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Humanised mouse models for haematopoiesis and infectious diseases

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Summary

"Humanised" mouse models have emerged over past years as powerful tools for investigating human haematopoiesis and immunity. They allowed the identification of key factors for the maintenance and function of normal and leukaemic human haematopoietic stem cells. These findings have been widely used to dissect the pathogenesis of multiple myeloid and lymphoid neoplasms, such as acute myeloid leukaemia and acute lymphoblastic leukaemia. Furthermore, these models can serve as a stepping-stone to clinical trials by testing novel drugs that target leukaemic stem cells. The investigation of human immunity *in vivo* is also of great interest in both the context of understanding the innate and adaptive immune system and responses to viral infections with exclusive human tropism, such as Epstein-Barr virus and human immunodeficiency virus. This review focuses on recent advances in the study of human haematopoiesis and immunity in humanised mouse models, underlining their relevance and limitations.

Key words: *humanised mouse models, xenograft, leukaemia, HIV, EBV, PDX*

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Introduction

The need to understand normal or malignant human cell development and disease heterogeneity, and to investigate novel therapeutic strategies in order to accelerate their transition into clinical trials, strongly supported the development of "humanised" mouse models [1, 2]. Another important driving force for the development of these models is the ethical concerns raised by clinical trials in humans.

Three major advancements over past decades have significantly shaped the field (fig. 1). The first was the discovery of a spontaneous autosomal recessive mutation in the Prkdc gene, termed *Prkdc^{scid} (Prkdc*: protein kinase, DNA activated, catalytic polypeptide; *scid*: severe combined immunodeficiency) in C.B-17 mice (called SCID mice). This permitted low engraftment of human fetal tissues, peripheral blood mononuclear cells and haematopoietic stem cells (HSCs) owing to a T and B cell deficiency [3–5]. Low human haematopoietic engraftment in C.B-17 mice is due to a tendency to develop functional mouse T and B cell immunity (termed "leakiness") and normal natural killer (NK) cell activity [6]. Furthermore, mutations in the locus of one of the recombination-activating genes (RAG1 or RAG2) resulted in *Rag1^{tm1Mom}* or *Rag2$^{tm1.1Cgn}$* mice with a T- and B-cell deficiency similar to that seen in C.B-17 mice, but in addition rendered them resistant to radiation and leakiness [7].

The second advancement was the development of NOD.CB17-*Prkdc^{scid}*/J mice, generated by backcrossing mice with the *Prkdc^{scid}* mutation to mice on the NOD (non-obese diabetic) background [8]. In comparison to the C.B-17 mice, these mice showed improved engraftment of HSCs as a result of lower NK cell activity and defects in their innate immunity, although they had short life-spans due to spontaneous development of thymic lymphomas [8].

The introduction of a targeted mutation in the locus of the interleukin-2 receptor (IL-2R) γ-chain, which leads to NK cell depletion, was the third key achievement that led to a substantially higher engraftment of functional human HSCs [9–12]. Today, the NOD.*Cg-Prkdc^{scid} Il2rg^{tm1Wjl}*/SzJ (NSG) model that resulted represents the most commonly used host strain for xenograft experiments. Although the introduction of NSG mice has significantly advanced the field, NSG mice fail to support maturation of human B cells into memory and plasma cells. In addition, T cell development lacks thymic support and the development of innate immunity (i.e., NK cell and macrophage development) is limited [13].

A crucial immune determinant of human haematopoietic cell survival identified in xenograft models is macrophage tolerance [14, 15]. Binding of human CD47 on human haematopoietic cells to mouse signal regulatory protein-alpha (SIRPα) on resident host macrophages generates a "don't eat me" signal that leads to inhibition of phagocytosis, protects the transplanted human cells and consecutively contributes to the human cell survival *in vivo* [15, 16]. The interaction between human CD47 and mouse SIRPα is present in mice on a NOD background since the mouse

SIRPα IgV domain is very similar to the human homologue.

In addition, the recognition of the importance of cytokines and growth factors secreted by bone marrow stromal cells in supporting HSC development led to the development of "next-generation" humanised mouse strains, such as MISTRG mice [17]. These mice were genetically engineered to express human macrophage colony stimulating factor (M-CSF), interleukin-3 (IL-3), SIRPα, thrombopoietin and granulocyte-macrophage-CSF (GM-CSF) (abbreviated MISTRG) to allow efficient human haematopoietic development. In contrast to previous models, these support the development of functional human macrophages and NK cells [17]. Moreover, these mice favour myelomonocytic differentiation and the engraftment of mobilised peripheral blood mononuclear cells, which is limited in the NOD.CB17-*Prkdc*^{scid}/J and NSG mouse models [18]. Recently, an improved "next-generation" humanised mouse strain of MISTRG that harbours an additional knock-in of human interleukin-6 (IL-6) was developed. These mice support efficient engraftment of human plasma cell neoplasms, which particularly rely on the presence of IL-6 [19, 20]. Improved models that support multilineage engraftment of human HSCs while minimising the mouse HSC compartment by ablating mouse cells were also developed. These models were generated by the introduction of mutations in the Kit receptor, which is vital for HSC maintenance and function, in immunodeficient mouse strains on the BALB/c (BRg *Kit*$^{Wv/Wv}$) or NOD (NSG *Kit*$^{Wv/Wv}$) background [21].

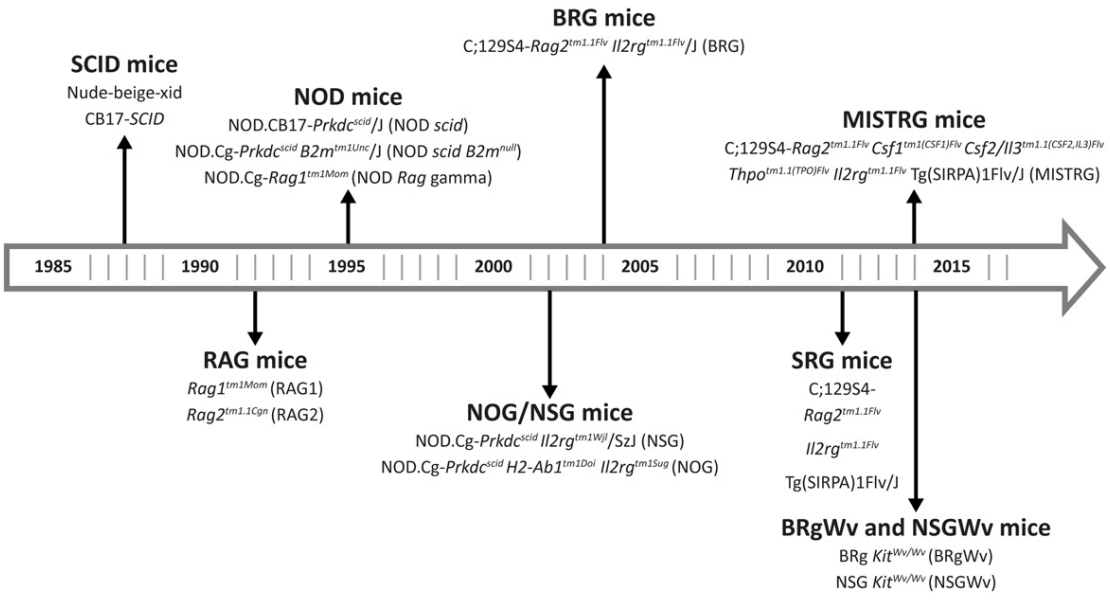
Although the humanised mice field has advanced extensively, there are still obstacles that need to be overcome in order to generate a model that fully recapitulates the complexity of the human haematopoietic system. This review focuses on current advances in the development of humanised mouse models for human haematopoiesis and immunity, highlighting their relevance and limitations.

Haematopoiesis

Normal human haematopoiesis

The haematopoietic system is a hierarchically organised cellular system, in which HSCs reside at the apex and are characterised by their ability to self-renew and to differentiate into mature blood cells of the myeloid and the lymphoid lineage (fig. 2 and table 1). By definition, human HSCs are capable of repopulating immune-compromised mice and are enriched in the lineage-marker negative (Lin^-), $CD34^+$, $CD38^-$, $CD90^+$ ($Thy1^+$) and $CD45RA^-$ population in the human fetal liver / fetal bone marrow, umbilical cord blood and adult bone marrow [24–30]. Later, CD49f integrin was identified as a marker that further enriches for human HSCs in umbilical cord blood samples. In combination with the high efflux of the mitochondrial dye rhodamin 123, 14 to 28% (i.e., 1 in 3.5–7 cells) of functional umbilical cord blood human HSCs that express the surface phenotype mentioned above are capable of engrafting NSG mice at the single cell level [22]. HSCs give rise to multipotent progenitors (MPPs), which in turn give rise to oligopotent multilymphoid progenitors (MLPs) and common myeloid progenitors (CMPs), which further differentiate into unipotent progenitors (fig. 2). For example, CMPs give rise to lineage-committed megakaryocyte/erythrocyte progenitors (MEPs) and granulocyte/monocyte progenitors (GMPs). MPPs are distinguished from HSCs by the lack of CD49f expression (table 1) [22]. Recently, the surface marker profile of CMPs, MEPs and MPPs was re-defined by the addition of CD71 and the thrombopoi-

Figure 1: Historical development of humanised mouse strains. The first major advancement was the discovery of a mutation in the Prkdc gene that led to development of SCID mice. The second was the development of NOD.CB17-*Prkdc*^{scid}/J (NOD *scid*) mice, generated by backcrossing mice with the *Prkdc*^{scid} mutation to mice on the NOD (non-obese diabetic) background. The introduction of a targeted mutation in the locus of the interleukin-2 receptor (IL-2R) γ-chain led to the development of the NOD.Cg-*Prkdc*^{scid} *Il2rg*^{tm1Wjl}/SzJ (NSG) mice, which represents the most widely used host strain so far. Further discoveries, took into account the importance of cytokines and growth factors in supporting HSC development, which led to the development of "next-generation" humanised mouse strains, such as MISTRG mice.



etin receptor, which allowed better purification from the rest of the populations in the haematopoietic tree [31]. Furthermore, it was shown that cells with megakaryocyte/erythroid potential branch out directly from HSCs, rather than from MEPs, a concept that resembles the mouse haematopoietic tree [32].

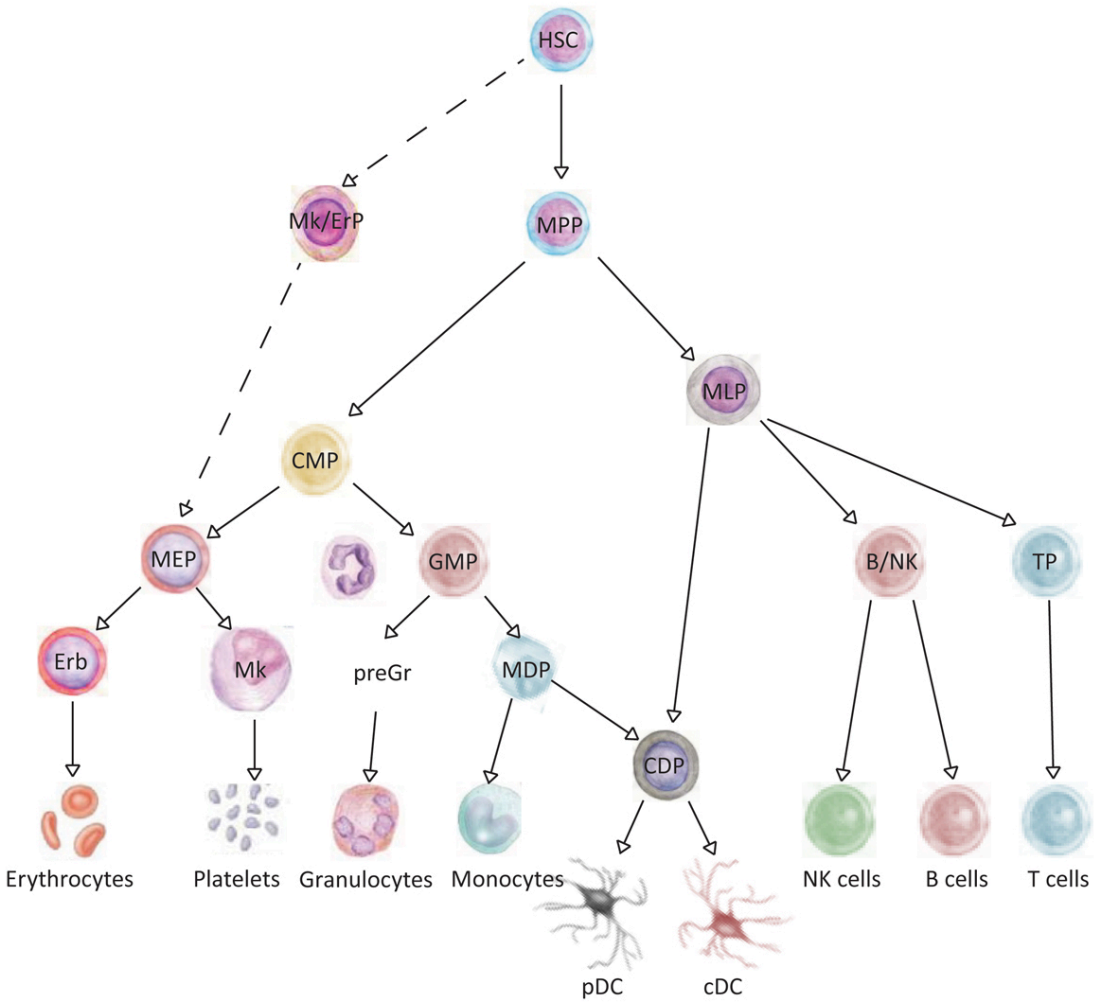
The regulation of HSC self-renewal and differentiation by cell-intrinsic and cell-extrinsic factors has been extensively investigated in the mouse system using knockout models, whereas their human analogues have not been explored because of the lack of suitable humanised model. Nonetheless, some key human HSCs factors have been cross-confirmed and/or newly identified. HSCs are a quiescent cell population that mostly reside in the G0 phase of the cell cycle [33]. Recently, it was demonstrated that cyclin-dependent kinase C (CDK6) levels regulate the kinetics of quiescence exit of human HSC subsets in xenograft models [34]. Further intrinsic factors that have been associated with the human HSC state include the transcription factors *SOX8, SOX18, HoxB4, HES1* and *BMI1*. However, their functional role remains to be validated in xenograft studies. Extrinsic factors that support human HSC function and engraftment in xenograft models include the macrophage receptor SIRPα (see introduction) and thrombopoietin. Both human factors have been genetically introduced into immune-compromised mice in order to facilitate normal and malignant human HSC engraftment [35, 36]. Overall, there are still many gaps in the current knowledge of normal human HSC maintenance and function, which with the development of new humanized mouse models can be further elucidated.

Malignant human haematopoiesis

Myeloid neoplasms

Patient-derived xenograft (PDX) models have been extensively used to dissect the pathogenesis of acute myeloid leukaemia (AML) at the clonal level and for preclinical testing of novel therapeutic regimens. One major contribution of PDX models to the AML field is the identi-

Figure 2: Hierarchy within human haematopoietic system. The tree-like organisation of the haematopoietic system is composed of haematopoietic stem cells (HSCs) with long-term self-renewal capacity that reside at the apex of the hierarchy and through multipotent and lineage-committed progenitors generate the differentiated mature cells with no or limited self-renewal capacity.B/NK = B and NK cell progenitor; cDC = conventional dendritic cells; CDP = common dendritic cell progenitor; CMP = common myeloid progenitor; Erb = erythroblast; GMP = granulocyte monocyte progenitor; HSC = haematopoietic stem cells; MDP = monocyte and dendritic cell progenitor; MEP = megakaryocyte-erythrocyte progenitor; Mk = megakaryocytes; Mk/ErP = megakaryocyte/erythrocyte progenitor; MLP = multi-lymphoid progenitor; MPP = multi-potent progenitor; pDC = plasmocytoid dendritic cells; PreGr = pre granulocytes; TP = T cell progenitor



fication of preleukaemic (pre-LSCs) and leukaemic stem cells (LSCs) (fig. 3). By definition, LSCs can repopulate immune-compromised mice and propagate disease when transplanted from the primary into secondary recipients. It is well accepted that LSCs initiate disease and their persistence after therapy causes relapse in patients [38, 39]. Thus, the identification and therapeutic targeting of LSCs is of high clinical relevance.

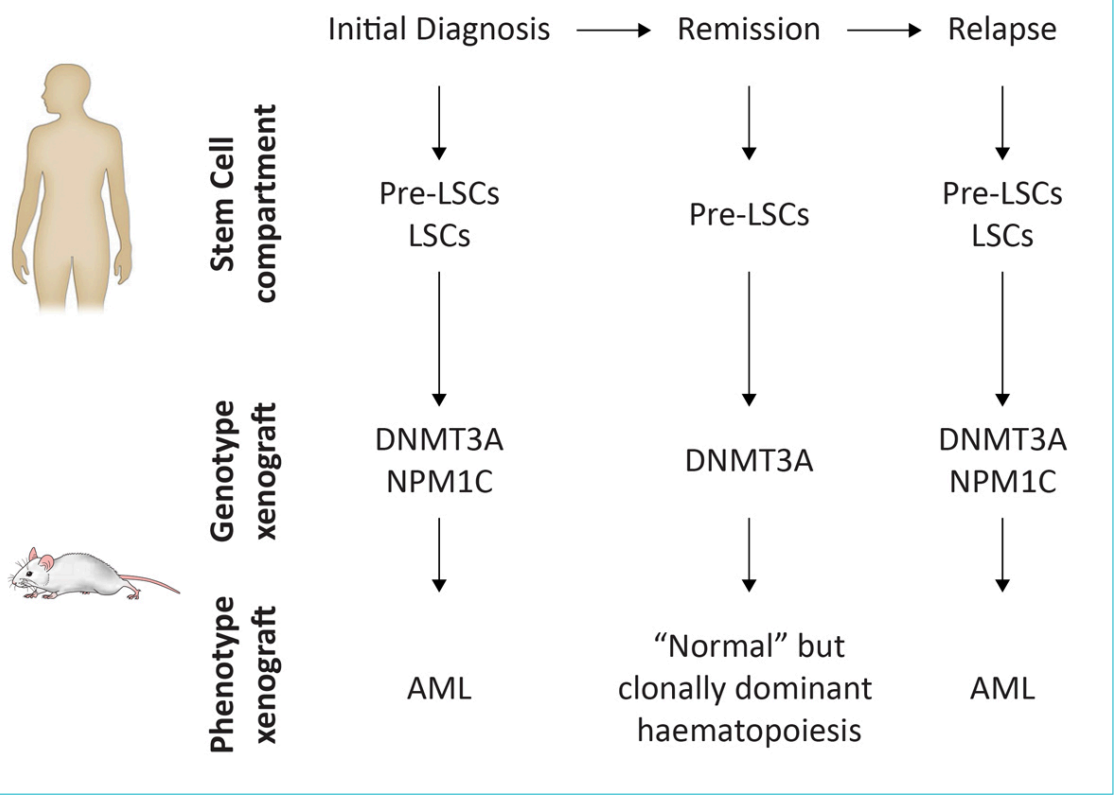
More recently, AML PDX models led to the identification of pre-LSCs. Pre-LSCs are HSCs that harbour recurrent somatic mutations and, with the acquisition of additional genetic hits, can lead to AML initiation and relapse [37, 40]. Mutations present in pre-LSCs are mostly found in genes involved in chromatin remodelling, whereas mutations that confer growth advantage to the AML clone occur later in AML development [41]. In contrast to LSCs,

Table 1: Human haematopoietic stem and progenitor cell phenotypes.

Haematopoietic cell population	Cell surface marker combination
HSPC	$Lin^-CD34^+CD38^-$
HSC	$Lin^-CD34^+CD38^-CD45RA^-CD90^+Rho^{-/low}CD49f^+$
MPP	$Lin^-CD34^+CD38^-CD45RA^-CD90^-CD49f^-$
*MPP F1	$Lin^-CD34^+CD38^-CD45RA^-CD90^-CD49f^-CD71^-BAH1^-$
*MPP F2/3	$Lin^-CD34^+CD38^-CD45RA^-CD90^-CD49f^-CD71^+BAH1^{-/+}$
MLP	$Lin^-CD34^+CD38^-CD45RA^+CD90^{-/low}$
CMP	$CD34^+CD38^+CD45RA^-CD10^-Flt3^+$
*CMP F1	$CD34^+CD38^+CD45RA^-CD10^-Flt3^+CD71^-BAH1^-$
*CMP F2/3	$CD34^+CD38^+CD45RA^-CD10^-Flt3^+CD71^+BAH1^{-/+}$
MEP	$CD34^+CD38^+CD45RA^-CD10^-Flt3^-$
*MEP F1	$CD34^+CD38^+CD45RA^-CD10^-Flt3^-CD71^-BAH1^-$
*MEP F2/3	$CD34^+CD38^+CD45RA^-CD10^-Flt3^-CD71^+BAH1^{-/+}$
GMP	$CD34^+CD38^+CD45RA^+CD10^-Flt3^+$
B/NK	$CD34^+CD38^+CD45RA^+CD10^+$

B/NK = B and NK cell progenitor; CMP = common myeloid progenitor; GMP = granulocyte monocyte progenitor; HSC = hematopoietic stem cells; HSPC = hematopoietic stem and progenitor cells; MEP = megakaryocyte-erythrocyte progenitor; MPP = multi-potent progenitor; MLP = multi-lymphoid progenitor; CMPs are found in cells that are $CD34^+CD38^+CD10^-Flt3^+CD45RA^-$, MLPs are $CD34^+CD38^-Thy1^-CD45RA^+$, whereas MEPs and GMPs are $CD34^+CD38^+CD10^-Flt3^-CD45RA^-$ and $CD34^+CD38^+CD10^-Flt3^+CD45RA^+$ respectively [22, 23].

Figure 3: Concept of acute myeloid leukaemia (AML) xenograft models as an example for myeloid neoplasms. PDX models reflect phenotypic as well as genetic features of myeloid neoplasms. In AML, the HSC compartment consists of preleukaemic stem cells (pre-LSCs) and leukaemic stem cells (LSCs) that harbour somatic mutations. Transplantation of AML cells from the initial diagnosis into PDX models leads to AML development *in vivo*. Genetically aberrant pre-LSCs can persist in patient remission, but lead to multilineage (normal) repopulation in PDX models. Frequently, the persistence of pre-LSCs that acquire additional somatic mutations is at the origin of disease relapse, which can be reproduced in PDX models (adapted from [37]).



pre-LSCs lead to multilineage repopulation in PDX models, which makes it an excellent model to study the progression of preleukaemic lesions to overt AML.

Although PDX models significantly contributed to the AML field by enabling LSCs to be identified and characterised, the knowledge extracted from these models also answered more clinically related questions. In particular, potentially critical therapeutic factors for the survival of LSCs have been identified [42]. Among these, the immunoglobulin superfamily member CD47, which is highly expressed on LSCs, has been shown to protect LSCs from innate immune surveillance [43, 44]. Targeting CD47 in the AML PDX models demonstrated significant efficacy and constituted the basis for currently on-going clinical trials in AML, other haematopoietic neoplasms and solid tumours [45]. Furthermore, the gene expression profile of AML stem and progenitor fractions was correlated with AML development in the PDX model and led to the identification of an AML gene expression signature that correlates with clinical outcome and may be used for risk stratification in future clinical trials [46].

The relevance of AML PDX models has been questioned in the past since immune-compromised hosts such as NOD.CB17-*Prkdc*^{scid}/J and NSG mice support engraftment of only a fraction of all AML patient samples. In particular, AML with a favourable risk profile was shown to poorly engraft conventional models. In an attempt to extend the spectrum of transplantable AML, a defined subgroup of AML characterised by the presence of an inversion of chromosome 16, excellent response to conventional chemotherapy, but poor engraftment in NSG mice, was investigated in MISTRG mice (see introduction) [47]. In contrast to NSG mice, MISTRG mice support robust engraftment of this AML subtype. The same study identified M-CSF as a critical factor for AML engraftment, a finding that could potentially be exploited therapeutically. In another study, favourable risk AML and acute promyelocytic leukaemia were successfully transplanted into engineered human bone fragments, so called ossicles, implanted into immune-deficient mice [48].

Only a few studies have successfully demonstrated engraftment of less aggressive diseases such as myelodysplastic syndromes and myeloproliferative neoplasms in immune-compromised mice. Engraftment of certain human myelodysplastic syndrome subtypes was improved by transplantation into newborn animals and by direct intrafemoral transplantation into adults [49, 50]. Other approaches include the co-transplantation of myelodysplastic syndrome samples along with healthy mesenchymal stromal cells and transplantation into ossicles (see above) [48, 51]. Similar approaches have also been undertaken for the development of myeloproliferative neoplasm PDX models.

In conclusion, PDX models for myeloid neoplasms have significantly contributed to understanding the pathogenesis of AML in recent decades and today have become a standard in preclinical testing of novel therapeutic regimens. Future studies will show whether the knowledge gained from these models can be directly translated into clinical practice and help elucidate the pathogenesis of less aggressive diseases.

Lymphoid neoplasms

Lymphoid neoplasms include acute and chronic leukaemia as well as a wide range of lymphomas that are characterised by variable stages of B and T cell maturation [52]. Acute B-precursor lymphoblastic leukaemia is a malignant disease most commonly found in children; in adulthood chronic lymphoblastic leukaemia (CLL) is more common [53]. The first leukaemia xenograft model was established more than two decades ago with AML patient samples, and provided the first functional insights into the haematopoietic hierarchy of this disease [38]. Systematic studies for acute lymphoblastic leukaemia (ALL) later set the stage for comprehensive preclinical testing [54, 55].

The development of NSG mice (see introduction) substantially improved the engraftment capacities of human lymphoma, B and T cell leukaemia [56–58]. In contrast to AML, emerging evidence indicates that most ALL cells can propagate the disease in this xenotransplantation model [59–61]. Between 50 and 100 cells are sufficient to reconstitute the leukaemic phenotype observed in the diagnostic sample [60–63]. In fact, leukaemia can be reconstituted from bone marrow samples with minimal residual disease [64] (fig. 4).

These models also contributed to the understanding of leukaemic stem cell plasticity found in certain ALL subtypes, in particular mixed lineage leukaemia (MLL)-rearranged leukaemia [65]. They further clarified the role of the MLL-rearranged ALL lineage switch to escape immunotherapeutic approaches that target CD19 on ALL cells [66]. Residual subclones that retained CD19 expression and displayed a myeloid phenotype in patients could reconstitute both a myeloid and lymphoid leukaemia in xenografts. Thus, this model can be used to address essential functional questions with leukaemia cells that are directly derived from patients. In fact, several publications have shown that leukaemia xenografts can recapitulate the clonal complexity of ALL with reasonable fidelity [60, 63, 64, 67, 68].

Recent studies indicate that this approach is also effective for adult leukaemia samples, although conditioning using total body irradiation of NSG may improve the engraftment capacities [69]. Lymphomas represent one of the most complex and heterogeneous set of malignancies and although a high diversity of PDX models are available, they fall short in truly representing patient heterogeneity [70, 71]. Additionally, the injection of lymphoma cells into specific tissues might introduce potential bias. However, tumour injection models are useful for studying the impact of different mutations and drugs on tumour aggressiveness [72–74]. Furthermore, repositories of AML, ALL and lymphoma PDXs will be available that should facilitate translational research forward [42].

The use of xenograft ALL models for preclinical testing of predictive biomarkers and drug efficacies offers new possibilities for translational research and is a big step towards clinical trials [64, 68, 75–77]. Finally, given the high degree of phenotypic and genotypic conservation between patient samples and their corresponding xenografts, this model will enable us to study the interaction of leukaemic cells within their complex niche in the bone marrow, which may provide improved and more precise rationales for therapeutic targeting of resistant leukaemia clones in their microenvironment [61, 78] (fig. 4). Xenograft models for

chronic lymphoid malignancies are also being developed using "next-generation" humanised mouse strains and will be extremely useful for preclinical testing of novel therapies [79].

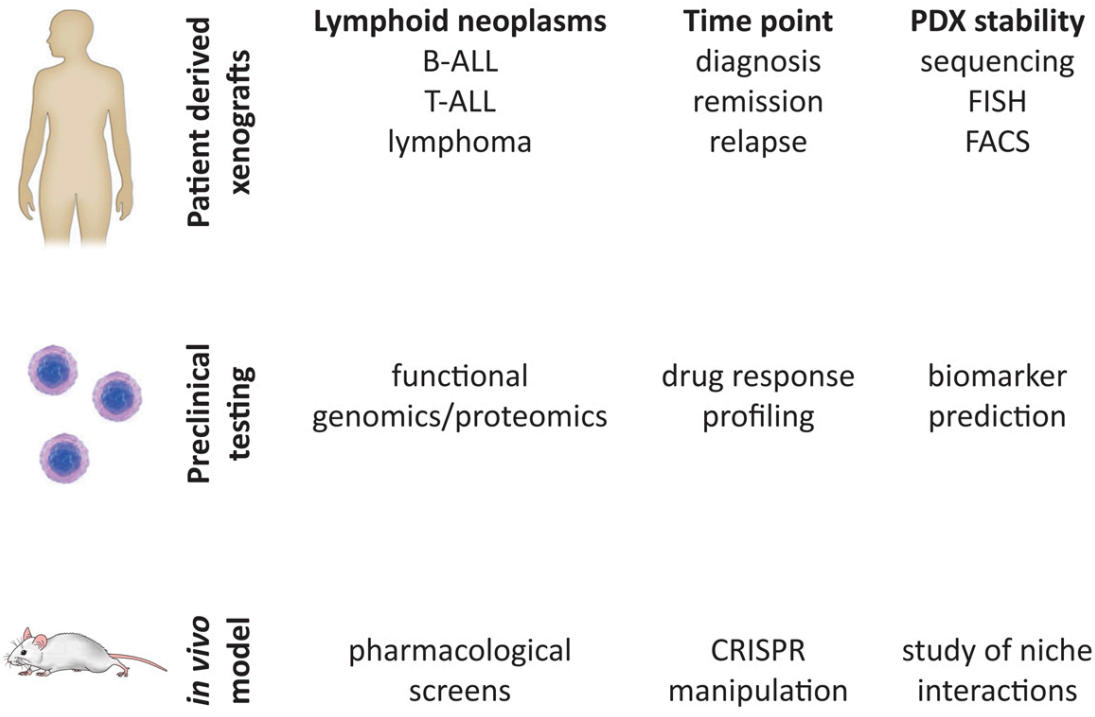
Immune system and infectious diseases

Human innate and adaptive immune responses in humanised mice

The human leucocytes of mice with human immune system components reconstituted from transferred human haematopoietic progenitor cells (HIS mice) show similarities to cord blood immune cells. Most B cells have a transitional or naïve phenotype, NK cells have a low frequency of killer immunoglobulin-like receptor (KIR) expression and still contain a CD56 negative subpopulation, and most T cells are naïve [80–83]. In addition, lymph nodes and mucosal secondary lymphoid tissues are poorly developed as a result of blocked lymphoid tissue inducer (LTi) cell differentiation in the absence of the cytokine receptor common gamma chain (γc) in most mouse strains that support efficient human immune compartment reconstitution [84]. Accordingly, cell-mediated immune responses can be mounted, while humoral immune responses are compromised, in HIS mice. Dendritic cells (DCs), the antigen-presenting cells that coordinate both cell-mediated and humoral immune responses, are reconstituted at frequencies and with a subset representation similar to that of human secondary lymphoid tissues [85–87]. Furthermore, they react to adjuvant exposure in the form of defined toll-like receptor (TLR) ligands similar to human peripheral blood DCs, whereas mouse DCs within HIS mice react with a different hierarchy that is consistent with the dissimilar TLR distribution between mouse and man [85]. Antigen that is targeted to these human DCs with suitable adjuvants can elicit $CD4^+$ T cell responses [85, 88]. These are HLA restricted and can recognise a diverse set of antigen-derived epitopes. In addition, primed $CD4^+$ and $CD8^+$ T cell clones can target EBV-transformed B cells and, in HLA transgenic HIS mice, specificities develop which are also found in human EBV carriers [80, 89–91]. These T cell responses restrict viral loads not only during EBV infection, but also after human immunodeficiency virus (HIV) and hepatic adenovirus infection of HIS mice [80, 89, 92–94]. Therefore, protective human T cell responses can be reproduced in the humanised mouse model.

In addition to priming T cells, DCs are also capable to activate innate lymphocytes. Indeed, human DC-activating adjuvants render NK cells of HIS mice more cytotoxic [81]. The early differentiated NK cell compartment of HIS mice can restrict lytic EBV infection [95] and recognises tumour cells with their distinct set of human NK cell-specific receptors [81, 96]. Furthermore, reconstituting two human immune systems in parallel from two donors that are mismatched in the HLA ligands of inhibitory NK cell receptors changes the education of these innate cytotoxic lym-

Figure 4: Generation of PDX models for preclinical studies. Patient-derived xenograft models of B-acute lymphocytic leukaemia (ALL), T-ALL and lymphoma can be generated from different disease time points like diagnosis, remission or relapse. PDX stability and complexity are highly conserved and can be proven by targeted sequencing, FISH (fluorescence *in situ* hybridisation) or FACS (fluorescence activated cell sort) analysis. PDX models can be used for a broad range of translation studies. Use of a co-culture system with mesenchymal stem cellss enables *in vitro* approaches for drug efficacy testing or biomarker identification. The high conservation of patient samples and xenografts enables the development of *in vivo* models for pharmacological screens, cell manipulations with CRISPR or the study of niche interactions in the microenvironment of the cancer cells.

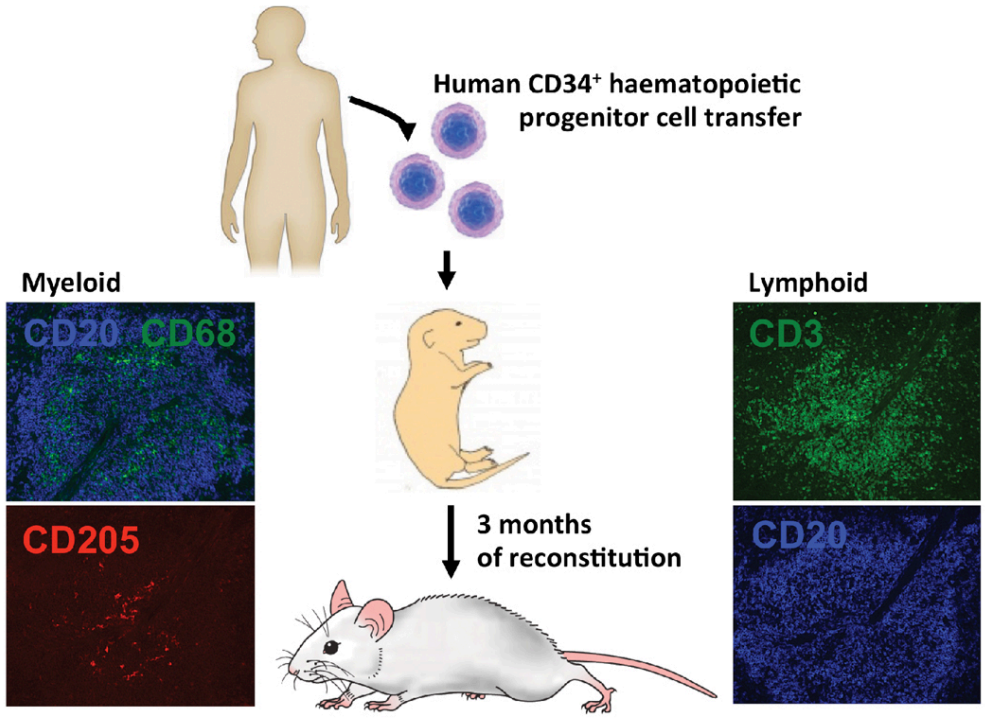


phocytes and allows the resulting NK cell population to more efficiently control EBV infection in the mixed B cell compartment [83]. This improved innate immune control most likely results from efficient targeting of EBV-infected B cells of one donor by the less inhibited NK cell compartment of the other donor. Thus, NK cell responses that are similar to those in small children can be modelled in HIS mice and manipulated for improved EBV-specific immune control.

In contrast to functional DC, T and NK cell responses, humoral immune responses are difficult to achieve. Usually only a minority of HIS mice develop specific isotype-switched antibody responses after viral infection [97]. Furthermore, the steady-state levels of human IgG in HIS mice are with below one microgram per millilitre more than a thousand-fold lower than in human serum [9, 82]. Accordingly, germinal centres are rarely observed, but even so the B and T cell zone is segregated in secondary lymphoid tissues of HIS mice (fig. 5) [85]. However, most B cells are of transitional and naïve phenotype, and follicular helper T cells that assist B cells in the germinal centre reaction are absent [82]. Interestingly, providing additional DCs either by adoptive transfer or possibly also by expansion of the reconstituted human DC populations might at least partially overcome these deficiencies and lead to B cell plasmablast differentiation, follicular helper T cell priming and antigen specific IgG responses [82]. Therefore, humoral immune responses are largely absent in HIS mice, but further manipulation of this model system might allow them to develop.

In addition to the compromised B cell compartment, it remains unclear if there is sufficient human myeloid reconstitution in HIS mice. Although DCs are found at considerable frequencies, it has been argued that macrophage populations are poorly developed [98]. Along these lines, human alveolar macrophages were able to protect HIS mice from lung proteinosis only when they developed in a human IL-3 and GM-CSF transgenic mouse background [98]. However, some lung infections could be studied in the absence of this enhanced human myeloid cell reconstitution in HIS mice. Influenza A virus infection caused the induction of virus-specific T cell responses and *Mycobacterium tuberculosis* infection led to granuloma formation [99, 100]. Therefore, whether enhanced myeloid cell reconstitution is beneficial or not might depend on the particular application of HIS mice. With respect to immune responses, it has to be kept in mind that the immunoregulatory function of some myeloid cells might be detrimental for the induction of immune responses. It has been shown that stem cell factor, IL-3 and GM-CSF transgenic mice allow elevated myeloid cell reconstitution, although at the same time expanded FoxP3 positive regulatory T cells could dampen the induction of immune responses [101]. Thus, HIS mice reconstitute a wide variety of human immune system compartments. Some of these already demonstrate functionality in the currently available models, whereas others, especially B cells, might benefit from additional manipulations of the model system in order to obtain the ability to execute their full range of immune functions. The resulting novel HIS mice may allow for a

Figure 5: Human immune compartments of HIS mice. HIS mice reconstitute most human immune compartments within 3 months after neonatal intrahepatic human CD34$^+$ haematopoietic progenitor cell transfer. In the spleen of NSG mice, macrophages (CD68), dendritic cells (CD205), T cells (CD3) and B cells (CD20) can be found in the white pulp.



more comprehensive investigation of human immune responses.

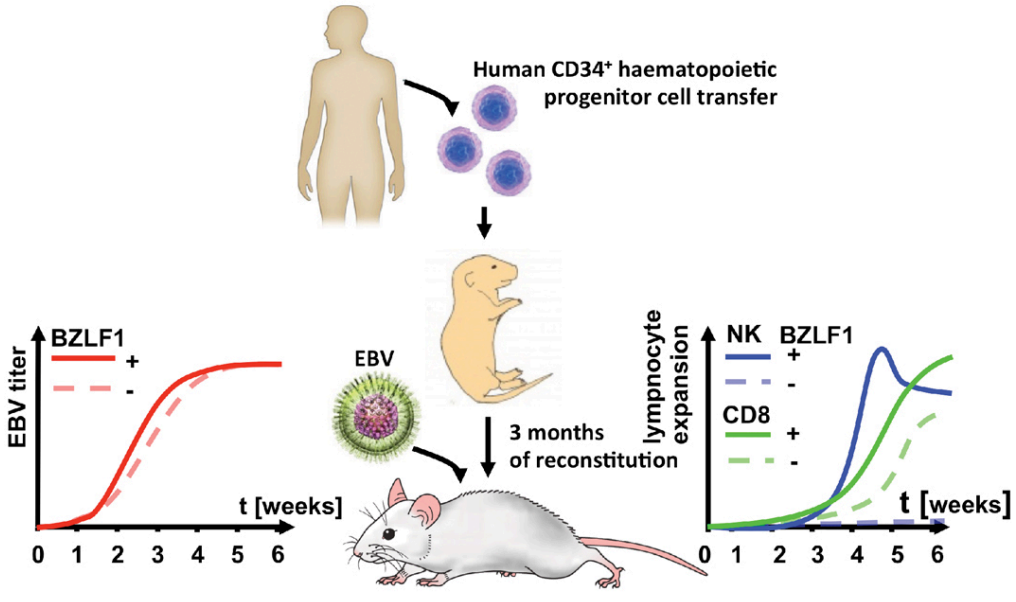
Espstein-Barr virus research with humanised mice

The ubiquitous gamma-herpes virus, Espstein-Barr virus (EBV), is the cause of infectious mononucleosis, and is associated with lymphomas predominantly of B cell origin and certain epithelial cell malignancies. Owing to its exclusive tropism for humans, studying this virus *in vivo* is difficult and was until recently restricted to related viruses in old-world monkeys [102]. However, EBV was not only the first human oncogenic virus to be discovered, fifty years ago [103], but it was also the first pathogenic agent to be studied in HIS mice [9]. EBV infection of B cells in HIS mice can result in asymptomatic infection or lymphoproliferative disease, depending on the infectious dose [80, 104]. Both latent and lytic EBV infection can be modelled in HIS mice (fig. 6). Depending on the viral strain, the majority of infected B cells either express all eight latent EBV proteins and only a minority initiates lytic replication (latency III predominates after B95-8 infection), or the frequency of lytically EBV replicating cells can be significantly increased (high lytic replication after M81 infection) [105]. The Hodgkin's and Burkitt's lymphoma-associated latencies I and II have been suggested to be present after HIS mouse infection with EBV [106–108], but they probably contribute to infection only at very low frequencies.

Apart from these virological parameters, some hallmarks of EBV specific immune control can also be recapitulated in HIS mice (fig. 6). The importance of NK cells in protecting from EBV-associated diseases, especially in the innate restriction of primary infection, was first demonstrated in HIS mice. Depletion of NK cells prior to infection revealed a protective effect against lytic EBV replication [83, 95]. The importance of NK cells in recognising lytic replication during acute EBV infection was then also confirmed in longitudinal studies of paediatric patients with infectious mononucleosis [109], indicating the predictive nature of the humanised mouse model for EBV-associated diseases.

After the transient and protective NK cell proliferation, $CD8^+$ T cells massively expand during EBV infection of HIS mice [80, 89]. Both $CD4^+$ and $CD8^+$ T cells control viral loads, whereas $CD8^+$ T cells are more important in preventing EBV-associated lymphomas [80]. Among these expanding $CD8^+$ T cells, both latent and lytic EBV antigen specificities can be detected in HLA transgenic HIS mice [80, 90, 91]. Furthermore, a protective effect of EBV-specific T cell clones, which target lytic antigens against high viremia and extra-lymphoid lymphoma genesis, could be documented [91], suggesting the importance of these specificities for future vaccine development against EBV. Indeed, targeting of EBV antigens to DC populations is under investigation in HIS mice [85, 110]. However, these distinct antigen specificities are so far still difficult to modulate and the role of their effector functions cannot be assessed. The generation of recombinant viral vectors to manipulate the expression of certain genes in the developing haematopoietic lineage would constitute a valuable tool for assessing the importance of specific human immune functions during EBV-associated lymphoma genesis. For the investigation of adaptive cellular immune responses to EBV, T cell receptor transgenes would allow the development of T cell populations that could not only be tracked during the course of EBV challenge, but also further investigated for their protective potential during viral infection. Together with recombinant manipulation of the EBV

Figure 6: HIS mice can be persistently infected with Epstein-Barr virus (EBV) and develop immune responses to the infection. Mice that have been reconstituted by means of neonatal intrahepatic injection with human $CD34^+$ haematopoietic progenitor cells develop viraemia upon intraperitoneal EBV infection. Lytic replication deficient virus (BZLF1-) has a lower viral titre at 3 weeks after infection. NK cells expand to lytic EBV infection at 4 weeks after infection and $CD8^+$ T cells react to both lytic and latent infection with expansion by 5 weeks of infection.



genome [91, 95, 107, 111], the aforementioned strategies might lead to predictive preclinical models for relevant human diseases, which would provide insight that can be more readily translated into targeted clinical research.

Human immunodeficiency virus infection in humanised mice

The human immunodeficiency virus (HIV) type-1 belongs to the lentivirus genus (family of retroviruses), comprising five serogroups that can infect a number of vertebrates. Clinical hallmarks of lentiviral infections are chronicity, intra-host evolution and cellular tropism [112]. Their basic genomic structure comprises the long-terminal repeat (LTR; transcription start), three genes encoding the structural proteins – gag, pol and env – and regulatory genes (Tat and Rev) [113]. Notably, accessory genes contribute to their virulence [114, 115].

HIV is a human-specific virus; the HIV pandemic counts currently 36.7 million of HIV-infected human (http://www.who.int/hiv/en/). HIV infects mainly $CD4^+$ T-cells and macrophages, leading to their progressive depletion [116, 117] and exhaustion [118, 119]. Moreover, HIV can remain latent by integrating its genome into the host chromosome, allowing viral rebound upon interruption of antiretroviral treatment (ART). Many *in vivo* aspects of HIV infection remain an enigma, e.g., HIV transmission [120], HIV latency [121, 122], tissue target cells [123–125], role of the HIV accessory genes [126], immune activation [127] and co-infection pathogenesis [128–130]. Of course, a key in any research efforts is the identification of novel therapeutic approaches [131–136].

The lack of a small HIV animal model has hampered HIV research since its discovery. The feline immunodeficiency virus (FIV), the simian immunodeficiency virus (SIV) and hybrid viruses between SIV and HIV (SHIV) have been used as a substitute for HIV in domestic cats or nonhuman primates, respectively. Findings with these models must further be validated with HIV itself. Notably, the "first" generation of hu mice, already used in the 1980s i.e., the SCID hu thy/liv or hu PBL SCID [137–142], lacked *de novo* multilineage haematopoiesis and/or suffered from graft-versus-host disease (GvHD), limiting their use in HIV research.

The "new" generation of hu mice (see introduction) are highly sensitive to HIV infection (figs 7 and 8) [97, 143–146] and recapitulate key features of HIV infection in human, e.g., dissemination [146], pathogenesis [147] and latency [148]. In the following, we will give a short update about the various humanized mouse models currently used in HIV research, including the pros and cons (figs 7 and 8).

NSG mice intraperitoneally transplanted with adult human peripheral blood mononuclear cells s comprise a simple and direct lymphoid-based model that allows HIV infection over 10 weeks [149]. This model is similar to the hu PBL SCID mouse model [4] and has similar limitations, i.e., lack of multilineage haematopoiesis and risk of GvHD. Thus, we would like to highlight only one study, which showed the value of this model to assess the presence of residual HIV reservoir in immunosuppressed patients, employed as a murine viral outgrowth assay [150].

Hu mice based on the transplantation of human $CD34^+$ HSPCs develop long-term *de novo* multilineage haematopoiesis and immunity [9] with low prevalence of GvHD. They are very valuable for long-term studies, studying HIV infection in deep tissues, HIV latency establishment [146, 148] and virus-specific $CD8^+$ T-cell development [92].

These mice have been very useful for dissecting the pathogenic role of the HIV accessory proteins and for studying HIV-associated immune activation. For example, by use of these mice, the destructive potential of a distinct motif of the Nef gene, the secretory modification region (SMR) by promoting CD4 apoptosis and a cytokine storm was convincingly demonstrated [151]. As related to HIV-associated immune activation, a recent study reported how exosomes containing transactivation response (TAR) RNA-stimulated macrophages and plasmacytoid dendritic cells (pDC), thereby promoting interferon-beta (IFN-β) and IL-6 secretion [152].

Notably, NSG mice have been successfully used to investigate novel treatment approaches (fig. 7), pre-exposure prophylaxis (PreP) against vaginal transmission [153, 154], testing novel innovative compounds [155], optimised long-acting ART [156–158] or broadly neutralising antibodies (bNAb) [159]. Importantly, bNAb also reduced the latent reservoir size, either through FcγR engagement [160] or by additional treatment with latency reversing agents [161] – the latter was the first study that successfully targeted the latent reservoir for its eradication and thus a potential cure of HIV. Similarly, gene therapy-based approaches resulted in functional cure in NSG mice.

Bone marrow, liver, thymus mice (BLT) produced by concomitant transplantation of fetal liver/thymus tissues with $CD34^+$ cells into adult immunodeficient mice are a widely

Figure 7: Humanised mice are highly susceptible to human immunodeficiency virus (HIV) and allow the examination investigational drugs. Humanised mice were infected with the CCR5-tropic HIV strain, YU-2, and viral load was monitored over time with quantitative real-time PCR. The left panel shows untreated mice. The middle panel shows mice treated with antiretroviral therapy (tenofovir, lamivudine and raltegravir). The right panel shows mice treated with an investigational compound. The grey shaded area indicates the treatment period. The filled and empty circles in the untreated group represent mice from two independent cohorts.

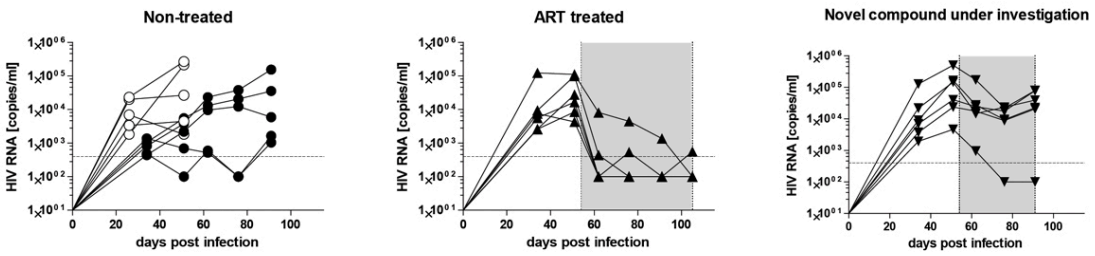
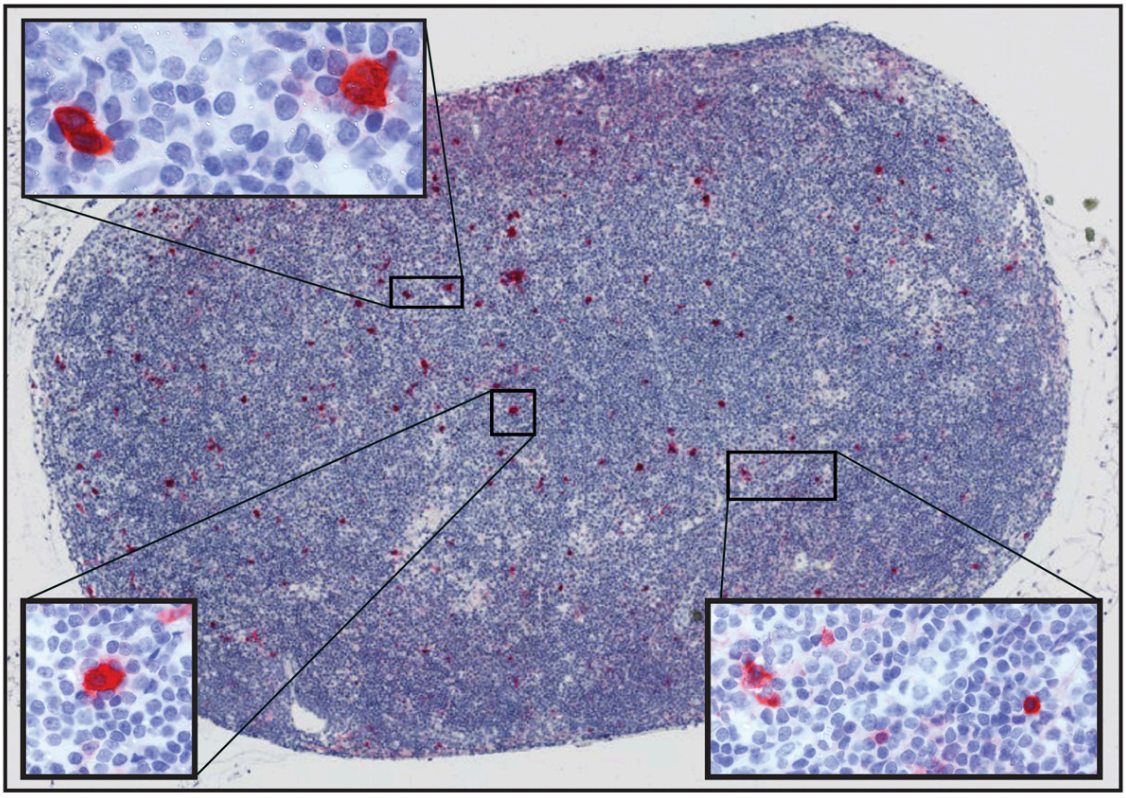


Figure 8: Human cells expressing p24 (dark red) in the lymph node of an HIV-infected NSG mouse. The amplified image at the top left corner shows multi-nucleated giant cells, i.e., infected T cells that fuse with each other inducing syncytia. The lower left image shows a single HIV-infected macrophage. The lower right image depicts HIV-infected T cells among other HIV-infected cells.



used model. The thymic tissue resulted in an increase of T cell reconstitution, maturation and selection, enabling the generation of an MHC-restricted adaptive immune response from which HIV shows immune escape [162–164]. Notably, engraftment of human cells in the gastrointestinal tract and female reproductive tract is very prominent as compared with NSG mice, which permits study of HIV transmission and replication in mucosal tissues [165–169]. Viral replication was found preferentially in intestinal crypts, which released a pool of both immature and mature virions [170]. These findings were in contradiction to *in vitro* findings, where virion maturation occurred quickly, emphasising the value of *in vivo* investigations with the corresponding microenvironment [171].

Furthermore, BLT mice facilitated broad HIV transmission research; for example, ART reduced the amount of cell-free virus in the female reproductive tract and in the breast milk, overall decreasing the prevalence of HIV transmission [172, 173]. Another study showed that early ART initiation was able to preserve the immune system and functionally control productive HIV infection thanks to the development of a HIV-specific immune response [174, 175]. These results corroborated findings from the Visconti study in humans, where 14 patients treated during primary infection were able to constrain HIV infection for several years without treatment [174, 175].

Moreover, numerous prophylactic approaches have been validated using this model. For instance, lentiviral modification of HSPCs with a polymeric anti-HIV IgA construct or on adeno-associated virus (AAV) containing a bNAb (VRC07) hampered mucosal HIV transmission [176, 177].

Last, but not least, a study examining cross-species transmission events from chimpanzees to humans: Yuan et al. decoded critical viral envelop mutations required to allow HIV zoonosis [178]. Altogether, the BLT model is highly valuable, especially for mucosal tissues and adaptive immune response analysis.

More and more sophisticated humanised mice models are generated in order to recapitulate the human cell distribution and functionality in animals and which are examined for studying HIV. For example, MITRG and MISTRG mice demonstrated superior myeloid and NK cell development and function, in comparison to preceding models [17]. Deng et al., using MIS^{KI}TRG mice demonstrated the potential of cytotoxic CD8^{+} T cells (CTL) obtained from chronically infected patients, to constrain HIV released from reactivated latent cells [179].

To address more precise questions, highly specific humanised mouse models are used, such as, the T cell only and macrophage only mouse model (TOM and MOM, respectively) that permits development of only a specific human lineage. TOM can be readily infected with HIV and establishment of latency occurred in absence of other cell types [180]. Similarly, MOM confirmed the capacity of some strains of HIV to replicate and infect macrophages *in vivo* [181].

The studies mentioned above represent only a small fraction of insights gained in HIV infection/pathogenesis and cure using humanised mice that underscores their value for studying HIV. They likely represent the long awaited small animal model for the HIV research community. Since various models are available, and more will be available soon,

the HIV researcher will be faced with the challenge to identify the most suitable model to address their research question.

Conclusions and outlook

Our understanding of human haematopoiesis and immunity has extensively evolved with the help of humanised mouse models. Currently, there are an increasing number of models for more aggressive neoplasms, such as AML and ALL, and for infectious diseases, such as EBV and HIV. Experimental strategies used in order to improve these and create new models include the expression of supportive human factors by genetic modification of mice, the co-transplantation of human mesenchymal stem cells, and the xenotransplantation of humanised ossicles. One particularly interesting approach is the use of the CRISPR/Cas9 technology for fast genetic modification of humanised host strains to express specific human factors. Despite on-going efforts, modelling less aggressive neoplasms, reproducing disease heterogeneity seen in patients, and probing certain immune cell pathologies is still a challenge that researchers face. Future models will need to support engraftment of the majority of patient samples for any given neoplasm or infection to allow researchers to draw substantial conclusions and predict accurate therapy responses in humans.

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References

1 Mestas J, Hughes CC. Of mice and not men: differences between mouse and human immunology. J Immunol. 2004;172(5):2731–8. doi: http://dx.doi.org/10.4049/jimmunol.172.5.2731. PubMed.

2 Rongvaux A, Takizawa H, Strowig T, Willinger T, Eynon EE, Flavell RA, et al. Human hemato-lymphoid system mice: current use and future potential for medicine. Annu Rev Immunol. 2013;31(1):635–74. doi: http://dx.doi.org/10.1146/annurev-immunol-032712-095921. PubMed.

3 Bosma GC, Custer RP, Bosma MJ. A severe combined immunodeficiency mutation in the mouse. Nature. 1983;301(5900):527–30. doi: http://dx.doi.org/10.1038/301527a0. PubMed.

4 Mosier DE, Gulizia RJ, Baird SM, Wilson DB. Transfer of a functional human immune system to mice with severe combined immunodeficiency. Nature. 1988;335(6187):256–9. doi: http://dx.doi.org/10.1038/335256a0. PubMed.

5 Lapidot T, Pflumio F, Doedens M, Murdoch B, Williams DE, Dick JE. Cytokine stimulation of multilineage hematopoiesis from immature human cells engrafted in SCID mice. Science. 1992;255(5048):1137–41. doi: http://dx.doi.org/10.1126/science.1372131. PubMed.

6 Nonoyama S, Smith FO, Bernstein ID, Ochs HD. Strain-dependent leakiness of mice with severe combined immune deficiency. J Immunol. 1993;150(9):3817–24. PubMed.

7 Mombaerts P, Iacomini J, Johnson RS, Herrup K, Tonegawa S, Papaioannou VE. RAG-1-deficient mice have no mature B and T lymphocytes. Cell. 1992;68(5):869–77. doi: http://dx.doi.org/10.1016/0092-8674(92)90030-G. PubMed.

8 Shultz LD, Schweitzer PA, Christianson SW, Gott B, Schweitzer IB, Tennent B, et al. Multiple defects in innate and adaptive immunologic function in NOD/LtSz-scid mice. J Immunol. 1995;154(1):180–91. PubMed.

9 Traggiai E, Chicha L, Mazzucchelli L, Bronz L, Piffaretti JC, Lanzavecchia A, et al. Development of a human adaptive immune system in cord blood cell-transplanted mice. Science. 2004;304(5667):104–7. doi: http://dx.doi.org/10.1126/science.1093933. PubMed.

10 Kerre TC, De Smet G, De Smedt M, Zippelius A, Pittet MJ, Langerak AW, et al. Adapted NOD/SCID model supports development of phenotypically and functionally mature T cells from human umbilical cord blood CD34(+) cells. Blood. 2002;99(5):1620–6. doi: http://dx.doi.org/10.1182/blood.V99.5.1620. PubMed.

11 Ito M, Hiramatsu H, Kobayashi K, Suzue K, Kawahata M, Hioki K, et al. NOD/SCID/gamma(c)(null) mouse: an excellent recipient mouse model for engraftment of human cells. Blood. 2002;100(9):3175–82. doi: http://dx.doi.org/10.1182/blood-2001-12-0207. PubMed.

12 Ishikawa F, Yasukawa M, Lyons B, Yoshida S, Miyamoto T, Yoshimoto G, et al. Development of functional human blood and immune systems in NOD/SCID/IL2 receptor gamma chain(null) mice. Blood. 2005;106(5):1565–73. doi: http://dx.doi.org/10.1182/blood-2005-02-0516. PubMed.

13 Theocharides AP, Rongvaux A, Fritsch K, Flavell RA, Manz MG. Humanized hemato-lymphoid system mice. Haematologica. 2016;101(1):5–19. doi: http://dx.doi.org/10.3324/haematol.2014.115212. PubMed.

14 Terpstra W, Leenen PJ, van den Bos C, Prins A, Loenen WA, Verstegen MM, et al. Facilitated engraftment of human hematopoietic cells in severe combined immunodeficient mice following a single injection of Cl2MDP liposomes. Leukemia. 1997;11(7):1049–54. doi: http://dx.doi.org/10.1038/sj.leu.2400694. PubMed.

15 Takenaka K, Prasolava TK, Wang JC, Mortin-Toth SM, Khalouei S, Gan OI, et al. Polymorphism in Sirpa modulates engraftment of human hematopoietic stem cells. Nat Immunol. 2007;8(12):1313–23. doi: http://dx.doi.org/10.1038/ni1527. PubMed.

16 Takizawa H, Manz MG. Macrophage tolerance: CD47-SIRP-alpha-mediated signals matter. Nat Immunol. 2007;8(12):1287–9. doi: http://dx.doi.org/10.1038/ni1207-1287. PubMed.

17 Rongvaux A, Willinger T, Martinek J, Strowig T, Gearty SV, Teichmann LL, et al. Development and function of human innate immune cells in a humanized mouse model. Nat Biotechnol. 2014;32(4):364–72. doi: http://dx.doi.org/10.1038/nbt.2858. PubMed.

18 Saito Y, Ellegast JM, Rafiei A, Song Y, Kull D, Heikenwalder M, et al. Peripheral blood CD34(+) cells efficiently engraft human cytokine knock-in mice. Blood. 2016;128(14):1829–33. doi: http://dx.doi.org/10.1182/blood-2015-10-676452. PubMed.

19 Verma R, Strowig T, Das R, Koduru S, Hafemann A, Hopf S, et al. Humanized Mouse Model of Myeloma Reveals Clinically Occult Genomic Changes in Primary Tumor Cells. Blood. 2015;126(23).

20 Das R, Strowig T, Verma R, Koduru S, Hafemann A, Hopf S, et al. Microenvironment-dependent growth of preneoplastic and malignant plasma cells in humanized mice. Nat Med. 2016;22(11):1351–7. doi: http://dx.doi.org/10.1038/nm.4202. PubMed.

21 Cosgun KN, Rahmig S, Mende N, Reinke S, Hauber I, Schäfer C, et al. Kit regulates HSC engraftment across the human-mouse species barrier. Cell Stem Cell. 2014;15(2):227–38. doi: http://dx.doi.org/10.1016/j.stem.2014.06.001. PubMed.

22 Notta F, Doulatov S, Laurenti E, Poeppl A, Jurisica I, Dick JE. Isolation of single human hematopoietic stem cells capable of long-term multilineage engraftment. Science. 2011;333(6039):218–21. doi: http://dx.doi.org/10.1126/science.1201219. PubMed.

23 Laurenti E, Doulatov S, Zandi S, Plumb I, Chen J, April C, et al. The transcriptional architecture of early human hematopoiesis identifies multilevel control of lymphoid commitment. Nat Immunol. 2013;14(7):756–63. doi: http://dx.doi.org/10.1038/ni.2615. PubMed.

24 Baum CM, Weissman IL, Tsukamoto AS, Buckle AM, Peault B. Isolation of a candidate human hematopoietic stem-cell population. Proc Natl

Acad Sci USA. 1992;89(7):2804–8. doi: http://dx.doi.org/10.1073/pnas.89.7.2804. PubMed.
25 Craig W, Kay R, Cutler RL, Lansdorp PM. Expression of Thy-1 on human hematopoietic progenitor cells. J Exp Med. 1993;177(5):1331–42. doi: http://dx.doi.org/10.1084/jem.177.5.1331. PubMed.
26 Bhatia M, Wang JC, Kapp U, Bonnet D, Dick JE. Purification of primitive human hematopoietic cells capable of repopulating immune-deficient mice. Proc Natl Acad Sci USA. 1997;94(10):5320–5. doi: http://dx.doi.org/10.1073/pnas.94.10.5320. PubMed.
27 Larochelle A, Vormoor J, Hanenberg H, Wang JC, Bhatia M, Lapidot T, et al. Identification of primitive human hematopoietic cells capable of repopulating NOD/SCID mouse bone marrow: implications for gene therapy. Nat Med. 1996;2(12):1329–37. doi: http://dx.doi.org/10.1038/nm1296-1329. PubMed.
28 Kondo M, Wagers AJ, Manz MG, Prohaska SS, Scherer DC, Beilhack GF, et al. Biology of hematopoietic stem cells and progenitors: implications for clinical application. Annu Rev Immunol. 2003;21(1):759–806. doi: http://dx.doi.org/10.1146/annurev.immunol.21.120601.141007. PubMed.
29 Majeti R, Park CY, Weissman IL. Identification of a hierarchy of multipotent hematopoietic progenitors in human cord blood. Cell Stem Cell. 2007;1(6):635–45. doi: http://dx.doi.org/10.1016/j.stem.2007.10.001. PubMed.
30 McKenzie JL, Takenaka K, Gan OI, Doedens M, Dick JE. Low rhodamine 123 retention identifies long-term human hematopoietic stem cells within the Lin-CD34+CD38- population. Blood. 2007;109(2):543–5. doi: http://dx.doi.org/10.1182/blood-2006-06-030270. PubMed.
31 Notta F, Zandi S, Takayama N, Dobson S, Gan OI, Wilson G, et al. Distinct routes of lineage development reshape the human blood hierarchy across ontogeny. Science. 2016;351(6269):aab2116. doi: http://dx.doi.org/10.1126/science.aab2116. PubMed.
32 Yamamoto R, Morita Y, Ooehara J, Hamanaka S, Onodera M, Rudolph KL, et al. Clonal analysis unveils self-renewing lineage-restricted progenitors generated directly from hematopoietic stem cells. Cell. 2013;154(5):1112–26. doi: http://dx.doi.org/10.1016/j.cell.2013.08.007. PubMed.
33 Catlin SN, Busque L, Gale RE, Guttorp P, Abkowitz JL. The replication rate of human hematopoietic stem cells in vivo. Blood. 2011;117(17):4460–6. doi: http://dx.doi.org/10.1182/blood-2010-08-303537. PubMed.
34 Laurenti E, Frelin C, Xie S, Ferrari R, Dunant CF, Zandi S, et al. CDK6 levels regulate quiescence exit in human hematopoietic stem cells. Cell Stem Cell. 2015;16(3):302–13. doi: http://dx.doi.org/10.1016/j.stem.2015.01.017. PubMed.
35 Rongvaux A, Willinger T, Takizawa H, Rathinam C, Auerbach W, Murphy AJ, et al. Human thrombopoietin knockin mice efficiently support human hematopoiesis in vivo. Proc Natl Acad Sci USA. 2011;108(6):2378–83. doi: http://dx.doi.org/10.1073/pnas.1019524108. PubMed.
36 Strowig T, Rongvaux A, Rathinam C, Takizawa H, Borsotti C, Philbrick W, et al. Transgenic expression of human signal regulatory protein alpha in Rag2-/-gamma(c)-/- mice improves engraftment of human hematopoietic cells in humanized mice. Proc Natl Acad Sci USA. 2011;108(32):13218–23. doi: http://dx.doi.org/10.1073/pnas.1109769108. PubMed.
37 Shlush LI, Zandi S, Mitchell A, Chen WC, Brandwein JM, Gupta V, et al.; HALT Pan-Leukemia Gene Panel Consortium. Identification of pre-leukaemic haematopoietic stem cells in acute leukaemia. Nature. 2014;506(7488):328–33. doi: http://dx.doi.org/10.1038/nature13038. PubMed.
38 Lapidot T, Sirard C, Vormoor J, Murdoch B, Hoang T, Caceres-Cortes J, et al. A cell initiating human acute myeloid leukaemia after transplantation into SCID mice. Nature. 1994;367(6464):645–8. doi: http://dx.doi.org/10.1038/367645a0. PubMed.
39 Hope KJ, Jin L, Dick JE. Acute myeloid leukemia originates from a hierarchy of leukemic stem cell classes that differ in self-renewal capacity. Nat Immunol. 2004;5(7):738–43. doi: http://dx.doi.org/10.1038/ni1080. PubMed.
40 Thomas D, Majeti R. Biology and relevance of human acute myeloid leukemia stem cells. Blood. 2017;129(12):1577–85. doi: http://dx.doi.org/10.1182/blood-2016-10-696054. PubMed.
41 Corces-Zimmerman MR, Hong WJ, Weissman IL, Medeiros BC, Majeti R. Preleukemic mutations in human acute myeloid leukemia affect epigenetic regulators and persist in remission. Proc Natl Acad Sci USA. 2014;111(7):2548–53. doi: http://dx.doi.org/10.1073/pnas.1324297111. PubMed.
42 Townsend EC, Murakami MA, Christodoulou A, Christie AL, Köster J, DeSouza TA, et al. The Public Repository of Xenografts Enables Discovery and Randomized Phase II-like Trials in Mice. Cancer Cell. 2016;29(4):574–86. doi: http://dx.doi.org/10.1016/j.ccell.2016.03.008. PubMed.
43 Theocharides AP, Jin L, Cheng PY, Prasolava TK, Malko AV, Ho JM, et al. Disruption of SIRPα signaling in macrophages eliminates human acute myeloid leukemia stem cells in xenografts. J Exp Med. 2012;209(10):1883–99. doi: http://dx.doi.org/10.1084/jem.20120502. PubMed.
44 Majeti R, Chao MP, Alizadeh AA, Pang WW, Jaiswal S, Gibbs KD, Jr, et al. CD47 is an adverse prognostic factor and therapeutic antibody target on human acute myeloid leukemia stem cells. Cell. 2009;138(2):286–99. doi: http://dx.doi.org/10.1016/j.cell.2009.05.045. PubMed.
45 Petrova PS, Viller NN, Wong M, Pang X, Lin GH, Dodge K, et al. TTI-621 (SIRPαFc): A CD47-Blocking Innate Immune Checkpoint Inhibitor with Broad Antitumor Activity and Minimal Erythrocyte Binding. Clin Cancer Res. 2017;23(4):1068–79. doi: http://dx.doi.org/10.1158/1078-0432.CCR-16-1700. PubMed.
46 Ng SW, Mitchell A, Kennedy JA, Chen WC, McLeod J, Ibrahimova N, et al. A 17-gene stemness score for rapid determination of risk in acute leukaemia. Nature. 2016;540(7633):433–7. doi: http://dx.doi.org/10.1038/nature20598. PubMed.
47 Ellegast JM, Rauch PJ, Kovtonyuk LV, Müller R, Wagner U, Saito Y, et al. inv(16) and NPM1mut AMLs engraft human cytokine knock-in mice. Blood. 2016;128(17):2130–4. doi: http://dx.doi.org/10.1182/blood-2015-12-689356. PubMed.
48 Reinisch A, Thomas D, Corces MR, Zhang X, Gratzinger D, Hong WJ, et al. A humanized bone marrow ossicle xenotransplantation model enables improved engraftment of healthy and leukemic human hematopoietic cells. Nat Med. 2016;22(7):812–21. doi: http://dx.doi.org/10.1038/nm.4103. PubMed.
49 Pang WW, Pluvinage JV, Price EA, Sridhar K, Arber DA, Greenberg PL, et al. Hematopoietic stem cell and progenitor cell mechanisms in myelodysplastic syndromes. Proc Natl Acad Sci USA. 2013;110(8):3011–6. doi: http://dx.doi.org/10.1073/pnas.1222861110. PubMed.
50 Woll PS, Kjällquist U, Chowdhury O, Doolittle H, Wedge DC, Thongjuea S, et al. Myelodysplastic syndromes are propagated by rare and distinct human cancer stem cells in vivo. Cancer Cell. 2014;25(6):794–808. doi: . Correction in: Cancer Cell. 2015;27(4)603–5. http://www.cell.com/cancer-cell/fulltext/S1535-6108(15)00091-4 http://dx.doi.org/10.1016/j.ccr.2014.03.036. PubMed.
51 Medyouf H, Mossner M, Jann JC, Nolte F, Raffel S, Herrmann C, et al. Myelodysplastic cells in patients reprogram mesenchymal stromal cells to establish a transplantable stem cell niche disease unit. Cell Stem Cell. 2014;14(6):824–37. doi: http://dx.doi.org/10.1016/j.stem.2014.02.014. PubMed.
52 Campo E, Swerdlow SH, Harris NL, Pileri S, Stein H, Jaffe ES. The 2008 WHO classification of lymphoid neoplasms and beyond: evolving concepts and practical applications. Blood. 2011;117(19):5019–32. doi: http://dx.doi.org/10.1182/blood-2011-01-293050. PubMed.
53 Dores GM, Devesa SS, Curtis RE, Linet MS, Morton LM. Acute leukemia incidence and patient survival among children and adults in the United States, 2001-2007. Blood. 2012;119(1):34–43. doi: http://dx.doi.org/10.1182/blood-2011-04-347872. PubMed.
54 Liem NL, Papa RA, Milross CG, Schmid MA, Tajbakhsh M, Choi S, et al. Characterization of childhood acute lymphoblastic leukemia xenograft models for the preclinical evaluation of new therapies. Blood. 2004;103(10):3905–14. doi: http://dx.doi.org/10.1182/blood-2003-08-2911. PubMed.
55 Jones L, Carol H, Evans K, Richmond J, Houghton PJ, Smith MA, et al. A review of new agents evaluated against pediatric acute lymphoblastic leukemia by the Pediatric Preclinical Testing Program. Leukemia. 2016;30(11):2133–41. doi: http://dx.doi.org/10.1038/leu.2016.192. PubMed.
56 Imada K. Immunodeficient mouse models of lymphoid tumors. Int J Hematol. 2003;77(4):336–41. doi: http://dx.doi.org/10.1007/BF02982640. PubMed.
57 Shultz LD, Lyons BL, Burzenski LM, Gott B, Chen X, Chaleff S, et al. Human lymphoid and myeloid cell development in NOD/LtSz-scid IL2R gamma null mice engrafted with mobilized human hemopoietic stem cells. J Immunol. 2005;174(10):6477–89. doi: http://dx.doi.org/10.4049/jimmunol.174.10.6477. PubMed.
58 Agliano A, Martin-Padura I, Mancuso P, Marighetti P, Rabascio C, Pruneri G, et al. Human acute leukemia cells injected in NOD/LtSz-scid/IL-2Rgamma null mice generate a faster and more efficient disease compared to other NOD/scid-related strains. Int J Cancer. 2008;123(9):2222–7. doi: http://dx.doi.org/10.1002/ijc.23772. PubMed.

59 le Viseur C, Hotfilder M, Bomken S, Wilson K, Röttgers S, Schrauder A, et al. In childhood acute lymphoblastic leukemia, blasts at different stages of immunophenotypic maturation have stem cell properties. Cancer Cell. 2008;14(1):47–58. doi: http://dx.doi.org/10.1016/j.ccr.2008.05.015. PubMed.

60 Schmitz M, Breithaupt P, Scheidegger N, Cario G, Bonapace L, Meissner B, et al. Xenografts of highly resistant leukemia recapitulate the clonal composition of the leukemogenic compartment. Blood. 2011;118(7):1854–64. doi: http://dx.doi.org/10.1182/blood-2010-11-320309. PubMed.

61 Ebinger S, Özdemir EZ, Ziegenhain C, Tiedt S, Castro Alves C, Grunert M, et al. Characterization of Rare, Dormant, and Therapy-Resistant Cells in Acute Lymphoblastic Leukemia. Cancer Cell. 2016;30(6):849–62. doi: http://dx.doi.org/10.1016/j.ccell.2016.11.002. PubMed.

62 Rehe K, Wilson K, Bomken S, Williamson D, Irving J, den Boer ML, et al. Acute B lymphoblastic leukaemia-propagating cells are present at high frequency in diverse lymphoblast populations. EMBO Mol Med. 2013;5(1):38–51. doi: http://dx.doi.org/10.1002/emmm.201201703. PubMed.

63 Clappier E, Gerby B, Sigaux F, Delord M, Touzri F, Hernandez L, et al. Clonal selection in xenografted human T cell acute lymphoblastic leukemia recapitulates gain of malignancy at relapse. J Exp Med. 2011;208(4):653–61. doi: http://dx.doi.org/10.1084/jem.20110105. PubMed.

64 Fischer U, Forster M, Rinaldi A, Risch T, Sungalee S, Warnatz HJ, et al. Genomics and drug profiling of fatal TCF3-HLF-positive acute lymphoblastic leukemia identifies recurrent mutation patterns and therapeutic options. Nat Genet. 2015;47(9):1020–9. doi: http://dx.doi.org/10.1038/ng.3362. PubMed.

65 Barabé F, Kennedy JA, Hope KJ, Dick JE. Modeling the initiation and progression of human acute leukemia in mice. Science. 2007;316(5824):600–4. doi: http://dx.doi.org/10.1126/science.1139851. PubMed.

66 Lin S, Luo RT, Ptasinska A, Kerry J, Assi SA, Wunderlich M, et al. Instructive Role of MLL-Fusion Proteins Revealed by a Model of t(4;11) Pro-B Acute Lymphoblastic Leukemia. Cancer Cell. 2016;30(5):737–49. doi: http://dx.doi.org/10.1016/j.ccell.2016.10.008. PubMed.

67 Notta F, Mullighan CG, Wang JC, Poeppl A, Doulatov S, Phillips LA, et al. Evolution of human BCR-ABL1 lymphoblastic leukaemia-initiating cells. Nature. 2011;469(7330):362–7. doi: http://dx.doi.org/10.1038/nature09733. PubMed.

68 Frismantas V, Dobay MP, Rinaldi A, Tchinda J, Dunn SH, Kunz J, et al. Ex vivo drug response profiling detects recurrent sensitivity patterns in drug resistant ALL. Blood. 2017; 129(11):e26–37. doi: http://dx.doi.org/10.1182/blood-2016-09-738070. PubMed.

69 Patel B, Dey A, Castleton AZ, Schwab C, Samuel E, Sivakumaran J, et al. Mouse xenograft modeling of human adult acute lymphoblastic leukemia provides mechanistic insights into adult LIC biology. Blood. 2014;124(1):96–105. doi: http://dx.doi.org/10.1182/blood-2014-01-549352. PubMed.

70 Macor P, Secco E, Zorzet S, Tripodo C, Celeghini C, Tedesco F. An update on the xenograft and mouse models suitable for investigating new therapeutic compounds for the treatment of B-cell malignancies. Curr Pharm Des. 2008;14(21):2023–39. doi: http://dx.doi.org/10.2174/138161208785294591. PubMed.

71 Donnou S, Galand C, Touitou V, Sautès-Fridman C, Fabry Z, Fisson S. Murine models of B-cell lymphomas: promising tools for designing cancer therapies. Adv Hematol. 2012;2012:701704. doi: http://dx.doi.org/10.1155/2012/701704. PubMed.

72 Birkenmeier K, Moll K, Newrzela S, Hartmann S, Dröse S, Hansmann ML. Basal autophagy is pivotal for Hodgkin and Reed-Sternberg cells' survival and growth revealing a new strategy for Hodgkin lymphoma treatment. Oncotarget. 2016;7(29):46579–88. doi: http://dx.doi.org/10.18632/oncotarget.10300. PubMed.

73 Chu Y, Hochberg J, Yahr A, Ayello J, van de Ven C, Barth M, et al. Targeting CD20+ Aggressive B-cell Non-Hodgkin Lymphoma by Anti-CD20 CAR mRNA-Modified Expanded Natural Killer Cells In Vitro and in NSG Mice. Cancer Immunol Res. 2015;3(4):333–44. doi: http://dx.doi.org/10.1158/2326-6066.CIR-14-0114. PubMed.

74 Deutsch AJA, Rinner B, Pichler M, Prochazka K, Pansy K, Bischof M, et al. NR4A3 suppresses lymphomagenesis through induction of proapoptotic genes. Cancer Res. 2017;77(9):2375–86. doi: http://dx.doi.org/10.1158/0008-5472.CAN-16-2320. PubMed.

75 Suryani S, Carol H, Chonghaile TN, Frismantas V, Sarmah C, High L, et al. Cell and molecular determinants of in vivo efficacy of the BH3 mimetic ABT-263 against pediatric acute lymphoblastic leukemia xenografts. Clin Cancer Res. 2014;20(17):4520–31. doi: http://dx.doi.org/10.1158/1078-0432.CCR-14-0259. PubMed.

76 Bonapace L, Bornhauser BC, Schmitz M, Cario G, Ziegler U, Niggli FK, et al. Induction of autophagy-dependent necroptosis is required for childhood acute lymphoblastic leukemia cells to overcome glucocorticoid resistance. J Clin Invest. 2010;120(4):1310–23. doi: http://dx.doi.org/10.1172/JCI39987. PubMed.

77 McComb S, Aguadé-Gorgorió J, Harder L, Marovca B, Cario G, Eckert C, et al. Activation of concurrent apoptosis and necroptosis by SMAC mimetics for the treatment of refractory and relapsed ALL. Sci Transl Med. 2016;8(339):339ra70. doi: http://dx.doi.org/10.1126/scitranslmed.aad2986. PubMed.

78 Hawkins ED, Duarte D, Akinduro O, Khorshed RA, Passaro D, Nowicka M, et al. T-cell acute leukaemia exhibits dynamic interactions with bone marrow microenvironments. Nature. 2016;538(7626):518–22. doi: http://dx.doi.org/10.1038/nature19801. PubMed.

79 Herman SE, Wiestner A. Preclinical modeling of novel therapeutics in chronic lymphocytic leukemia: the tools of the trade. Semin Oncol. 2016;43(2):222–32. doi: http://dx.doi.org/10.1053/j.seminoncol.2016.02.007. PubMed.

80 Strowig T, Gurer C, Ploss A, Liu YF, Arrey F, Sashihara J, et al. Priming of protective T cell responses against virus-induced tumors in mice with human immune system components. J Exp Med. 2009;206(6):1423–34. doi: http://dx.doi.org/10.1084/jem.20081720. PubMed.

81 Strowig T, Chijioke O, Carrega P, Arrey F, Meixlsperger S, Rämer PC, et al. Human NK cells of mice with reconstituted human immune system components require preactivation to acquire functional competence. Blood. 2010;116(20):4158–67. doi: http://dx.doi.org/10.1182/blood-2010-02-270678. PubMed.

82 Salguero G, Daenthanasanmak A, Münz C, Raykova A, Guzmán CA, Riese P, et al. Dendritic cell-mediated immune humanization of mice: implications for allogeneic and xenogeneic stem cell transplantation. J Immunol. 2014;192(10):4636–47. doi: http://dx.doi.org/10.4049/jimmunol.1302887. PubMed.

83 Landtwing V, Raykova A, Pezzino G, Béziat V, Marcenaro E, Graf C, et al. Cognate HLA absence in trans diminishes human NK cell education. J Clin Invest. 2016;126(10):3772–82. doi: http://dx.doi.org/10.1172/JCI86923. PubMed.

84 Nochi T, Denton PW, Wahl A, Garcia JV. Cryptopatches are essential for the development of human GALT. Cell Reports. 2013;3(6):1874–84. doi: http://dx.doi.org/10.1016/j.celrep.2013.05.037. PubMed.

85 Meixlsperger S, Leung CS, Rämer PC, Pack M, Vanoaica LD, Breton G, et al. CD141+ dendritic cells produce prominent amounts of IFN-α after dsRNA recognition and can be targeted via DEC-205 in humanized mice. Blood. 2013;121(25):5034–44. doi: http://dx.doi.org/10.1182/blood-2012-12-473413. PubMed.

86 Ishikawa F, Niiro H, Iino T, Yoshida S, Saito N, Onohara S, et al. The developmental program of human dendritic cells is operated independently of conventional myeloid and lymphoid pathways. Blood. 2007;110(10):3591–660. doi: http://dx.doi.org/10.1182/blood-2007-02-071613. PubMed.

87 Ding Y, Wilkinson A, Idris A, Fancke B, O'Keeffe M, Khalil D, et al. FLT3-ligand treatment of humanized mice results in the generation of large numbers of CD141+ and CD1c+ dendritic cells in vivo. J Immunol. 2014;192(4):1982–9. doi: http://dx.doi.org/10.4049/jimmunol.1302391. PubMed.

88 Gurer C, Strowig T, Brilot F, Pack M, Trumpfheller C, Arrey F, et al. Targeting the nuclear antigen 1 of Epstein-Barr virus to the human endocytic receptor DEC-205 stimulates protective T-cell responses. Blood. 2008;112(4):1231–9. doi: http://dx.doi.org/10.1182/blood-2008-03-148072. PubMed.

89 Yajima M, Imadome K, Nakagawa A, Watanabe S, Terashima K, Nakamura H, et al. T cell-mediated control of Epstein-Barr virus infection in humanized mice. J Infect Dis. 2009;200(10):1611–5. Published online October 17, 2009. doi: http://dx.doi.org/10.1086/644644. PubMed.

90 Shultz LD, Saito Y, Najima Y, Tanaka S, Ochi T, Tomizawa M, et al. Generation of functional human T-cell subsets with HLA-restricted immune responses in HLA class I expressing NOD/SCID/IL2r gamma(null) humanized mice. Proc Natl Acad Sci USA. 2010;107(29):13022–7. doi: http://dx.doi.org/10.1073/pnas.1000475107. PubMed.

91 Antsiferova O, Müller A, Rämer PC, Chijioke O, Chatterjee B, Raykova A, et al. Adoptive transfer of EBV specific CD8+ T cell clones can transiently control EBV infection in humanized mice. PLoS Pathog. 2014;10(8):e1004333. doi: http://dx.doi.org/10.1371/journal.ppat.1004333. PubMed.

92 Gorantla S, Makarov E, Finke-Dwyer J, Gebhart CL, Domm W, Dewhurst S, et al. CD8+ cell depletion accelerates HIV-1 immunopatholo-

gy in humanized mice. J Immunol. 2010;184(12):7082–91. doi: http://dx.doi.org/10.4049/jimmunol.1000438. PubMed.
93 Billerbeck E, Horwitz JA, Labitt RN, Donovan BM, Vega K, Budell WC, et al. Characterization of human antiviral adaptive immune responses during hepatotropic virus infection in HLA-transgenic human immune system mice. J Immunol. 2013;191(4):1753–64. doi: http://dx.doi.org/10.4049/jimmunol.1201518. PubMed.
94 Chijioke O, Marcenaro E, Moretta A, Capaul R, Munz C. Role of the 2B4 Receptor in CD8+ T-Cell-Dependent Immune Control of Epstein-Barr Virus Infection in Mice With Reconstituted Human Immune System Components. J Infect Dis. 2015;212(5):803–7. doi: http://dx.doi.org/10.1093/infdis/jiv114.
95 Chijioke O, Müller A, Feederle R, Barros MH, Krieg C, Emmel V, et al. Human natural killer cells prevent infectious mononucleosis features by targeting lytic Epstein-Barr virus infection. Cell Reports. 2013;5(6):1489–98. Published online December 24, 2013. doi: . Correction published in: Cell Reports. 2015;12(5):901. http://dx.doi.org/10.1016/j.celrep.2013.11.041. PubMed.
96 Jandus C, Boligan KF, Chijioke O, Liu H, Dahlhaus M, Démoulins T, et al. Interactions between Siglec-7/9 receptors and ligands influence NK cell-dependent tumor immunosurveillance. J Clin Invest. 2014;124(4):1810–20. doi: http://dx.doi.org/10.1172/JCI65899. PubMed.
97 Baenziger S, Tussiwand R, Schlaepfer E, Mazzucchelli L, Heikenwalder M, Kurrer MO, et al. Disseminated and sustained HIV infection in CD34+ cord blood cell-transplanted Rag2-/-gamma c-/- mice. Proc Natl Acad Sci USA. 2006;103(43):15951–6. doi: http://dx.doi.org/10.1073/pnas.0604493103. PubMed.
98 Willinger T, Rongvaux A, Takizawa H, Yancopoulos GD, Valenzuela DM, Murphy AJ, et al. Human IL-3/GM-CSF knock-in mice support human alveolar macrophage development and human immune responses in the lung. Proc Natl Acad Sci USA. 2011;108(6):2390–5. doi: http://dx.doi.org/10.1073/pnas.1019682108. PubMed.
99 Yu CI, Becker C, Wang Y, Marches F, Helft J, Leboeuf M, et al. Human CD1c+ dendritic cells drive the differentiation of CD103+ CD8+ mucosal effector T cells via the cytokine TGF-β. Immunity. 2013;38(4):818–30. doi: http://dx.doi.org/10.1016/j.immuni.2013.03.004. PubMed.
100 Heuts F, Gavier-Widén D, Carow B, Juarez J, Wigzell H, Rottenberg ME. CD4+ cell-dependent granuloma formation in humanized mice infected with mycobacteria. Proc Natl Acad Sci USA. 2013;110(16):6482–7. doi: http://dx.doi.org/10.1073/pnas.1219985110. PubMed.
101 Billerbeck E, Barry WT, Mu K, Dorner M, Rice CM, Ploss A. Development of human CD4+FoxP3+ regulatory T cells in human stem cell factor-, granulocyte-macrophage colony-stimulating factor-, and interleukin-3-expressing NOD-SCID IL2Rγ(null) humanized mice. Blood. 2011;117(11):3076–86. doi: http://dx.doi.org/10.1182/blood-2010-08-301507. PubMed.
102 Wang F. Nonhuman primate models for Epstein-Barr virus infection. Curr Opin Virol. 2013;3(3):233–7. doi: http://dx.doi.org/10.1016/j.coviro.2013.03.003. PubMed.
103 Epstein MA, Achong BG, Barr YM. Virus Particles in Cultured Lymphoblasts from Burkitt's Lymphoma. Lancet. 1964;1(7335):702–3. doi: http://dx.doi.org/10.1016/S0140-6736(64)91524-7. PubMed.
104 Yajima M, Imadome K, Nakagawa A, Watanabe S, Terashima K, Nakamura H, et al. A new humanized mouse model of Epstein-Barr virus infection that reproduces persistent infection, lymphoproliferative disorder, and cell-mediated and humoral immune responses. J Infect Dis. 2008;198(5):673–82. doi: http://dx.doi.org/10.1086/590502. PubMed.
105 Tsai MH, Raykova A, Klinke O, Bernhardt K, Gärtner K, Leung CS, et al. Spontaneous lytic replication and epitheliotropism define an Epstein-Barr virus strain found in carcinomas. Cell Reports. 2013;5(2):458–70. doi: http://dx.doi.org/10.1016/j.celrep.2013.09.012. PubMed.
106 Cocco M, Bellan C, Tussiwand R, Corti D, Traggiai E, Lazzi S, et al. CD34+ cord blood cell-transplanted Rag2-/- gamma(c)-/- mice as a model for Epstein-Barr virus infection. Am J Pathol. 2008;173(5):1369–78. doi: http://dx.doi.org/10.2353/ajpath.2008.071186. PubMed.
107 Ma SD, Hegde S, Young KH, Sullivan R, Rajesh D, Zhou Y, et al. A new model of Epstein-Barr virus infection reveals an important role for early lytic viral protein expression in the development of lymphomas. J Virol. 2011;85(1):165–77. doi: http://dx.doi.org/10.1128/JVI.01512-10. PubMed.
108 Heuts F, Rottenberg ME, Salamon D, Rasul E, Adori M, Klein G, et al. T cells modulate Epstein-Barr virus latency phenotypes during infection of humanized mice. J Virol. 2014;88(6):3235–45. doi: http://dx.doi.org/10.1128/JVI.02885-13. PubMed.
109 Azzi T, Lünemann A, Murer A, Ueda S, Béziat V, Malmberg KJ, et al. Role for early-differentiated natural killer cells in infectious mononucleosis. Blood. 2014;124(16):2533–43. doi: http://dx.doi.org/10.1182/blood-2014-01-553024. PubMed.
110 Gurer C, Strowig T, Brilot F, Pack M, Trumpfheller C, Arrey F, et al. Targeting the nuclear antigen 1 of Epstein-Barr virus to the human endocytic receptor DEC-205 stimulates protective T-cell responses. Blood. 2008;112(4):1231–9. doi: http://dx.doi.org/10.1182/blood-2008-03-148072. PubMed.
111 White RE, Rämer PC, Naresh KN, Meixlsperger S, Pinaud L, Rooney C, et al. EBNA3B-deficient EBV promotes B cell lymphomagenesis in humanized mice and is found in human tumors. J Clin Invest. 2012;122(4):1487–502. Published online March 13, 2012. doi: http://dx.doi.org/10.1172/JCI58092. PubMed.
112 Clapham PR, McKnight A. Cell surface receptors, virus entry and tropism of primate lentiviruses. J Gen Virol. 2002;83(Pt 8):1809–29. doi: http://dx.doi.org/10.1099/0022-1317-83-8-1809. PubMed.
113 Gifford RJ. Viral evolution in deep time: lentiviruses and mammals. Trends Genet. 2012;28(2):89–100. doi: http://dx.doi.org/10.1016/j.tig.2011.11.003. PubMed.
114 Goff SP. Intracellular trafficking of retroviral genomes during the early phase of infection: viral exploitation of cellular pathways. J Gene Med. 2001;3(6):517–28. doi: http://dx.doi.org/10.1002/1521-2254(200111)3:6<517::AID-JGM234>3.0.CO;2-E. PubMed.
115 Boeke JD, Stoye JP. Retrotransposons, Endogenous Retroviruses, and the Evolution of Retroelements. In: Coffin JM, Hughes SH, Varmus HE, editors. Retroviruses. Cold Spring Harbor (NY): 1997.
116 Doitsh G, Greene WC. Dissecting How CD4 T Cells Are Lost During HIV Infection. Cell Host Microbe. 2016;19(3):280–91. doi: http://dx.doi.org/10.1016/j.chom.2016.02.012. PubMed.
117 Nasi M, Pinti M, Mussini C, Cossarizza A. Persistent inflammation in HIV infection: established concepts, new perspectives. Immunol Lett. 2014;161(2):184–8. doi: http://dx.doi.org/10.1016/j.imlet.2014.01.008. PubMed.
118 Moir S, Fauci AS. B-cell exhaustion in HIV infection: the role of immune activation. Curr Opin HIV AIDS. 2014;9(5):472–7. doi: http://dx.doi.org/10.1097/COH.0000000000000092. PubMed.
119 Seddiki N, Brezar V, Draenert R. Cell exhaustion in HIV-1 infection: role of suppressor cells. Curr Opin HIV AIDS. 2014;9(5):452–8. doi: http://dx.doi.org/10.1097/COH.0000000000000087. PubMed.
120 Sagar M. Origin of the transmitted virus in HIV infection: infected cells versus cell-free virus. J Infect Dis. 2014;210(Suppl 3):S667–73. doi: http://dx.doi.org/10.1093/infdis/jiu369. PubMed.
121 Battistini A, Sgarbanti M. HIV-1 latency: an update of molecular mechanisms and therapeutic strategies. Viruses. 2014;6(4):1715–58. doi: http://dx.doi.org/10.3390/v6041715. PubMed.
122 Mbonye U, Karn J. Transcriptional control of HIV latency: cellular signaling pathways, epigenetics, happenstance and the hope for a cure. Virology. 2014;454-455:328–39. doi: http://dx.doi.org/10.1016/j.virol.2014.02.008. PubMed.
123 Churchill MJ, Deeks SG, Margolis DM, Siliciano RF, Swanstrom R. HIV reservoirs: what, where and how to target them. Nat Rev Microbiol. 2016;14(1):55–60. doi: http://dx.doi.org/10.1038/nrmicro.2015.5. PubMed.
124 Fois AF, Brew BJ. The Potential of the CNS as a Reservoir for HIV-1 Infection: Implications for HIV Eradication. Curr HIV/AIDS Rep. 2015;12(2):299–303. doi: http://dx.doi.org/10.1007/s11904-015-0257-9. PubMed.
125 Kandathil AJ, Sugawara S, Balagopal A. Are T cells the only HIV-1 reservoir? Retrovirology. 2016;13(1):86. doi: . Correction published in: Retrovirology. 2017;14:11. http://dx.doi.org/10.1186/s12977-016-0323-4. PubMed.
126 Giri MS, Nebozhyn M, Showe L, Montaner LJ. Microarray data on gene modulation by HIV-1 in immune cells: 2000-2006. J Leukoc Biol. 2006;80(5):1031–43. doi: http://dx.doi.org/10.1189/jlb.0306157. PubMed.
127 Younas M, Psomas C, Reynes J, Corbeau P. Immune activation in the course of HIV-1 infection: Causes, phenotypes and persistence under therapy. HIV Med. 2016;17(2):89–105. doi: http://dx.doi.org/10.1111/hiv.12310. PubMed.
128 Pawlowski A, Jansson M, Sköld M, Rottenberg ME, Källenius G. Tuberculosis and HIV co-infection. PLoS Pathog. 2012;8(2):e1002464. doi: http://dx.doi.org/10.1371/journal.ppat.1002464. PubMed.
129 Desai DV, Kulkarni SS. Herpes Simplex Virus: The Interplay Between HSV, Host, and HIV-1. Viral Immunol. 2015;28(10):546–55. doi: http://dx.doi.org/10.1089/vim.2015.0012. PubMed.
130 Carbone A, Gloghini A, Caruso A, De Paoli P, Dolcetti R. The impact of EBV and HIV infection on the microenvironmental niche underlying

Hodgkin lymphoma pathogenesis. Int J Cancer. 2017;140(6):1233–45. doi: http://dx.doi.org/10.1002/ijc.30473. PubMed.
131 Kuritzkes DR. Hematopoietic stem cell transplantation for HIV cure. J Clin Invest. 2016;126(2):432–7. doi: http://dx.doi.org/10.1172/JCI80563. PubMed.
132 Wang HB, Mo QH, Yang Z. HIV vaccine research: the challenge and the way forward. J Immunol Res. 2015;2015:503978. doi: http://dx.doi.org/10.1155/2015/503978. PubMed.
133 Brockman MA, Jones RB, Brumme ZL. Challenges and Opportunities for T-Cell-Mediated Strategies to Eliminate HIV Reservoirs. Front Immunol. 2015;6:506. doi: http://dx.doi.org/10.3389/fimmu.2015.00506. PubMed.
134 Shang HT, Ding JW, Yu SY, Wu T, Zhang QL, Liang FJ. Progress and challenges in the use of latent HIV-1 reactivating agents. Acta Pharmacol Sin. 2015;36(8):908–16. doi: http://dx.doi.org/10.1038/aps.2015.22. PubMed.
135 Chun TW, Stuyver L, Mizell SB, Ehler LA, Mican JA, Baseler M, et al. Presence of an inducible HIV-1 latent reservoir during highly active antiretroviral therapy. Proc Natl Acad Sci USA. 1997;94(24):13193–7. doi: http://dx.doi.org/10.1073/pnas.94.24.13193. PubMed.
136 Finzi D, Blankson J, Siliciano JD, Margolick JB, Chadwick K, Pierson T, et al. Latent infection of CD4+ T cells provides a mechanism for lifelong persistence of HIV-1, even in patients on effective combination therapy. Nat Med. 1999;5(5):512–7. doi: http://dx.doi.org/10.1038/8394. PubMed.
137 McCune JM. The SCID-hu mouse: a small animal model for the analysis of human hematolymphoid differentiation and function. Bone Marrow Transplant. 1992;9(Suppl 1):74–6. PubMed.
138 Namikawa R, Weilbaecher KN, Kaneshima H, Yee EJ, McCune JM. Long-term human hematopoiesis in the SCID-hu mouse. J Exp Med. 1990;172(4):1055–63. doi: http://dx.doi.org/10.1084/jem.172.4.1055. PubMed.
139 McCune JM, Namikawa R, Shih CC, Rabin L, Kaneshima H. Suppression of HIV infection in AZT-treated SCID-hu mice. Science. 1990;247(4942):564–6. doi: http://dx.doi.org/10.1126/science.2300816. PubMed.
140 Shih CC, Kaneshima H, Rabin L, Namikawa R, Sager P, McGowan J, et al. Postexposure prophylaxis with zidovudine suppresses human immunodeficiency virus type 1 infection in SCID-hu mice in a time-dependent manner. J Infect Dis. 1991;163(3):625–7. doi: http://dx.doi.org/10.1093/infdis/163.3.625. PubMed.
141 Stoddart CA, Bales CA, Bare JC, Chkhenkeli G, Galkina SA, Kinkade AN, et al. Validation of the SCID-hu Thy/Liv mouse model with four classes of licensed antiretrovirals. PLoS One. 2007;2(7):e655. doi: http://dx.doi.org/10.1371/journal.pone.0000655. PubMed.
142 Pettoello-Mantovani M, Kollmann TR, Raker C, Kim A, Yurasov S, Tudor R, et al. Saquinavir-mediated inhibition of human immunodeficiency virus (HIV) infection in SCID mice implanted with human fetal thymus and liver tissue: an in vivo model for evaluating the effect of drug therapy on HIV infection in lymphoid tissues. Antimicrob Agents Chemother. 1997;41(9):1880–7. PubMed.
143 Berges BK, Wheat WH, Palmer BE, Connick E, Akkina R. HIV-1 infection and CD4 T cell depletion in the humanized Rag2-/-gamma c-/- (RAG-hu) mouse model. Retrovirology. 2006;3(1):76. doi: http://dx.doi.org/10.1186/1742-4690-3-76. PubMed.
144 Watanabe S, Terashima K, Ohta S, Horibata S, Yajima M, Shiozawa Y, et al. Hematopoietic stem cell-engrafted NOD/SCID/IL2Rgamma null mice develop human lymphoid systems and induce long-lasting HIV-1 infection with specific humoral immune responses. Blood. 2007;109(1):212–8. doi: http://dx.doi.org/10.1182/blood-2006-04-017681. PubMed.
145 Zhang L, Kovalev GI, Su L. HIV-1 infection and pathogenesis in a novel humanized mouse model. Blood. 2007;109(7):2978–81. doi: http://dx.doi.org/10.1182/blood-2006-07-033159. PubMed.
146 Araínga M, Su H, Poluektova LY, Gorantla S, Gendelman HE. HIV-1 cellular and tissue replication patterns in infected humanized mice. Sci Rep. 2016;6(1):23513. doi: http://dx.doi.org/10.1038/srep23513. PubMed.
147 Berges BK, Rowan MR. The utility of the new generation of humanized mice to study HIV-1 infection: transmission, prevention, pathogenesis, and treatment. Retrovirology. 2011;8(1):65. doi: http://dx.doi.org/10.1186/1742-4690-8-65. PubMed.
148 Choudhary SK, Archin NM, Cheema M, Dahl NP, Garcia JV, Margolis DM. Latent HIV-1 infection of resting $CD4^+$ T cells in the humanized $Rag2^{-/-}$ $\gamma c^{-/-}$ mouse. J Virol. 2012;86(1):114–20. doi: http://dx.doi.org/10.1128/JVI.05590-11. PubMed.
149 Kim KC, Choi BS, Kim KC, Park KH, Lee HJ, Cho YK, et al. A Simple Mouse Model for the Study of Human Immunodeficiency Virus. AIDS Res Hum Retroviruses. 2016;32(2):194–202. doi: http://dx.doi.org/10.1089/aid.2015.0211. PubMed.
150 Metcalf Pate KA, Pohlmeyer CW, Walker-Sperling VE, Foote JB, Najarro KM, Cryer CG, et al. A Murine Viral Outgrowth Assay to Detect Residual HIV Type 1 in Patients With Undetectable Viral Loads. J Infect Dis. 2015;212(9):1387–96. doi: http://dx.doi.org/10.1093/infdis/jiv230. PubMed.
151 Konadu KA, Anderson JS, Huang MB, Ali SA, Powell MD, Villinger F, et al. Hallmarks of HIV-1 pathogenesis are modulated by Nef's Secretion Modification Region. J AIDS Clin Res. 2015;6(7):476. doi: http://dx.doi.org/10.4172/2155-6113.1000476. PubMed.
152 Sampey GC, Saifuddin M, Schwab A, Barclay R, Punya S, Chung MC, et al. Exosomes from HIV-1-infected Cells Stimulate Production of Pro-inflammatory Cytokines through Trans-activating Response (TAR) RNA. J Biol Chem. 2016;291(3):1251–66. doi: http://dx.doi.org/10.1074/jbc.M115.662171. PubMed.
153 Neff CP, Ndolo T, Tandon A, Habu Y, Akkina R. Oral pre-exposure prophylaxis by anti-retrovirals raltegravir and maraviroc protects against HIV-1 vaginal transmission in a humanized mouse model. PLoS One. 2010;5(12):e15257. doi: http://dx.doi.org/10.1371/journal.pone.0015257. PubMed.
154 Mandal S, Prathipati PK, Kang G, Zhou Y, Yuan Z, Fan W, et al. Tenofovir alafenamide and elvitegravir loaded nanoparticles for long-acting prevention of HIV-1 vaginal transmission. AIDS. 2017;31(4):469–76. doi: http://dx.doi.org/10.1097/QAD.0000000000001349. PubMed.
155 Campos N, Myburgh R, Garcel A, Vautrin A, Lapasset L, Nadal ES, et al. Long lasting control of viral rebound with a new drug ABX464 targeting Rev - mediated viral RNA biogenesis. Retrovirology. 2015;12(1):30. doi: http://dx.doi.org/10.1186/s12977-015-0159-3. PubMed.
156 Nischang M, Sutmuller R, Gers-Huber G, Audigé A, Li D, Rochat MA, et al. Humanized mice recapitulate key features of HIV-1 infection: a novel concept using long-acting anti-retroviral drugs for treating HIV-1. PLoS One. 2012;7(6):e38853. doi: http://dx.doi.org/10.1371/journal.pone.0038853. PubMed.
157 Dash PK, Gendelman HE, Roy U, Balkundi S, Alnouti Y, Mosley RL, et al. Long-acting nanoformulated antiretroviral therapy elicits potent antiretroviral and neuroprotective responses in HIV-1-infected humanized mice. AIDS. 2012;26(17):2135–44. doi: http://dx.doi.org/10.1097/QAD.0b013e328357f5ad. PubMed.
158 Gnanadhas DP, Dash PK, Sillman B, Bade AN, Lin Z, Palandri DL, et al. Autophagy facilitates macrophage depots of sustained-release nanoformulated antiretroviral drugs. J Clin Invest. 2017;127(3):857–73. doi: http://dx.doi.org/10.1172/JCI90025. PubMed.
159 Pardi N, Secreto AJ, Shan X, Debonera F, Glover J, Yi Y, et al. Administration of nucleoside-modified mRNA encoding broadly neutralizing antibody protects humanized mice from HIV-1 challenge. Nat Commun. 2017;8:14630. doi: http://dx.doi.org/10.1038/ncomms14630. PubMed.
160 Lu CL, Murakowski DK, Bournazos S, Schoofs T, Sarkar D, Halper-Stromberg A, et al. Enhanced clearance of HIV-1-infected cells by broadly neutralizing antibodies against HIV-1 in vivo. Science. 2016;352(6288):1001–4. doi: http://dx.doi.org/10.1126/science.aaf1279. PubMed.
161 Halper-Stromberg A, Lu CL, Klein F, Horwitz JA, Bournazos S, Nogueira L, et al. Broadly neutralizing antibodies and viral inducers decrease rebound from HIV-1 latent reservoirs in humanized mice. Cell. 2014;158(5):989–99. doi: http://dx.doi.org/10.1016/j.cell.2014.07.043. PubMed.
162 Lan P, Tonomura N, Shimizu A, Wang S, Yang YG. Reconstitution of a functional human immune system in immunodeficient mice through combined human fetal thymus/liver and CD34+ cell transplantation. Blood. 2006;108(2):487–92. doi: http://dx.doi.org/10.1182/blood-2005-11-4388. PubMed.
163 Brainard DM, Seung E, Frahm N, Cariappa A, Bailey CC, Hart WK, et al. Induction of robust cellular and humoral virus-specific adaptive immune responses in human immunodeficiency virus-infected humanized BLT mice. J Virol. 2009;83(14):7305–21. doi: http://dx.doi.org/10.1128/JVI.02207-08. PubMed.
164 Dudek TE, No DC, Seung E, Vrbanac VD, Fadda L, Bhoumik P, et al. Rapid evolution of HIV-1 to functional $CD8^+$ T cell responses in humanized BLT mice. Sci Transl Med. 2012;4(143):143ra98. doi: http://dx.doi.org/10.1126/scitranslmed.3003984. PubMed.
165 Hofer U, Baenziger S, Heikenwalder M, Schlaepfer E, Gehre N, Regenass S, et al. RAG2-/- gamma(c)-/- mice transplanted with CD34+ cells from human cord blood show low levels of intestinal engraftment and are resistant to rectal transmission of human immunodeficiency virus. J Virol. 2008;82(24):12145–53. doi: http://dx.doi.org/10.1128/JVI.01105-08. PubMed.

166 Sun Z, Denton PW, Estes JD, Othieno FA, Wei BL, Wege AK, et al. Intrarectal transmission, systemic infection, and CD4+ T cell depletion in humanized mice infected with HIV-1. J Exp Med. 2007;204(4):705–14. doi: http://dx.doi.org/10.1084/jem.20062411. PubMed.

167 Denton PW, Nochi T, Lim A, Krisko JF, Martinez-Torres F, Choudhary SK, et al. IL-2 receptor γ-chain molecule is critical for intestinal T-cell reconstitution in humanized mice. Mucosal Immunol. 2012;5(5):555–66. PubMed.

168 Deruaz M, Luster AD. BLT humanized mice as model to study HIV vaginal transmission. J Infect Dis. 2013;208(Suppl 2):S131–6. doi: http://dx.doi.org/10.1093/infdis/jit318. PubMed.

169 Stoddart CA, Maidji E, Galkina SA, Kosikova G, Rivera JM, Moreno ME, et al. Superior human leukocyte reconstitution and susceptibility to vaginal HIV transmission in humanized NOD-scid IL-2Rγ(-/-) (NSG) BLT mice. Virology. 2011;417(1):154–60. doi: http://dx.doi.org/10.1016/j.virol.2011.05.013. PubMed.

170 Ladinsky MS, Kieffer C, Olson G, Deruaz M, Vrbanac V, Tager AM, et al. Electron tomography of HIV-1 infection in gut-associated lymphoid tissue. PLoS Pathog. 2014;10(1):e1003899. doi: http://dx.doi.org/10.1371/journal.ppat.1003899. PubMed.

171 de Marco A, Müller B, Glass B, Riches JD, Kräusslich HG, Briggs JA. Structural analysis of HIV-1 maturation using cryo-electron tomography. PLoS Pathog. 2010;6(11):e1001215. doi: http://dx.doi.org/10.1371/journal.ppat.1001215. PubMed.

172 Olesen R, Swanson MD, Kovarova M, Nochi T, Chateau M, Honeycutt JB, et al. ART influences HIV persistence in the female reproductive tract and cervicovaginal secretions. J Clin Invest. 2016;126(3):892–904. doi: http://dx.doi.org/10.1172/JCI64212. PubMed.

173 Wahl A, Swanson MD, Nochi T, Olesen R, Denton PW, Chateau M, et al. Human breast milk and antiretrovirals dramatically reduce oral HIV-1 transmission in BLT humanized mice. PLoS Pathog. 2012;8(6):e1002732. doi: http://dx.doi.org/10.1371/journal.ppat.1002732. PubMed.

174 Li Q, Tso FY, Kang G, Lu W, Li Y, Fan W, et al. Early Initiation of Antiretroviral Therapy Can Functionally Control Productive HIV-1 Infection in Humanized-BLT Mice. J Acquir Immune Defic Syndr. 2015;69(5):519–27. doi: http://dx.doi.org/10.1097/QAI.0000000000000687. PubMed.

175 Sáez-Cirión A, Bacchus C, Hocqueloux L, Avettand-Fenoel V, Girault I, Lecuroux C, et al.; ANRS VISCONTI Study Group. Post-treatment HIV-1 controllers with a long-term virological remission after the interruption of early initiated antiretroviral therapy ANRS VISCONTI Study. PLoS Pathog. 2013;9(3):e1003211. doi: http://dx.doi.org/10.1371/journal.ppat.1003211. PubMed.

176 Hur EM, Patel SN, Shimizu S, Rao DS, Gnanapragasam PN, An DS, et al. Inhibitory effect of HIV-specific neutralizing IgA on mucosal transmission of HIV in humanized mice. Blood. 2012;120(23):4571–82. doi: http://dx.doi.org/10.1182/blood-2012-04-422303. PubMed.

177 Balazs AB, Ouyang Y, Hong CM, Chen J, Nguyen SM, Rao DS, et al. Vectored immunoprophylaxis protects humanized mice from mucosal HIV transmission. Nat Med. 2014;20(3):296–300. doi: http://dx.doi.org/10.1038/nm.3471. PubMed.

178 Yuan Z, Kang G, Ma F, Lu W, Fan W, Fennessey CM, et al. Recapitulating Cross-Species Transmission of Simian Immunodeficiency Virus SIVcpz to Humans by Using Humanized BLT Mice. J Virol. 2016;90(17):7728–39. doi: http://dx.doi.org/10.1128/JVI.00860-16. PubMed.

179 Deng K, Pertea M, Rongvaux A, Wang L, Durand CM, Ghiaur G, et al. Broad CTL response is required to clear latent HIV-1 due to dominance of escape mutations. Nature. 2015;517(7534):381–5. doi: http://dx.doi.org/10.1038/nature14053. PubMed.

180 Honeycutt JB, Wahl A, Archin N, Choudhary S, Margolis D, Garcia JV. HIV-1 infection, response to treatment and establishment of viral latency in a novel humanized T cell-only mouse (TOM) model. Retrovirology. 2013;10(1):121. doi: http://dx.doi.org/10.1186/1742-4690-10-121. PubMed.

181 Honeycutt JB, Wahl A, Baker C, Spagnuolo RA, Foster J, Zakharova O, et al. Macrophages sustain HIV replication in vivo independently of T cells. J Clin Invest. 2016;126(4):1353–66. doi: http://dx.doi.org/10.1172/JCI84456. PubMed.