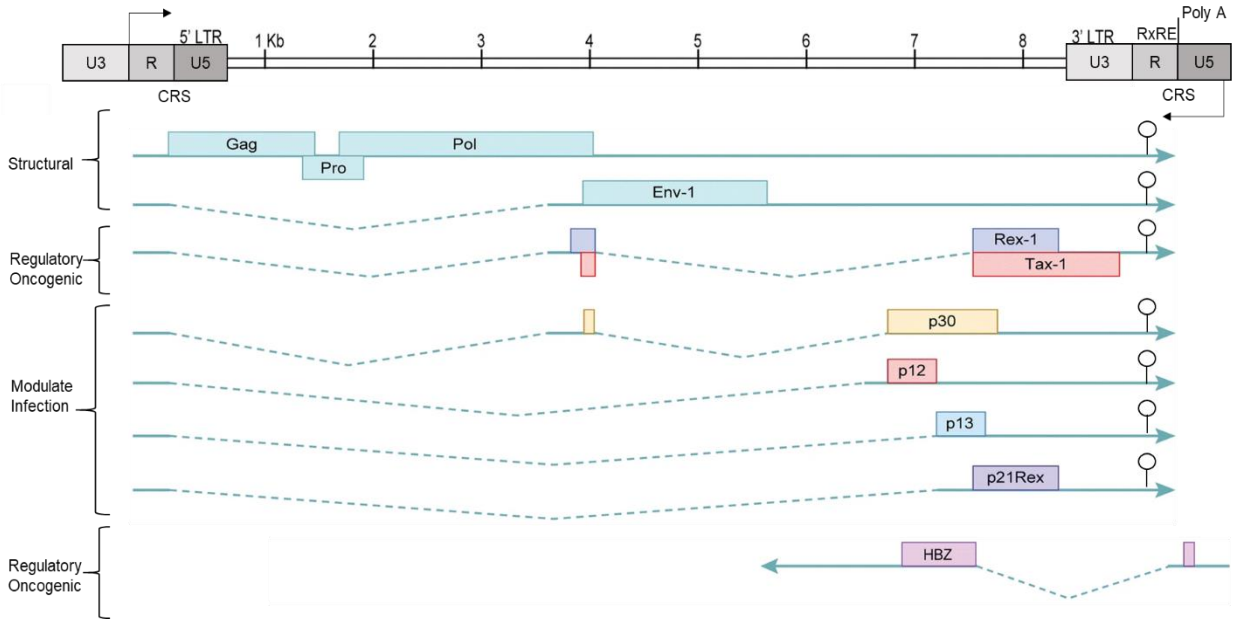


**Figure 3 - HTLV-1 mRNAs**. Different viral mRNA are transcribed from the viral genome and play important roles in either the structure or function of the HTLV-1 virus. Majority of the viral mRNA are transcribed in the sense direction. The important structural mRNA are the *Gag/Pol* and *Env* mRNA that are crucial for producing proteins responsible for the structure of the viral particle. The mRNA *Tax/Rex* is responsible for producing the proteins Tax and Rex that are important for viral replication and mRNA export respectively. Accessory mRNA such as; *p30*, *p12*, and *p13*, encode for proteins that are important for viral maintenance. The only antisense transcribed mRNA, *Hbz*, encodes for HBZ. Both *Hbz* mRNA and HBZ protein play important roles in regulating HTLV-1 transcription and cell functions including preventing apoptosis. The HBZ and Tax proteins both promote cell proliferation and are believed to be HTLV-1 oncoproteins. Image modified from (Martinez et al., 2019).

## 2. HTLV-1 Associated Diseases

### *2.1 Adult T-cell Leukemia/Lymphoma (ATLL)*

Adult T-cell Leukemia/Lymphoma (ATLL) is one of the most common HTLV-1 associated diseases. Discovered in 1977, ATLL is an aggressive and malignant $CD4^+$ T-cell leukemia/lymphoma (Matsuoka, 2005). The onset of ATLL typically occurs thirty to forty years after HTLV-1 infection. ATLL cell transformation is induced by HTLV-1 oncoproteins Tax and HBZ by promoting proliferation and avoiding apoptosis. Tax is believed to cause genomic stability by regulating different signaling pathways such as the IκB/NF-κB signaling pathway and DNA damage repair pathways and innate immune signaling pathways. The innate immune signaling pathways include; RIG-I/MDA5-dependent and TLR-independent pathways, TRIF-dependent TLR pathways and cGAS-STING pathway (Zhang et al., 2017). HBZ has been shown to promote cell proliferation by forming heterodimers with the host factor C/EBPα and activating ATF3. HBZ will also impair apoptosis and autophagy by preventing the binding of FoxO3a. As previously stated, Tax and HBZ both deactivate p53 by forming complexes with p300/CBP and HBO1 (Van Orden et al., 1999; Wright et al., 2016) and inducing NF-κB activation (Pise-Masison et al., 2000a). With the current available treatments, the prognosis for ATLL cases is poor.

The clinical features of ATLL include; malaise, fever, jaundice, drowsiness, and weight loss (Uchiyama et al., 1977). ATLL also presents distinctive features such as a high rate of osteolytic tumors and hypercalcemia leading to increased bone resorption. Around 40% of ATLL cases develop skin nodules, plagues, ulcers, or lesions on the face, limbs, or trunk. These skin manifestations are caused by the infiltration of the

transformed cells into the skin (Kawahira, 1999). ATLL cells also infiltrate other organs such as the liver, lungs, spleen, and the gastrointestinal tract. Abnormalities such as diffuse lymph node filtration and hepatosplenomegaly can occur in ATLL patients. As with other forms of cancer, ATLL is associated with immunosuppression and opportunistic infections.

The diagnosis of ATLL involves the detection of HTLV-1 antibodies in the serum of patients through the use of enzyme-linked immunosorbent assay (ELISA) or particle agglutination (PA) and confirmed with the use of western blots, immunofluorescence (IFA), or polymerase chain reaction (PCR) and by observing clinical manifestations. The presence of multilobulated nuclei cells, “flower cells,” can also be used for diagnosis. The current available treatment for ATLL includes combination of chemotherapy, stem cell transplants, and antiviral therapies such as Zidovudine (AZT) that inhibits viral reverse transcriptase and reactivates p53 and IFN-$\alpha$ that degrades Tax (Nasr et al., 2011; Tsukasaki et al., 2009). Treatments implemented are dependent on the patients age and condition. The prognosis can vary between 6 months or a decade.

*2.2 HTLV-1 Associated Myelopathy/Tropical Spastic Paraparesis*

Another common HTLV-1 associated disease is HAM/TSP, a neurodegenerative disease caused by the infiltration of HTLV-1 infected T-cells into the central nervous system crossing the blood brain barrier (Cavrois et al., 2000). The onset of HAM/TSP typically occurs within a decade after HTLV-1 infection as opposed to the decades of delay in ATLL onset. The full pathogenesis of HAM/TSP is not fully understood; however, it is believed that a strong immune response to the infiltrated cells initiates a

cytokine release that leads to axon demyelination that is typically localized in the thoracic spinal cord and the formation of brain lesions (Puccioni-Sohler et al., 2012).

Clinical manifestations of HAM/TSP present symptoms that are similar to other neurological disorders such multiple sclerosis, HIV vacuolar myelopathy, familial spastic paraparesis, and others. The overlapping symptoms make it important to properly diagnose the disease. Symptoms such as the loss of lower limb function can easily be misdiagnosed as multiple sclerosis. HAM/TSP patients also experience loss of internal functions in the bowels and bladders. Immunosuppressive drugs are standard treatment for HAM/TSP therapy in order to prevent T-cell proliferation. To reduce the signs and symptoms of HAM/TSP patients have been given corticosteroids, plasmapheresis, cyclophosphamide, and IFN-$\alpha$ (Oh & Jacobson, 2008). Antiretroviral therapies have been used in attempts to lower the proviral load in HAM/TSP patients. Though some treatments have shown some effect, relapse does occur in HTLV-1 individuals. There is evidence to suggest a host-to-virus interaction that determines predisposition to HAM/TSP development.

### *2.3 HTLV-1 Associated Disease Differentiation*

The transition from HTLV-1 to one of the associated diseases is not fully understood in terms of what causes a patient to develop one disease instead of another. Early on it was believed that disease onset was determined by what region of the body HTLV-1 infiltrated. Therefore, if the virus were to cross the blood brain barrier it would lead to HAM/TSP or if the virus infiltrated the skin it would cause infective dermatitis. However, recent studies suggest the differentiation is more complex. A focus

has been placed on the interaction between the human leukocyte antigen (HLA) and HTLV-1 subtypes leading to the onset of HAM/TSP (Saito, 2019).

HLA is responsible for coding the major histocompatibility complex (MHC) in the human immune system (Choo, 2007). Notably the HLA gene has been shown to vary amongst different ethnicities. In certain endemic areas, Japan and Jamaica, HTLV-1 patients are three times more likely to develop HAM/TSP than those in Africa or in Europe. Furthermore, Jamaican patients have a higher risk of developing HAM/TSP than Japanese patients.

Studies have revealed that certain HLA genes are associated with a lower risk of HAM/TSP while others have been shown to increase the risk of HAM/TSP. Populations with HLA-A*02 and HLA-Cw*08 possess a lower risk of HAM/TSP development. Those who possess HLA-B*5401 and HLA-DRB1*0101 are at a higher risk of developing HAM/TSP. HLA-A*02 may play a role in regulating HTLV-1 as a protective effect has been reported among different populations. However, possession of the genes alone does not determine HAM/TSP transformation. In southwest Japan the presence of HLA-DRB1*0101 and absence of HLA-A*02 makes patients susceptible to HAM/TSP; while Iranian patients with HLA-A*02 or HLA-Cw*08 have no lower risk of HAM/TSP (Saito, 2019).

Though HTLV-1 has a very stable genotype, there are subtypes that have evolved and that are distributed geographically around the globe. The major subtypes are; Cosmopolitan subtype A, Central African subtype B, Australo-Melanesia subtype C, and Central African/Pygmies subtype D. Other but rare strains of HTLV-1 are Central African subtypes E, F, and G. Notably, the Cosmopolitan subtype has genetically

divided into five different subgroups; Transcontinental (A), Japanese (B), West African (C), North African (D), and Black Peruvian (E) (Gessain & Cassar, 2012). One of the variations in the HTLV-1 subtype genomes is in the Tax-subgroups. Studies suggest the interaction with the Tax-subgroup and certain host factors could determine disease onset.

HTLV-1 patients in Jamaica have shown to have a higher rate of infection with the Cosmopolitan Transcontinental strain of HTLV-1 while Japanese individuals have a higher rate of Cosmopolitan Japanese strain. The variations in the Tax-subgroups within the strains and their interactions with the individual's HLA is theorized to play a role in the onset of HAM/TSP. Based on studies in HLA susceptibility residents in Jamaica have a higher presentation of HLA-B*5401 and HLA-DRB*0101. Though Japanese strain is more common in Japan the Transcontinental strain is present in Japan. The lower rate of HAM/TSP in the Japanese population can be contributed to the presence of the HLA-A*02 in the population that protects against Tax-subgroup b in the Cosmopolitan Japanese strain (Saito, 2019).

Though the studies suggest a correlation between an individual's HLA, the strand of HTLV-1 exposed to and the onset of HAM/TSP, there has been no other correlation established with other diseases such as ATLL. It is possible that these interactions exist with other host genetic factors. It is also possible that the onset of ATLL is dependent on the production proteins and the presence of HLA genes that lower the risk of HAM/TSP onset or just the production of oncoproteins alone.

Beyond the interaction between HLA and HTLV-1 there are two other hypotheses to explain the differences in viral transformation among different populations. One

explanation is that the variations in the target epitopes recognized by MHC-1 restricted cytotoxic T-cells. The other possible explanation is that patients encounter environmental factors, such as co-infections with other pathogens, that make them susceptible to HAM/TSP. These hypotheses need to be researched thoroughly in order to determine their validity (Saito, 2019).

## 3. Nuclear Export of mRNA

### *3.1 Methods of mRNA Export in Cells*

HTLV-1 mRNAs are dependent on their nuclear export for the production of viral proteins. Research has been focused into understanding the export process for HTLV-1 mRNAs with the goal of finding effective forms of treatment and potentially a cure for HTLV-1 and the diseases associated with it. The export of RNA in healthy cells is achieved through one of four different pathways: transcriptional export I and II (TREX and TREX2), exportin-5 (XPO-5), and exportin-1 (XPO-1) (Williams et al., 2018). Under normal conditions and for normal cell functions mRNAs are exported through TREX and TREX2 and XPO-5 only exports miRNA.

TREX is a multi-protein complex that exports mRNA associated with general cell function, hematopoiesis, pluripotency, and heat shock. TREX is recruited by the splicing machinery, assembly is ATP-dependent, and is composed of subunits ALY, UAP56, and THO. Different forms of TREX adaptors can bind directly to mRNA to promote nuclear export; including ALY and THOC5. The binding of ALY and THOC5 is important for the recruitment of NXF1, an RNA export protein.

TREX2 is a complex that links transcription to export that exports mRNA; however, it is unknown whether the mRNA exported by TREX2 is the same as TREX. TREX2 is based on the Germinal-centre Associated Nuclear Protein (GANP) that PCID2, DSS1, ENY2, and CETN2/3 bind to. As with TREX, TREX2 interacts with NXF1 but the mechanism of interaction is not as well defined. NFX1 binds to the GANP N-terminal domain that contains a cluster of nucleoporin-like FG repeats.

XPO-1 exports mRNA required for proliferation, survival, metastasis, and invasion. Along with mRNA, XPO-1 exports snRNA and rRNA. XPO-1 also exports the tumor suppressor protein p53. Unlike TREX and TREX2, XPO-1 does not contain NXF1 or ALY which means that XPO-1 plays the role of NXF-1 for the nuclear cargo it exports.

In cancer cells, the production of XPO-1 has been shown to be increased in order to increase the export of p53 to reduce the chance of the protein recognizing the damaged DNA and inducing apoptosis. XPO-1 has also been shown to export mRNA of viruses such as Human Immunodeficiency Virus (HIV) (Boons et al., 2015) and HTLV-1 (Bai et al., 2012) . In terms of HTLV-1 mRNA export, XPO-1 forms an export complex with Rex and RxRE.

Previous research suggests that not all HTLV-1 mRNA utilizes the XPO-1/RxRE/Rex export complex. *Gag/Pol* and *Env* mRNAs have been shown to consistently utilize the export complex (Hidaka et al., 1988). Previous research conflicts on whether *Tax/Rex* mRNA utilizes XPO-1 for nuclear export (Bai et al., 2012; Li et al., 2012). It would be expected that *Tax/Rex* mRNA utilizes the transport complex since it contains the RxRE RNA structure. *Hbz* mRNA has been shown not to utilize the export complex which would be expected since the mRNA does not contain the RxRE (Cavallari et al., 2016).

### *3.2 XPO-1 Export Process*

The export of XPO-1 is initiated by the binding of leucine-rich nuclear export signals from the cargo proteins to the residues on the C-terminal of the export protein (Figure 4). The export of mRNA through XPO-1 is mediated by binding adaptor protein such as eukaryotic translation initiation factor 4E (eIF4E) (Piserà et al., 2018). The

protein eIF4E also plays a role in initiating the translation of mRNA into their associated proteins. The export of XPO-1 from the nucleus is active and energy is acquired through XPO-1 interactions with Ran GTPase. RanGTP binds to the N-terminal of XPO-1 to mediate the export of XPO-1 through the nuclear pore complex (NPC). The hydrolysis of RanGTP to RanGDP supplies the energy for the export and leads to the destabilization of the export complex and leads to the release of the nuclear cargo (Azizian & Li, 2020). The reentry of XPO-1 into the cell is passive and occurs using the NPC.

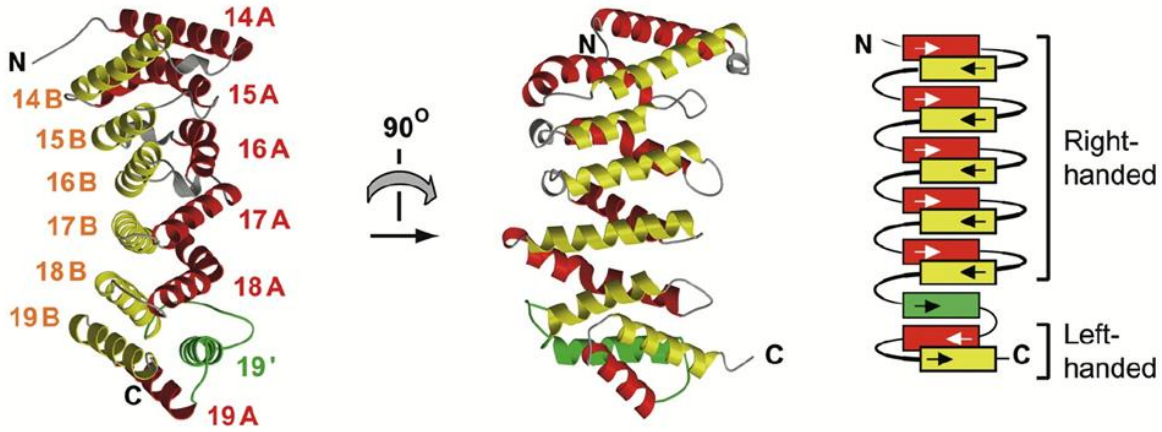


**Figure 4 - Ribbon structure of exportin-1 (XPO-1).** Nuclear cargo such as p53 and mRNA bind to the C-terminal and RanGTP binds to the N-terminal. The protein passes through a nuclear pore complex (NPC). Though the export of the protein is active the reentrance of the protein to the nucleus is passive. Image of XPO-1 structure from (Petosa et al., 2004)

*3.3 XPO-1 Inhibition*

Methods of XPO-1 inhibition involved a variety of compounds ranging from natural compounds to synthetic ones. XPO-1 inhibition research originated with the use of leptomycin B. Leptomycin B is a secondary metabolite from *Streptomyces* bacteria in

which it plays the role of an antifungal agent (Matthew & Ghildyal, 2017). When introduced to cancer cells, leptomycin B was shown to be effective in inducing apoptosis in cell cultures. This occurs by leptomycin B forming a permanent bond to the cysteine residues of XPO-1 in order to reduce the export of p53; however, leptomycin B is cytotoxic to cells. Leptomycin B never made it through clinical trials due to the side effects cause in patients including: dehydration, vomiting, and anorexia (Mahipal & Malafa, 2016). These side effects suggest that leptomycin B affects other important cell functions. These side effects have shifted the focus of XPO-1 inhibition to synthetic compounds such as Selective Inhibitors of Nuclear Export (SINE).

SINE compounds were originally developed to treat cancer through XPO-1 inhibition without the toxicity of leptomycin B. One such group of compounds, produced by Karyopharm Therapeutics, are designed to create a reversible bond with XPO-1 without the cytotoxic effect of leptomycin B. Karyopharm's compounds include KPT-185, KPT-301, and KPT-330 and were originally patented for treatment of renal cancer but have been tested in other forms of cancer. These compounds function by binding to the cysteine residue 528 of XPO-1, likely while the protein is in the cytoplasm (Figure 5). The compounds have been shown to be effective in retaining mRNA and p53 in the nucleus (Boons et al., 2015). The compound KPT-185 has also been shown to be an effective treatment for HIV (Boons et al., 2015).

When HIV positive cells were treated with KPT-185 a reduction in the export of mRNA and retention of p53 resulted in lower infection rates and induction of apoptosis (Boons et al., 2015). Due to the effect Karyopharm's SINE compound KPT-185 had on treating HIV and cancer cells in previous studies, we believed they could be effective in

treating HTLV-1. We hypothesized that treatment with KPT-185 will reduce the export of viral mRNA in HTLV-1 cells and therefore will reduce viral particle production. In theory, the decrease in viral particle production will lead to a reduction in HTLV-1 infectivity. KPT-185 treatment may also have an effect on the production of the viral oncoproteins Tax and HBZ and prevent the onset of ATLL. We also hypothesize that KPT-185 treatment will retain p53 in the nucleus. It is possible that some apoptosis could occur as it did in HIV; however, the retention of p53 would have to overcome the inhibitory effect that Tax and HBZ have on p53. Our primary goal is to reduce the transmission of the virus and potentially prevent the onset of HTLV-1 associated diseases. Establishing an effective form of treatment for HTLV-1 and HTLV-1 associated diseases is important due to the gaps in the knowledge of the diseases and how to treat them.

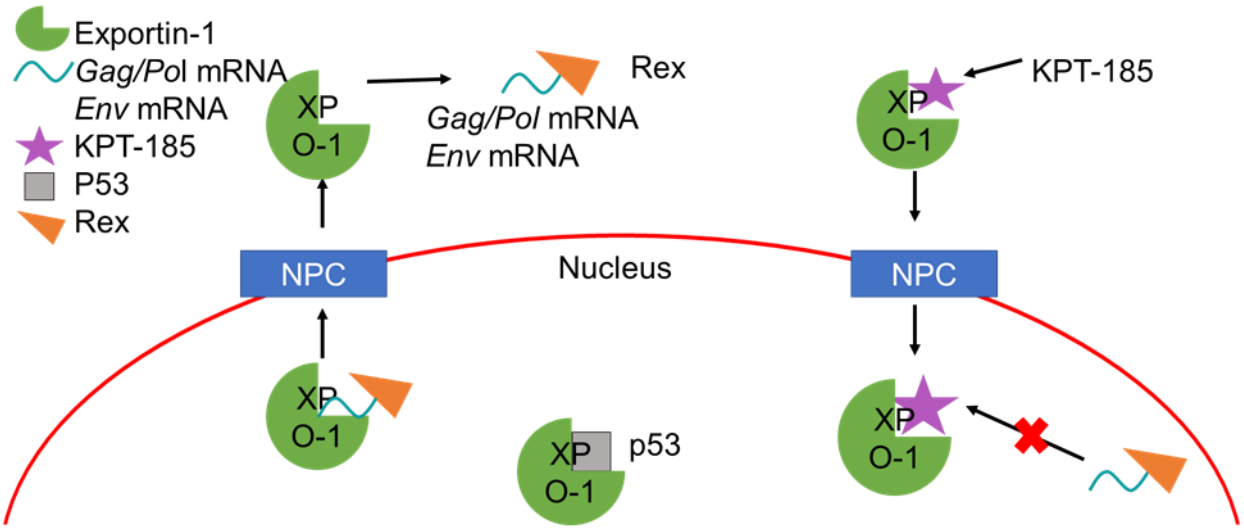


**Figure 5 - Pathway of exportin-1 (XPO-1) and inhibition**. Nuclear cargo such as the tumor suppressor protein p53 or mRNA/Rex complexes bind to the residues in XPO-1 for XPO-1 mediated export. The XPO-1/RxRE/Rex export is mediated by a nuclear export signal, RanGTP, which binds to the N-terminal of XPO-1. The complex exits the nucleus through nuclear pore complexes (NPC). The RanGTP is converted into RanGDP through hydrolysis and the nuclear cargo is released from XPO-1. The time XPO-1 is in the cytoplasm is likely when the protein is the most susceptible for inhibition. Inhibitors such as KPT-185 bind to the cysteine residue 528 of XPO-1. When the export protein reenters the cell passively through the NPC, the nuclear cargo is unable to bind to the protein. This should increase the concentration of the viral mRNA and p53 in the nucleus.

## 4. Materials and Methods

### *4.1 Cell Lines*

The HTLV-1 cell lines utilized in the experiments included *in vitro* and patient derived cell lines. *In vitro* cell lines used included MT-2 and SLB-1. Both cell lines are derived from coculturing normal human leukocytes with HTLV-1 positive cells (Koeffler et al., 1984; Miyoshi et al., 1981). MT-2 and SLB-1 cells carry multiple copies of the HTLV-1 provirus ranging from two to eight copies, but typically average around six copies and express all of the mRNA. The patient derived cell lines included ATL-2 and TL-Om1. ATL-2 cells express two copies of the provirus and express all viral mRNA. TL-Om1 cells only express *Hbz* mRNA due to heavy methylation on the 5' LTR that inhibit transcription (Kuramitsu et al., 2015).

The HTLV-1 negative cell lines utilized in the experiments include Jurkat and CHOK-1. Jurkat cells are patient derived, $CD4^+$ acute T-cell leukemia lymphocytes. CHOK-1 cells are Chinese hamster ovary cells. Furthermore, the HTLV-1 negative cells have been modified to express the luciferase gene from fireflies. In the Jurkat cells the gene is under the control of the HTLV-1 viral CRE sequence (Jurkat 3S3-Luc); while in the CHOK-1 cells the gene is under control of the entire HTLV-1 promoter (CHOK-1-Luc).

### *4.2 SINE Compounds*

Karyopharm Therapeutics gifted our lab with the SINE compounds KPT-185 and KPT-301 (Figure 6). The compounds are resuspended in DMSO at a stock concentration of 10mM. The two compounds are similar in structure but not in function.

KPT-185 is an active compound that, in theory, should bind to inhibit XPO-1 export. KPT-301 is an inactive compound; more specifically it is a trans-isomer of KPT-185. In theory KPT-301 will have no effect on XPO-1 export and produce similar results to the control, DMSO.

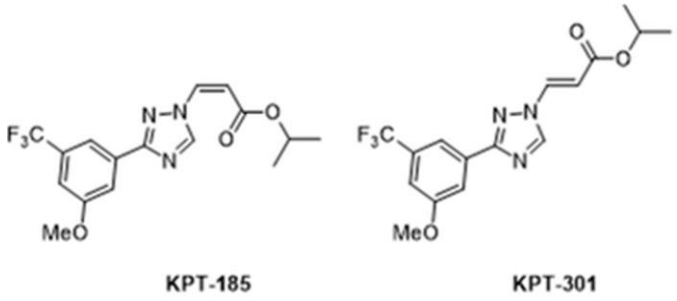


**Figure 6 - Structure of KPT-185 and KPT-301.** KPT-185 is the active drug that is designed to bind to the cysteine residue 528 of XPO-1 to prevent the binding of nuclear cargo such as mRNA or tumor suppressor protein, p53. In cancer and other viral studies KPT-185 has been shown to be effective in retaining p53 and viral mRNA in HIV. KPT-185 was also shown to induce apoptosis in cancer and HIV infected cells. KPT-301 has the same formula as KPT-185 but, with a different structure, specifically KPT-301 is a trans-isomer of KPT-185. The change in structure makes the compound inactive and unable to inhibit XPO-1. Compound structures from (Crochiere et al., 2016).

*4.3 Cell Viability Assay*

Cells were equalized and plated in 96 well plated at $4x10^5$ cells in 100μL. The cells were allowed to incubate in the plate overnight at 37ºC. The cells were then treated with 1nm, 10nm, 100nm, 1μm KPT-185, KPT-301, or DMSO alone as a control, then incubated overnight at 37ºC. Following the second incubation AlamarBlue (Bio-Rad) was added to the cells and incubated for 4hs at 37 ºC. AlamarBlue contains a fluorescent dye called reazurin. When taken into the cells, reazurin is metabolized into

resofurin and undergoes a colorimetric change producing a pink, highly fluorescent color (Figure 7). The intensity of the pink color in the well directly correlates to the amount of living cells in the well. After the 4hr incubation the plates were read in a SpectraMax Gemini.

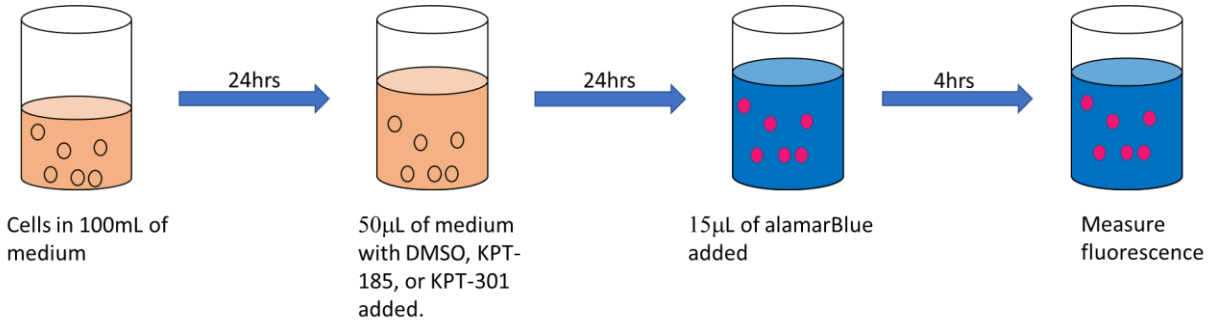


**Figure 7 - Cell viability assay process.** After cell equalization and plating in a 96 well plate, the cells were incubated overnight to acclimate to the plate. The cells were then treated with DMSO alone, KPT-185, or KPT-301 for a second overnight incubation. On the third day, AlamarBlue was added to the cells for 4hrs. The AlamarBlue contains reazurin that is metabolized by the cells, producing a vibrant red color. The amount of pigment produced is directly correlated to the number of healthy cells produced.

*4.4 RNA Extraction, DNA Synthesis, and Quantitative Real-Time PCR*

Cells were equalized, treated with 1µm KPT-185, KPT 301, or DMSO alone as a control. The cells were harvested, and RNA extracted using the Paris Kit (Thermo Fisher) following the manufacturer's instructions (Figure 8). Cells were incubated in Cell Fraction Buffer (<0.9% Nonidet P40 Ethylphenolpoly) for 10min on ice and centrifuged at 600g for 5min at 4ºC. The supernatant was then harvested as the cytoplasmic fraction. The nuclear pellet was lysed in Cell Disruption Buffer (1-2.4% Triton X-100) and kept on ice. The fractions were mixed with equal parts ethanol and 2x Lysis/Binding Buffer (55-88% Guanidine isothiocyanate, 1-2.4% Triton X-100, 1.4% 2-

mercaptoethanol). The cellular fractions were filtered with the supplied filters and tubes. The samples are then washed with Wash Solution 1 (18-24% Thiocyanic acid, compound with guanidine (1:1) and 0.1-1% 2-mercaptoethanol) and Wash Solution 2/3 (44% ethanol). The mRNA was collected in a fresh collected tube with the elution solution and quantified with a NanoDrop.

The cDNA were synthesized using the iScript cDNA Synthesis Kit (Bio-Rad) with random primers. Quantitative Real-Time PCR (qRT-PCR) were performed as previously described (Lemasson et al., 2007). Primer sequences utilized in the qRT-PCR were as follows: UBE2D2 (housekeeping gene); 5'-TGCCTGAGATTGCTCGGATCTACA-3' and 5'-ACTTCTGAGTCCATTCCCGAGCTA-3', Gag/Pol; 5′-GAGGGAGGAGCAAAGGTACTG-3′ and 5′-AGCCCCCAGTTCATGCAGACC-3′, and Tax/Rex; 5′-ACCAACACCATGG^CCCA-3′ and 5′-GAGTCGAGGGATAAGGAAC-3 (Li & Green, 2007).

qRT-PCR was performed using a CF96 Touch Real-Time PCR Detection System (Bio-Rad). Standard curves were generated for each PCR for all primer pairs on the plate using a serial dilution of an appropriate sample. Each sample was amplified in triplicate on each plate in 15μL reactions, each containing 7.5μL 2x Sybr Green (Bio-Rad) and 1μL 1:20 diluted cDNA. Results were analyzed using CFX Maestro Software (Bio-Rad).

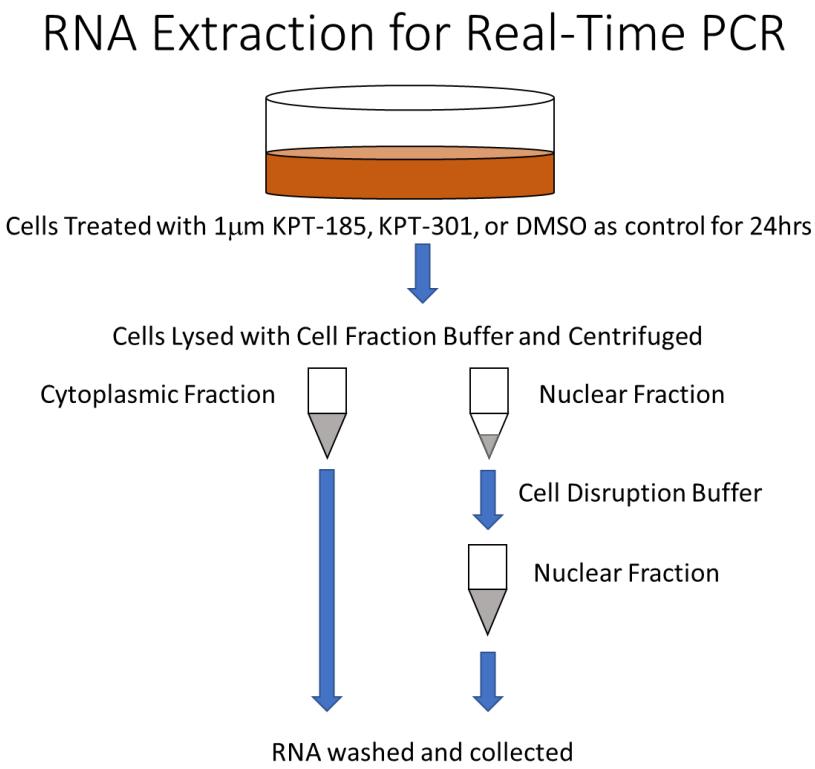


**Figure 8 - Paris Kit RNA extraction schematic.** After cells are equalized and treated with KPT-185, KPT-301, or DMSO alone for 24hrs, the cells were lysed with the Cell Fraction Buffer and centrifuged to separate the cells into the cellular fractions. The nuclear pellet was then suspended in Cell Disruption Buffer. The RNA is then washed three times with wash buffers. Lastly, the RNA were eluted with an elution solution.

*4.5 Protein Fractionation*

Cells were treated with 1μm KPT-185, KPT 301, or DMSO alone for 24hrs. Whole cells extracts were prepared by washing the cells in 1x PBS and lysed using RIPA+ buffer (50mM Tris HCl [pH 8.0], 1% Triton x-100, 100mM NaCl, 1mM $MgCl_2$, 1mM PMSF, 1mM benzamidine, 2μg/mL leupeptin, and 5μg/ml leupeptin). For cell fractionation, the cells were first suspended in 200μL hypotonic buffer (10% glycerol,

1.5mM $MgCl_2$, 10mM NaCl, 0.34M Sucrose, 20 mM Hepes pH 7.9, 1mM DTT, 2μg/mL leupeptin, 5μg/mL aprotinin, 0.5μg/mL pepstatin A, 1mM PMSF, and 0.1% Triton X-100) and chilled on ice for 10 minutes. The samples were then centrifuged at 3.7k RPM and 4ºC for 4 minutes. The supernatants, cytoplasmic fractions, were collected and stored in new tubes. Nuclear pellets were washed in the hypotonic buffer without the Triton X-100 and centrifuged as before. The supernatants were removed, and the nuclear pellets were suspended in 100μL of RIPA+ buffer, chilled on ice for 20 minutes, and centrifuged at 13,000 RPM and 4ºC for 15 minutes. The supernatants, nuclear fractions, were collected and stored in new tubes. Protein concentrations were determined by Bradford assays (Bio-Rad).

*4.6 Western Blot Assays*

Whole cell extracts and the cellular fractions were resolved on 10% SDS-PAGE polyacrylamide gels and transferred to nitrocellulose in a submerged apparatus at 300mA. After the transfer, the membranes were first stained with Ponceau S, to ensure equal protein amounts were loaded between samples, then probed with antibodies. Blots were developed using Pierce ECL Plus (Thermo Fisher) and scanned using a Typhoon 9410 (GE Healthcare). Image analysis was performed using Image Quant TL 1D software (version 8.1; GE Healthcare).

*4.7 Antibodies*

The following antibodies were used: anti-Actin clone C4 (MAB1501) purchased from Millipore-Sigma; anti-Mek1/2 L38C12 (#4694) purchased from cell Signaling

Technology; anti-Sp1 (21962-1-AP) purchased from Proteintech; anti-p53 (do-1) and anti-Gag-p19 (E-11) purchased from Santa Cruz Biotechnology; Tax monoclonal antibody (hybridoma 168B17-46-92) and Env antibody obtained from the National Institute of Health AIDS Research and Reference Reagent Program.

*4.8 Infection Assay*

HTLV-1 positive cells were plated and treated with 1μM KPT-185, KPT-301, or DMSO alone as a control. The cells were incubated overnight at 37ºC and the next day the cells were irradiated at 77Gy to prevent further growth of the cells. After irradiation the cells were washed in serum free IMDM and cocultured with Jurkat-3S3-Luc cells overnight at 37ºC (Figure 9). Following the co-culture incubation, the cells were centrifuged at 1300 RPM for 3 min and washed in 1x PBS. The cells were then washed in 1x passive cell lysis buffer and chilled on ice for 15 minutes. 20μL of sample was then mixed with 50μL of LAS Reagent (Promega) and luminescence was detected with a Glomax 20/20 Luminometer.

Another kind of luciferase assay was performed utilizing CHOK-1-Luc cells. These assays were performed similarly to the ones stated above with modifications (Figure 9). The CHOK-1 cells were plated the same day as the HTLV-1 positive cells to allow them to adhere to plate. The HTLV-1 positive cells were treated as described above, but after the overnight treatment, the cells were not irradiated before being co-cultured with the CHOK-1-Luc cells. The cells were co-cultured together for 90 min at 37 ºC. The media containing the infected cells was removed and the CHOK-1-Luc cells were washed three times in 1x PBS and incubated in fresh DMEM overnight at 37 ºC.

1x Passive Lysis Buffer was added to the cells and they were rocked at room temperature for 15 min. Sample preparation and reading was performed the same as stated above. A variation of this assay was also performed with different concentration of the SINE compounds: 1nM, 10nM, 50nM, 100nM, and 1μM.

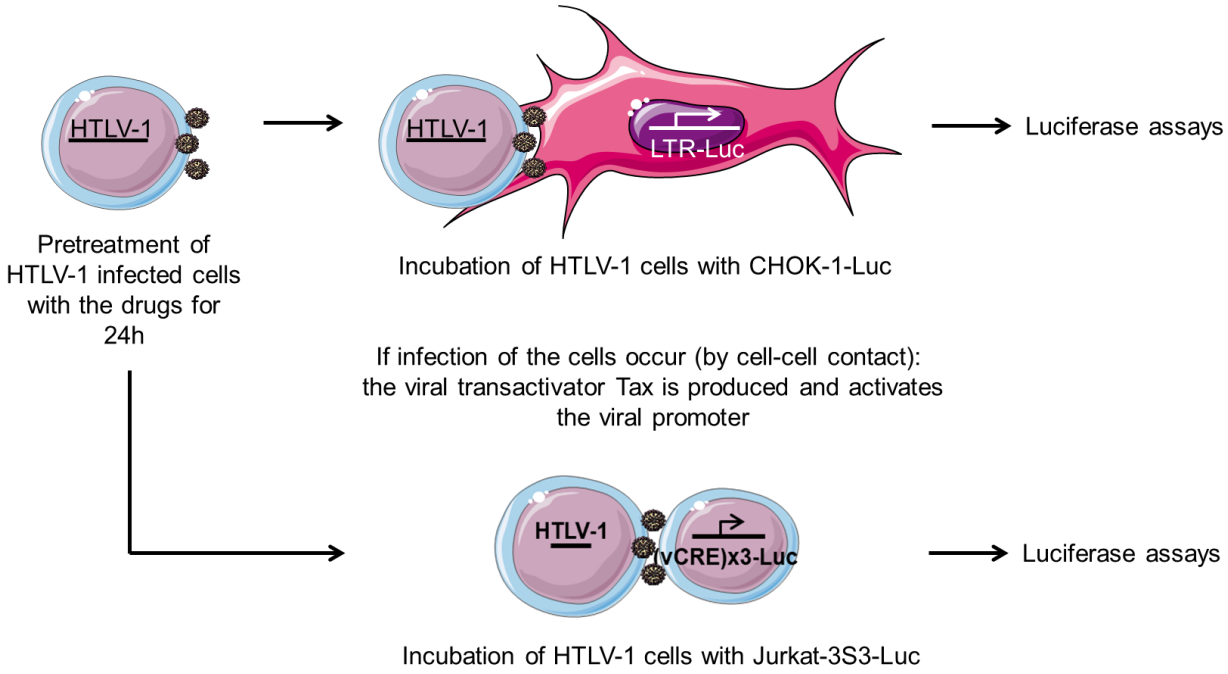


**Figure 9 - Infection assay model.** Effector cells (HTLV-1 infected cells) are treated with 1μM of KPT-185, KPT-301, and DMSO alone as control for 24hrs. The effector cells were then cocultured with the target cells (CHOK-1-Luc and Jurkat-3S3-Luc) for an another 24hrs. As HTLV-1 infects the target cells, the viral protein Tax activates the viral promoter directing transcription of the luciferase gene. Therefore, the greater the amount of target cells infected the greater the amount of luciferase produced.

*4.9 Enzyme-Linked Immunosorbent Assay (ELISA)*

The ELISA's were performed using the HTLV p19 Antigen ELISA (ZeptoMetrix). HTLV p19 is a protein that forms the matrix of the viral particle (Figure 1). This was

implemented using ATL-2 cells that were treated for 24 hours with 1μM KPT-185, KPT-301, or DMSO alone as a control. The cells were spun down at 1300 rpm for 3 minutes and the supernatants were collected. To deactivate the viral particles the samples were heated at 55ºC for 30 minutes. The samples were prepared at a 1:50 dilution using the lysis buffer (Triton X-100 in PBS and 2-chloracetamide). The standards for the ELISA were prepared using the supplied HTLV-1 antigen standard (heat deactivated viral antigen at a concentration of 16ng/mL, added proteins, Triton X-100, and sodium azide). The plate was washed with 200μL wash buffer six times (PBS, Tween 20, 2-chloroacetamide). The 200μL of the standards and specimens were placed in the well which were incubated at 37ºC for 2hrs. The samples and standards are aspirated, and the wells were washed again six times with wash buffer. 100μL of HTLV Detector Antibody (Antibodies to HTLV-1 and HTLV-2 p19 core proteins, added proteins, Triton X-100, and 2-chloroacetamide) was added and incubated at 37ºC for 1hr. The HTLV Detector Antibody is aspirated and the wells are washed again. 100μL of Peroxidase Reagent (Peroxidase conjugated IgG with added protein, Triton X-100, and 2-chloroacetamide) is added to each well and incubated at 37ºC for 1hr. The Peroxidase Reagent is removed, and the plate is washed again. Then 100μL Substrate Solution (Substrate; Tetramethylbenzide solution in dimethyl sulfoxide, and Substrate Buffer; Contains citrate/acetate buffer, hydrogen peroxide, and 2-chloroacetamide) and incubated uncovered at room temperature for 30min. After the last incubation the stop solution was added. Within 15min the plates were read at 450nm using the ThermoFisher Multiskan FC microplate reader.

*4.10 Statistics*

Microsoft Excel was used to perform unpaired t-test and ANOVA for statistical analysis. Unpaired t-tests were done between the DMSO treated groups to either the KPT-185 or KPT-301 treated groups in order to determine the effects of the compounds. ANOVA was done to determine if a statistical difference occurred across the entire treatment groups between DMSO, KPT-185, or KPT-301. Differences were considered significant at a $p$ value $<0.05$.

## 5. Results

### *5.1 SINE Compounds Did Not Induce Cell Death in Patient Derived Cell Lines*

We first determined the range of concentrations of the SINE compounds KPT-185 and KPT-301 that will not cause cytotoxic effects in different HTLV-1 infected cell lines. The goal is to avoid a concentration that is detrimental to the health of cells while determining the maximum effective concentration that can be used to inhibit HTLV-1 replication. Since KPT-185 could also retain p53 in the nucleus, it may also induce apoptosis which could alter the cells viability.

In the cell viability assay, KPT-185 an KPT-301 had minor impacts on the viability of the ATL-2 cells (cells derived from an ATL patient). The only statistically significant decrease in viability in the ATL-2 cells occurred using 1μM of KPT-185 inducing a reduction of 10 percent (Figure 10A) when compared to DMSO alone. In TL-Om1 cells (cells derived from an ATL patient), the treatment with KPT-185 and KPT-301 did not induce any statistical change across any of the concentrations tested (Figure 10B). In MT-2 cells (cells developed *in vitro*), there were statically significant reductions in cell viability in the KPT-185 treatment concentrations: 10nM, 100nM, and 1μM with the greatest decrease being around 22 percent (Figure 10C). Lastly, in SLB-1 cells (cells developed *in vitro*), the treatment with both KPT-185 lead to significant reductions in viable cells at the concentrations of 100nM and 1μM when compared to the DMSO control (Figure 10D). KPT-301 caused a significant reduction in the SLB-1 cells at the concentrations of 10nm, 100nM, and 1μM when compared to DMSO. The results suggest that the patient derived cell lines are less sensitive to the SINE compounds

than the *in vitro* derived cells. The sensitivity in the *in vitro* derived cells, MT-2 and SLB-1, could be due to the increased Tax produced in the cells inducing apoptosis due to more copies of the cells containing greater copies of the provirus. It is also possible that KPT-185 treatment leads to a reduction in proteins such as Tax and HBZ which deactivate tumor suppressor protein p53. The viability assay results suggest it is possible that the SINE compounds would have a less toxic effect in the host.

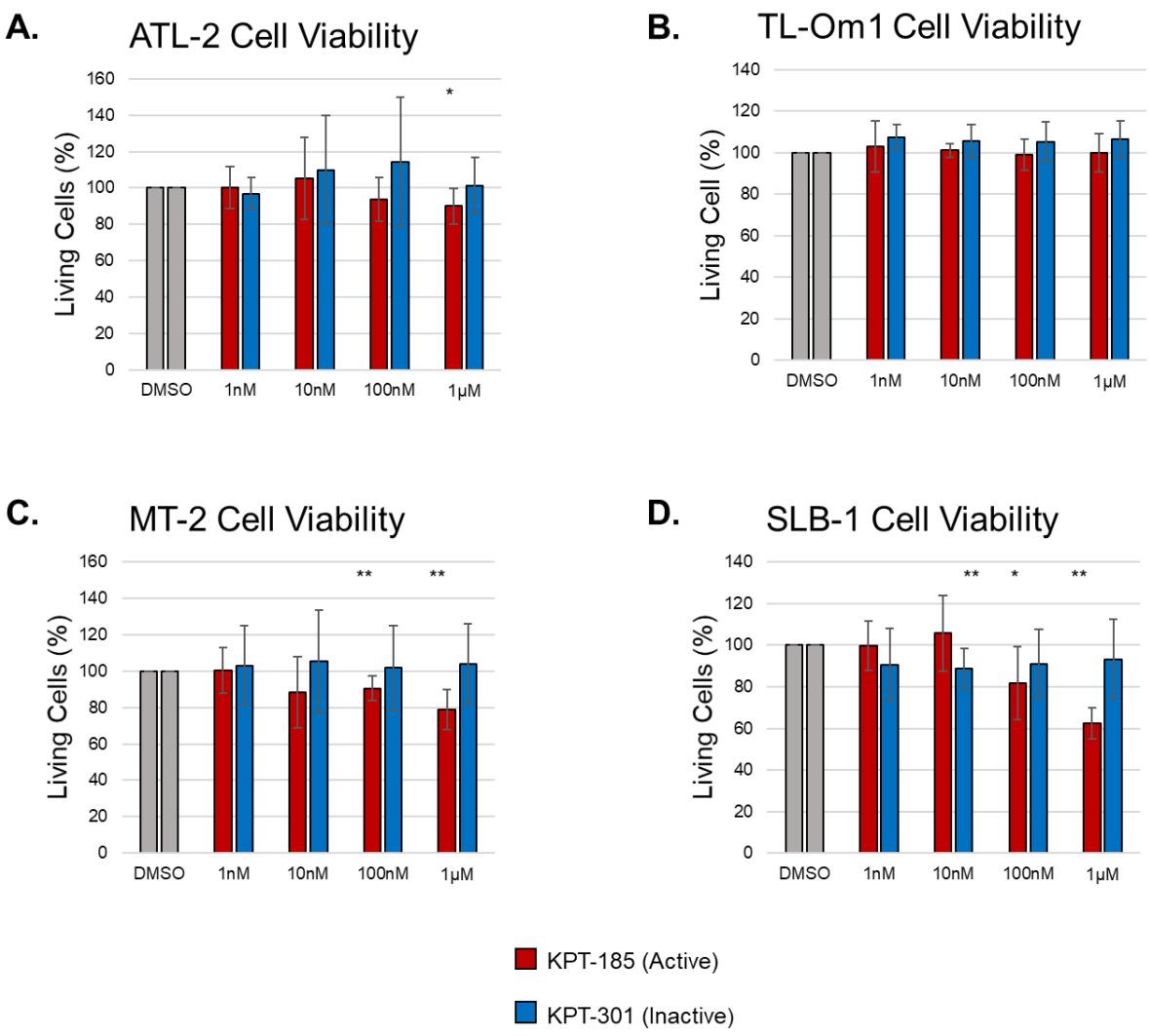


**Figure 10 - Treatment with the SINE compounds did not affect the cell viability of patient derived cells.** Relative viability of the indicated cells was quantified using AlamarBlue and normalized to DMSO treatment (set to 100%). Cells were treated with the indicated concentration of KPT-185, KPT-301, or DMSO alone for 24 hrs. Data for each cell line were averaged from three independent experiments where each treatment was performed in four replicates. **(A)** ATL-2 cells; t-test (*:$p$<0.05). **(B)** TLOM-1 cells; ANOVA ($p$<0.05). **(C)** MT-2 cells; t-test (**:$p$<0.0005), ANOVA ($p$<0.05). **(D)** SLB-1 cells; t-test (*:$p$<0.05, **: $p$<0.0005); ANOVA ($p$<0.0005).

*5.2 KPT-185 Treatment Reduces Gag/Pol mRNA Nuclear Export*

Previous studies have shown that certain HTLV-1 mRNA utilize the XPO-1/RxRE/Rex export complex, particularly singly- and unspliced mRNA. This would suggest that only structural mRNA such as *Gag/Pol* and *Env* primarily utilize this pathway. To determine which HTLV-1 mRNA is sensitive to KPT-185, we analyzed the export of unspliced (*Gag/Pol* mRNA) and doubly-spliced (*Tax/Rex* mRNA) mRNA by fractioning HTLV-1 infected cells treated with the SINE compounds and performing quantitative real-time PCR (qRT-PCR).

In the ATL-2 cells, the cytoplasmic fraction displayed lower levels of *Gag/Pol* mRNA when cells were treated with KPT-185 compared to the cells treated with KPT-301 or DMSO control. This observation suggested that the *Gag/Pol* mRNA does utilize the XPO-1/RxRE/Rex complex for nuclear export (Figure 11A). As was expected, there was no *Gag/Pol* mRNA expression in the TL-Om1 cells due to the heavy methylation on the 5' LTR. The opposite effect was observed when analyzing the effect on the *Tax/Rex* mRNA. KPT-185 treatment lead to an increase in the export of the *Tax/Rex* mRNA in ATL-2 cells (Figure 11B). As expected, TL-Om1 cell lines did not express any *Tax/Rex* mRNA. The inverse effects were observed in the nuclear portion of the ATL-2 cells, with an increase of *Gag/Pol* mRNA and decrease of *Tax/Rex* mRNA when the cells were treated to KPT-185 compared to the DMSO treated cells. The decrease in *Gag/Pol* mRNA and increase in *Tax/Rex* mRNA export suggests that *Tax/Rex* mRNA utilizes a different export pathway than *Gag/Pol* mRNA. The increase in *Tax/Rex* mRNA export was surprising and suggests that a common co-factor (or multiple co-factors) between XPO-1 system and the system used by *Tax/Rex* mRNA is redistributed to favor *Tax/Rex*

mRNA export. Another possible explanation for the increase in *Tax/Rex* mRNA export could be that KPT-185 reduces the export of the *p30* transcript, leading to a reduction of p30 in the cells. Previous studies have shown that p30 plays a role in the nuclear retention of *Tax/Rex* mRNA. Determination of p30 protein will be difficult as there is no antibody currently available that recognize the protein; however, the export of the mRNA can be determined through qRT-PCR. Finally, KPT-301 treatment lead to an increase in the nuclear export of both *Gag/Pol* and *Tax* mRNA. These results will need to be confirmed as they do not correlate with the results obtained with the nucleus fraction, which does not show a change of *Gag/Pol* and *Tax* mRNA with KPT-301 compared to DMSO alone.

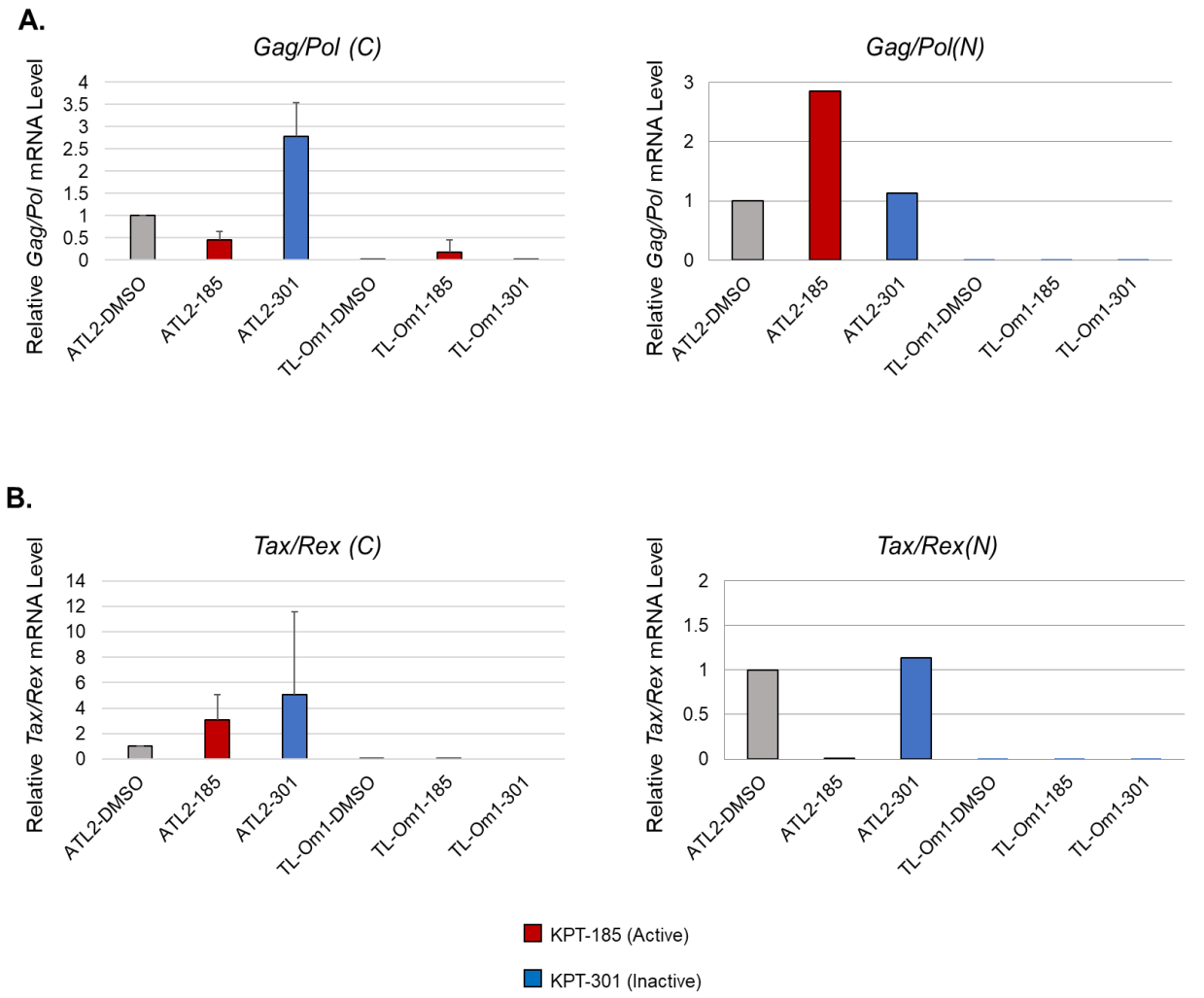


**Figure 11 - KPT-185 treatment reduces *Gag/Pol* mRNA nuclear export. (A)** Relative cytoplasm (C) and nucleus (N) *Gag/Pol* mRNA levels were analyzed by qRT-PCR. Data are normalized to ATL-2 DMSO value (set to 1). ATL-2 and TLOM-1 cells were treated with 1μM of KPT-185, KPT-301, or DMSO as a control for 24hrs prior to fractionation. Cytoplasmic data shown are representative of two independent experiments performed in triplicate and nuclear fractions are representative of one experiment performed in triplicate. ANOVA ($p$<0.05). **(B)** Relative cytoplasm (C) and nucleus (N) *Tax/Rex* mRNA levels were analyzed by qRT-PCR. Data are normalized to ATL-2 DMSO value (set to 1). ATL-2 and TLOM-1 cells were treated with 1μM of KPT-185, KPT-301, or DMSO as a control for 24hrs prior to fractionation. Cytoplasmic data shown are representative of two independent experiments performed in triplicate and nuclear fractions are representative of one experiment performed in triplicate.

*5.3 KPT-185 Treatment Reduces the Amount of Viral Proteins*

The retention of the *Gag/Pol* mRNA is an indicator that expression of the associated proteins could be altered due to the lack of available mRNA for translation. To determine whether KPT-185 affects viral proteins levels, ATL-2 and TL-Om1 cells were treated with the SINE compounds and the level of the Gag precursor protein p55 was analyzed by Western-blot. In ATL-2 cells, KPT-185 treatment lead to a reduction of Gag precursor when compared to the control protein actin (Figure 12). As expected, TL-Om1 cells did not express any Gag protein due to the heavy methylation of the 5' LTR preventing transcription of the sense mRNAs. The reduction of the precursor proteins will affect Gag proteins such as the matrix, the nucleocapsid, and the capsid that are produced when the virion is released from the cells. Without the structural proteins there would be a reduction in the amount of infectious viral particles.

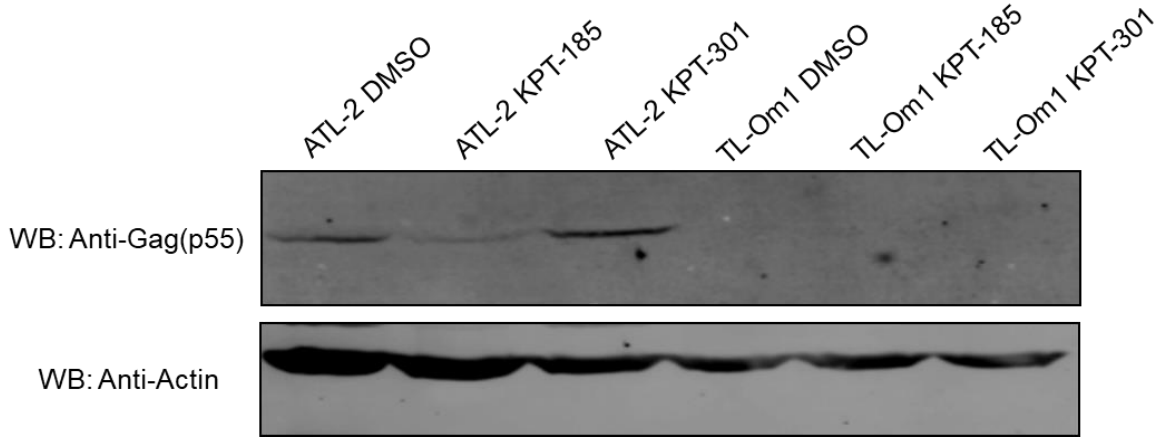


**Figure 12 - KPT-185 treatment reduces the amount of the structural Gag protein.** ATL-2 and TL-Om1 cells were treated with 1μM KPT-185, KPT-301, or DMSO alone as control for 24hrs. 50 μg of whole cell extracts were analyzed by Western-blot. The membrane was probed with an antibody against Gag, stripped, and reprobed with an antibody against actin.

We also analyzed the level of the viral protein Env in MT-2 cells treated with KPT-185. Env is the protein that forms the viral envelope of HTLV-1 and a protein that interacts with cell surface receptors for viral retention and initiating viral entry into a new cell. As the concentration of KPT-185 was increased, the level of the Env protein decreased, establishing a correlation between the amount of KPT-185 and the reduction of protein production (Figure 13). The Env protein is important for the formation of the envelope glycoproteins. Without the envelope proteins the structural integrity of the viral particle will be compromised and the ability for the ability for the viral particle to interact with cell surface receptors; GLUT-1, Neuropilin-1, and Heparan Sulfate Proteoglycan.

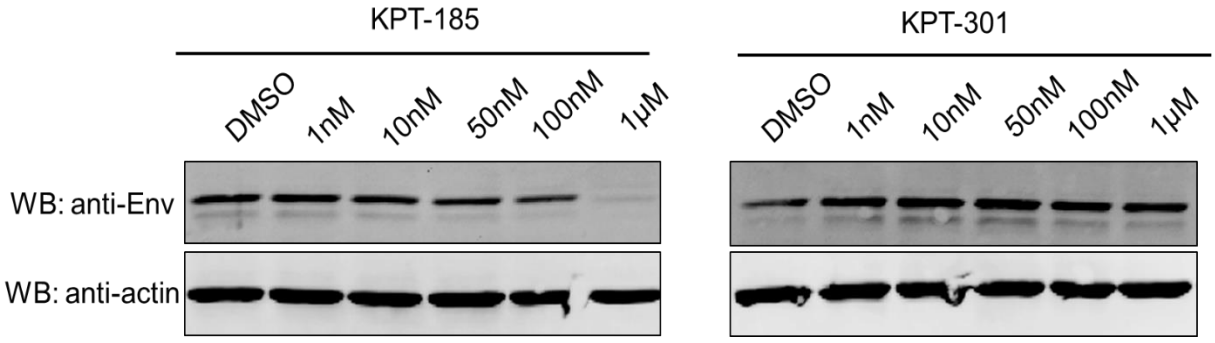


**Figure 13 - KPT-185 treatment reduces the amount of the envelope protein.** MT-2 cells were treated with 1nm, 10nm, 50nm, 100nm, and 1μM KPT-185, KPT-301, or DMSO alone as a control for 24hrs. 50μg of whole cell extracts were analyzed by Western-blot. The membranes were probed with an antibody against Env, stripped, and reprobed with an antibody against actin. Image prepared by Lemasson, I.

*5.4 KPT-185 Treatment Reduces Tax Protein*

Our preliminary results indicated that the export of *Tax/Rex* mRNA might be increased by treatment with KPT-185; therefore, it was not expected to affect the overall level of Tax protein. The Tax protein is typically located in the nucleus; however, nuclear

export occasionally occurs. Interestingly, KPT-185 treatment reduced the level of the Tax protein in both the cytoplasm and the nucleus of SLB-1 cells (Figure 14). As control of cellular fractionation, Sp1 was used as a marker for the nuclear fraction and MEK1/2 was used as a marker for the cytoplasmic fraction (Rushing et al., 2019). The reduction of the level of the Tax protein in both the nucleus and cytoplasm suggests that KPT-185 may have secondary effects on the translation of the *Tax/Rex* mRNA. Interestingly, the translation initiation factor eIF4E has been reported to be associated with XPO-1. Therefore, it is possible that KPT-185 also inhibits its nuclear export. This could be tested by probing fractionized cell western blots with an antibody for eIF4E.

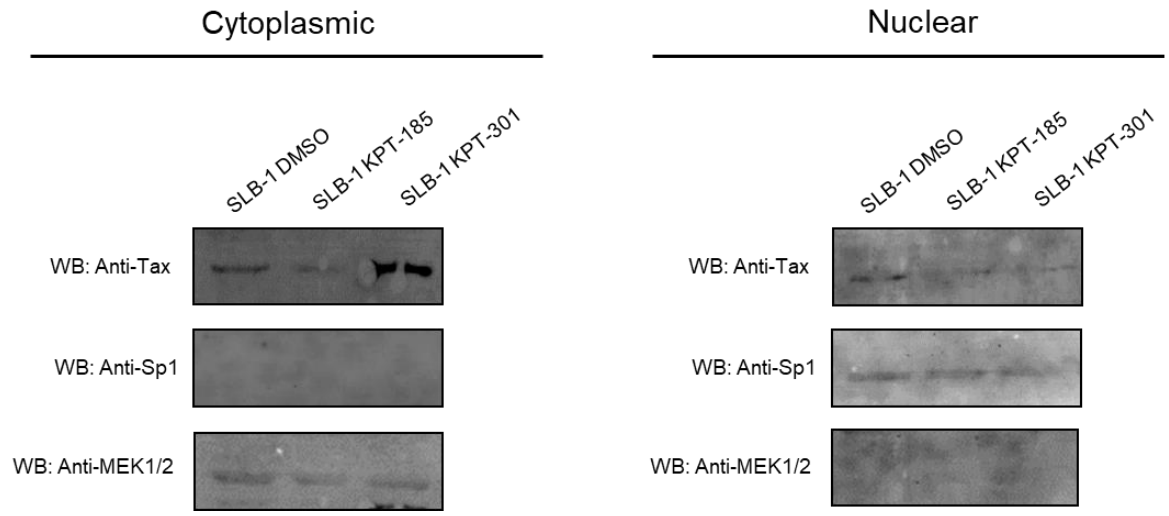


**Figure 14 - KPT-185 treatment reduces Tax protein level in the cytoplasm and nucleus.** Cells were treated with KPT-185, KPT-301, or DMSO alone for 24hrs. Cells were harvested and separated into cytoplasmic and nuclear fractions. 20μg of samples was analyzed by Western-blot. Membranes were probed with antibodies against Tax, SP-1, and MEK1/2. Sp1 and MEK1/2 served as nuclear and cytoplasmic markers respectively. Same membranes were stripped and reprobed.

*5.5 Treatment with KPT-185 Reduces Viral Particles Production*

Since we observed a reduction of viral proteins Gag and Env produced when the HTLV-1 infected cells are treated with KPT-185, we wondered whether a reduction in viral particles would be observed in the same treated cells. Though HTLV-1 infects cells through cell-to-cell contact, there are viral particles released from the infected cells that free float in medium of cultured cells. The amount of particles can be determined through the use of the HTLV p19 (matrix protein) Antigen ELISA kit. In this assay, supernatants of HTLV-1 infected cells were collected and virions were lysed before performing the ELISA.

Interestingly, KPT-185 treated cells displayed a lower amount of p19 than the KPT-301 and the DMSO alone treated cells. This observation suggests that treatment with KPT-185 reduced the amount of HTLV-1 viral particles (Figure 15). If the amount of free viral particles decreases, then it is likely that the amount of surface bound particles is reduced as well. Treatment with KPT-301, on the other hand, lead to no reduction in HTLV-1 p19, suggesting that this compound has no effect on viral particles production.

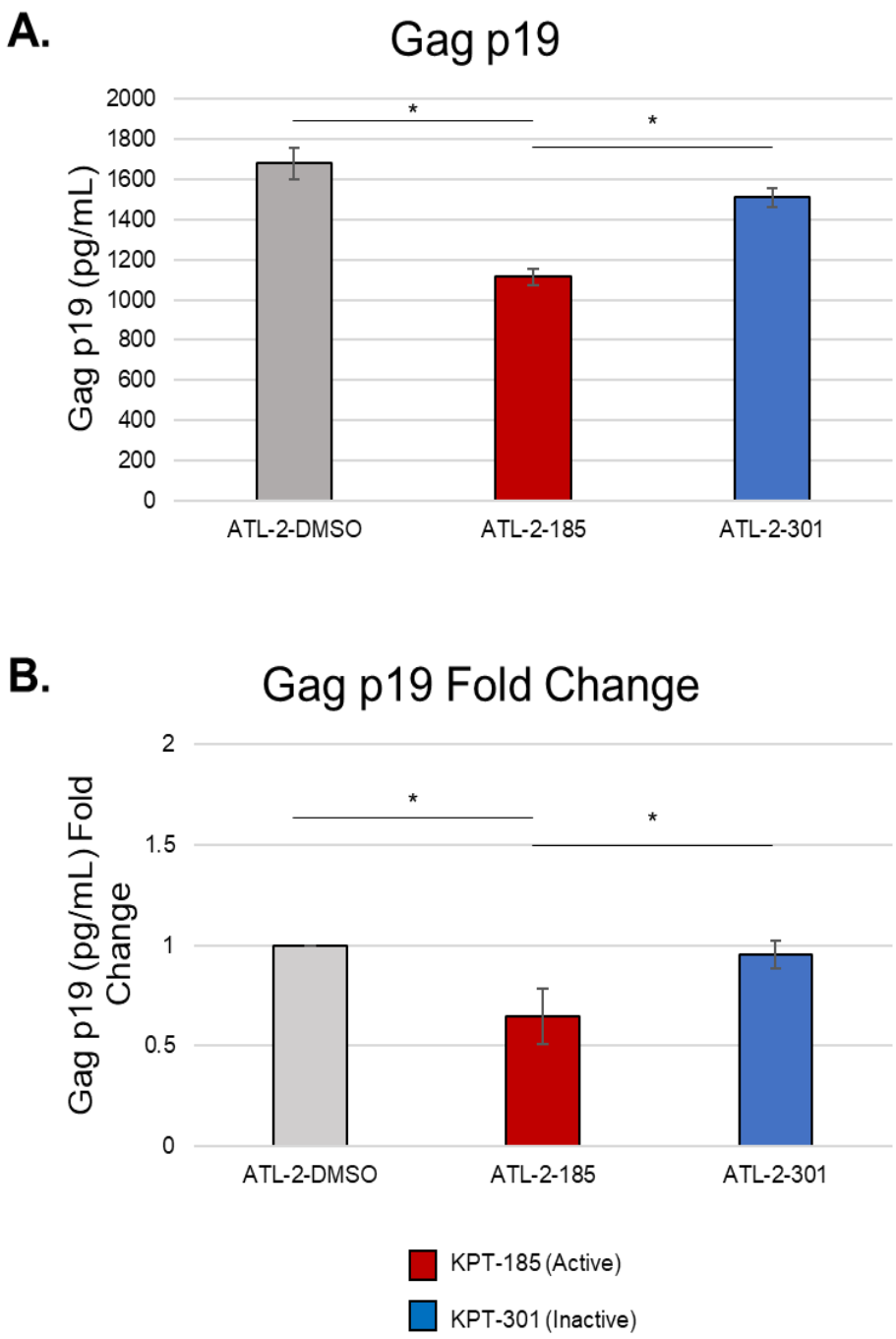


**Figure 15 - KPT-185 treatment reduced the amount of HTLV-1 viral particles produced.** After 24 hours of treatment the supernatant of ATL-2 cells was collected, heated at 55ºC to deactivate the viral particles and used in the HTLV p19 Antigen ELISA. **(A)** ELISA results from one individual experiment where samples were analyzed in duplicate. t-test (*:$p$<0.05) ANOVA ($p$<0.05). **(B)** Data show the average from two independent experiments performed in duplicate and were normalized to DMSO control (set to 1). t-test (*:$p$<0.05) ANOVA ($p$<0.05).

*5.6 KPT-185 Reduces HTLV-1 Infectivity*

Our results showing the retention of the *Gag/Pol* mRNA and the subsequent reduction of Gag protein, as well as the reduction of Env protein level and viral particles being produced, suggest that KPT-185 will affect HTLV-1 infectivity. In addition, we also observed that KPT-185 lead to a decrease in Tax protein level in the nucleus and cytoplasm. The decrease of Tax in the nucleus will result in HTLV-1 transcription inhibition (less HTLV-1 transcripts produced). The reduction of Tax in the cytoplasm will affect the rate of which the virus can spread as Tax is indispensable for the formation of the viral synapse (Gross et al., 2016). To examine the effect of KPT-185 on HTLV-1 infectivity, we performed infection assays with modified uninfected cells and treated HTLV-1 cells.

Infection assays with MT-2 as effector cells and CHOK-1-Luc as the target cells were performed (see Figure 9). In these assays, cells were treated with 1nm, 10nm, 50nm, 100nm, and 1μM of KPT-185, KPT-301, or DMSO alone to determine an ideal concentration for reducing the infectivity of HTLV-1. The results from this experiment revealed that, as the concentration of KPT-185 increases, up to 1μM, the infectivity of HTLV-1 decreases (Figure 16). As expected, KPT-301 did not lead to a decrease in HTLV-1 infectivity. Since 1μM of KPT-185 had the greatest reduction in infectivity we used this concentration in other cell lines.

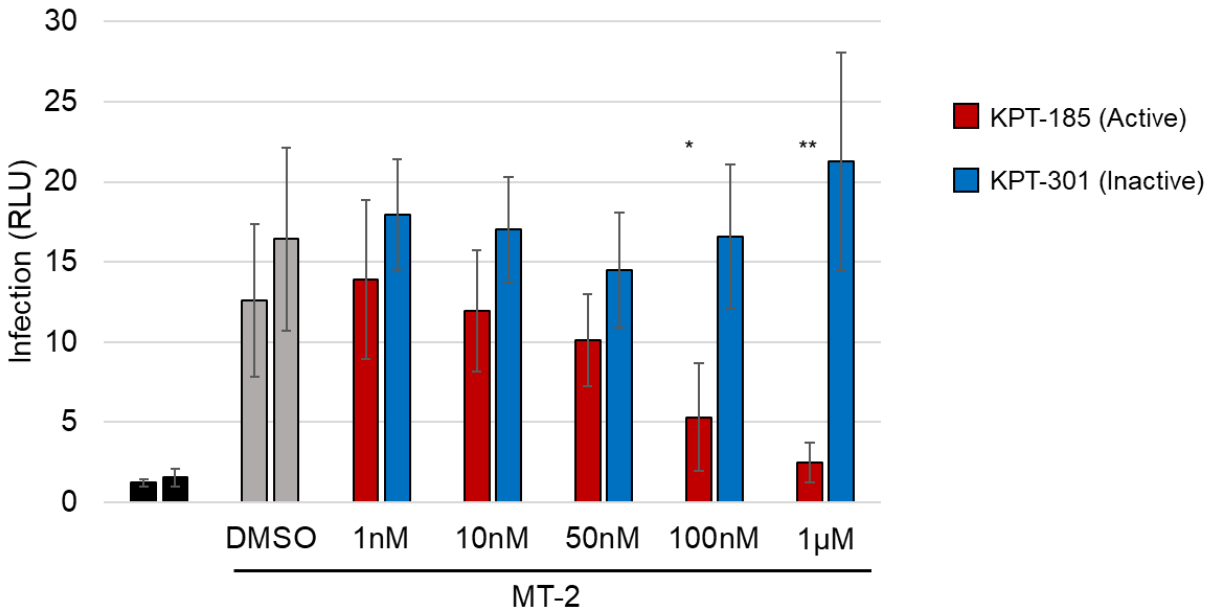


**Figure 16 – Increasing KPT-185 concentration decreases HTLV-1 infection.** MT-2 cells were treated with 1nM, 10nM, 50nM, 100nm, and 1μM of KPT-185 and KPT-301 or DMSO alone. Luciferase assays were performed 24hrs later. The graph shows the data from 2 independent experiments, each performed in triplicate. Infection is shown as a measure of relative luminescence unit (RLU). t-test (*:$p$<0.05, **:$p$<0.0005) ANOVA ($p$<0.0005). Image prepared by Lemasson, I.

KPT-185 was used at 1μM in infection assays with ATL-2, MT-2, and SLB-1 cells along with 1μM of KPT-301 or DMSO alone. In these experiments, the HTLV-1 cells were incubated with Jurkat-3S3-Luc, another $CD4^+$ T-cell line. Across all three cell lines, 1μM of KPT-185 lead to a significant reduction of HTLV-1 infectivity (Figure 17). As expected, KPT-301 had no impact on the infectivity of HTLV-1 in ATL-2 and MT-2 cell lines. Surprisingly, KPT-301 treatment lead to a decrease in HTLV-1 infectivity in the SLB-1 cells. The exact reason for this reduction is unknown.

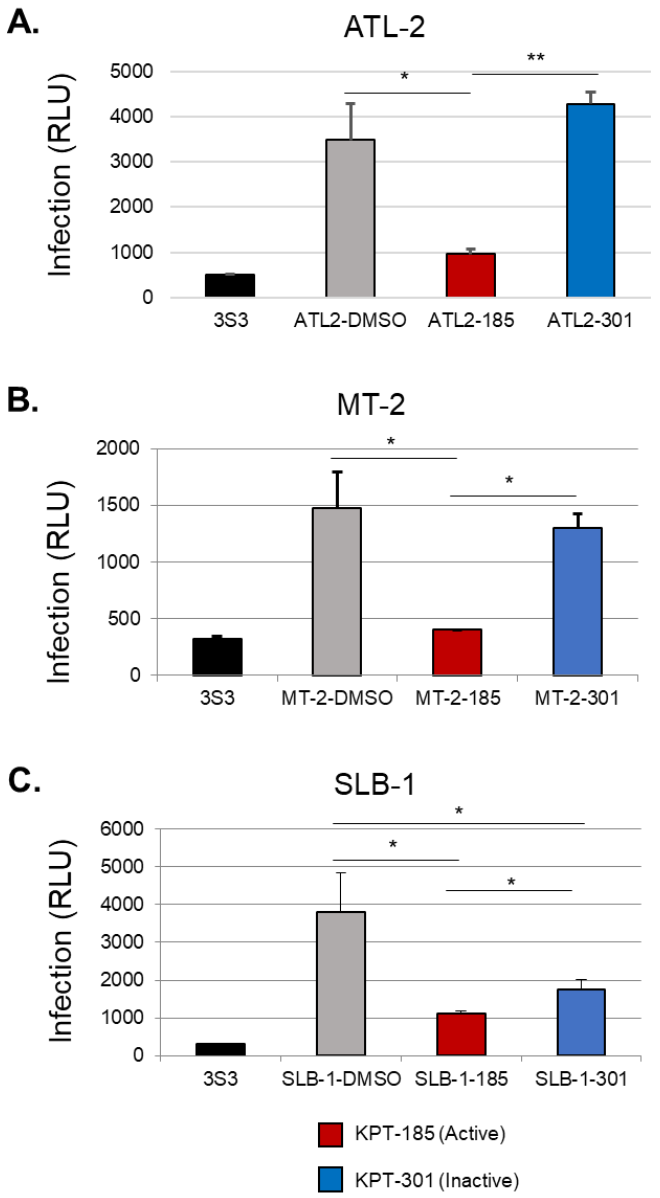


**Figure 17 - KPT-185 reduces HTLV-1 infectivity. (A)** ATL-2 cells were treated with 1μM KPT-185, KPT-301, or DMSO alone. After 24hrs a luciferase assay was performed. The graph displays the infection as relative luminsesence units (RLU). t-test (*:$p$<0.05, **: $p$<0.0005), ANOVA ($p$<0.05). **(B)** MT-2 cells were treated with 1μM KPT-185, KPT-301, or DMSO alone. After 24hrs a luciferase assay was performed. The graph displays the infection as relative luminsesence units (RLU). t-test (*:$p$<0.05), ANOVA ($p$<0.05). **(C)** SLB-1 cells were treated with 1μM KPT-185, KPT-301, or DMSO alone. After 24hrs a luciferase assay was performed. The graph displays the infection as relative luminsesence units (RLU). t-test (*:$p$<0.05), ANOVA ($p$<0.05). The graphs shown represent one experiment performed in triplicate and are representative of 3 independent experiments.

*5.7 KPT-185 Retains p53 in the Nucleus*

In cancer and HIV studies, treatment with KPT-185 has shown to be effective in both retaining p53 in the nucleus and inducing apoptosis. However, the same results may not be likely to occur in HTLV-1 cells with KPT-185 treatment due to the viral proteins Tax and HBZ inhibiting the activity of the tumor suppressor protein. Based on the results from the cell viability assays, KPT-185 treatment did not induce apoptosis at 24hrs in all cell lines, except SLB-1.

Therefore, we examined the p53 level in the SLB-1 cells after KPT-185 treatment. Our results revealed that KPT-185 treatment increased level of p53 in the nucleus and cytoplasm of SLB-1 cells (Figure 18). The retention of p53 in the nucleus correlates with an increase of cell death within the SLB-1 cell line (Figure 10), suggesting that p53 might be responsible for it. It would be of interest to investigate the p53 nuclear retention in other cell lines and determine if increasing the concentration above 1μM will also increase cell death in these cell lines. In addition, increasing the treatment time beyond 24hrs could also increase cell death.

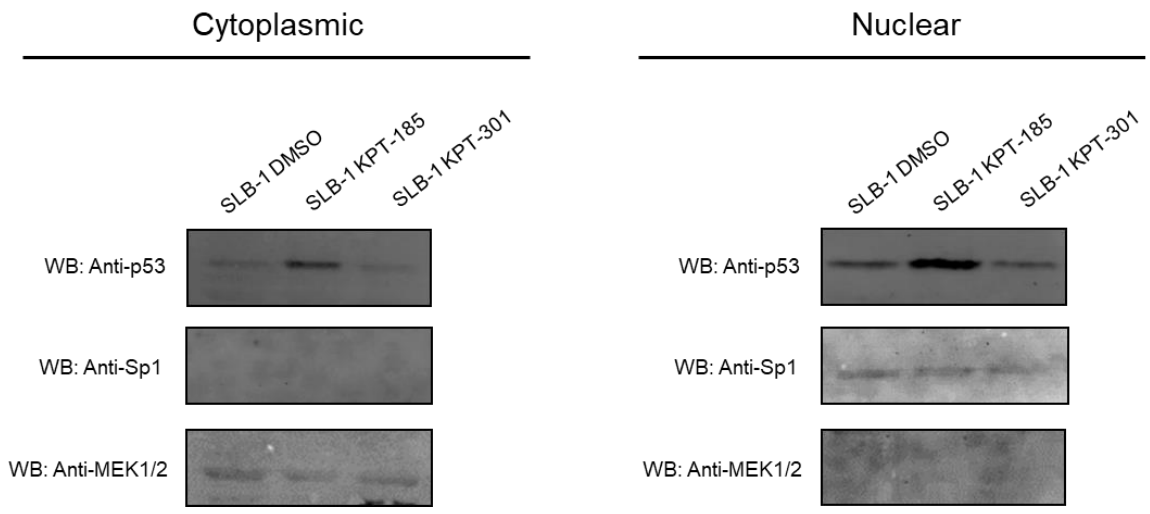


**Figure 18 - KPT-185 treatment increases p53 level in the nucleus.** SLB-1 cells were treated with 1μM KPT-185, KPT-185, or DMSO alone for 24hrs. The cells were fractionized and 20μg of the samples were analyzed by Western-blot. The membranes were probed with antibodies against p53, Sp1, and MEK1/2. Sp1 and MEK1/2 served as nuclear and cytoplasmic markers respectively. Same membranes were stripped and reprobed.

## 6. Discussion

Though HTLV-1 is typically asymptomatic in its host, out of the millions of those infected, around 5% will develop a serious and detrimental diseases. Certain diseases such as ATLL and HAM/TSP are severe and have no effective treatment or cure. The rapid proliferation and spread of the ATLL cells leads to a short prognosis (Matsuoka, 2005). HAM/TSP is a complex neurodegenerative disease associated with brain lesions and axon demyelination leading to loss of body functions. The exact onset of these diseases is not completely understood but it is believed that two viral proteins, Tax and HBZ, are important for their development and maintenance.

Current treatments aim at treating ATLL and HAM/TSP when the diseases are already developed. There are good evidences that HTLV-1 spread continuously inside an individual as there are between $10^4$ and $10^5$ distinct clones (cells with different HTLV-1 integration sites) that can be detected before a disease developed (Bangham, 2018). These clones are expanded by mitotic division. For ATLL, one of these clones will become malignant and is responsible for the disease development. For HAM/TSP, there is a strong correlation between HTLV-1 proviral load and the development of the disease (Yamano et al., 2002). Therefore, targeting HTLV-1 spread earlier in a carrier will be of interest to reduce the number of infected clones, and therefore, the chance of diseases development. HTLV-1 specific anti-retroviral inhibitors have not yet been developed. Studies using HIV anti-retroviral inhibitors such as nucleoside/nucleotide reverse transcriptase inhibitors (NRTIs) have shown some promise in *in vitro* studies (Pasquier et al., 2018). However, once these NRTIs are implemented in preclinical studies they are ineffective at inhibiting HTLV-1 replication. KPT-185 is a compound

designed to inhibit the nuclear export protein XPO-1. KPT-185 inhibits XPO-1 by binding to the cysteine 528 of the export protein to prevent the binding of nuclear cargo such as the tumor suppressor protein p53 and, in the case of HTLV-1, the viral mRNAs. Treatment of HTLV-1 with KPT-185 prior to disease development could be effective at reducing the spread of the virus and therefore progression of the diseases.

Though treatment with KPT-185 was shown not to cause a biologically significant reduction in the viability of HTLV-1 infected cells derived from patients, ATL-2 and TL-Om1, and in cells derived from *in vitro* culture, MT-2, the compound was successful in reducing the infectivity of HTLV-1 across different cell lines. The reduction in infectivity of HTLV-1 can be attributed to the effect of KPT-185 at reducing HTLV-1 mRNA nuclear export through XPO-1 inhibition. KPT-185 caused a retention of the *Gag/Pol* mRNA in the nucleus of ATL-2 cells (Figure 19). This observation confirms results from previous studies that unspliced mRNA *Gag/Pol* are exported by XPO-1 (Hidaka et al., 1988). Furthermore, treatment with KPT-185 lead to an increase in the export of *Tax/Rex* mRNA. There have been conflicting results of whether *Tax/Rex* mRNA is exported by XPO-1 (Bai et al., 2012; Li et al., 2012). Our preliminary results show that *Tax/Rex* mRNA does not utilize the XPO-1/RxRE/Rex complex. It is unclear why KPT-185 increased the export of this particular mRNA and several more experiments will need to be performed in order to confirm these results including replication of qRT-PCR. At this time, it is unknown which nuclear export system is used by the *Tax/Rex* mRNA. It is possible that in inhibiting XPO-1 nuclear export with KPT-185, a common protein (or several proteins) is redistributed to the *Tax/Rex* mRNA nuclear export system and therefore stimulates the export activity of this system. Another explanation could be that

KPT-185 treatment reduces the amount of the viral protein p30 that has been shown to retain *Tax/Rex* mRNA in the nucleus (Moles et al., 2019). Without p30, *Tax/Rex* mRNA export could occur more frequently. Though not observed, it would be expected that *HBZ* mRNA does not utilize XPO-1/RxRE/Rex complex since *HBZ* mRNA is not transcribed with the RxRE and should not be affected by KPT-185 treatment.

The nuclear retention of *Gag/Pol* mRNA correlates with the reduction of Gag p55 protein in the cells. We also observed a reduction of the Env protein which suggests that KPT-185 is also affecting the nuclear export of *Env* mRNA. Intriguingly, KPT-185 appeared to reduce the level of the Tax protein in both the nucleus and the cytoplasm fraction of SLB-1 cells, suggesting that this compound also affects the translation process of *Tax/Rex* mRNA. Additional experiments would need to verify the reduction of Tax protein level in other HTLV-1 infected cell lines. Interestingly, XPO-1 is involved in the transport of some ribosomal proteins (Williams et al., 2018); therefore, inhibition of XPO-1 by KPT-185 could reduce the levels of ribosomes available for translation. In addition, XPO-1 mRNA export is mediated by different adaptor proteins (Azizian & Li, 2020). The eukaryotic translation initiation factor eIF4E has been reported to be one of the adaptor proteins utilized in the XPO-1 nuclear export complex (Piserà et al., 2018) (Williams et al., 2018); consequently, inhibition of XPO-1 nuclear export might also affect the transport of eIF4E, reducing the amount of this factor available for translation, and explain the reduction of Tax level. Though not observed, it is possible that the protein of HBZ could be affected if *HBZ* mRNA translation is dependent upon eIF4E.

The reduction of the proteins Gag p55 and Env could have an impact on the formation of the viral particles and explain the reduction of virions observed in the ELISA assays; as Gag is important for the formation of the capsid, nucleocapsid, and matrix, and Env is responsible for the formation of the viral envelope (Figure 1). The reduction in viral particles as well as the reduction in Tax, which plays a role in the formation of the viral synapse (Gross & Thoma-Kress, 2016), could be responsible for the observed reduction of infection (Figure 19). In addition, the reduction of Tax protein in the nucleus would amplify this phenomenon by reducing the overall levels of HTLV-1 transcripts, as Tax is responsible for strong HTLV-1 transcription (Polakowski & Lemasson, 2010),

Treatment with KPT-185 on the SLB-1 cells was effective at inducing cell death which correlated to a nuclear retention of the tumor suppressor protein p53. This result suggests that the retention of the tumor suppressor protein is responsible for the cell death observed in the cell viability assays. Further experiments would be needed to determine if this occurs in the patient derived cells, ATL-2, and TL-Om-1, and the other *in vitro* cell line, MT-2, at a higher concentration of KPT-185 and longer treatment time. This will determine whether the compounds will have any cytotoxic effect on ATLL patient derived cells and could be a possible treatment to target transformed cells in patients.

Overall SINE compounds such as KPT-185 are a promising method of treatment for HTLV-1 carriers. Treatment with KPT-185 has a potential at being effective in slowing the rate of cell-to-cell transmission in the infected carriers and, therefore,

lowering the viral load within them. KPT-185 also shows to possibly reduce the translation of the viral oncoprotein Tax. Lower level of Tax could be important, not only in reducing viral synapse formation and inhibiting viral transcription, but also in affecting the process of cellular transformation, since Tax has oncogenic properties. Further directions will involve testing the SINE compounds on a larger panel of cell lines as well as using fresh cells isolated from carriers. Over time the experiments will have to transition to *in vivo* models such as the rabbit model, which is a good animal model to study HTLV-1 replication (Panfil et al., 2013). Though KPT-185 has not been yet implemented in clinical trials, another SINE compound from Karyopharm Therapeutic, KPT-330, is currently in phase 3 clinical trials for treating multiple myeloma.

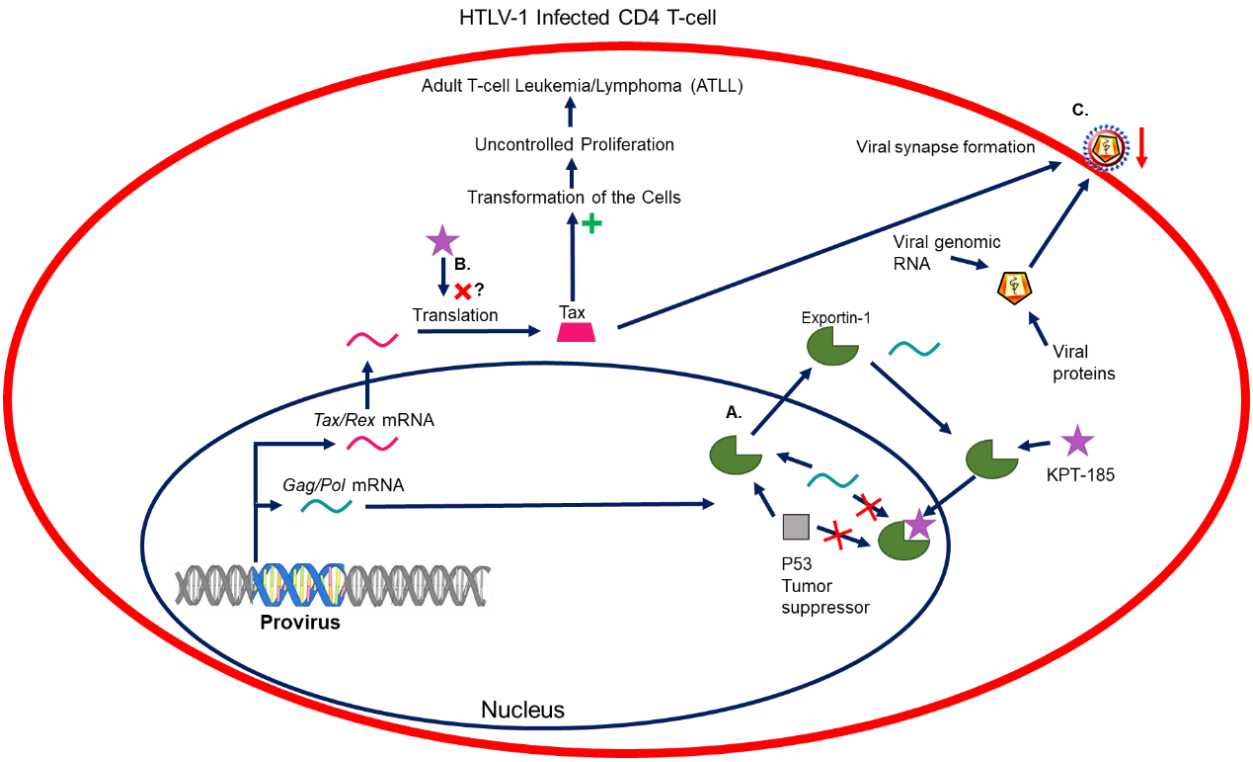


**Figure 19 - Treatment with KPT-185 is a potential therapeutic agent for HTLV-1.** **(A)** Treatment with KPT-185 has been shown through our research to inhibit the export of tumor suppressor protein p53 and *Gag/Pol* mRNA into the cytoplasm which subsequently reduced viral proteins. **(B)** Treatment with KPT-185 also possibly impacts the translation of *Tax/Rex* mRNA through an unknown mechanism. **(C)** KPT-185 treatment also lowered the infectivity of HTLV-1. This reduced infectivity could be attributed to the lack of structural mRNA and proteins available for viral particle packaging, as well as reduction of the viral synapse formation through reduction of Tax level.

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