μ-Lat: A High-Throughput Humanized Mouse Model for Preclinical Evaluation of Human Immunodeficiency Virus Eradication Strategies

Hannah S. Sperber[1,2,3*], Padma Priya Togarrati[1*], Kyle A. Raymond[1,3], Mohamed S. Bouzidi[1,3], Renata Gilfanova[1], Alan G. Gutierrez[1], Marcus O. Muench[1,3] and Satish K. Pillai[1,3+]

[1]Vitalant Research Institute, San Francisco, California, United States of America

[2]Free University of Berlin, Institute of Biochemistry, Berlin, Germany

[3]University of California, San Francisco, California, United States of America

*Hannah S. Sperber and Padma Priya Togarrati contributed equally to this work. Author order was determined both alphabetically and in order of increasing seniority.

+Corresponding author, e-mail: satish.pillai@ucsf.edu

Conflict of interest statement:

The authors have declared that no conflict of interest exists.

Keywords: Humanized mouse, HIV, HIV latency, shock and kill, antiviral gene therapy

Abstract

A critical barrier to the development of a human immunodeficiency virus (HIV) cure is the lack of an appropriate and scalable preclinical animal model that enables robust evaluation of candidate eradication approaches prior to testing in humans. Humanized mouse models typically involve engraftment of human fetal tissue and currently face ethical and political challenges. We established a fetal tissue-free humanized model of latent HIV infection, by transplanting "J-Lat" cells, Jurkat cells harboring a latent HIV provirus encoding an enhanced green fluorescent protein (GFP) reporter, into irradiated adult NOD.Cg-*Prkdc*^{scid} *Il2rg*^{tm1Wjl}/SzJ (NSG) mice. J-Lat cells exhibited successful engraftment in several tissue sites, including spleen, bone barrow, peripheral blood, and lung, mirroring the diverse tissue tropism of HIV in the human host. Administration of tumor necrosis factor (TNF)-α, an established HIV latency reversal agent, significantly induced GFP expression in engrafted cells across tissues (as measured by flow cytometry at necropsy), reflecting viral reactivation. These data suggest that the "**μ-Lat**" model enables efficient determination of how effectively viral eradication agents, including latency reversal agents, penetrate and function in diverse anatomical sites harboring HIV *in vivo*.

Importance

The search for an HIV cure has been impeded by the lack of convenient and cost-effective preclinical animal models that allow us to evaluate candidate cure approaches prior to testing in humans. In this study, we aim to address this critical gap and present a novel, highly scalable and standardizable HIV-infected humanized mouse model that enables rigorous preclinical testing of HIV cure strategies. In addition to basic safety and tolerability data, the model provides detailed information describing an agent's therapeutic efficacy as it navigates through tissue-specific barriers and the circulatory, respiratory and excretory systems. Development and dissemination of our humanized mouse platform will help us to move promising HIV cure approaches, including HIV latency reversal and anti-HIV gene therapy strategies, from the laboratory into the clinic.

Introduction

The advent of antiretroviral therapy (ART) has dramatically reduced morbidity and mortality for human immunodeficiency virus (HIV)-infected individuals with access to healthcare in resource-rich countries. However, despite years of potent therapy, eradication of infection is not achieved due to the persistence of HIV latently-infected cells during treatment(1). Accumulating evidence suggest that "non-AIDS" cardiovascular, renal and hepatic diseases are amplified by HIV infection, and the immune system may exhibit premature senescence even among patients with complete viral suppression(2). Moreover, although enormous progress has been made to provide ART in resource limited settings, there are huge economic, political and operational challenges to reach the goal of universal access to lifelong treatment. These realities have created a pronounced interest in developing HIV cure strategies.

Elimination of the latent HIV reservoir is critical to achieving HIV eradication *in vivo*. A number of approaches are currently under investigation, including therapeutic vaccination, immunomodulatory approaches, ART intensification, therapeutic HIV latency reversal (the "shock and kill" strategy), as well as a number of gene therapy approaches(3,4). In all scenarios, it will be critical to have proper diagnostic tools and models in place to comprehensively evaluate performance and safety prior to deployment in a clinical setting. A critical barrier to the development of an HIV cure is the lack of an appropriate, accessible, and scalable preclinical animal model that enables robust evaluation of candidate eradication approaches prior to testing in humans(5). As a result, many promising curative approaches never graduate past the petri dish stage. Infection of nonhuman primates (NHP) with simian immunodeficiency virus (SIV) is an option and has been utilized extensively to study HIV/AIDS pathogenesis(6,7). Recent

advancements have been made in optimizing ART regimens to achieve durable virus suppression and thus enable evaluation of HIV cure strategies in the SIV-NHP model(8–10). However, biological limitations remain since this model uses SIV and might not recapitulate human host-HIV interactions and HIV latency mechanisms(11–15). In addition, NHP experiments involve complex ethical considerations, and the high costs and labor requirements only allow small numbers of animals to be utilized in any given trial, limiting statistical power and generalizability(11).

Mouse models represent another alternative with lower costs, more convenient husbandry requirements, as well as greater scalability. In the context of HIV studies, a wide range of small animal models have been developed comprising knockout mouse models(16–18), transgenic mouse(19–23) and humanized mouse models(6,24–29). Humanized mice are established by xenotransplantation of human cells or tissues in immunodeficient mouse strains. Most strains used in HIV research are derivatives of severe combined immunodeficient (SCID) mice, which harbor mutations in the gene coding for a DNA-dependent protein kinase catalytic subunit (Prkdc). These mice are “humanized” using two approaches: 1) human cells are injected with or without prior irradiation of mice or 2) portions of tissue are surgically implanted. Different cells have been used for injection comprising human peripheral blood mononuclear cells (PBMCs), as in the *hu-PBL-SCID* mouse model(30), obtained from healthy(31) or HIV-infected ART suppressed individuals(32), or human hematopoietic stem cells (HSCs), as in the *hu-HSC* mouse model(33), or the more recently developed *T-cell-only*(34) and *myeloid-only*(35) mouse models (*ToM* and *MoM*). Implantation of fetal thymus and liver tissue fragments are used for the *SCID-hu thy/liv*(36,37) and *bone marrow/liver/thymus* (BLT)(38,39) mouse models. Although all these

model systems have contributed to our understanding of HIV pathogenesis and persistence, key limitations remain that need to be addressed in order to fully exploit the potential of these small animal models in HIV cure research. *Hu-PBL-SCID* mice struggle with the development of graft versus host disease (GVHD) which renders this model inapplicable for long-term studies involving HIV persistence. The generation of *SCID-hu thy/liv* mice and BLT mice is limited due to the need for surgical implantation and a limited supply of tissue. Moreover, these models as well as *hu-HSC*, *ToM* and *MoM* mice rely on the engraftment of cells or tissues typically derived from human fetal specimens, which in light of recent changes in federal policies, now face significant challenges, as ethical, legal, and political considerations surrounding the use of fetal tissue in scientific research have made it increasingly difficult to obtain such material(40,41). A major limitation shared by all these models remains the low frequency of HIV-latently infected cells, which impacts the applicability of these models as robust preclinical *in vivo* test bases for HIV cure strategies.

In the present study, we therefore pursued a new approach and transplanted J-Lat 11.1 cells (J-Lat cells), Jurkat cells harboring a latent HIV provirus encoding an enhanced green fluorescent protein (GFP) reporter, into irradiated adult NOD.Cg-*Prkdc*^{scid} *Il2rg*^{tm1Wjl}/SzJ (NSG) mice. By applying an established and widely-utilized HIV latency reporter cell line(42–44), we circumvent the need for fetal or any other donor-derived tissue, and achieve high frequencies of HIV latently-infected cells *in vivo* on a short experimental time scale. In addition, the presence of the GFP reporter cassette in the integrated viral genome provides for quick and convenient assessment of viral reactivation using flow cytometry or microscopy. Our data show robust engraftment of J-Lat cells in several tissues three weeks after injection as well as significant reactivation of these

latently infected cells *in vivo* upon intravenous administration of the established latency reversal agent (LRA) tumor necrosis factor (TNF)-α. We hereby show that our "μ-Lat" humanized mouse model enables rapid and efficient testing of HIV latency reversal approaches *in vivo*. Although not intended to serve as a pathophysiology model, we present this approach as a scalable, accessible, and cost-effective preclinical testbed to evaluate the safety, tolerability, and performance of HIV cure strategies in distinct anatomical niches.

Results

The human cell surface proteins CD147 and CD29 enable clear and reliable discrimination of J-Lat cells from mouse cells. Our model involves transplantation of J-Lat cells (immortalized human T cells harboring latent HIV provirus with a GFP reporter reflecting viral transcriptional activity) into irradiated adult NSG mice(42). We therefore searched for cell surface markers that reliably identify engrafted J-Lat cells in a background of mouse cells, exhibiting three key features: 1) universal expression across J-Lat cells, 2) high per-cell expression on J-Lat cells, and 3) absence of expression on the surface of mouse cells.

Our candidate panel included seven cell surface proteins commonly known to be expressed in human CD4+ T cells: CD45, CD4, TCR α/β, CD27, CD147, CD29, and HLA-ABC. Among these, four cell surface markers were found to be expressed universally among the J-Lat population: CD45, CD147, CD29 and HLA-ABC (Fig. 1A). The mean fluorescence intensity (MFI) of these four proteins was measured, reflecting the relative abundance of each protein on the cell surface. CD147 exhibited the highest MFI, followed by CD29 (Fig. 1B). We next tested if the CD29 and CD147 antibodies (used in combination to achieve maximum signal to noise ratio in the APC-channel) showed detectable binding to mouse cells obtained from bone marrow (BM), spleen, lung, and peripheral blood (PB). In preparation for subsequent engraftment experiments, PB harvest was performed in three different ways: by retro-orbital bleed (r.-o.), tail vein bleed, or heart bleed, based on Hoggatt et al.(45), who reported that blood parameters including cell composition significantly varied depending on sampling manner and site. Additional staining with antibodies targeting mouse-specific lineage markers CD45, TER-119, and H-2K^{d} was performed for a positive identification of mouse cells. While 100% of cultured J-Lat cells expressed CD29 and CD147, the

frequency of $CD147^+/CD29^+$ cells was negligible or absent across mouse tissues (Fig. 1C). These results show that among the tested cell surface proteins, human CD147 combined with CD29 function as a reliable and specific marker pair for the identification of J-Lat cells engrafted in mouse tissues.

J-Lat cells engraft in several tissues three weeks post transplantation in NSG mice. We initially examined the effects of varying cell doses and irradiation of mice prior to cell transplantation on the kinetics of J-Lat cell engraftment in mouse tissues. We determined that irradiation of recipient mice and a cell dose of 10×10^6 J-Lat cells per mouse administered through intravenous injection resulted in efficient and reproducible cell engraftment, which peaked at 3 weeks following cell transplantation (data not shown). Using these experimental conditions, we first investigated J-Lat cell engraftment levels and background GFP expression upon injection in 5 mice. Single live cells were gated for the analysis of the expression of J-Lat markers on harvested mice tissues (Fig. 2A). Engraftment of J-Lat cells was observed in the BM (mean = 35.2%), lung (mean = 6.1%), and spleen (mean = 0.6%) along with a GFP background signal not exceeding 5% in these tissues (Fig. 2B). Regarding PB harvest, highest engraftment levels of J-Lat cells were found after heart bleed with an average of 21.4%, followed by r.-o. bleed with a mean of 15%, and GFP background signals less than 2%, while on average 0.5% engrafted J-Lats were detected after tail vein bleed without exhibiting GFP background signal (Fig. 2C). Due to considerable engraftment levels and practicality of collection, we decided to harvest PB in subsequent experiments via r.-o. bleeding. We thus established a suitable engraftment and sample collection protocol to drive the model (Fig. 3).

TNF-α treatment leads to GFP expression in tissue engrafted J-Lat cells reflecting reactivation of latent provirus *in vivo*. Following refinement of the protocol for J-Lat engraftment in NSG mice, we sought to determine if HIV LRA administration would result in viral reactivation *in vivo*. Viral reactivation was measured as %GFP expressing cells within engrafted cells following LRA treatment (Fig. 3).

The proviruses within the J-Lat family of clones were selected to be responsive to TNF-α stimulation, resulting in viral LTR-driven GFP expression(42). 24h *in vitro* treatment of J-Lat cells with 20 ng/µl TNF-α yielded up to 40.8% GFP-positive cells compared to 63.2% GFP-positive cells upon 20 nM PMA / 1 µM Ionomycin treatment as positive control, whereas mock-treated cells (0.5% DMSO) were largely GFP-negative (Fig. 4A). Therefore, we performed *in vivo* reactivation experiments using 20 µg of TNF-α as an LRA (with PBS as a vehicle control)(46). 24h following TNF-α tail vein injection, effects on tissue engraftment and reactivation of latent provirus were estimated in a cross-sectional manner at necropsy, comparing 9 animals treated with TNF-α versus 10 vehicle control animals. Analyses of all tissues demonstrated no significant effect of TNF-α treatment on engraftment within the respective compartment (Fig. 4B) but revealed significant increases in the frequency of GFP-positive cells, reflecting viral reactivation (Fig. 4C). Engrafted J-Lat cells obtained from TNF-α treated mice exhibited on average 16.4% GFP-positive cells in the BM, 22.3% in spleen, 21.9% in the lung, and 21.8% in PB (Fig. 4C). In contrast, engrafted J-Lat cells obtained from mice treated with PBS as vehicle control comprised 6.9% GFP-positive cells in the BM, 6.1% in spleen, 2.2% in the lung and 1.6% in PB (Fig. 4C). Comparing these two populations (TNF-α vs vehicle control) demonstrated a GFP expression fold-change of 2.3 in the BM (adjusted p-value = 0.02), 3.7 in the spleen (adjusted p-value < 0.0001), 10 in the lung

(adjusted p-value < 0.0001) and 13.6 in the PB (adjusted p-value < 0.0001), illustrating potent and significant viral reactivation *in vivo*.

Discussion

The development of an HIV cure will be accelerated by the deployment of a convenient and cost-effective preclinical animal model that enables determination of an agent's therapeutic efficacy as it navigates through tissue-specific barriers and the circulatory, respiratory and excretory systems. We present a novel humanized mouse model of HIV latency that aims to address this gap. Although not intended to serve as a pathophysiology model, the μ-Lat platform offers key advantages over existing preclinical models focused on HIV eradication. Firstly, the model is highly efficient; the timeframe to generate mouse colonies that are ready for administration of experimental therapies is approximately three weeks. In comparison, even the simplified humanized mouse model introduced by Kim et al.(31) requires four weeks for robust engraftment of intraperitoneally injected human PBMCs in NSG mice and an additional 5 weeks of incubation upon HIV infection. Secondly, the presence of the GFP reporter cassette in the integrated J-Lat viral genome allows for rapid and convenient assessment of HIV latency reversal *in vivo*, eliminating the requirement for PCR or culture-based diagnostics to determine LRA potency. Thirdly, the model is characterized by high frequencies of HIV latently-infected cells (e.g. exceeding 30% engraftment in BM), and these cells are distributed in a number of relevant anatomical sites that are central to HIV persistence. This stands in contrast to organoid-based models (e.g. the *SCID-hu thy/liv* mouse model) that exclusively involves viral colonization within a xenografted tissue(47). Lastly, the model is highly scalable due to the low costs and limited labor requirements of the method involving the easily and widely available J-Lat cell line (provided free of charge via the NIH AIDS Reagent Program), and the clonal nature of these cells promotes consistency and reproducibility across laboratories.

Similar to any other animal model system, there are certain caveats associated with the μ-Lat model that should be considered. The efficiency of the model is largely driven by the administration of an HIV latently-infected cell line, rather than differentiated primary cells. As presented here, a single latent clone with a single proviral integration site was injected into mice. Proviral integration site is known to affect viral latency and responsiveness to LRA administration(48–50). However, this issue could be ameliorated by mixing different HIV latently-infected cell clones at specific ratios, thereby achieving higher integration site heterogeneity to more accurately represent *in vivo* variability. For instance, there are 11 J-Lat clones currently available via the NIH AIDS Reagent Program, each of which is characterized by a distinct proviral integration site. These clones often behave differently from each other as well when exposed to LRAs *in vitro*, likely reflecting a diversity of molecular mechanisms reinforcing viral latency across clones(43). This mechanistic diversity could further enhance the predictive potential of the μ-Lat model.

Beyond concerns regarding integration site heterogeneity, the immortalized nature of the J-Lat cell line may impact molecular and regulatory pathways that affect HIV latency. However, the widespread usage of the J-Lat model and its derivative clones to examine viral latency and to evaluate the efficacy of HIV cure strategies *in vitro* speak to the model's utility(51–60). Importantly, ample data from primary cell-based models of latency and experiments involving *ex vivo* administration of LRAs to cells obtained from HIV-infected individuals on ART suggest that differences between applied models and between individuals can have dramatic effects on the establishment, maintenance, and reversal of HIV latency(43,61,62). Moreover, profiling of HIV latency in multiple tissue sites has demonstrated that the nature of viral latency may even vary

extensively within a single infected individual(63–65). Therefore, it needs to be considered that any applied model system or cells from any given individual will influence predicted responses for the general population with a certain bias just as a cell line-based system(26).

Our work described here represents a proof of concept, demonstrating that the engraftment of a cell line-based model of HIV latency may constitute a useful testbed for HIV cure strategies. This general approach is highly versatile and should allow for a broad range of infected cell types to be examined *in vivo*. For example, HIV latency in the myeloid compartment is likely critical to viral persistence(35,66–69), and multiple reports suggest this compartment may respond quite differently to curative approaches, as compared to lymphoid reservoirs(70,71). It may be fruitful to inject the U1 HIV chronically-infected promonocytic cell line(72) into NSG mice to examine LRA responses in myeloid cells. Building further on the myeloid theme, the central nervous system (CNS) compartment is an important viral sanctuary site in the setting of ART(71,73–76), and HIV eradication approaches will almost certainly face unique challenges in this niche(76,77). As direct injection of human cells into the murine CNS has been used successfully as an engraftment approach(78–82), site-specific injection of the recently developed HC69.5 HIV latently-infected microglial cell line(83,84) into the brains of NSG mice may provide a convenient platform to gauge CNS-focused cure strategies.

Beyond preclinical investigation of LRAs, the μ-Lat framework may provide a convenient model system to evaluate gene therapy-based HIV eradication approaches. Gene therapy approaches targeting HIV infection generally fall into two categories: 1) Gene editing can be used to target or "excise" the HIV provirus directly in infected cells as an eradication approach(85,86) or 2) Editing can be used to modulate host cells to render them refractory to HIV infection and/or potentiate

antiviral immune responses(87). In the former case, *in vivo* delivery of the gene therapy modality will likely be necessary to pervasively attack the HIV reservoir within a broad range of tissue sites in infected individuals. The μ-Lat model is well-suited to investigate gene delivery in this context, as it is characterized by robust engraftment of HIV latently-infected cells into diverse anatomical niches. This will allow for efficient assessment of gene therapy vector dissemination and antiviral function across anatomic sites. The LTR-driven GFP cassette within the J-Lat integrated provirus may facilitate this assessment; direct targeting of the GFP sequence may be used to examine vector trafficking and delivery, while specific targeting of the HIV LTR as a cure approach would be associated with a convenient readout (relative loss of GFP expression upon induced latency reversal).

In summary, the μ-Lat model is optimized for efficient and scalable evaluation of select HIV eradication approaches *in vivo*, allowing determination of therapeutic efficacy in addition to essential safety, tolerability, and pharmacokinetic parameters. Further development and diversification of the μ-Lat model system may enable convenient preclinical testing of HIV eradication approaches, including antiviral gene therapy strategies, in a range of cell types and tissue sites *in vivo*.

Materials and Methods

Cell culture and treatment: J-Lat 11.1 cells (kindly provided by Dr. Eric Verdin's lab) contain integrated latent full-length HIV genome harboring a mutation in the *env* gene and GFP in place of the *nef* gene as reporter for transcriptional activity of the provirus(88). J-Lat 11.1 cells were grown in media composed of RPMI 1640 (Gibco) supplemented with 10% fetal bovine serum (FBS) (Corning) and 1% Penicillin-Streptomycin (Gibco). Cells were cultured at 37°C in a humidified incubator containing 5% CO_2. To test potency of latency reversal agents (LRAs), 1 x 10^6 cells in 1 ml RPMI were incubated in 0.5% DMSO (negative control), 20 nM PMA/1 µM Ionomycin (positive control) or 20 ng/µl TNF-α for 24h.

Mice: The work was approved by the Institutional Animal Care and Use Committee guidelines at Covance Laboratories, Inc. (San Carlos, CA) under Animal Welfare Assurance A3367-01 and protocol number IAC 2185 / ANS 2469. Animal husbandry was carried out according to the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. Mice were sacrificed in accordance with the guidelines from the American Veterinary Medical Association.

Adult female and male mice (≥8 weeks old) were included in this study and were maintained at the Vitalant Research Institute (VRI). Breeding pairs of NSG mice were obtained from Jackson Laboratories (Bar Harbor, ME), and were bred and maintained at VRI in a vivarium free from >40 murine pathogens as determined through biannual nucleic acid testing (Mouse Surveillance Plus PRIA; Charles River) of sentinel mice exposed to mixed bedding. Mice were maintained in sterile, disposable microisolator cages (Innovive, Inc.), which were changed every 14 days.

Environmental enrichment was provided by autoclaved cotton Nestlets (Ancare Corp.) and GLP-certified Bio-Huts (Bio-Serv). Feed consisted of sterile, irradiated diet of Teklad Global 19% protein diet (Envigo) with free access to sterile-filtered, acidified water (Innovive, Inc.). Several days prior to radiation and 3 weeks following radiation, mice were fed with irradiated Global 2018 rodent diet with 4100 ppm Uniprim® (Envigo).

J-Lat cell surface marker staining: For each staining, 1 x 10^6 J-Lat cells were washed once with PBS (Gibco), resuspended in 100 µl PBS, and stained with Zombie dye (Cat. #423105, BioLegend) according to manufacturer's protocol (1:100 dilution) to enable subsequent discrimination between live and dead cells. 10 min after incubation with the Zombie dye, human CD45-PE (clone HI30, Cat. #304039, BioLegend), CD4-PE (clone OKT4, Cat. #317410, BioLegend), TCR α/β-PE (clone IP26, Cat. #306708, BioLegend), CD27-PE (clone M-T271, Cat. #356406, BioLegend), CD147-APC (clone HIM6, Cat. #306214, BioLegend), CD29-APC (clone TS2/16, Cat. #303008, BioLegend), and HLA-ABC-APC/Cy7 (clone W6/32, Cat. #311426, BioLegend) antibodies were added respectively, and were incubated for another 20-30 min at room temperature (RT) in the dark. Cells were then washed with 2 ml of cell staining buffer (Cat. #420201, BioLegend), resuspended in 300 µl PBS and measured using an LSR II flow cytometer (BD Biosciences).

J-Lat cell transplantation into mice: Mice were irradiated with 175 cGy radiation dose using a RS2000 X-Ray Biological Irradiator (RAD Source Technologies, Inc.) 3 hours prior to cell transplantation. Mice were transplanted with 10 x 10^6 J-Lat cells in a volume of 200 µl by tail-vein injection. J-Lat cell transplanted mice as well as control mice (left untransplanted) were sacrificed and analyzed within 25 days of cell transplantation (when optimal engraftment levels were achieved in our preliminary experiments).

Engraftment analysis of J-Lat cells in mice by flow cytometry: Transplanted mice were sacrificed by cervical dislocation 3 weeks post-injection. BM, spleen, lung tissues and PB were harvested. Single cell suspensions from the BM, spleen, and PB were prepared as described previously by Beyer et al.(89). Lung specimens were processed as follows: after harvest, lung samples were stored in PBS on ice. Lung specimens were washed twice with PBS and then cut into 3-4 mm^2 pieces in a petri dish in 1 ml digestion solution consisting of 1 mg/ml DE Collagenase (Cat. #011-1040, VitaCyte) and 100 U/ml DNase I (Cat. #D5025-15KU, Sigma-Aldrich) final concentration in HBSS (Gibco). Lung fragments were transferred to 50 ml falcon tubes, digestion solution was added to a final volume of 5 ml, and samples were incubated at 37°C for 50 min. Afterwards, 5 ml of stop solution, consisting of 0.5% BSA (Cat. #A2153, MilliPoreSigma) and 2 mM EDTA (Cat. #E0306, Teknova) final concentration in PBS, was added to each sample to end the enzymatic digestion reaction. Single cell suspensions were prepared by passage through a 70 µm cell strainer and washed with PBS. For the analysis of J-Lat cell engraftment, single cells from harvested mouse tissues were stained with human CD147-APC, human CD29-APC, mouse CD45-Pacific Blue (clone 30-F11, Cat. #103126, BioLegend), TER-119-Pacific Blue (clone TER-119, Cat. #116232, BioLegend), and H-2K^D-Pacific Blue antibodies (clone SF1-1.1, Cat. #116616, BioLegend) and incubated for 30 min at RT in the dark. Zombie dye staining detected in the APC-Cy7 channel was used for discrimination of live and dead cells. Following staining, cells were washed and run on an LSR II flow cytometer (BD Biosciences). Flow cytometry data were analyzed using FlowJo software (FlowJo, Inc.).

Treatment of J-Lat engrafted mice with latency reversal agent TNF-α: Mice engrafted with J-Lat cells were treated with recombinant human TNF-α (Cat. #PHC3011, Gibco), a potent LRA. Briefly,

NSG mice were transplanted with J-Lat cells and 3 weeks post-transplantation, mice received TNF-α (diluted in PBS) intravenously at a dose of 20 µg/mouse(46). NSG mice injected with J-Lat cells and treated with PBS were used as vehicle control group.

After 24h of TNF-α treatment, mice were sacrificed to determine viral reactivation in PB, BM, lung and spleen tissues. Latency reversal of the HIV provirus was analyzed by comparing GFP expression of J-Lat cells in tissues of TNF-α treated mice vs the vehicle control group. GFP expression of engrafted J-Lat cells was determined by flow cytometry.

Data analysis: All data were analyzed using GraphPad Prism version 8.2 and are presented as mean ± the standard error of the mean (SEM). Outliers were identified following the software's recommendation by applying the ROUT method with Q = 1%. One-way (column statistics) or two-way ANOVA (grouped statistics) Sidak's multiple comparisons test was used for statistical analyses. An adjusted p-value≤0.05 was considered as statistically significant. *, **, *** and **** in the graphs represent adjusted p-value ≤0.05, adjusted p-value ≤0.01, adjusted p-value ≤0.001 and adjusted p-value ≤0.0001, respectively.

Author contributions

HSS, PPT, KAR, MSB conceived and performed experiments; PPT, RG, AGG, MOM performed *in vivo* experiments; HSS, PPT collected and analyzed the data; HSS, PPT, MOM, SKP designed the study and wrote the manuscript.

Acknowledgments

This study was supported by the National Institutes of Health grants R01AI150449 (SKP) and R01MH112457 (SKP), and was additionally supported by a grant from the National Institutes of Health, University of California San Francisco-Gladstone Institute of Virology & Immunology Center for AIDS Research (P30 AI027763). We thank Dr. Roland Schwarzer for valuable input and careful review of the manuscript.

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Figures and figure legends

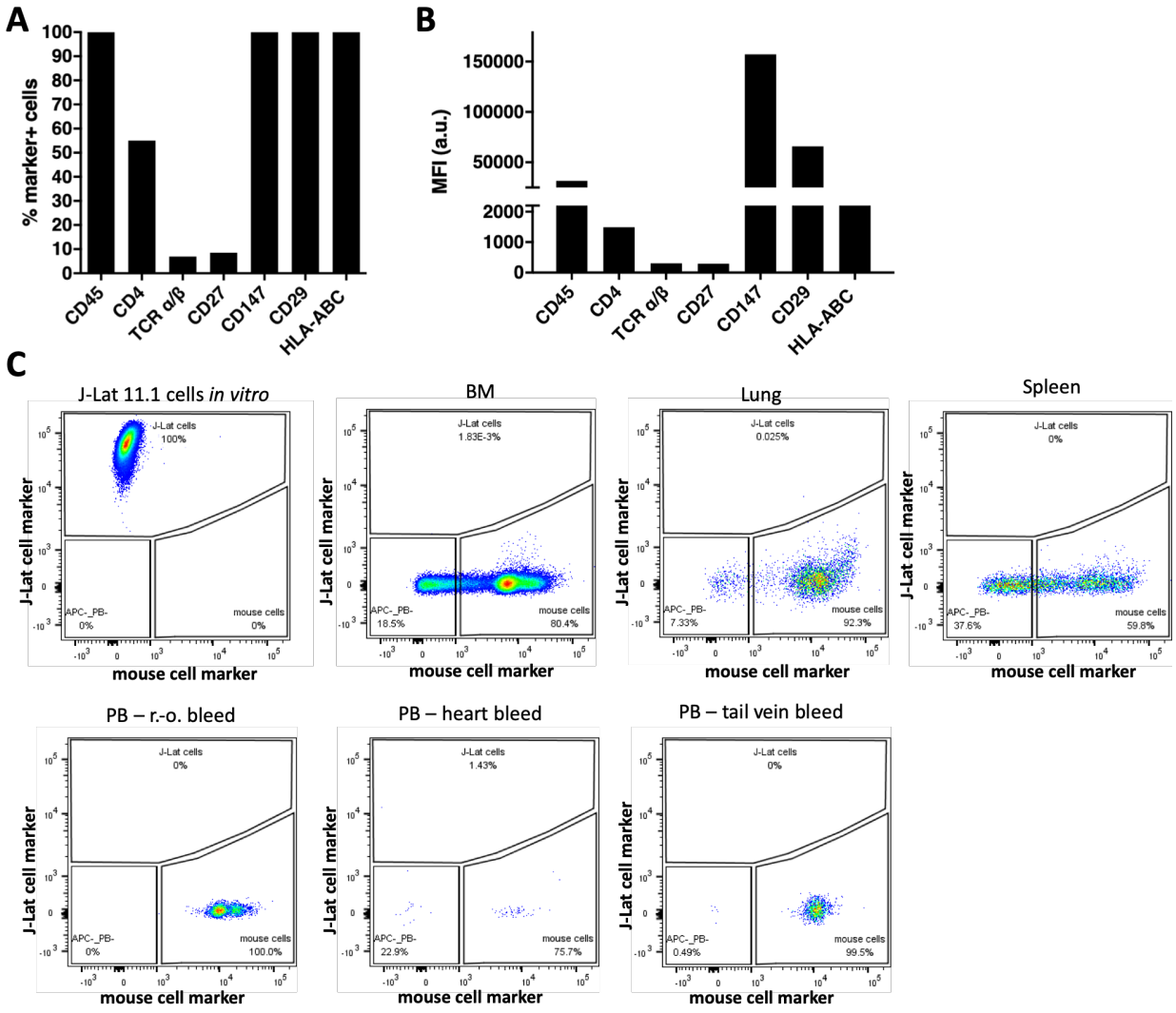

Fig. 1: The cell surface proteins CD147 and CD29 are abundantly and specifically expressed in J-Lat 11.1 cells. 1 x 10^6 J-Lat cells were stained with antibodies targeting select cell surface proteins to (A) measure the frequency of marker-positive cells and (B) MFI of proteins on the cell surface using flow cytometry. (C) Representative flow plots show multicolor staining of J-Lat cells and mouse cells harvested from different tissues. Cells were stained with CD147/CD29-APC (J-Lat cell marker) and CD45/TER-119/H-$2K^d$-Pacific Blue (mouse cell marker) to evaluate specificity and background signal of human CD29 and CD147 antibodies and to determine its applicablity for subsequent engraftment studies. BM = bone marrow, PB = peripheral blood, r.-o. = retro-orbital.

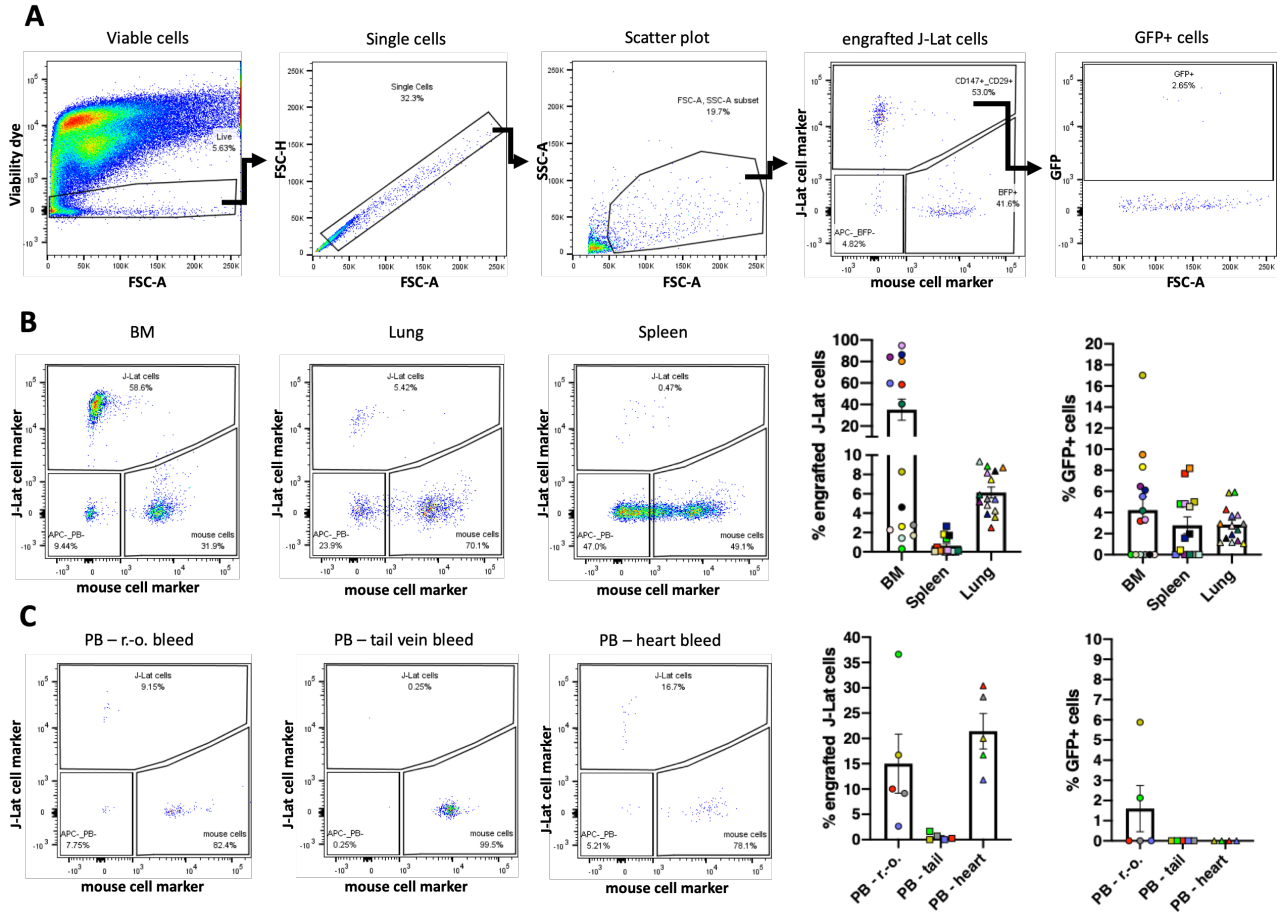

Fig. 2: J-Lat cells engraft successfully in several tissues in transplanted NSG mice. (A) Gating strategy for identifying human J-Lat cells in harvested mouse tissues based on CD147 and CD29 expression exemplified here with BM. Representative flow plots are shown for (B) engraftment levels across tissue sites (n = 15) observed at necropsy and (C) engraftment levels in PB (n = 5) using three different harvest approaches: r.-o. bleeding, tail vein bleeding, and heart bleeding. Bar graphs summarize J-Lat engraftment (left) and GFP background signal (right) of engrafted J-Lat cells. Each data point represents an individual animal. Colors indicate tissues harvested from the same animal. Outliers were identified in two cases: engraftment in the spleen (B) , and %GFP+ cells in PB – heart (C) and removed from graphs. Error bars show the standard error of the mean (SEM). BM = bone marrow, PB = peripheral blood, r.-o. = retro-orbital.

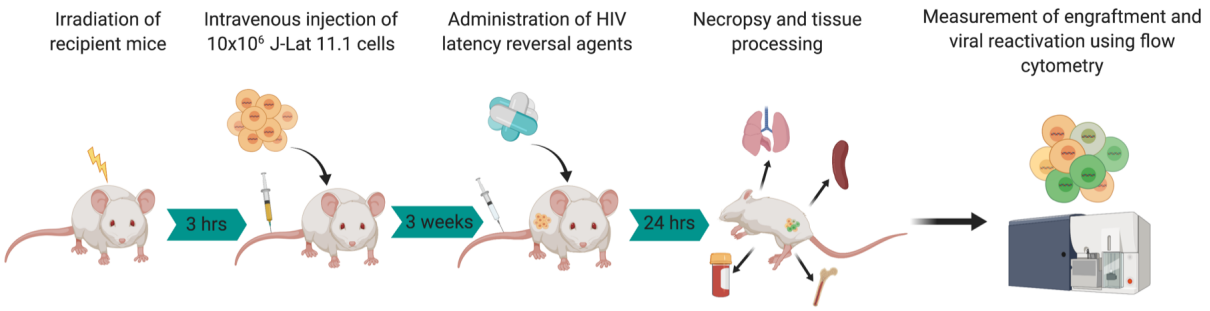

Fig. 3: Schematic representation of the procedure to test LRAs *in vivo* using the µ-Lat model. Mice receiving cells of interest for transplantation are irradiated three hours prior to cell injection. Each recipient mouse receives 10 x 10^6 J-Lat cells via intravenous injection. 3 weeks post injection (21 days) mice are treated for 24h with LRAs of interest, followed by necropsy, tissue harvest and processing, and preparation of single-cell suspensions. Single-cell suspensions are stained for J-Lat and mouse cell markers to assess engraftment levels via flow cytometry. Reactivation of latent provirus following LRA treatment is assessed by measuring GFP expression via flow cytometry (the J-Lat provirus contains an LTR-driven GFP reporter).

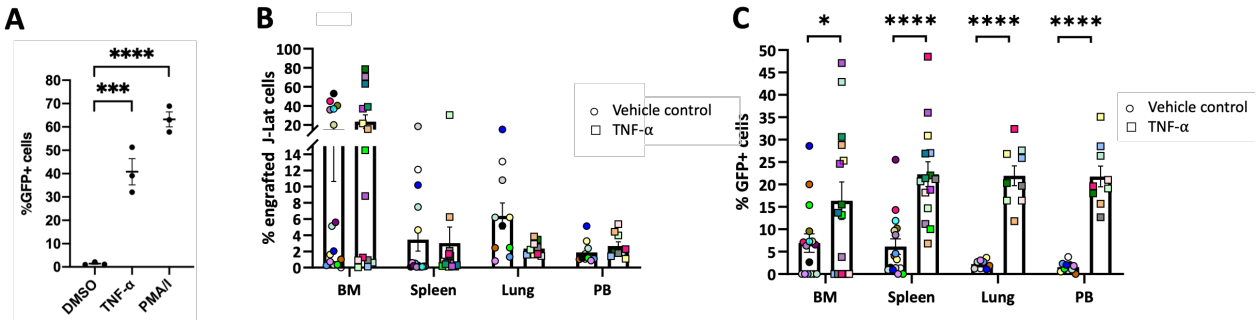

Fig. 4: TNF-α treatment reactivates latent HIV *in vivo* in the μ-Lat model. (A) Effect of 20 ng/μl TNF-α and 20 nM PMA/1 μM Ionomycin (positive control) on viral reactivation (GFP expression) in J-Lat cells *in vitro* 24h post treatment. (B) Effect of 20 μg TNF-α treatment (n = 9) or vehicle control (PBS, n = 10) on J-Lat engraftment in BM, spleen, lung and PB of mice 24h post tail vein injection. (C) Effect of TNF-α on viral reactivation (GFP expression), comparing animals treated with TNF-α versus vehicle control. Colors indicate specific animals. One-way ANOVA Sidak's multiple comparisons test was used to analyze *in vitro* reactivation data (A) and two-way ANOVA Sidak's multiple comparisons test was used for *in vivo* engraftment (B) and reactivation data (C). * = adjusted p-value<0.05; *** = adjusted p-value<0.001; **** = adjusted p-value <0.0001. Error bars represent SEM. BM = bone marrow, PB = peripheral blood.