Animal Models Utilized in HTLV-1 Research

Amanda R. Panfil1,2, Jacob J. Al-Saleem1,2 and Patrick L. Green1–4

1Center for Retrovirus Research; 2Department of Veterinary Biosciences; 3Comprehensive Cancer Center and Solove Research Institute; 4Department of Molecular Virology, Immunology, and Medical Genetics, The Ohio State University Columbus, OH, USA.

ABSTRACT: Since the isolation and discovery of human T-cell leukemia virus type 1 (HTLV-1) over 30 years ago, researchers have utilized animal models to study HTLV-1 transmission, viral persistence, virus-elicited immune responses, and HTLV-1-associated disease development (ATL, HAM/TSP). Non-human primates, rabbits, rats, and mice have all been used to help understand HTLV-1 biology and disease progression. Non-human primates offer a model system that is phylogenetically similar to humans for examining viral persistence. Viral transmission, persistence, and immune responses have been widely studied using New Zealand White rabbits. The advent of molecular clones of HTLV-1 has offered the opportunity to assess the importance of various viral genes in rabbits, non-human primates, and mice. Additionally, over-expression of viral genes using transgenic mice has helped uncover the importance of Tax and Hbzs in the induction of lymphoma and other lymphocyte-mediated diseases. HTLV-1 inoculation of certain strains of rats results in histopathological features and clinical symptoms similar to that of humans with HAM/TSP. Transplantation of certain types of ATL cell lines in immuno-compromised mice results in lymphoma. Recently, “humanized” mice have been used to model ATL development for the first time. Not all HTLV-1 animal models develop disease and those that do vary in consistency depending on the type of monkey, strain of rat, or even type of ATL cell line used. However, the progress made using animal models cannot be understated as it has led to insights into the mechanisms regulating viral replication, viral persistence, disease development, and, most importantly, model systems to test disease treatments.

KEYWORDS: HTLV-1, animal model, primate, rabbit, rat, mouse, ATL, HAM/TSP

CITATION: Panfil et al. Animal Models Utilized in HTLV-1 Research. Virology: Research and Treatment 2013:4 49–59 doi:10.4137/VrT.s12140.

TYPE: Review

FUNDING: This work was supported by grants from the National Institutes of Health P01 CA100730 (PLG).

COMPETING INTERESTS: Authors disclose no potential conflicts of interest.

COPYRIGHT: © the authors, publisher and licensee Libertas Academica Limited. This is an open-access article distributed under the terms of the Creative Commons CC-BY-NC 3.0 License.

CORRESPONDENCE: green.466@osu.edu

Introduction

Human T-cell leukemia virus type 1 (HTLV-1) is a tumorigenic retrovirus that infects an estimated 15–20 million people worldwide. Geographic areas with the highest infection prevalence include the Caribbean, southwestern Japan, Africa, South America, and the Pacific islands. HTLV-1 is well-known as the causative infectious agent of an extremely aggressive and fatal disease of CD4+ T-lymphocytes known as adult T-cell leukemia/lymphoma (ATL)3-2 and other inflammatory disorders such as HTLV-1-associated myelopathy/ tropical spastic paraparesis (HAM/TSP).3,4 However, these diseases associated with HTLV-1 infection often occur following an extensive clinical latency period of up to 3–4 decades. In addition, approximately only 2–5% of infected individuals will develop any symptoms over their lifetime. Since the isolation and discovery of HTLV-1 from ATL patients in the late 1970s, significant progress has been made regarding viral determinants of cellular transformation and host immune responses, as well as host and viral factors involved in HTLV-1 transmission and spread. While many aspects of HTLV-1 biology have been revealed over the years, the detailed mechanism(s) of ATL development after an extensive latency period in only a subset of individuals remains unclear.

HTLV-1 is a member of deltaretroviridae, a family of retroviruses that includes both simian T-lymphotropic virus (STLV-1) and bovine leukemia virus (BLV). HTLV-1 is a complex pathogenic retrovirus with a genome structure containing structural, enzymatic, regulatory, and accessory genes. Accumulating evidence suggests that expression of viral proteins early in infection plays a major role in
disease development. One widely accepted theory is that the pleiotropic functions of the HTLV-1 viral transcriptional activator, Tax, which include deregulating mitotic checkpoints, inducing nuclear factor (NF)-κB activation, and inactivating tumor suppressors, play a critical role in the early stages of leukemogenesis.\(^5\)\(^6\) However, Tax expression may require strict regulation during clinical latency and may be expendable during the later stages of disease development. For example, >70% of ATL cells lack Tax expression due to genetic and or epigenetic changes in the HTLV-1 provirus that include deletion or methylation of the promoter located in the 5' long terminal repeat (LTR).\(^7\) These changes abolish expression of other viral genes with the exception of the HTLV-1 β-Zip factor gene, Hbz. Hbz is unique among the HTLV-1 genes; it is encoded on the complementary strand of the HTLV-1 provirus and is regulated by an independent promoter in the 3' LTR. This promoter is not repressed during the leukemogenic process, thus resulting in consistent Hbz expression over time, including in the malignant tumor cell. The HBZ protein has been shown to interact with several cellular transcription factors, such as CREB and CBP, p300,\(^8\) JunD,\(^9\) JunB, and c-Jun,\(^8\)\(^10\) and is a negative regulator of Tax-mediated HTLV-1 transcription.\(^11\) It has been reported that the HBZ protein is required for enhanced viral infectivity and persistence in inoculated rabbits\(^12\) and promotes cell proliferation and tumor cell growth in cell culture and transplantation models.\(^13\) There is also evidence that the Hbz mRNA supports proliferation of ATL tumor cells,\(^14\) suggesting that the gene has bimodal functions in two different molecular forms (RNA and protein). HBZ is also tumorigenic in transgenic mice.\(^14\)

Shortly after the discovery and isolation of HTLV-1 particles from an ATL patient cell line, researchers began using animal models to help understand the biology of HTLV-1 in the context of macro- and micro-environments. Animal models are useful for developing vaccines, studying course of disease transmission and development, and testing emerging therapies. The long-term goal of HTLV-1 researchers is to understand how HTLV-1 alters T-cell physiology and thus gain insight into the mechanisms regulating viral replication, the early phases of lymphocyte activation, and cellular transformation. Recent advances in cell culture should ultimately be tested and/or confirmed in animal models. This review focuses on the various animal model systems available for HTLV-1 research and the progress made using each system.

**Non-human Primate Models**

Establishment of persistent infection and disease incidence. HTLV-1 infection in non-human primates was first reported in 1984 by Miyoshi et al.\(^15\) The HTLV-1-producing cell line, Ra-1 (established from rabbit lymphocytes co-cultivated with human HTLV-1 producing MT-2 cells), was inoculated intravenously into Japanese monkeys, which resulted in antibody production and seroconversion.\(^15\) Cynomolgus and squirrel monkeys were also inoculated with an HTLV-1-producing cell line. Specific antibodies against HTLV-1 were detected in the plasma, along with the recovery of transformed, infected cells from the peripheral blood up to 6 months post-inoculation. No signs of ATL development were present up to 2 years following inoculation.\(^16\) Numerous studies in squirrel monkeys found that early in infection, the peripheral lymphocytes, spleen, and lymph nodes were major reservoirs for HTLV-1.\(^17\)\(^19\) It was also determined that infection of squirrel monkeys with HTLV-1 could be divided into two phases of infection: reverse transcription of the viral genome followed by persistent clonal expansion of infected cells.\(^20\)

Rhesus macaques inoculated with HTLV-1-producing cells developed arthritis, uveitis, and polymyositis.\(^21\) Antibodies to membrane antigens were found in macaques with malignant lymphoma or lymphoproliferative disease.\(^22\) Infection of pig-tailed macaques with the ACH HTLV-1 molecular clone is associated with multiple signs of disease characteristic of both HAM/TSP and ATL.\(^23\) A subsequent report found that in the first few months after infection of pig-tailed macaques, there was activation of both innate and adaptive immunity, limited virus-specific cellular responses, sustained immune system activation, and, in some cases, immunodeficiency.\(^24\) A recent report examined HTLV-1 infection in macaques using a variety of molecular clones. HTLV-1 viruses with mutations in functional HBZ reverted to the wild-type genotype in 3 out of 4 animals within weeks after initial infection. Animals infected with a p30 mutant virus also experienced reversion to wild-type in 2 out of 4 animals, while all 4 animals exposed to a p12 mutant virus remained seronegative. This report established p12, p30, and HBZ as essential for the establishment and maintenance of HTLV-1 infection in macaques.\(^25\)

**Immunization and vaccination studies.** Immunization studies in non-human primates began in 1987 with the successful immunization of cynomolgus monkeys with envelope peptides synthesized in bacteria.\(^26\) Since then, Japanese macaques have been immunized with hyperimmune globulin from healthy blood donors with high titers for HTLV-1 and 27 and cynomolgus monkeys were passively immunized 24 hours before viral inoculation with purified human immunoglobulin against HTLV-1.\(^28\) Successful vaccination has also been recorded using recombinant vaccinia virus with HTLV-1 env and gag in both cynomolgus and squirrel monkeys.\(^29\)\(^30\)

**BLV and STLV.** Large bodies of work exist for retroviruses closely related to HTLV-1, including bovine leukemia virus (BLV) and simian T-cell lymphotropic virus (STLV). BLV naturally infects cattle and shares a series of structural and functional properties with many HTLV-1 viral gene products. Similarly to HTLV-1, 3–5% of infected animals develop leukemia or lymphoma, but unlike HTLV-1, the latency period for disease is relatively short at 4–10 years. Additionally, the permissive cell types for HTLV-1 infection
include CD4+ and CD8+ T cells, while BLV infection is restricted to B lymphocytes.31

STLV naturally infects Asian and African non-human primates, with more than 30 documented species infected to date. This related virus can also cause T-cell leukemia/lymphoma, with many of these tumors displaying all of the hallmarks of ATL found in humans.32 While both BLV and STLV provide useful models for infection, seroconversion, and/or disease pathogenesis, this review will focus on animal models for HTLV-1.

Rabbit Model

Transmission of HTLV-1. The rabbit model for HTLV-1 research was first established in 1984 when it was shown that intravenously inoculating rabbits with Ra-1 cells would lead to seropositivity for HTLV-1. This initial study also demonstrated that HTLV-1 was transferable via blood transfusions in rabbits.15,33 Over the next few years, several studies demonstrated other possible routes of transmission for HTLV-1, including breast milk and semen.34 Another study demonstrated that cell-free plasma was not capable of transmitting the virus to rabbits, while whole blood and washed blood cells were capable of infecting healthy rabbits.35 Rabbit immune responses to HTLV-1 infection, as well as the dose of blood necessary to infect a healthy rabbit have also been well-characterized.36,37 Several studies have used passive immunization in an attempt to halt HTLV-1 transmission.36,38,39 Rabbits have been used to test possible vaccines using both synthetic peptides and chimeric pox viruses.40–43

Haines et al44 recently developed a new method to analyze the early immunological events in orally acquired infections using the rabbit model. Female New Zealand White (NZW) rabbits were orally or intravenously inoculated with R-49 cells (rabbit lymphocyte cell line immortalized by HTLV-1 infection) and then monitored for hematologic and virologic parameters over an 8-week period. Researchers found a delay in HTLV-1 antibody response and p19 production from 1–4 weeks in orally inoculated rabbits compared to those that were intravenously inoculated. This delay was diminished by 8 weeks post-infection. The results suggested that this protocol can be utilized to better understand parameters of mother-to-child transmission via breast milk.

Cell tropism of HTLV-1. HTLV-1 can infect many different cell types; however, CD4+ T cells are the permissive cell type for transformation in vivo. Recently, the rabbit model was used to determine whether the transformation tropism of HTLV-1 for CD4+ T cells was established at the time of infection or during the transformation process.45 Male NZW rabbits were inoculated intravenously with a lethally irradiated HTLV-1 producing cell line. One week post-inoculation, HTLV-1 was detected in both CD4+ and CD8+ T cell populations, demonstrating that HTLV-1 does not exhibit cellular preference during the initial infection stage. However, beyond 5 weeks post-infection, the CD4+ T cell population became the predominant cell type, leading to the conclusion that preferential tropism of CD4+ is established via a post-infection clonal selection/expansion.

Molecular clones of HTLV-1. Molecular clones of HTLV-1 were developed in the mid-1990s.46,47 After the generation of one of the HTLV-1 molecular clones, termed ACH, rabbit studies demonstrated that the molecular clone acted similarly to previously characterized virus isolated from infected humans.48,49 Molecular clones were subsequently generated with mutations that ablated the expression of certain viral genes. Virus-infected NZW rabbits were used to analyze the importance of these genes during in vivo infection. The regulatory gene, Rex, and accessory genes, p12 and p30, have all been demonstrated as dispensable for viral immortalization in vitro. However, when compared to wild-type virus in the rabbit model, infection with these mutants led to a diminished anti-HTLV-1 antibody response, a lack of p19 production in peripheral blood mononuclear cells, and only a transient ability to detect proviral DNA via PCR. These findings suggest that all three viral genes play a role in viral persistence and spread within rabbits.50–52 Using the same approach, the anti-sense transcript protein, HBZ, and the PDZ domain of Tax were found to be necessary for viral persistence in vivo.12,53 While studying the effect of ablating the expression of the HTLV-1 accessory gene, p30, evidence for reversion to wild-type sequence was found. As early as 2 weeks post inoculation, the wild-type sequence of the provirus was found in some rabbits that were infected with mutant virus. This finding demonstrates the importance of p30 for persistence and the necessity to carefully sequence/verify all mutants in such studies to ensure that reversion does not obscure the results.52

Advantages and disadvantages of rabbits. The rabbit model has been used for many studies and has driven the progress of HTLV research due to its ease of use. This model is extensively used due to the low cost and the little maintenance necessary for rabbit upkeep. Major strengths of the rabbit model include the ease of infection, different methods for testing the immune response, multiple routes of transmission, and the ability to use molecular clones to analyze genetically variant viruses. However, there are some limitations to this model. The most prominent is the inability to efficiently establish disease. While disease has been reported several times in rabbits, including an ATL-like disease, uveitis, and a cutaneous lymphoma, these cannot be replicated reliably.54–59

Rat Model

A manageable model for HAM/TSP. The rat animal model utilized for HTLV-1 research was first published in 1991, approximately 15 years after the discovery of ATL.60 The ATL-derived, HTLV-1-producing cell line, MT-2, was injected intravenously into female F344 rats to produce HTLV-1 carrier rats that produced antibody titers against a variety of HTLV-1 antigens and proviral loads consistent with chronic infection. In the years that followed, researchers
found that while HTLV-1 can be efficiently transmitted to several inbred strains of newborn and adult rats (ACI, F344, SDJ, WKA, BUF, LEJ, LEW) regardless of age at transmission, the production of HTLV-1 antibodies differed significantly among strain and age at the time of transmission. Multiple studies concluded that HTLV-1-infected Wistar-King-Aptekman (WKA) strain of rats not only produced an antibody response to HTLV-1, but these rats also developed spastic paraparesis of the hind legs with degenerative thoracic spinal cord and peripheral nerve lesions. The histopathological features and clinical symptoms of these infected rats mimicked that of humans with HAM/TSP, and thus a manageable model for HAM/TSP was born.

Transmission and immunological studies in rats. Rat models are a valuable tool for studying mother-to-child transmission of HTLV-1—the major route of HTLV-1 infection. By mating an HTLV-1-infected female F344 rat with a non-infected male rat, researchers were able to show vertical transmission of the virus in 2 of 9 offspring. Pregnant female rats were also infected with HTLV-1 and were able to pass the virus via intrauterine transmission to their offspring, also validating the use of rats as a model for mother-to-child transmission. Immunological studies suggest that T-cell unresponsiveness to HTLV-1 is a risk factor for HTLV-1-infected cell propagation, but this risk may be reduced by re-immunization after infection. Komori et al found that subcutaneous re-immunization (using mitomycin C-treated syngeneic HTLV-1-infected cells) restored the HTLV-1-specific T-lymphocyte response and decreased the proviral load in viral-infected rats.

Rat HAM/TSP disease pathology. The disease pathology in rats has been characterized over time, beginning with apoptotic cell death by 9 months in the spinal cord of HTLV-1-infected WKA rats, followed by demyelination at 12 months, macrophage infiltration in response to demyelination at 15 months, and activation of astrocytes at 20 months. Apoptosis of oligodendrocytes and Schwann cells is thought to be the primary event leading to demyelination. Initial reports indicated that the pathology of rat HAM/TSP differed from the HAM/TSP reported in humans. Human HAM/TSP lesions showed distinct T-cell infiltration, while the HAM/TSP lesions in rats did not. However, a recent report found that HTLV-1-infected Wistar rats showed diffuse lymphocyte infiltration in some cases. Other studies using HTLV-1-infected rats reported selective activation of HTLV-1 pX and tumor necrosis factor (TNF)-α mRNA in target spinal cord and peripheral nerves, altered expression of bcl-2, bax, and p53 apoptosis-regulating genes in lesions, and interferon (IFN)-γ production in spinal cord lesions. HTLV-1 proviral DNA has also been found in lesion-associated microglial cells and macrophages of infected animals. Taken together, these data reveal several major pathogenetic pathways of HTLV-1-induced myeloneuropathy in the WKA rat.

ATL and tumorigenic disease model in rats. Transgenic rats have shown promise as a suitable model for oncogenesis and immune pathogenesis. In 1997, a transgenic rat with the HTLV-1 pX gene under control of the mouse H-2Kd promoter was created and shown to develop mammary carcinomas. Ten years later, a transgenic rat expressing the human viral RNA transporter, cellular cofactor of Rex (CRM1 gene) was established. HTLV-1 viral production in the CRM1 transgenic rat was 100–10,000-fold higher than in wild-type rats. Interestingly, the CRM1 transgenic rats showed similar viral loads to those found in human T-lymphocytes, indicating an essential role for CRM1 in proper HTLV-1 replication and its importance in the rat model. Immunodeficient rats have also provided the opportunity to develop models for clinical ATL. Inoculation of adult nude rats with HTLV-1 immortalized cell lines resulted in a lymphoproliferative disease similar to ATL. Protection against tumor development was examined in immunodeficient rats using adoptive transfer of T-lymphocytes, Tax-specific peptide vaccines, and Tax-specific small interfering RNAs (siRNA).

The past and future of rat model research. Laboratory rats provide a model HTLV-1 animal system that is low in cost, easy to maintain, and provides useful parameters of infection (immune response, viral loads). Over 20 years of research using rats has proven their usefulness as tools for the study of the neurological disease HAM/TSP, provided the correct strain is utilized. While the disease induced from HTLV-1 infection of rats does not completely follow the histology of human disease, recent advances have shown that some pathological features are similar. Like other rodent models, rats can be genetically modified; however, immunodeficient rats must be used to model ATL—a feature that eliminates a functional immune system. While rats are not as popular as other small animal models, they continue to provide a useful tool for the study of HAM/TSP and immune regulation of HTLV-1.

Mouse Model

Immunocompetent HTLV-1 carrier mice. As with rabbit and rat models, HTLV-1 does not efficiently infect murine cells and therefore must be manipulated using intra-peritoneal inoculation to establish persistent infection. In 1997, Kushida and colleagues published one of the first studies demonstrating persistent infection in mice. Using C3H/HeJ and BALB/c strains of immune competent mice, they injected HTLV-1-producing MT-2 cells intraperitoneally into neonatal mice. After 3 months, provirus was detected in the peripheral blood mononuclear cells, lymph nodes, thymus, spleen, and liver of the mice. However, antibody responses against viral Gag were minimal and found only in BALB/c mice. Further studies confirmed this finding of persistent infection, but without the presence of an antibody response. In many ways, inoculation of immunocompetent mice is similar to in vivo human infection. Infected mice demonstrate a broad spectrum of infected cell types including CD4+, CD8+, B cells, and granulocyte fractions, similar to
those observed in HTLV-1-infected individuals. Similarly to HTLV-1-infected individuals, infected mice present polyclonal proliferation of spleen infected cells and lymphatic tissues that prove a reservoir for infected and proliferating cell clones. Although easy to maintain and care for, immunocompetent HTLV-1 carrier mice have provided very little evidence of the in vivo spread of infection and no apparent disease following infection, although there has been one report of tumor induction.

**Chimeric murine/HTLV-1 virus.** In an effort to improve murine infection by HTLV-1, Delebecque and colleagues created a chimeric murine/HTLV-1 provirus (infectious for murine cells in culture), which replaced the original HTLV-1 envelope precursor sequence with that of the modified ecotropic Moloney murine leukemia virus. This chimeric virus retained the replication characteristics of HTLV-1, but could now be used to persistently infect adult mice. Infected mice presented with persistent infection in the spleen, thymus, lung, brain, and spinal cord, as well as oligoclonal integration resembling human retroviral integrations and humoral and cellular immune responses, although no lesion development was observed. Using a dendritic cell (DC) depleted transgenic mouse (CD11c-diphtheria toxin receptor transgenic), infection with cell-free chimeric virus led to higher proviral mRNA loads and diminished cellular immune responses compared to mice infected with cell-associated virus, demonstrating that DCs control cell-free virus at an early stage of viral infection. More recent studies utilizing this transgenic mouse and chimeric virus demonstrated that the role of DCs during the early phase of infection with cell-free virus is to up-regulate the expression of various interferon-stimulated genes (ISGs), an array of antiviral cytokines, and transcription factors that help to control viral infection. In addition, down-regulation of critical chemokines by the virus acts as an attempt to counter the threat posed by DCs. These studies represent a working model for productive murine viral infection that has already resulted in significant findings on viral immunogenetics.

**SCID mice and leukemic potential.** Despite advances with rats and rabbits as model systems for HTLV-1-associated diseases, the need for an effective tumorigenic small animal model remained. First reported in 1992 by Ishihara and colleagues, severe combined immunodeficiency (SCID) mice were used to successfully model the proliferative and tumorigenic mouse (CD11c-diphtheria toxin receptor transgenic), which replaced the original HTLV-1 envelope precursor sequence with that of the modified ecotropic Moloney murine leukemia virus. This chimeric virus retained the replication characteristics of HTLV-1, but could now be used to persistently infect adult mice. Infected mice presented with persistent infection in the spleen, thymus, lung, brain, and spinal cord, as well as oligoclonal integration resembling human retroviral integrations and humoral and cellular immune responses, although no lesion development was observed. Using a dendritic cell (DC) depleted transgenic mouse (CD11c-diphtheria toxin receptor transgenic), infection with cell-free chimeric virus led to higher proviral mRNA loads and diminished cellular immune responses compared to mice infected with cell-associated virus, demonstrating that DCs control cell-free virus at an early stage of viral infection. More recent studies utilizing this transgenic mouse and chimeric virus demonstrated that the role of DCs during the early phase of infection with cell-free virus is to up-regulate the expression of various interferon-stimulated genes (ISGs), an array of antiviral cytokines, and transcription factors that help to control viral infection. In addition, down-regulation of critical chemokines by the virus acts as an attempt to counter the threat posed by DCs. These studies represent a working model for productive murine viral infection that has already resulted in significant findings on viral immunogenetics.

**SCID mice and leukemic potential.** Despite advances with rats and rabbits as model systems for HTLV-1-associated diseases, the need for an effective tumorigenic small animal model remained. First reported in 1992 by Ishihara and colleagues, severe combined immunodeficiency (SCID) mice were used to successfully model the proliferative and tumorigenic potential of ATL cells in mice. BALB/c nu/nu SCID mice were treated with an anti-asialo GM-1 antibody, to abolish NK activity and enhance growth of lymphoma, and inoculated with MT-2 cells. Some of the mice developed tumors at or near the injection site, which were positive for viral p19 and positive detection of proviral DNA. Similarly, repression of SCID mouse NK cells using an anti-murine interleukin 2 receptor beta chain monoclonal antibody followed by injection with ATL patient cells resulted in tumor and leukemia development 5–7 weeks after inoculation. In this study, proliferating leukemic cells in the mouse were verified as deriving from the original inoculating ATL cell clone; infiltration of ATL cells into various mouse organs was similar to what is observed in ATL patients. While these experiments utilized reduced NK cell function to establish tumors, a report by Ohsugi et al demonstrated successful tumorigenesis from MT-2 cell engraftment in SCID mice with normal NK cell function.

Differences between the tumorigenic potential of in vitro immortalized cells and ATL leukemic cells were first revealed when SCID mice were infected with peripheral blood lymphocytes (PBLs) from ATL patients or cell lines created in vitro from HTLV-1-immortalized T-lymphocytes. Interestingly, lymphoblastic lymphomas developed in mice injected with PBLs from ATL patients, but not with the in vitro generated immortalized/transformed T-lymphocyte cell lines. In addition to verifying that cells of non-leukemic origin cannot induce tumorigenicity, other reports have shown that immunosuppression of SCID mice with whole-body irradiation or treatment with antiserum to reduce/eliminate NK cell activity is needed to derive tumors from HTLV-1 cell lines. In fact, a report by Ohsugi et al showed that even age can affect tumor formation; SCID mice less than 5 weeks old with low NK cell activity developed rapid tumor formation that resulted in death.

The success of ATL leukemic cells vs in vitro transformed cell lines in establishing tumorigenesis may also reside in their lack of detectable viral gene expression compared to cell lines that display measurable quantities of all viral gene transcripts. A HTLV-1 superinfected ATL cell line, HT-1RV, showed a reduced tumorigenic phenotype compared to the parental ATL cells, RV-ATL, as the cells were more sensitive to NK cell cytolysis. The absence of viral gene expression in ATL cells helps these cells evade detection by the immune system and establish tumors. Taken together, these data suggest that cells originating from a non-leukemic origin have not gathered the required genetic changes, such as the ability to evade NK cell-mediated cytotoxicity, needed to reach the full tumorigenic potential. Further support for the notion that genetic environment plays a role in tumorigenesis was the use of A lymphoplasia (aly/aly) mice. These mice have a mutation in NF-kB inducing kinase (NIK), which is critical for non-canonical activation of NF-kB, in addition to defects in lymphoid organ development and deficiencies in immune function. A lymphoplasia mice have impaired viral proliferation, lack of provirus, and undetectable clonal proliferation in secondary lymphoid organs, which may provide a reservoir for HTLV-1 persistence.

The use of NOD/SCID gamma c (null), or NOG mice, which have immunological dysfunctions of T, B, and NK cells, has been beneficial for examining viral-induced cell proliferation. Successful transplantation of peripheral blood mononuclear cells (PBMCs) from NOG mice with primary HTLV-1 engraftment into a 2nd group of NOG mice resulted in a higher
The presence of proviral load and multiple proviral integrations indicative of new infections.\textsuperscript{106} The Hbz anti-sense viral transcript and protein has also been shown to enhance the proliferative capacity of HTLV-1-infected T cells in NOG mice.\textsuperscript{11} SLB-1 cells, an HTLV-1 in vitro transformed cell line, with HBZ knockdown showed significantly reduced tumor formation and viral organ infiltration in NOG mice compared to wild-type SLB-1 cells.

**Therapeutic potential of SCID mice.** In addition to developing lymphomas following transplantation, SCID mice provide a testable model for diverse therapeutic reagents against HTLV-1-induced tumors. Various inhibitors and small molecules such as the proteasome inhibitor, PS-341,\textsuperscript{106} humanized anti-CD2 monoclonal antibody, MEDI-507,\textsuperscript{107} NF-κB inhibitor, Bay 11-7082,\textsuperscript{108} reverse transcriptase inhibitors, azidothymidine and tenofovir,\textsuperscript{109} biphosphonic cyclopentadecanone, C7a,\textsuperscript{110} and the small molecule inhibitor of Bcl-2, Bcl-X(L), and Bcl-w, ABT-737,\textsuperscript{111} have been successfully used to examine primary viral infection, tumor growth, leukemic infiltration in various organs, and overall survival of HTLV-1-infected cells in SCID mice.

The SCID mouse model for ATL was also shown to be associated with humoral hypercalcemia of malignancy (HHM), a life threatening complication of ATL.\textsuperscript{112} Parathyroid hormone-related protein (PTHrP), an important factor in the pathogenesis of HHM, was elevated in SCID mice, independently of Tax expression. Expression of PTHrP can be activated by NF-κB, which is well-known to be constitutively activated in ATL cells. By using the proteasomal inhibitor PS-341 to disrupt NF-κB, researchers found that SCID mice had a reduced tumor burden and reduced PTHrP expression in tumor cells compared to the control treatment groups.\textsuperscript{113}

Until the use of SCID mice, attempts to propagate ATL tumor cells in animals were unsuccessful. HTLV-1 can immortalize T lymphocytes in culture, but the factors leading to tumor progression after infection remained elusive. Over the past 20 years, the microenvironment of SCID mice has proved conducive for leukemic tumor growth and clues as to the factors involved in ATL tumor development, viral proliferation, and a testable model for therapeutic drugs.

**Tax transgenic mice.** Shortly after establishment of the rabbit model of HTLV-1 persistence, transgenic (Tg) mice were used to provide an understanding of the role of Tax and Tax-mediated disruption of lymphocyte function. HTLV-1 promoter (LTR)-Tax transgenic mice were first described by Nerenberg et al\textsuperscript{114} in 1987. Tax protein expression efficiently induced mesenchymal tumors in the Tg mice and had tissue-specific expression patterns. In other Tax Tg mice, Tax protein was expressed in the brain, salivary gland, spleen, thymus, skin, muscle, and mammary gland tissues.\textsuperscript{115} These mice had a higher incidence of mesenchymal tumors and adenocarcinomas with activated c-Fos and c-Jun expression in the tumors. Interestingly, LTR-c-myc mice mated with Ig promoter/enhancer-driven Tax Tg mice produced offspring with both CD4+ T-cell lymphomas and central nervous system tumors.\textsuperscript{116}

In order to restrict Tax expression to the lymphoid cell compartment, the use of alternative promoters was investigated. Over-expression of Tax under the human granzyme B promoter to target expression to the mature T-lymphocyte compartment causes large granular lymphocytic leukemia/lymphomas in mice.\textsuperscript{117} These Tax Tg mice also spontaneously developed hypercalcemia, high frequency osteolytic bone metastases, and enhanced osteoclast activity, symptoms frequently associated with ATL development.\textsuperscript{118} Upon further examination, Tax-positive tumor cells expressed transcripts for distinct osteoclast-activating factors and mice doubly transgenic for Tax and the osteoclast inhibitory factor, osteoprotegerin, were protected from osteolytic bone disease and developed fewer soft tissue tumors. IFN-γ was also shown to play a role in tumor-induced bone loss, hypercalcemia, and host-induced osteolysis in Tax Tg mice.\textsuperscript{119} Using Gz b-Tax and LTR-luc transgenic mice, inflammation and lymphoma can be monitored using non-invasive bioluminescent imaging. Studies using these doubly transgenic mice found that development of lymphoma is promoted by an inflammatory stimulus and that Tax expression causes chronic inflammation that begins as microscopic intraepithelial lesions and progresses into inflammatory nodules, subcutaneous tumors, and large granular lymphocytic leukemia.\textsuperscript{120,121} Other Tax Tg models have used Tax under the control of the CD3-epsilon promoter-enhancer sequence to show that Tax expression in tumors is closely associated with apoptosis\textsuperscript{122} and Tax under the control of the Lck proximal promoter (restricts expression to developing thymocytes) to demonstrate lymphomas characterized by constitutive NF-κB activation and increased cytokine and growth factor expression.\textsuperscript{123,124} These bodies of work confirm HTLV-1 as a transforming virus with Tax as its oncogenic protein.

In addition to Tax, other mechanisms of carcinogenesis have also been investigated, including the p53 pathway. Mutations in p53 apoptotic pathways are commonly found in Tax-induced tumors and tumor-derived cell lines. Mating Tax Tg mice with p53-deficient mice resulted in minimal acceleration of initial tumor formation, but when mated with p53 heterozygous mice, there was significant acceleration of disease and rapid progression to death.\textsuperscript{125} This showed that functional inactivation of p53 is not critical for initial tumor formation, but does contribute to late-stage tumor progression. A recent report using both Tax and p53 Tg mice showed that Tax expression does not achieve a functional equivalence of p53 inactivation, but did point out a role for the cellular phosphatase protein, Wip1, in tumor formation.\textsuperscript{126} Cytokine expression analyses in Tax transgenic mice have also provided evidence that Tax may be responsible for the up-regulation of certain cytokines and adhesion molecules that affect the infiltrating capabilities of HTLV-1-infected cells.\textsuperscript{127} Recently, a small portion (8/297) of Tax Tg mice developed HAM/TSP-like disease with symmetrical paraparesis of the hind limbs.\textsuperscript{128}
HBZ transgenic mice. The viral anti-sense transcript, Hbz, is expressed in all ATL tumor cells and plays a role in viral proliferation. In order to better understand its role during ATL development, Tg mice expressing Hbz in CD4+ T cells were developed. These mice developed T-cell lymphomas and systemic inflammation. HBZ Tg mice also displayed elevated levels of CD4+ Foxp3+ T(reg) cells and effector/memory CD4+ T cells, and HBZ expression directly induced Foxp3 gene transcription. Foxp3 is a key transcription factor for T(reg) cells found in approximately 60% of ATL cases. HBZ Tg mice are also more vulnerable to both HSV-2 and listeria monocytogenes infections, likely due to suppressed IFN-γ production by CD4+ T cells in these mice.

Therapeutic potential of transgenic mice. Similarly to SCID mice, Tg mice provide a valuable tool to test ATL treatments, particularly those targeted for Tax or Tax-related activities. The proteasome inhibitor PS-341, CXCR4 antagonist AMD3100, NF-kB inhibitor DHMEQ, and HSP90 inhibitor 17-DMAG have shown promise as novel therapies or in some cases as part of combination therapies for ATL. Recently, an HTLV-1/hepatitis B virus core chimeric particle was also found to be capable of inducing strong cellular immune responses in HLA-A*201-transgenic mice without adjuvants via effective maturation of dendritic cells.

Transgenic mice and RA. Another common feature of Tax Tg mice is the development of chronic arthritis at 2–3 months. The many histopathologic features of the joints in the murine form of chronic arthritis, including synovial and periarticular inflammation, articular erosion from invasion of granulation tissues, etc., are similar to symptoms of human rheumatoid arthritis (RA). In patients with RA, anti-Fas antibody (RK-8) can be used to treat RA symptoms and in Tax Tg mice treated with RK-8 there is reduction of paw swelling and arthritis with apoptosis of many reactive cells in the synovium and no systemic side effects. However, a recent report found that the portion of different splenic T-cell subsets in Tax Tg mice was completely different from those of the other commonly used animal models of RA.

“Humanized” mice. Humanized mouse models have been used extensively for both HIV-1 and EBV models of infection and disease state. A modified humanized mouse model for HTLV-1 was first used by Miyazato et al. in 2006, in which NOG mice were inoculated with human PBMCs followed by inoculation with HTLV-1-virus producing cells resulting in a readily detectable proviral load in both the CD4+ and CD8+ T cells. In a similar experiment, researchers established HTLV-1 infection in mice with PBMCs from HTLV-1-infected individuals. Although the approaches were different, the end result was the same: mice can harbor HTLV-1-infected human cells and permit clonal proliferation of these cells.

In order to develop a more humanized model of ATL progression and initiation, two different methods of HTLV-1 infected mice were established using NSG mice, or NOD-SCID/γnull mice, with HTLV-1 ex vivo and then inoculated these cells into NSG mice. These mice developed CD4+ T cell lymphomas with characteristics similar to ATL such as elevated T cell proliferation. Additionally, they also purified CD4+ HP/HSCs from HTLV-1-infected patient PBMCs and found proviral integrations, suggesting viral infection of bone marrow-derived stem cells. Their conclusions suggest that HSCs may provide a viral reservoir in vivo and act as cellular targets for cell transformation in humans. In 2011, Villaudy et al. repopulated NSG mice with CD34+ hematopoietic progenitor cells (reconstitution with CD34+ stem cells results in phenotypically normal human lymphocytes), and then infected the mice with HTLV-1. Efficient viral infection was established in this model system as measured by proviral integration in thymocytes and increased proviral load during the course of infection. Alterations in human T cell development and accumulation of activated human CD4+ and CD8+ cells were found in the thymus and spleen of the infected mice. The mice also developed hepatosplenomegaly, lymphadenopathy, and lymphoma/thymoma several months after infection, with Tax expression present in all tumors.

The advent of humanized mice for HTLV-1 research has provided an invaluable tool for the study of ATL development and testing of potential ATL therapeutics. Recent advances have demonstrated that infection of humanized mice with molecularly cloned HTLV-1 leads to CD4+ T-cell proliferation (unpublished data, Niewiesk et al). The ability to incite disease in this model with HTLV-1 allows for not only a detailed investigation of the pathogenesis of CD4+ T-cell transformation and tumorigenesis, but also the interaction of tumor cells with the microenvironment and importance of various viral genes on transformation and tumorigenesis. Limitations with humanized mice include the cost associated with their generation, maintenance, and care, but more importantly the absence of a functional immune system.

Summary

Over the years, the use of animal models in HTLV-1 research has provided invaluable information regarding viral transmission, persistence of viral infection, immunological responses, the role of viral accessory proteins in disease, disease development, and potential therapeutics (Table 1). The use of non-human primates as a model system offers the advantage of phylogenetic similarity to humans and has been
Virology: research and Treatment 2013:4

HAM/TSP (in certain strains), transmission, immune proliferation and tumorigenic potential to ATL tumor cells, Role of Tax and HBZ during ATL development, Tax-mediated disruption of lymphocyte function, ATL treatments, chronic arthritis

LIMITATIONS
Transmission, persistent infection, immune response, vaccination/immunization, lymphoma/leukemia (in certain types of monkeys) High cost, inconsistent disease development

Rats
HAM/TSP (in certain strains), transmission, immune response, lymphoproliferative disease (in immunodeficient rats) Induced disease does not completely follow histology of human disease, strain dependent disease development, immunodeficient rats are required to model ATL

Mouse (immune competent)
Persistent infection Lock of disease and in vivo spread of virus following infection

SCID mouse
Proliferation and tumorigenic potential to ATL tumor cells, viral proliferation, therapeutic drugs No immune system, high maintenance costs, use of certain ATL cell lines to induce disease

Transgenic mouse
Role of Tax and HBZ during ATL development, Tax-mediated disruption of lymphocyte function, ATL treatments, chronic arthritis High production and maintenance costs, overexpression of viral gene products is not representative of true viral infection of disease

“Humanized” mouse
ATL development, ATL therapeutics, tumor microenvironment, role of viral and cellular genes in tumorigenesis Absence of a functional immune system, higher cost associated with generation, maintenance, and care

Table 1. Summary of the different types of animal models used for HTLV-1 research, the aspects of viral infection and tumorigenesis they are most useful to study, and limitations associated with each animal model.

| ANIMAL MODEL                        | USED TO STUDY...                                                                 | LIMITATIONS                                                                 |
|-------------------------------------|---------------------------------------------------------------------------------|---------------------------------------------------------------------------|
| Non-human primate                   | Persistent infection, immune response, vaccination/immunization, lymphoma/leukemia (in certain types of monkeys) | High cost, inconsistent disease development                               |
| Rabbit                              | Transmission, persistent infection, immune response                             | Inability to establish persistent disease                                  |
| Rat                                 | HAM/TSP (in certain strains), transmission, immune response, lymphoproliferative disease (in immunodeficient rats) | Induced disease does not completely follow histology of human disease, strain dependent disease development, immunodeficient rats are required to model ATL |
| Mouse (immune competent)            | Persistent infection                                                            | Lock of disease and in vivo spread of virus following infection            |
| SCID mouse                          | Proliferation and tumorigenic potential to ATL tumor cells, viral proliferation, therapeutic drugs | No immune system, high maintenance costs, use of certain ATL cell lines to induce disease |
| Transgenic mouse                    | Role of Tax and HBZ during ATL development, Tax-mediated disruption of lymphocyte function, ATL treatments, chronic arthritis | High production and maintenance costs, overexpression of viral gene products is not representative of true viral infection of disease |
| “Humanized” mouse                   | ATL development, ATL therapeutics, tumor microenvironment, role of viral and cellular genes in tumorigenesis | Absence of a functional immune system, higher cost associated with generation, maintenance, and care |

The use of many different animal models in HTLV-1 research has enabled researchers to better understand HTLV-1 infection and the multiple genetic and cellular changes that occur during HTLV-1-associated disease development. Animal models enable the continued development and testing of vaccines against key viral proteins that mediate cellular transformation. While progress has been made concerning therapies to treat ATL, it remains a deadly disease that is highly chemotherapeutic and drug resistant. Various drugs and novel small molecule inhibitors can be tested for efficacy in animal models to determine their value before preclinical trials. Animal models, in particular the humanized mouse, will allow researchers to understand the role of viral gene products in the maintenance and development of ATL by using molecular viral clones and inducible viral gene expression systems. The importance of animal models in HTLV-1 research has been and will continue to advance the field towards understanding HTLV-1 biology and disease progression.

Acknowledgements
We thank Kate Hayes-Ozello for editorial comments on the manuscript.

Author Contributions
Wrote the first draft of the manuscript: ARP, JJA. Contributed to the writing of the manuscript: ARP, JJA, PLG. Agree with manuscript results and conclusions: ARP, JJA, PLG. Made critical revisions and approved final version: ARP, PLG. All authors reviewed and approved of the final manuscript.

DISCLOSURES AND ETHICS
As a requirement of publication the authors have provided signed confirmation of their compliance with ethical and legal obligations including but not limited to compliance
REFERENCES

1. Foezes BJ, Ruscetti FW, Gazdar AF, Bunn PA, Minna JD, Gallo RC. Detection and isolation of type C retrovirus particles from fresh and cultured lymphocytes of a patient with cutaneous T-cell lymphoma. Proc Natl Acad Sci U S A. 1980;77(12):7415–7419.

2. Yoshida M, Miyoshi I, Himura Y. Isolation and characterization of retrovirus from cell lines of human adult T-cell leukemia and its implication in the disease. Proc Natl Acad Sci U S A. 1982;79(6):2031–2035.

3. Osame M, Izumo S, Igata A, et al. Blood transfusion and HTLV-I associated myelopathy. Lancet. 1986;2(8498):104–105.

4. Gessain A, Barin F, Vernant JC, et al. Antibodies to human T-lymphotropic virus type I in patients with tropical spastic paraparesis. Lancet. 1985;2(8452):440–441.

5. Tawara T, Inai Y, Yodoi J, et al. Immune activation by human T lymphotropic virus type I. Nature. 1985;318(6041):792–795.

6. Matsuoka M, Jeang KT. Human T-cell leukemia virus type 1 infection. In: Boshoff C, ed. Retroviruses. New York: Cold Spring Harbor Laboratory Press; 2000:407–460.

7. Barbeau B, Mesnard JM. Making sense out of antisense transcription in human T-cell lymphotropic viruses. J Virol. 2002;76(24):12813–12822.

8. B Basbous J, Arpin C, Gaudray G, Piechaczyk M, Devaux C, Mesnard JM. The HBZ factor of human T-cell leukemia virus type I dimerizes with transcription factors JunB and Fos, and modulates their transcriptional activity. J Biol Chem. 2003;278(44):43620–43627.

9. Theuhb S, Basbous J, Hivin P, Devaux C, Mesnard JM. HBZ interacts with JunD and stimulates its transcriptional activity. FEBS Lett. 2004;562(1–3):165–170.

10. Matsuoka M, Ichihara Y, Oono T, Shimotohno K. HTLV-1 HBZ suppresses AP-1 activity by impairing both the DNA-binding ability and the stability of c-Jun protein. Oncogene. 2005;24(6):1001–1010.

11. Gaudray G, Gachon F, Basbous J, Biard-Piechaczyk M, Devaux C, Mesnard JM. The complementary strand of the human T-cell leukemia virus type 1 RNA genome encodes a bipartite transcription factor that down-regulates viral transcription. J Virol. 2002;76(24):12813–12822.

12. Arnold J, Yamamoto B, Li M, et al. Enhancement of infectivity and persistence in vivo by HBZ, a natural antisense-coded protein of HTLV-1. J Virol. 2006;80(10):3976–3982.

13. Arnold J, Yamamoto B, Li M, Lairmore MD, Green PL. Human T-cell leukemia virus type I antisense-encoded gene, Hbz, promotes T-lymphotropic proliferation. Blood. 2008;112(9):3070–3079.

14. Gaudray G, Arpin C, Basbous J, Biard-Piechaczyk M, Devaux C, Mesnard JM. The HBZ factor of human T-cell leukemia virus type I dimerizes with transcription factors JunB and Fos, and modulates their transcriptional activity. J Biol Chem. 2003;278(44):43620–43627.

15. Miyoshi I, Yoshimoto S, Kubonishi I, et al. Infectious transmission of human T-cell leukemia virus to animals. Int J Cancer. 1985;35(1):81–85.

16. Iwahara T, Takehara N, Kataoka R, et al. Transmission of HTLV-I to rabbits via semen and breast milk from seropositive healthy persons. J Infect. 1990;24(5):980–983.

17. Kotani S, Yoshimoto S, Yamato K, et al. Serial transmission of human T-cell leukemia virus type I by blood transfusion in rabbits and its prevention by use of X-irradiated stored blood. Int J Cancer. 1986;37(6):843–847.

18. Kataoka R, Takehara N, Iwahara T, et al. Transmission of HTLV-I by blood transfusion and its prevention by passive immunization in rabbits. Blood. 1990;76:1657–1661.

19. Cockerell GL, Lairmore MD, De B, Rovnak J, Hartley TM, Miyoshi I. Persistent infection of rabbits with HTLV-I: patterns of anti-viral antibody reactivity and detection of virus by gene amplification. Int J Cancer. 1990;45(1):127–130.

20. Sawada T, Iwahara Y, Isihi K, Taguchi H, Hoshino H, Miyoshi I. Immunoglobulin prophylaxis against milkborne transmission of HTLV-I in rabbits. J Infect Dis. 1991;164(6):1193–1196.

21. Miyoshi I, Takehara N, Sawada T, et al. Immunoglobulin prophylaxis against HTLV-I in a rabbit model. Leukemia. 1992;6(1):24–26.

22. Conrad SF, Byon IJ, DiGeorge AM, Lairmore MD, Taii MD, Kaumaya PT. Immunogenicity and conformational properties of an N-linked glycosylated peptide epitope of human T-lymphotropic virus type 1 (HTLV-I). Biomed Immunol. 1995;1(1):83–92.

23. Cockerell GL, Beebe M, Rose RT, Kaumaya PT, Schwendeman SP. Microencapsulation of a synthetic peptide epitope for HTLV-I in biodegradable poly(D, L-lactide-co-glycolide) microspheres using a novel encapsulation technique. J Microencapul. 2001;18(5):663–677.

24. Lairmore MD, Roberts B, Frank D, Rovnak J, Weiser MG, Cockerell GL. Comparative biological response of rabbits infected with human T-lymphotropic virus type I isolates from patients with lymphoproliferative and neurodegenerative disease. Int J Cancer. 1992;50(1):124–130.

25. Franchini G, Taggaglia J, Markham P, et al. Highly attenuated HTLV type I provirus vaccines induce protection against a cell-associated HTLV type I challenge in rabbits. AIDS Res Hum Retroviruses. 2005;21(5):307–313.

26. Haines R, Urbizondo Urbiztondo R, Hayes R, Niewiesk S, Lairmore MG. Development of a model of HTLV-I oral transmission in the rabbit. Retrovirology. 2011;8(Suppl 1):A19.

27. Kappan P, Yin H, Davis R, Lairmore MD, Fernandez S, Green PL. Distinct transmission tropism exhibited by human T-lymphotropic virus type 1 (HTLV-1) and HTLV-2 is the result of postinfection T-cell clonal expansion. J Virol. 2012;86(7):3757–3766.

28. Kinuta JT, Wong FH, Wang JJ, Ratner L. Construction and characterization of infectious human T-cell leukemia virus type I molecular clones. Virology. 1994;204(2):656–664.

29. Derse D, Mixovitov J, Polianova M, Felber BK, Ruscetti F. Virions released from cells transfected with a molecular clone of human T-cell leukemia virus type I give rise to primary and secondary infections of T cells. J Virol. 1995;69(5):1907–1915.
74. Tomaru U, Ikeda H, Jiang X, Ohya O, Yoshiki T. Provirus expansion and deregulation of apoptosis-related genes in the spinal cord of a rat model for human T-lymphotropic virus type I (HTLV-I)-induced myelopathy. J Neurovirol. 2003;9(3):530–538.

75. Miyake Y, Ikeda H, Ishizuru A, et al. Role of neuronal interferon-gamma in the development of myelopathy in rats infected with human T-cell leukemia virus type I. Am J Pathol. 2006;169(1):189–199.

76. Kausa T, Ikeda H, Tomaru U, et al. A rat model of human T lymphocyte virus type I (HTLV-I)-infected in situ detection of HTLV-I provirus DNA in microgliacerebrophagocytes in affected spinal cords of rats with HTLV-I-induced chronic progressive myeloneuropathy. Acta Neuropath. 1999;97(2):107–112.

77. Shikishima H, Ikeda H, Yamada S, et al. HTLV-I pX transgenic rats: development of cytotoxic-producing mammary carcinomas and establishment of the pX mammary carcinoma cell line. Leukemia. 1997;11 Suppl 3:70–72.

78. Takayama R, Ohashi T, Yoshida E, et al. Enhanced replication of human T-cell leukemia virus type 1 in T cells from transgenic rats expressing human CRM1 that is regulated in a natural manner. J Virol. 2007;81(11):5908–5918.

79. Ohta T, Hanabuchi S, Kato H, et al. Induction of adult T-cell leukemia-like lymphoproliferative disease and its inhibition by adoptive immunotherapy in T-cell-deficient nude rats inoculated with syngeneic human T-cell leukemia virus type I-immortalized cells. J Virol. 1999;73(7):6631–6640.

80. Kannagi M, Ohashi T, Hanabuchi S, et al. Immunological aspects of rat models of HTLV type I-infected T lymphoproliferative disease. AIDS Res Hum Retrovir. 2000;16(16):1737–1740.

81. Hanabuchi S, Ohashi T, Koya Y, et al. Regression of human T-cell leukemia virus type 1 (HTLV-I)-associated lymphomas in a rat model: peptide-induced T-cell immunity. J Natl Cancer Inst. 2001;93(23):1773–1783.

82. Nomura M, Ohashi T, Ishiguro N, et al. Retention of tax expression is associated both with resistance of human T-cell leukemia virus type 1-infected T cells to killing by tax-specific cytotoxic T lymphocytes and with impaired tumorigenicity in a rat model. J Virol. 2004;78(4):3827–3836.

83. Kusuda S, Mauds L, Fang J, Uchida K, Miwa M. Establishment of HTLV-I carrier rat cells by injection with HTLV-I-producing T cells. Leukemia. 1997;11 Suppl 3:260–262.

84. Fang J, Kusuda S, Feng R, et al. Transmission of human T-cell leukemia virus type 1 to mice. J Virol. 1998;72(5):3952–3957.

85. Fang R, Tanaka M, Abe H, et al. Human T-cell leukemia virus type 1 can infect a wide variety of cells in mice. J Virol. 1999;73(9):48–54.

86. Tanaka M, Sun B, Fang J, et al. Human T-cell leukemia virus type 1 (HTLV-I) infection of mice: proliferation of cell clones with integrated HTLV-I provirus in lymphoid organs. J Virol. 2001;75(9):4420–4423.

87. Tanaka M, Nitta T, Yoshida T, et al. Clonal proliferation of HTLV-I-infected cells is associated with spontaneous malignant tumor formation in mice. Int J Oncol. 2009;34(5):701–707.

88. Delebecque F, Pramberger K, Prévoit MC, Braich T, Tangy F. A chimeric human T-cell lymphoproliferative virus type 1 with the glycoprotein of human T-cell leukemia virus is infectious for murine cells. J Virol. 2002;76(15):7883–7889.

89. Delebecque F, Combrebet C, Gabet AS, Wattel E, Braich T, Tangy F. A chimeric human T-cell leukemia virus type I bearing a deltaretal Moloney-murine leukemia virus envelope infects mice persistently and induces humoral and cellular immune responses. J Virol. 2005;79(12):7518–7525.

90. Rahman S, Manuel SL, Khan ZK, et al. Depletion of dendritic cells enhances susceptibility to cell-free infection of human T cell leukemia virus type 1 in CD11c-diphtheria toxin receptor transgenic mice. J Immunol. 2010;184(1):40–49.

91. Tomasello JJ, Menon S, Wadsworth J, et al. Pancreatic and islet-specific expression of human T-cell leukemia virus type 1 in T cells from transgenic rats with diabetes. J Virol. 2011;85(5):2553–2557.

92. Kusuda S, Matsuda S, Seto A. Induction of leukemic infiltration by allogeneic HTLV-1 carrier rat. Jpn J Cancer Res. 1988;79:335–340.

93. Simpson RM, Leno M, Hubbard BS, Kindt TJ. Cutaneous manifestations of human T-cell leukemia virus type I infection in an experimental model. J Infect Dis. 1996;173(3):722–726.

94. Kushida S, Kojima Y, Nitta T, et al. Chronic progressive myeloneuropathy in WKAH rats induced by HTLV-I pX transgenic rats: development of cytotoxic-producing mammary carcinomas and establishment of the pX mammary carcinoma cell line. Leukemia. 1997;11 Suppl 3:70–72.

95. Ohya O, Yamashita I, et al. HTLV-I induced myeloneuropathy in WKAH rats: apoptosis and local activation of the HTLV-I pX and TNF-alpha gene in spinal cord tissue. Leukemia. 1995;9(3):339–406.

96. Ishiguro N, Abe M, Seto K, et al. A rat model of human T lymphocyte virus type I (HTLV-1)-associated myelopathy. J Virol. 1992;66(3):1114–1146.

97. Sugita T, Kameyama T, Kinoshita T, et al. Infection of rats with HTLV-I: a small-animal model for HTLV-I carriers. Int J Cancer. 1991;49(5):764–769.

98. Iwahiguro N, Abe M, Seto K, et al. A rat model of human T lymphocyte virus type I (HTLV-1)-associated myelopathy. J Immunol. 1992;149(3):1008–1011.

99. Taguchi H, Sawada T, Fukushima A, et al. Bilateral uveitis in a rabbit experimentally infected with HTLV-I. Lab Invest. 1993;69(3):336–339.

100. Ohya O, Tomaru U, Yamashita I, et al. HTLV-I induced myeloneuropathy in WKAH rats: apoptosis and local activation of the HTLV-I pX and TNF-alpha gene in spinal cord tissue. Leukemia. 1995;9(3):339–406.

101. Silverman LR, Phipps AJ, Montgomery A, Ratner L, Lairmore MD. Human T-cell lymphotropic virus type 1 open reading frame II-encoded p30II is required for in vivo replication: evidence of in vivo reversion. J Virol. 2004;78(8):3837–3845.

102. Kimura M, Kawamura K, et al. Induction of myeloneuropathy of T-cell leukemia virus type I in rats by intravenous injection of HTLV-I-producing rabbit cells. Leukemia. 1995;9(3):339–406.

103. Panfil J, Pleskac V, et al. HTLV-I infection of T lymphocytes in a rabbit model with a molecular clone of human T-cell lymphotropic virus type I. J Virol. 1996;70(7):7241–7246.

104. Ye J, Silveirman L, Lairmore MD, Green PL. HTLV-I X is required for viral spread and persistence in vivo but is dispensable for cellular immortalization in vitro. Blood. 2003;102(12):3963–3969.

105. Seto A, Kawasaki M, Matsuda S, Ohya O, Yoshihara A. Provirus expansion and deregulation of apoptosis-related genes in the spinal cord of a rat model for human T-lymphotropic virus type I (HTLV-I)-associated myelopathy. J Neurovirol. 2003;9(3):530–538.

106. Silverman LR, Phipps AJ, Montgomery A, Ratner L, Lairmore MD. Human T-cell lymphotropic virus type I open reading frame II-encoded p30II is required for in vivo replication: evidence of in vivo reversion. J Virol. 2004;78(8):3837–3845.

107. Xie L, Yamamoto B, Haoudi A, Semmes OJ, Green PL. PDZ binding motif of HTLV-1 Tax promotes virus-mediated T-cell proliferation in vitro and persistence in vivo. Blood. 2006;107(5):1980–1988.

108. Seto A, Kawasaki M, Matsuda S, Oyga K, Miyoshi I. Adult T-cell leukemia-like disease experimentally induced in rats. Jpn J Cancer Res. 1998;79:335–341.

109. Zhao TM, Robinson MA, Bowers FS, Kinchin TJ. Characterization of an infectious molecular clone of human T-cell leukemia virus type I. J Virol. 1995;69(4):2024–2030.

110. Collins ND, Newbound GC, Ratner L, Lairmore MD. In vitro CD4+ lymphocyte transformation and infection in a rabbit model with a molecular clone of human T-cell lymphotropic virus type I. J Virol. 1996;70(7):7241–7246.
108. Guimaraes-Correa AB, Crawford LB, Figueiredo CR, et al. C7a, a biphosphonic cyclopalladated compound, efficiently controls the development of a lymphoproliferative disease induced by human T-cell leukemia virus type 1-infected cell lines in novel NOD-SCID/gamma(null) mice. Int J Cancer. 2003;103(6):528–534.

109. Zhang Z, Zhang M, Ravetch JV, Goldman C, Waldmann TA. Effective therapy for a murine model of adult T-cell leukemia with the humanized anti-CD2 monoclonal antibody. J Virol. 2003;77(20):10425–10431.

110. Guimaraes-Correa AB, Crawford LB, Figueiredo CR, et al. C7a, a biphosphinic cyclopalladated compound, efficiently controls the development of a patient-derived xenograft model of adult T cell leukemia/lymphoma. Viruses. 2011;3(7):1041–1058.

111. Iwakura Y, Tosu M, Yoshida E, et al. Induction of inflammatory arthritis resembling rheumatoid arthritis in mice transgenic for HTLV-I. Arthritis Rheum. 1991;34(5):1234–1239.

112. Iwakura Y, Tosu M, Yoshida E, et al. Induction of inflammatory arthritis resembling rheumatoid arthritis in mice transgenic for HTLV-I. Arthritis Rheum. 1991;34(5):1234–1239.

113. Kozako T, Fukada K, Hirata S, et al. Efficient induction of human T-cell leukemia virus type 1-specific CTL by chimeric peptide without adjuvant as a prophylactic for adult T-cell leukemia. Mol Immunol. 2007;44(6-7):606–613.

114. Ikeda E, Kagawauchi A, Tsuchida K, et al. Oral administration of an SDF-1alpha/CXCR4 axis by the CXCR4 antagonist AMD3100 suppresses the migration of cultured cells from ATL patients and murine lymphoblastoid cells from HTLV-I transgenic mice. Blood. 2009;114(14):2961–2968.

115. Nitta T, Tanaka M, Sun B, et al. Reduction of human T-cell leukemia virus type-I in HTLV-1 transgenic mice by the therapeutic antibody on arthritis in HTLV-1 tax transgenic mice. Blood. 2004;103(4):802–809.

116. Boulignier JP, Gervois A, Rotter M, et al. Effective induction of human T-cell leukemia virus type-1-specific CTL by chimeric peptide without adjuvant as a prophylactic for adult T-cell leukemia. Mol Immunol. 2007;44(6-7):606–613.

117. Iwakura Y, Tosu M, Yoshida E, et al. Induction of inflammatory arthritis resembling rheumatoid arthritis in mice transgenic for HTLV-I. Arthritis Rheum. 1991;34(5):1234–1239.

118. Iwakura Y, Tosu M, Yoshida E, et al. Induction of inflammatory arthritis resembling rheumatoid arthritis in mice transgenic for HTLV-I. Arthritis Rheum. 1991;34(5):1234–1239.

119. Kozako T, Fukada K, Hirata S, et al. Efficient induction of human T-cell leukemia virus type 1-specific CTL by chimeric peptide without adjuvant as a prophylactic for adult T-cell leukemia. Mol Immunol. 2007;44(6-7):606–613.

120. Iwakura Y, Tosu M, Yoshida E, et al. Induction of inflammatory arthritis resembling rheumatoid arthritis in mice transgenic for HTLV-I. Arthritis Rheum. 1991;34(5):1234–1239.

121. Iwakura Y, Tosu M, Yoshida E, et al. Induction of inflammatory arthritis resembling rheumatoid arthritis in mice transgenic for HTLV-I. Arthritis Rheum. 1991;34(5):1234–1239.

122. Hall AP, Irvine J, Blyth K, Cameron ER, Onions DE, Campbell ME. Tumours derived from HTLV-I tax transgenic mice are characterized by enhanced levels of apoptosis and oncogene expression. J Pathol. 1998;186(2):209–214.

123. Hasegawa H, Sawa H, Lewis MJ, et al. Thymus-derived leukemia/lymphoma in mice transgenic for the Tax gene of human T-lymphotropic virus type I. Nat Med. 2006;12(4):446–447.

124. Watters KM, Dean J, Hasegawa H, Sawa H, Hall W, Sheehy N. Cytokine and growth factor expression by HTLV-I Lck-tax transgenic cells in SCID mice. AIDS Res Hum Retroviruses. 2010;26(5):593–603.

125. Portis T, Grossman WJ, Harding JC, Hess JL, Ratner L. Analysis of p53 inactivation in a human T-cell leukemia virus type 1 Tax transgenic mouse model. J Virol. 2001;75(5):2185–2193.

126. Zane I, Yasunaga J, Mitamgi Y, et al. Wp1 and p53 contribute to HTLV-I Tax-induced tumorigenesis. Retrovirology. 2012;9:114.

127. Grossman WJ, Ratner L. Cytokine suppression and tumorigenicity of large granular lymphocytic leukemia cells from mice transgenic for the tax gene of human T-cell leukemia virus type I. Blood. 1997;90(2):783–794.

128. Ohshu T, Takahashi M, Morikawa S, et al. Invasion of histiocytic sarcoma into the spinal cord of HTLV-I tax transgenic mice with HTLV-I-associated myeloproliferative syndrome in patients. Med Oncol Res. 2013;3:413.

129. Menard JM, Barbeau B, Devaux C, HBZ, a new player in the mystery of adult T-cell leukemia. Blood. 2006;108(13):3979–3982.

130. Murata K, Hayashibara T, Sugahara K, et al. A novel alternative splicing isoform of human T-cell leukemia virus type I BZIP factor (HBZ-S4) targets distinct subcellular localization. J Virol. 2006;80(9):4344–4344.

131. Mitra-Kaushik S, Harding JC, Hess JL, Ratner L. Effects of the pro tease inhibitor PSI-341 on tumor growth in HTLV-I transgenic mice and Tumor transplants. Blood. 2004;104(3):802–809.

132. Sugata K, Satou Y, Yasunaga J, et al. HTLV-I BZIP factor impairs cell-mediated immunity by suppressing production of Th1 cytokines. Blood. 2012;119(11):2129–2136.

133. Iwakura Y, Tosu M, Yoshida E, et al. Induction of inflammatory arthritis resembling rheumatoid arthritis in mice transgenic for HTLV-I. Science. 1991;253(5023):1026–1028.

134. Ikebe E, Kagawauchi A, Tsuchida K, et al. Oral administration of an HSFP90 inhibitor, 17-DMAG, inhibits tumor-cell infiltration into multiple organs and improves survival period for ATL model mice. Blood. Cancer J. 2013;3:e132.

135. Kozako T, Fukada K, Hirata S, et al. Efficient induction of human T-cell leukemia virus type 1-specific CTL by chimeric peptide without adjuvant as a prophylactic for adult T-cell leukemia. Mol Immunol. 2009;46(3-4):606–613.

136. Ikeda E, Kagawauchi A, Tsuchida K, et al. Oral administration of an HSFP90 inhibitor, 17-DMAG, inhibits tumor-cell infiltration into multiple organs and improves survival period for ATL model mice. Blood. Cancer J. 2013;3:e132.

137. Kozako T, Fukada K, Hirata S, et al. Efficient induction of human T-cell leukemia virus type 1-specific CTL by chimeric peptide without adjuvant as a prophylactic for adult T-cell leukemia. Mol Immunol. 2009;46(3-4):606–613.

138. Iwakura Y, Tosu M, Yoshida E, et al. Induction of inflammatory arthropathy resembling rheumatoid arthritis in mice transgenic for HTLV-I. Science. 1991;253(5023):1026–1028.