Humanized Mice as Unique Tools for Human-Specific Studies

Kylie Su Mei Yong1,2 · Zhisheng Her1 · Qingfeng Chen1,3,4

Received: 15 August 2017 / Accepted: 4 January 2018 / Published online: 7 February 2018
© The Author(s) 2018. This article is an open access publication

Abstract

With an increasing human population, medical research is pushed to progress into an era of precision therapy. Humanized mice are at the very heart of this new forefront where it is acutely required to decipher human-specific disease pathogenesis and test an array of novel therapeutics. In this review, “humanized” mice are defined as immunodeficient mouse engrafted with functional human biological systems. Over the past decade, researchers have been conscientiously making improvements on the development of humanized mice as a model to closely recapitulate disease pathogenesis and drug mechanisms in humans. Currently, literature is rife with descriptions of novel and innovative humanized mouse models that hold a significant promise to become a panacea for drug innovations to treat and control conditions such as infectious disease and cancer. This review will focus on the background of humanized mice, diseases, and human-specific therapeutics tested on this platform as well as solutions to improve humanized mice for future clinical use.

Keywords Humanized mice · Human specificity · Precision therapy · Human diseases · Drug testing

Introduction

Fundamental understandings of many biological processes that occur in humans have evolved from experimental studies on animal models, particularly non-human rodents and non-human primates (NHPs) (Hatziioannou and Evans 2012; Phillips et al. 2014). A major technical barrier in translating these discoveries to treatments is caused by differences in the biological systems between animals and humans (Greek and Rice 2012; Mestas and Hughes 2004; Shanks et al. 2009; Van der Worp et al. 2010). For example, functional Toll-like receptor 10 (TLR10) is absent in mice (Oosting et al. 2014) and cell expression marker CD28 is expressed on 100% of CD4+ and CD8+ T cells in mice but only on 80% of CD4+ and 50% CD8+ T cells in humans (Beyersdorf et al. 2015). Due to these differences, it is common that animal models are refractory to many infectious (Bäumler and Fang 2013; Carlton et al. 2008; Fauci 1988; Pain et al. 2008; Ploss et al. 2009), therapeutic (McKenzie et al. 1995; Rehman et al. 2011), or immunomodulatory agents (Attarwala 2010; Tsoneva et al. 2017) that are human-specific.

To address the limitations of translating discoveries on non-human animal models to clinical applications, a platform known as “humanized mice” was engineered to simulate humans at a cellular and molecular level (Bosma et al. 1983; Pearson et al. 2008). Humanized mice generated in recent years encompass functional human immune systems with expansive capabilities (Rongvaux et al. 2014) and are unprecedented platforms used for understanding disease pathogenesis and evaluation of compounds to treat a variety of human diseases which include but are not limited to, cancer (Her et al. 2017; Ito et al. 2009; Miyakawa et al. 2004; Pan et al. 2017), infectious disease (Amaladoss et al. 2015; Frias-Staheli et al. 2014; Keng et al. 2015; Yajima et al. 2008), autoimmune disease (Gunawan et al. 2017; Viehmann Milam et al. 2014; Young et al. 2015; Zayoud et al. 2013), and graft-versus-host disease (GvHD) (King et al. 2014).
et al. 2008; Kirkiles-Smith et al. 2009; Tobin et al. 2013; Zhao et al. 2015).

This review covers the background of humanized mice, diseases modelled on these platforms, human-specific therapeutics tested, and suggestions for overcoming remaining challenges to improve humanized mouse models for clinical applications.

**Evolving History of Humanized Mice**

There has been a constant pursuit to engineer novel immunodeficient mouse models via gene deletion or backcrossing strains with mutations in essential molecular compartments such as, T cells, B cells, macrophages, natural killer (NK) cells, cytokines, TLRs, and transcription factors (Pearson et al. 2008). The aim of introducing these mutations is to reduce murine cells and increase the engraftment of human cells and tissues to better recapitulate human immune responses (Aryee et al. 2014; Billerbeck et al. 2011; Chen et al. 2009; Rongvaux et al. 2014; Yao et al. 2016).

Tracing the roots of humanized mice, the discovery of non-human animal models xenotransplanted with cells and tissues of human origin was credited to the invention of C.B-17-Prkdc<sup>scid</sup> (CB17-scid) mice (Bosma et al. 1983). Derived from backcrossing C57BL/Ka and BALB/c, this mouse features loss of function mutation in a gene known as protein kinase, DNA-activated, catalytic polypeptide (PRKDC). In normal physiological conditions, PRKDC is essential for resolving breaks in DNA strands during variable, diversity, and joining [V(D)J] recombination for the development of T and B cells (Blunt et al. 1996; Finnie et al. 1996; Lieber et al. 1988; Taccioni et al. 1998). Non-functional PRKDC gene leads to impaired development of T and B cells resulting in syndrome known as severe combined immunodeficiency (scid) (Bosma and Carroll 1991). Despite efforts in creating CB17-scid mice, this model was not used in many experiments due to the poor engraftment of human hematopoietic stem cells (HSCs) (Bosma et al. 1983).

Further research saw the transfer of scid mutation onto a mouse of non-obese diabetic (NOD) background, creating NOD-scid mice which lacked T cells, B cells, and NK cells. This mouse allowed a slightly higher level of human cell reconstitution (Van der Loo et al. 1998). However, the biggest breakthrough in humanized mice only occurred when mutant interleukin 2 receptor α (IL2ra) gene was introduced into NOD-scid mice, creating NOD-scid-y<sup>em</sup> mice (NSG or NOG), which exhibited defective mouse cytokines IL-2, IL-4, IL-7, IL-9, and IL-15 (Ishikawa et al. 2005; Ito et al. 2002; Shultz et al. 2005). Knock-out of recombination activating gene (RAG) 1 or 2 (RAG<sup>1</sup> and RAG<sup>2</sup>) caused even greater immunodeficiencies including an absence of NK cells, T cells, B cells, and impaired macrophage and dendritic cell (DC) subsets (Harris and Badowski 2014; Watanabe et al. 2007). However, an absence of human leukocyte antigen (HLA) in these models resulted in engrafted human pre-T cells being “educated” and selected on mouse thymic epithelium and major histocompatibility complexes (MHCs) (Shultz et al. 2010). Due to this limitation, engrafted human T cells were unable to recognise human antigen-presenting cells, and hence, these mice had impaired immunoglobulin (Ig) class switching and disorganised secondary lymphoid structures (Shultz et al. 2010, 2012). To overcome this hurdle, HLA class I and II transgenes were added into NSG mice allowing the development of human T-cell repertoires and responses (Brehm et al. 2013; Shultz et al. 2010).

Improved models of immunodeficient mice enabled an increase in well-differentiated multilineage human hematopoietic cells, high levels of functional human cell reconstitution and an ability to be engrafted with tissues such as thymus, skin, liver, islets, solid tumors, and blood cancers (Ito et al. 2002). These inventions cascaded into a series of immunodeficient mice and their variants (BRG, NOG, NRG) (Ali et al. 2012; Grover et al. 2017; Ishikawa et al. 2005; Katano et al. 2014; Koboziev et al. 2015; Shultz et al. 2005) being innovated which enabled in-depth analysis in research areas, such as human hematopoiesis (Rongvaux et al. 2011; Yong et al. 2016), innate and adaptive immunity (Brehm et al. 2010; Pearson et al. 2008), autoimmunity (Gunawan et al. 2017; Viehmann Milam et al. 2014), infectious disease (Keng et al. 2015; Lüdtke et al. 2015; Wege et al. 2012), cancer biology (Chang et al. 2015; Her et al. 2017; Morton et al. 2016), and GvHD (King et al. 2008; Kirkiles-Smith et al. 2009; Zhao et al. 2015), in-turn, facilitating the development of therapeutic agents and novel vaccines. An overview of genotypic and physiological characteristics of each model is outlined in Tables 1 and 2.

The conventional ways to engraft immunodeficient mice with functional human cells include, intravenous (i.v.) injection of human peripheral blood mononuclear cells (PBMCs) into mice (Hu-PBL<sup>-scid</sup>) (Duchosal et al. 1992; Harui et al. 2011; King et al. 2008; Tary-Lehmann et al. 1995), injecting CD34<sup>+</sup> HSCs obtained from human fetal liver (FL), umbilical cord blood (UBC), bone marrow (BM) or granulocyte-colony-stimulating factor (G-CSF) mobilised peripheral blood (Hu-SRC-<sup>-scid</sup>) (Brehm et al. 2010; Chen et al. 2009, 2012, 2015; Keng et al. 2015; Yong et al. 2016), or i.v. injection of FL HSCs and BM cells paired with transplantation of matching FL and thymus under the kidney capsule to obtain a BM/liver/thymus (BLT) mouse model (Brainard et al. 2009; Covassin et al. 2013; Denton et al. 2008; Lan et al. 2004, 2006; Melkus et al. 2006; Tomomura et al. 2008). Advantages and drawbacks of each method are compared in Table 3. However, despite efforts in optimising humanized mice, critical challenges that remain include: limited fetal...
| Name                        | C.B-17-scid | NOD-scid | BRG | NOG | NSG™, NOD-scid-γ | NRG, NOD Rag-1™/Mm |
|-----------------------------|------------|----------|-----|-----|------------------|---------------------|
| Nomenclature                | C.B-Igh-1/korta Prkdc<sup>−/−</sup>/IcrTac<sup>−/−</sup> | NOD.CB17-Prkdc<sup>−/−</sup>/IcrTac<sup>−/−</sup> | C.B<sup>-</sup>-Rag2<sup>−/−</sup>/Il2rg<sup>−/−</sup>/SzJ/JicTac | NOD.Cg-Prkdc<sup>−/−</sup>/Il2rg<sup>−/−</sup>/SzJ/JicTac | NOD.Cg-Prkdc<sup>−/−</sup>/Il2rg<sup>−/−</sup>/SzJ/JicTac | NOD.Cg-Rag1<sup>−/−</sup>/MmMom<sup>−/−</sup>/Il2rg<sup>−/−</sup>/SzJ |
| Engraftment method for humanization | HSPCs, BM cells, Spleen cells | HSPCs, PBMCs | HSPCs, PBMCs | HSPCs, PBMCs | Thymus and liver under kidney capsule with matching engraftment of HSPCs from FL Cancer derived from patients and cell lines | Thymus and liver under kidney capsule with matching engraftment of HSPCs from FL Cancer derived from patients and cell lines |
| Limitations                 | Low tolerance for irradiation, Intact innate immune system, Rejection of engraftments, Spontaneous development of thymic lymphomas, Short lifespan | Low tolerance for irradiation, Spontaneous development of thymic lymphomas | Low tolerance for irradiation, Spontaneous development of thymic lymphomas | Low tolerance for irradiation, Spontaneous development of thymic lymphomas | Low tolerance for irradiation, Spontaneous development of thymic lymphomas | Requires a higher dose of irradiation, Not all cancers can be engrafted |
| Applications                | GvHD | Autoimmune type 1 diabetes, Infectious diseases, Oncological studies | Autoimmune type 1 diabetes, Infectious diseases, Oncological studies | Autoimmune type 1 diabetes, Infectious diseases, Oncological studies | Autoimmune type 1 diabetes, Infectious diseases, Oncological studies | Autoimmune type 1 diabetes, Infectious diseases, Oncological studies |
| Dendritic cells            | Yes | Impaired | Impaired | Impaired | Impaired | Impaired |
| Macrophages                 | Yes | Impaired | Impaired | Impaired | Impaired | Impaired |
| NK cells                    | Yes | No | No | No | No | No |
| Mature B cells              | No | No | No | No | No | No |
| Mature T cells              | No | No | No | No | No | No |
| Complement                  | Yes | No | No | No | No | No |
| Leakiness                   | Low | Low | Low | Low | Low | Low |
| Irradiation tolerance       | Low | High | Low | Low | High | Low |
| Lymphoma incidence          | High | High | High | High | High | High |
| Median lifespan             | < 12 months | < 10 months | Not determined | > 18 months | > 18 months | < 12 months |
| References                  | Schneider et al. (1997) | Bastide et al. (2002) | Traggiai et al. (2004) | Watanabe et al. (2009) | Yong et al. (2016) | Harris et al. (2013) |
|                            | Sheng-Tanner et al. (2000) | Brehm et al. (2013) | Ali et al. (2012) | Akkina (2013) | Her et al. (2017) | Shultz et al. (2012) |
|                            | Xia et al. (2006) | | | | | Maykel et al. (2014) |
### Table 2 Platforms for human immune system engrafted mice

| Name                | HuNOG-EXL                  | NSG-SGM3                  | NSG-HLA-A2                  | NSG-Ab DR4                  | MISTRG                  | NSGW41                  |
|---------------------|----------------------------|----------------------------|----------------------------|----------------------------|--------------------------|--------------------------|
| **Nomenclature**    | NOD.Cg-Pkd<sup>−/−</sup>Il2r<sup>−/−</sup>Tg(CMV, IL3, CSF2, KITLG)1Easf/MloySz | NOD.Cg-Pkd<sup>−/−</sup>Il2r<sup>−/−</sup>Tg(FL-HLA-A2.1)1Enge/SzJ | NOD.Cg-Pkd<sup>−/−</sup>Il2r<sup>−/−</sup>Tg(HLA-DRB1)3IDmz/SzJ | C129S4-Reg-Tg<sup>2mt.1(CSF1)</sup>Csf1<sup>−/−</sup>Tg(FL-Perox2<sup>−/−</sup>)Il2r<sup>−/−</sup>Tg(TIRPα<sup>−/−</sup>)1Dmj/FlvJ | NOD.Cg-KitW<sup>−/−</sup>-41JPrkdc<sup>−</sup>Il2rgtm1Wjl/Tg(SV40/HTLV-IL3, CSF2)10-7Jic/JicTac |
| **Engraftment method for humanization** | HSPCs PBMCs | HSPCs PBMCs | HSPCs PBMCs | HSPCs PBMCs | HSPCs Human melanoma cell line (Me290) | HSPCs |
| **Limitations**     | Not all cancers can be engrafted | Human cell engraftment does not last more than five months | Low tolerance for irradiation | Low CD45<sup>+</sup> human cell engraftment compared to NSG mice | Short lifespan post-engraftment (~10–12 weeks) but may be prolonged by avoiding irradiation, using less potent and lower number of stem cells | Not reported |
| **Applications**    | Stem cells Immune system Infectious diseases Oncological studies Drug tests | Stem cells Immune system Infectious diseases Oncological studies Drug tests | Immune system Oncological studies Vaccine development | GvHD | Stem cells Immune system Oncological studies | Stem cells |
| **Dendritic cells** | Impaired | Impaired | Impaired | Impaired | Impaired | Impaired |
| **Macrophages**     | Impaired | Impaired | Impaired | Impaired | Impaired | Impaired |
| **NK cells**        | No | No | No | No | No | No |
| **Mature B cells**  | No | No | No | No | No | No |
| **Mature T cells**  | No | No | No | No | No | No |
| **Complement**      | No | No | No | No | No | No |
| **Leakiness**       | No | No | Low | Low | No | No |
| **Irradiation tolerance** | Not determined | Not determined | Low | High | Low | Not determined |
| **Lymphoma incidence** | Not determined | Not determined | No | Not determined | Not determined | Not determined |
| **Median lifespan** | > 7 months | > 4 months | > 18 months | Not determined | Not determined | Not determined |
| **References**      | Fukuchi et al. (1998) | Biellerbeck et al. (2011) | Whitfield-Larry et al. (2011) | Covassin et al. (2011) | Rongvaux et al. (2014) | Rahmig et al. (2016) |

**HSPCs** hematopoietic stem and progenitor cells, FL fetal liver, GvHD graft-versus-host disease, PBMCs peripheral blood mononuclear cells, BM bone marrow
Table 3 Methods used to establish humanized mouse models

| Model | Human PBMCs engrafted into immunodeficient mice | Human HSCs engrafted into immunodeficient mice | Human HSCs, BM, liver, and thymus engrafted into immunodeficient mice |
|-------|-----------------------------------------------|-----------------------------------------------|---------------------------------------------------------------|
| Alternative name | Hu-PBL-scid | Hu-SRC-scid | BLT |
| Source of cells | Obtained from consented adult donors | FL | FL |
| Method of engraftment | Intravenous injection of mice | Intrahepatic injection of newborn mice within 72 h of birth | Intravenous injection of mice |
| Advantages | Easy techniques applied | Multilineage development of hematopoietic cells | Complete and fully functional human immune system |
| | Fast to establish | Generation of a naïve immune system | HLA-restricted T cells |
| | Presence of functional immune cells such as memory T cells | Injection to pups increase human cell reconstitution | Development of a mucosal system similar to humans |
| | Excellent in modelling GvHD | | Highest level of human cell reconstitution among all the models |
| Drawbacks | Lack B and myeloid cell engraftment | Cell differentiation takes a minimum of 10 weeks | Time-consuming and difficult as surgical implantation is required |
| | Engrafted T cells are activated | Engrafted human T cells are H2 restricted | Cell differentiation takes a minimum of 10 weeks |
| | May develop GvHD | Contains low levels of human RBCs, polymorphonuclear leukocytes, and megakaryocytes | Weak immune responses to xenobiotics |
| | Only suitable for short-term experiments (< 3 months) | | Poor class switching |
| | | | May develop GvHD |

BLT bone marrow/liver/thymus, HSCs hematopoietic stem cells, FL fetal liver, GvHD graft-versus-host disease, PBMCs peripheral blood mononuclear cells, UBC umbilical cord blood, BM bone marrow, G-CSF granulocyte-colony-stimulating factor, RBC red blood cells

samples due to ethical restrictions (Geraghty et al. 2014; Kapp 2006), absence of erythrocytes and neutrophils within reconstituted human immune system (Hu et al. 2011), low and impaired human myeloid cells, dominance of immature B cells (Chen et al. 2012; Lang et al. 2013), and minimal production of antigen-specific IgG class antibodies in humanized mice (Jangalwe et al. 2016).

To overcome technical barriers, a few methods to improve the functional human biological systems in mice is to inject humanized mice with recombinant proteins (Huntington et al. 2009; Van Lent et al. 2009), hydrodynamically inject DNA plasmids (Chen et al. 2009), induce lentivirus expression of cytokines (Van lent et al. 2009), or introduce knock-in gene replacement as so to increase the repertoire of cytokines to support human cells (Billerbeck et al. 2011; Lim et al. 2017; Nicotini et al. 2004; Rongvaux et al. 2011). An example of a technique that is effective does not require complex procedures and can be readily applied in any laboratory is the injection of plasmid DNA (IL-15 and Fms-like tyrosine kinase 3/fetal liver kinase-2 (FLT3/FLK2) ligand) via hydrodynamic tail-vein injection (Chen et al. 2009).

Upon application of this method, the expression levels of human cytokines were present for 2–3 weeks, while the levels of functional NK cells remained high for more than a month (Chen et al. 2009). Unlike mice induced to constitutively express cytokines which may activate cells and skew them toward unideal lineages, hydrodynamic injection enables researchers to control the exact timing of cytokine induction, allowing flexible manipulation of the model. On top of this, cytokine-stimulated NK cells expressed activation and inhibitory receptors; attacked in vitro target cells, and responded well to viral infections within an in vivo setting (Chen et al. 2009).

Another method which requires more time and resources to create but eliminates the need for cytokine plasmid injection is the use of transgenic mice with knock-in genes, encoding for cytokines. Four examples of these enhanced immunodeficient mouse are, first, NOD.Cg-Prkdscid Il2rgtm1SugTg (SV40/HTLV-IL3, CSF2) 10-7Jic/JicTac (huNOG-EXL mouse), this strain of super immunodeficient mouse has a high rate of human cell engraftment and expresses both granulocyte/macrophage colony-stimulating factor (GM-CSF) and human IL-3 cytokines, controlled by SV40 promoter, which induces myeloid reconstitution and differentiation.

Second, NOD.Cg-Prkdscid Il2rgtm1Wjt Tg (CMV-IL3, CSF2, KITLG) 1Eav/MloySzJ (NSG-SGM3 mouse) are knock-in mice expressing IL-3, GM-CSF and stem cell factor (SCF) under the control of human-specific cytomegalovirus (CMV) (Billerbeck et al. 2011; Yao et al. 2016). Even though this combination of genes supports human HSC engraftment, formation of myeloid leukocytes, and reduces
B-lymphopoiesis post-BM transplantation this model lacks an improved red blood cell (RBC) reconstitution and the presence of SCF may destructively affect human stem cell compartments by supporting the growth and competitive repopulation of mouse cells (Billerbeck et al. 2011; Yao et al. 2016).

Third, C;129S4-Rag2tm1.1Flv/Csf1rtnm1(CSF1)Flv/Csf2H2m1.1(CSF2)Flv/Il2rgtm1.1Flv/ThpoFvIl2rgtm1.1Flv/Tg (SIRPα)1Flv/J (MISTRG mouse) was designed to support a greater level of human cell reconstitution, particularly in the myeloid compartment by transgenically inducing human GM-CSF, IL-3, macrophage colony-stimulating factor (M-CSF), thrombopoietin (TPO), and signal-regulatory protein alpha (SIRPα). SIRPα produces anti-phagocytic signals upon interaction with human CD47 cells which inhibits murine macrophages from phagocytosing human cells (Rongvaux et al. 2014). However, due to poor erythropoiesis of both mouse and human cells especially post-irradiation preconditioning, MISTRG mice developed severe anemia resulting in its short lifespan and was eventually discontinued commercially (Rongvaux et al. 2014).

Fourth, NOD.Cg-KitW6.12/PrkdcscidIl2rgtm1WjflWaskJ (NSG/W41) was created to overcome a lack of erythro-megakaryopoiesis in humanized mouse models. Without the need for irradiation, this KIT-deficient mouse demonstrated improved erythropoiesis and platelet formation as compared to other models of mice (Cosgun et al. 2014; Rahmig et al. 2016). After reconstitution, significant numbers of mature thrombocytes were present in the peripheral blood while human erythroblasts were seen in the BM. In addition, the morphology, composition, and enucleation ability of de novo generated human erythroblasts were similar with those in the human BM (Rahmig et al. 2016). However, as this model is relatively new, more studies are needed to further characterise the advances and limitations of this platform. Details of immunodeficient mouse models are listed in Tables 1 and 2. As existing models are far from perfect, it is important to work on components that enhance cell–cell interactions, support differentiation, and induce maturation of human cells, particularly that of myeloid and B cell compartments to create a model that faithfully recapitulates the human immune system.

Models of Human Diseases Established on Humanized Mice

The introduction of humanized mice provides immeasurable opportunities to advance medical research. These increasingly important pre-clinical models are not only easy to handle due to their small sizes, but they also have short reproductive cycles, an exceptional ability to produce a large number of young and are relatively affordable to maintain in animal facilities as they do not require highly specialised infrastructures that are used by NHPs (Fischer and Austad 2011). In addition, humanized mice allow human-specific pathogens to infect and replicate within them and are able to develop functional human-specific immune responses to an array of diseases.

Many mechanisms underlying diseases are not completely dissected; therefore, utilization of humanized mice allows researchers to understand important factors that facilitate the development of medical issues including infectious disease, cancer, autoimmunity, and GvHD. Currently, a mouse model that completely mimics every single human disease does not exist; therefore, research aims such as the consideration of specific parameters to be analyzed including genotype, phenotype of the model, and scientific budget must be thought through carefully to select a suitable platform.

Infectious Disease

Since the invention of humanized mice, multitudinous attempts have been made to recapitulate infectious diseases within these mice. A particular human-specific infectious pathogen that has been successfully studied on humanized mice is a retrovirus known as human immunodeficiency virus (HIV) (Arainga et al. 2016; Berge and Rowan 2011; Choudhary et al. 2009; Duyne et al. 2011; Li et al. 2014). Before humanized mice were introduced, the only non-human animal model available for dissecting HIV pathogenesis was the chimpanzee (Vanden Haesevelde et al. 1996). Because of cellular and molecular differences between HIV pathogenesis in humans and chimpanzees, restricted tropism of HIV and high-expense of using NHPs, the small, cost-effective, and widely available humanized mice were used in place of the NHPs (Denton and Garcia 2011; Hatziioannou and Evans 2012; Miller et al. 2000).

Humanized mice infected with HIV recapitulated the disease’s progression, latency and virology, permitted long-term immunological studies and helped identify crucial factors such as viral infectivity factor, viral protein u, and negative factor which are essential for in vivo HIV replication (Yamada et al. 2015).

Of all the models (Hu-PBL-scid, Hu-SRC-scid and BLT) (Choudhary et al. 2012; Dash et al. 2011; Gorantla et al. 2010; Ince et al. 2010; Long and Stoddart 2012; Sato et al. 2010; Zhang et al. 2011) used to characterise HIV, BLT mice (Carter et al. 2011; Denton et al. 2012; Marsden et al. 2012) had the most accurate representation of the human mucosal system (Brainard et al. 2009; Denton et al. 2010; Sun et al. 2007), allowing the study of vaginal and rectal transmission and prevention of HIV by enabling evaluations of many prophylactic therapeutics (Balazs et al. 2011), anti-HIV antibodies (Choudhary et al. 2009; Joseph et al. 2010), and cellular therapeutic inventions for inhibiting or eliminating
HIV (Holt et al. 2010; Kumar et al. 2008; Neff et al. 2011; Shimizu et al. 2010).

Humanized mouse model with a fully functional human immune system has also been infected with Dengue virus (DENV) (Frias-Staheli et al. 2014; Kuruvilla et al. 2007; Sridharan et al. 2013; Subramanya et al. 2010). These mice demonstrated fever, rash, viremia, erythema, thrombocytopenia, and production of anti-DENV IgM, IgG and a range of cytokines as observed in patients (Mota and Rico-Hesse 2009, 2011). Another human-specific infectious pathogen studied on humanized, Plasmodium falciparum, is a causative agent of malaria (Amaladoss et al. 2015; Carlton et al. 2008; Chen et al. 2014; Good et al. 2015; Jiménez-Díaz et al. 2009; Soulard et al. 2015; Vaughan et al. 2012). For years, our understanding of malaria had been impeded by the lack of human-specific small animal models which can be infected by highly host-specific human Plasmodium species (Amaladoss et al. 2015; Chen et al. 2014; Pain et al. 2008) to recapitulate both erythrocytic and immunological disease pathogenesis observed in patients. Due to this, most in vivo experimental studies of malaria were conducted in rodents with mouse or rat-specific Plasmodium strains (Goodman et al. 2013). Differences in invasion and disease pathology between human and rodent parasite species hindered the translation of findings and evaluation of new therapeutics from rodents to humans (Amaladoss et al. 2015; Chen et al. 2014). This challenge has been tackled by incorporating RBC supplemented, immune cell-optimised (enhanced by hydrodynamic expression of human cytokines, IL-15, and FLT3/FLK2 ligand) humanized mice that supports multiple cycles of P. falciparum infection (Amaladoss et al. 2015; Chen et al. 2014).

Utilizing this model, research teams were able to identify the importance of human NK cells, DCs, and B cells in the control of parasitemia. Notably, how NK cells preferentially interacts with infected RBCs (iRBCs), resulting in the activation of NK cells, release of interferon (IFN)-γ, perforin, and granzyme to lyse and eliminate iRBCs in a contact-dependent manner and the importance of adhesion molecule lymphocyte-associated antigen-1 and DNAX accessory molecule-1 which are required for NK cell interaction and clearance of iRBCs (Amaladoss et al. 2015; Chen et al. 2014). Besides facilitating the understanding of human immune responses to Malaria infection, the use of humanized mice also assists in evaluation of new therapeutics and vaccines (Good et al. 2015; Tsuji et al. 1995).

In addition to the human immune system, recent progress has been made to introduce humanization of the liver in humanized mice to support the study of hepatotropic pathogens such as hepatitis B virus and hepatitis C virus (HCV) (Bility et al. 2012; Keng et al. 2015; Strick-Marchand et al. 2015; Tan-Garcia et al. 2017; Washburn et al. 2011). It has been shown that these new humanized mice could be infected with human strains of hepatitis viruses and exhibit leukocyte infiltrations, liver inflammation, fibrosis, cirrhosis, and elevated cytokines similar to HCV-infected patients (Bility et al. 2014; Keng et al. 2015; Tan-Garcia et al. 2017; Washburn et al. 2011). Mouse models with human liver cells and matched human immune system provides an important platform for understanding disease pathogenesis of hepatitis viruses through human-specific cytokines, chemokines and immune cell regulations involved, potentially translating this knowledge into creation of anti-fibrotic and immune-modulatory therapeutics (Ba et al. 2015; Keng et al. 2015).

Other examples of infectious pathogens studied on humanized mice include, *Mycobacterium tuberculosis* (Calderon et al. 2013; Nusbaum et al. 2016), influenza (Yu et al. 2008; Zheng et al. 2015), *Borrelia hermsii* (Vuyyuru et al. 2011), human CMV (Daenthanasanmak et al. 2015; Smith et al. 2010), Ebola virus (Bird et al. 2016; Lüdtke et al. 2015), Epstein-Barr virus (Cocco et al. 2008; Sato et al. 2011; Yajima et al. 2008) and Kaposi’s sarcoma-associated herpesvirus (Boss et al. 2011; Chang et al. 2009; Wang et al. 2014). Further details on infectious pathogens that have been studied using humanized mice as a platform are detailed in Table 4.

### Cancer

Immunodeficient mice that lack innate and adaptive immune cell compartments enable successful engraftment of many human tumors including tumor cell lines and primary solid and hematological tumors. Currently, there are three ways to study tumor growth and cancer immunology in humanized mice. First, tumor cell lines can be engrafted into humanized mice reconstituted with HSCs or PBMCs (Ito et al. 2009; Tsoneva et al. 2017; Wege et al. 2014). Breast cancer was modelled in mice by concurrently transplanting CD34+ HSCs and tumor cells into newborn mice or engrafting both PBMCs and tumor cells into BRG mice (Wege et al. 2014). In these models, human immune cells were able to traffic and infiltrate the microenvironment, enabling human tumor-immune system interactions to be studied (Wege et al. 2014). To more closely recapitulate human immune responses to tumor cell lines, MISTRG mice engrafted with CD34+ human FL cells were subcutaneously transplanted with a melanoma cell line, Me290 (Rongvaux et al. 2014). Similar to clinical scenarios, it was observed that myeloid cells infiltrated the tumor, numerous cells within the tumor expressed CD14 and CD163 which are commonly associated as macrophage markers, and CD163+ cells were most likely M2-like macrophages as they were HLA-DR<sup>low</sup> and CD206<sup>high</sup>. It was hypothesised that tumor growth may have been mediated by M2-like macrophages that can induce cytokine production or release enzymes to promote vascularisation and immune suppression. Therefore, these mice...
Table 4 Infectious diseases modelled in humanized mice

| Infectious disease | Model | Main findings | References |
|--------------------|-------|---------------|------------|
| *Borreli hermsii*  | Newborn NSG engrafted with human CD34+ UBC cells within 48 h of birth and intravenously or intraperitoneally infected with *B. hermsii* | Similar to clinical scenarios, infection of humanized mice with *B. hermsii* resulted in recurrent episodes of bacteremia which was resolved with *B. hermsii* specific IgM production. Anti-*B. hermsii* responses were diminished and persistent bacteremia recurred upon administration of anti-human CD20 antibody | Vuyyuru et al. (2011) |
| DENV              | NOD/scid engrafted with human fetal thymus and liver tissue under the kidney capsule and intravenously injected with CD34+ human FL cells to create huBLT mice. Mice were intravenously infected with DENV-2 | Intravenous inoculation of DENV-2 resulted in sustained viremia and infection of leukocytes in lymphoid and non-lymphoid organs. Serum cytokine levels and DENV-2-neutralising human IgM antibodies were detected in infected mice. In re-stimulation with DENV-infected DCs, in vivo primed T cells were activated and had effector functions | Frias-Staheli et al. (2014) |
| Ebola virus       | NSG-A2 intravenously (retro-orbital) injected with human CD34+ UBC from HLA-A2 donors and intraperitoneally infected with Ebola virus | Similar to clinical scenarios, mice showed signs of viremia, cell damage, liver steatosis, and hemorrhage | Lüddke et al. (2015) |
| EBV               | NOG mice intravenously injected with human CD34+ UBC and EBV | B cell lymphoproliferative disorder was observed with high dose of EBV. Low dose of EBV resulted in asymptomatic persistent infection, increased levels of CD8+ T in the peripheral blood, EBV-specific T cell responses and IgM specific to EBV-encoded protein BFRF3 | Yajima et al. (2008) |
| HBV               | NSG-A2 mice were intrahepatically injected with autologous CD34+ HSC and hepatic progenitor cells to create A2/NSG-hu HSC/Hep mice. These mice were intravenously infected with clinical isolates of HBV | Mice were able to demonstrate persistent infection for up to 4 months after HBV inoculation. Similar to clinical scenarios, chronic liver inflammation, liver fibrosis and immune responses were observed in infected mice. Neutralising antibody (anti-HBsAg scFv) was able inhibit liver disease | Bility et al. (2014) |
| HCV               | Newborn NSG were intrahepatically injected with human CD34+ FL cells within 72 h of birth and intravenously infected with HCV | Humanized mice were able to support HCV infection and demonstrated clinical symptoms and immune responses (innate and adaptive) commonly observed in HCV-infected patients | Keng et al. (2015) |
| hAdV              | HLA-A2 mice were engrafted with autologous human CD34+ HSPCs from UCB via intra-orbital injection and intravenously infected with hAdV | Humanized mice recapitulated the pathology of acute and persistent hAdV infection. In acute infection, high mortality, weight loss, liver pathology and expression of viral protein within organs were observed. Chronic infection was asymptomatic and resulted in the development of hAdV-specific adaptive immunity and expression of early viral genes within the BM | Rodríguez et al. (2017) |
| hCMV              | NRG mice engrafted with CD34+ human cells isolated from adult PBMCs and UBC and infected with hCMV | When a tetravalent integrase-defective lentiviral vector (co-expressing GM-CSF, IFN-α, and hCMV pp65 antigen) which induced self-differentiation of monocytes in PBMCs and UCB into DCs with pp65 (“SmyleDCpp65”) was administered, humanized mice infected with hCMV demonstrated remodeling of LNs, upregulation of thymopoiesis in CD4+ and CD8+ T cell precursors, polyclonal effector memory CD8+ T cells expansion in blood, spleen, and BM, PP65-specific CTL, and IgG responses | Daenthanasanmak et al. (2015) |
| HIV               | Newborn NSG intrahepatically infected with CD34+ human FL cells and infected with HIV-1ADA via intraperitoneal injection | Cell distribution and HIV viral life cycle were dependent on tissue compartment and time of infection. HIV-1 in cells was found as forms of integrated DNA and multi- and un-spliced RNA | Araínga et al. (2016) |
| HTLV1             | NOG mice engrafted with human CD133+ UBC cells by IBMI to create IBMI-huNOG mice which were intraperitoneally infected with HTLV-1 | Infected mice recapitulated symptoms of adult T-cell leukemia and HTLV-1-specific adaptive immune responses including, elevation of CD4+ T cells, and signs of atypical lymphocytes with lobulated nuclei | Tezuka et al. (2014) |
### Table 4 (continued)

| Infectious disease | Model                                                                 | Main findings                                                                                                                                                                                                 | References             |
|--------------------|------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------|
| Influenza          | Rag2<sup>−/−</sup>γc<sup>−/−</sup> mice intraperitoneally injected with human PBMCs and intranasally infected with Influenza | Intraperitoneal injection of pamidronate induced Vδ2-T cells to secrete IFN-γ and kill virus infected host cells which helped to control viral replication and suppressed inflammation in lungs of H7N9-infected mice, reducing their morbidity and mortality | Zheng et al. (2015)    |
| KSHV               | NSG mice engrafted with human fetal thymus and liver tissue under the kidney capsule and intravenously injected with CD34<sup>+</sup> human FL cells to create huBLT mice. Mice were infected with KSHV via the oral mucosa | Mice were infected with KSHV via the oral mucosa and established a robust infection by targeting human macrophages and B cells                                                                                     | Wang et al. (2014)     |
| Leishmania major   | Newborn NSG intrahepatically injected with human CD34<sup>+</sup> UBC cells and infected with Leishmania major via subcutaneous footpad injection | At the site of injection, human macrophages were infected with Leishmania parasites and Leishmania-specific human T cell responses were detected. Miltefosine reduced parasitic load and induced side-effects as observed in clinical scenarios | Wege et al. (2012)     |
| Malaria            | Newborn NSG intracardially injected with human CD34<sup>+</sup> UBC cell and intravenously infected with malaria | NSG mice were supplemented human erythropoietin and IL-3 via hydrodynamic tail-vein injection. Human RBCs generated de novo were infected with *P. falciparum* and it was observed that different strains of parasites varied in their infection rates | Amaladoss et al. (2015) |
| NiV                | NSG mice engrafted with human lung tissue and intra graft injected with NiV | Human fetal lung xenografts were able to form human adult lung structures. NiV replicated to high titer and infected human lung tissues resulting in the production of cytokines and chemokines including IL-6, G-CSF, and GM-CSF which commonly causes acute lung injury | Valbuena et al. (2014)  |
| *Mycobacterium tuberculosis* | NSG mice engrafted with human fetal thymus and liver tissue under the kidney capsule and intravenously injected with CD34<sup>+</sup> FL cells to create huBLT mice. These mice were intranasally infected with *tdTomato M. tuberculosis* H37Rv | Mice infected with *M. tuberculosis* demonstrated progressive bacterial infection within the lung which disseminated to the spleen and liver. Pathological analysis of the infected lung displayed obstruction of the bronchial granulomatous lesions, caseous necrosis and crystallised cholesterol deposits. Human T cells were detected at sites of inflammation and bacterial growth, within the lung, liver, and spleen | Calderon et al. (2013)  |
| VZV                | NOD<sup>scid</sup> id mice engrafted with human fetal thymus and liver tissue under the kidney capsule or subcutaneously implanted with fetal skin. MRC-5 cells infected with wild-type VZV/Oka strain was injected into the implants | Varicella-zoster viral proteins were expressed in CD4<sup>+</sup> and CD8<sup>+</sup> T cells which have a capacity to cause viremia. Similar to clinical scenarios, skin implants infected with VZV showed lesions of varicella | Moffat et al. (1995)    |

**Provide definitions, abbreviations, and acronyms:**

- **DEN**: Dengue virus
- **EBV**: Epstein–Barr virus
- **HBV**: hepatitis B virus
- **HCV**: hepatitis C virus
- **hAdV**: human adenovirus
- **hCMV**: human cytomegalovirus
- **HIV**: human immunodeficiency virus
- **HTLV1**: human T-lymphotropic virus 1
- **KSHV**: Kaposi’s sarcoma-associated herpesvirus
- **NiV**: Nipah virus
- **VZV**: Varicella-zoster virus

**Provide expansions of abbreviations and acronyms:**

- **BMSCs**: bone marrow stromal cells
- **PBMC**: peripheral blood mononuclear cells
- **IV**: intravenous
- **IP**: intraperitoneal
- **i.p.**: intraperitoneally
- **i.v.**: intravenously
- **i.m.**: intramuscularly
- **s.c.**: subcutaneous
- **i.d.**: intradermal
- **i.n.**: intranasal

**Provide relevant section titles:**

- **Table 4**: Summary of models and main findings for infectious diseases

---

**Additional notes or clarifications:**

- The table provides a comprehensive overview of models used to study various infectious diseases, detailing main findings and references for each model.

---

**References:**

- Zheng et al. (2015)
- Wang et al. (2014)
- Wege et al. (2012)
- Amaladoss et al. (2015)
- Valbuena et al. (2014)
- Calderon et al. (2013)
- Moffat et al. (1995)
were treated with human-vascular endothelial growth factor (VEGF) inhibitor, Avastin®. Humanized mice engrafted with Me290 responded to treatment by inhibiting tumor growth, suggesting that myeloid cells may support tumor growth via VEGF activity (Rongvaux et al. 2014).

Second, immunodeficient mice can be engrafted with patient-derived xenografts (PDX) (Bankert et al. 2011; Her et al. 2017; Simpson-Abelson et al. 2008). Engraftment of patient-derived acute myeloid leukemia (AML) cells into newborn NSG resulted in high levels of human cell engraftment in the peripheral blood, spleen and BM of recipient mice (Her et al. 2017). Similar to observations in the clinics, these mice also had enlarged spleens and infiltration of AML cells into multiple organs. Even though AML remained unaltered during serial transplantation, many studies with engrafted PDXs into immunodeficient mice have demonstrated that heterogeneity of parental tumor was often only maintained in primary engraftment (Cassidy et al. 2015). Over time and tumor passage, human stromal was frequently compromised by infiltration and replacement with mouse-derived cells (Cassidy et al. 2015; Maykel et al. 2014). This model is ideal for understanding stroma–tumor interactions, which is integral for tumor growth and an important target for cancer therapy.

Third, for a comprehensive study of interactions between human immune cells and tumor in vivo, immunodeficient mice should be engrafted with PDX and human immune cells (Pan et al. 2017; Roth and Harui 2015). This humanized PDX model would not only have a complete tumor microenvironment but also an ability to display heterogeneity lost in tumors (Pan et al. 2017). However, a drawback of this model is the scarcity of autologous HSCs which affects the capacity to generate cohorts for research. To overcome this challenge, HSCs isolated from UBC, FL or G-CSF mobilised PBMCs can be expanded either by transduction with tat-MYC and tat-Bcl2 fusion proteins or cultured with a validated cocktail of growth factors to induce in vitro proliferation of HSCs (Bird et al. 2014; Yong et al. 2016). An example of this model is XactMice which are engrafted with in vitro expanded HSCs and autologous PDX samples from head and neck squamous cell carcinoma patients (Morton et al. 2016). Even though these mice had low levels of humanization in their peripheral blood, they demonstrated an increase in lymphatic vessels and the presence of CD45*CD151+ cells, suggesting that these mice were able to recapitulate immune and stromal cell compartments of the tumor microenvironment (Morton et al. 2016).

While the current immunodeficient mouse strains are able to support the engraftment of most tumor cell lines, not all primary tumors for example prostate cancer can be easily engrafted (Roth and Harui 2015). Novel humanized oncological models are being innovated to address important questions on tumor-immune system interactions, mechanisms of tumor escape, therapeutic potential of immune modulation, as well as refining therapeutic solutions such as chemotherapy, NK cell therapy, checkpoint inhibitors and cytokine therapy. Tumor cell lines, and solid and hematological cancers tested on humanized mice are listed in Table 5.

Autoimmunity

Disparities in the immune system between mice and men restrict the use of mouse models which develops spontaneous autoimmunity (Covassin et al. 2013). To overcome this challenge, Gunawan et al. (2017) engrafted PBMCs from systemic lupus erythematosus (SLE) patients to create a human-specific disease-based immune system which demonstrated that human T and B cells were present in the peripheral blood and spleen of humanized mice and were important to lupus development. Similar to patients, when these mice were treated with dexamethasone, spleen weight, and proteinuria decreased. Mice with a human immune system xenografted with patient samples allow a spectrum of disorders such as SLE (Andrade et al. 2011; Gunawan et al. 2017) and type I diabetes (Shultz et al. 2007; Unger et al. 2012; Viehmann Milam et al. 2014) to be evaluated for the identification of screening markers, retrieval of antigen-specific autoantibodies, and drug tests. Autoimmune diseases that have been studied using humanized mice as a platform are listed in Table 6.

Graft-versus-host Disease

The occurrence of GvHD is a life-threatening complication that may develop following transplantsations (Hu et al. 2011; Hu and Yang 2012). Even though GvHD has been intensively analyzed in non-humanized animal models, many human-specific mechanisms and treatments cannot be tested due to incongruence between humans and mice. Humanized mice are excellent substitutes to investigate exact human immune responses of GvHD and its related therapeutics (Ali et al. 2012; King et al. 2008; Kirkiles-Smith et al. 2009; Tobin et al. 2013; Wang et al. 2011; Zhao et al. 2015). An example of a humanized mouse model applied in GvHD studies is the engraftment of human PBMCs into immunodeficient mice (Ali et al. 2012). Post-transplantation, these mice demonstrated human lymphocytes infiltration into peripheral blood, spleen, lymph nodes, and BM of the mice, had enhanced tissue homing cells with a T-effector memory (TEM) phenotype and high levels of cutaneous lymphocyte antigen, recapitulating the exact pathogenesis of GvHD as observed in patients (Ali et al. 2012; Wang et al. 2011). Utilizing humanized mice to understand human-specific mechanisms of rejection provides a strong pre-clinical platform for the design of novel immunotherapies (Fogal et al. 2011; Onoe et al. 2011; Tobin et al. 2012; Viehmann Milam et al. 2014).
| Cancer     | Model                                                                 | Main findings                                                                                                                                                                                                 | References       |
|------------|-----------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------|
| Bladder    | NSG mice were injected with CD34+ hematopoietic progenitor cells and subcutaneously engrafted with patient-derived bladder cancer cells | Major human immune cell subsets were reconstituted in humanized mice, no xenograft-versus-host disease was observed and PDX retained morphological and genetic fidelity of parental patient cancer                      | Pan et al. (2017) |
| Breast     | NSG were intrahepatically engrafted with human breast carcinoma cell line (SK-BR-3) | Mice were engrafted with functional human immune system and human breast cancer cells. MHC-mismatched tumor cells resulted in activated immune cells, but no clinical signs of rejection were observed                  | Wege et al. (2014) |
| Cervical   | Human cervical carcinoma cell line (C33a) was subcutaneously engrafted into scid mice | Herpes simplex virus type I-based oncolytic treatment in combination with radiation therapy may be an effective treatment for cervical cancer                                                                   | Blank et al. (2002) |
| Colorectal | Rag2−/−γc−/− mice were injected with human PBMCs and subcutaneously engrafted on the flank with colorectal carcinoma cell line (HT-29) | Co-administration of Urelumab and Nivolumab slowed down tumor growth by elevating activated human T lymphocytes which produced IFN-γ and decreased levels of human regulatory T cells in tumor xenografts | Sanmamed et al. (2015) |
| Gastric    | Patient-derived xenografts of gastric cancer were subcutaneously engrafted into the right hind flank of scid and nude mice | Mice engrafted with patient-derived gastric cancers demonstrated identical histological and genetic diversities which corresponded to parental patient tumors                                                         | Zhang et al. (2015) |
| HNSCC      | NSG mice were injected with expanded HSPCs and engrafted with patient-derived HNSCC | Human immune and stromal cells produced in XactMice mimics patient's tumor microenvironment. This model was able to reverse genetic drift of tumors that usually occur after serial transplantation in non-humanized mice | Morton et al. (2016) |
| Kidney     | NSG mice were engrafted with human RCC cell line (SKRC-59 cells) in the left subrenal capsule of their kidney | Human anti-CAIX mAbs inhibit RCC growth by halting migration and triggering immune-mediated killing of RCC. Improvements to anti-CAIX mAbs demonstrated enhanced antibody-dependent cell-mediated cytotoxicity against RCC | Chang et al. (2015) |
| Leukemia   | Newborn NSG were intravenously engrafted with patient-derived AML cells | High levels of AML engraftment were observed in the peripheral blood, spleen and BM of recipient mice. Similar to clinical scenarios, mice had enlarged spleen and infiltration of AML cells into multiple organs. Serial transplantation did not alter AML cells | Her et al. (2017)  |
| Lung       | NSG and C.B-17-scid subcutaneously engrafted with patient-derived xenograft at a position caudal to the xiphoid process | NSG mice were successfully engrafted with patient-derived primary lung tumors. Mice retained parental tumor architecture such as tumor-associated leukocytes, stromal fibroblasts, and had limited xenograft-versus-host disease. Tumor-associated T cells migrated from the microenvironment of xenografts toward the lung, liver, and spleen of mice | Simpson-Abelson et al. (2008) |
| Lymphoma   | NOG mice were subcutaneously engrafted with human PBMCs and injected with Hodgkin lymphoma cell line (L-428) or cutaneous T-cell lymphoma cell line (HH) | Anti-CCR4 mAb KM2760 demonstrated anti-tumor activity in humanized mouse models of lymphoma. Upon treatment of KM2760, tumor-infiltrating CD56+ NK cells were increased and T-regulatory cells were decreased | Ito et al. (2009) |
| Melanoma   | Newborn NSG were intrahepatically injected with CD34+ UBC and injected with human melanoma cell lines (1935-MEL and 888-MEL) | Mice were successfully engrafted with a functional human immune system. Oncolytic vaccinia virus therapy, particularly CTLA4 scAb increased CD56+ NK cells and decreased virus titers | Tsoneva et al. (2017) |
Human-Specific Drug Tests on Humanized Mouse Models

Non-human animal models are commonly used to test an array of human-specific therapeutics during pre-clinical trials. Due to a lack of human specificity, it is common for pre-clinical trials to inadequately identify exact pharmacokinetics, pharmacodynamics, and side-effects of therapeutics, which may result in debilitating and life-threatening situations when tested on humans (Horvath et al. 2012; Rehman et al. 2011; Xu et al. 2014). To improve from unsuccessful clinical trials, it is important to use validated and cost-effective animal models with high human specificity such as humanized mouse models to expand the traditional armamentarium of therapeutics for treatment of patients with complicated and progressive conditions.

Therapeutics successfully tested in mice with a functional human immune system includes an antiviral drug, peginterferon alpha-2a (Peg-IFNα2a) which demonstrated signs of HCV inhibition such as decreased human IFN-γ production, level of serum alanine aminotransferase, copies of HCV ribonucleic acid (RNA), and absence of leukocyte infiltration or fibrosis in the liver (Keng et al. 2015). Similar to clinical scenarios, humanized mice administered with Ipilimumab developed autoimmune disease with signs of weight loss, anti-nuclear antibodies, and adrenalitis. In addition, a biologic highly specific for human CD28, theralizumab, was tested in humanized mice engrafted with PBMCs (Weißmüller et al. 2016). These mice demonstrated severe reduction in CD45+ human cells, rapid drop of body temperature, elevated levels of cytokines, and succumbed to treatment within 6 h after antibody administration, recapitulating adverse effects observed in clinical scenarios (Weißmüller et al. 2016).

Considering the strengths, limitations, and potential developments of humanized mice, the current data indicate that these models are beneficial tools for researchers to investigate short and long-term studies of in vivo therapeutic interactions and toxicities to mitigate risks and ensure the safety of healthy volunteers and patients exposed to candidate agents during clinical trials. Therapeutics that has been tested on humanized mice is listed in Table 8.

Future Directions and Conclusion

To address gaps in humanized mice, scientists working in different biomedical disciplines are attempting a myriad of approaches including boosting human cell reconstitution,
| Autoimmunity     | Models                                                                 | Main findings                                                                                                                                                                                                 | References                          |
|------------------|------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------|
| Multiple sclerosis | NSG mice engrafted with PBMCs and injected with myelin antigens in Freund's adjuvant and antigen-pulsed autologous DCs | Mice demonstrated subclinical CNS inflammation. Human T cells (CD4^+ and CD8^+) were specific to the soluble domain of myelin oligodendrocyte glycoprotein and produced proinflammatory cytokines | Zayoud et al. (2013)                |
| SLE              | NSG mice engrafted with FL HSCs and injected with pristane            | Humanized mice recapitulated key clinical and immunological features of SLE including production of human anti-nuclear autoantibodies, lupus nephritis, pulmonary serositis, decreased human lymphocytes in peripheral blood, hyperactivated B and T cells and increased proinflammatory cytokines | Gunawan et al. (2017)               |
| SjS              | NSG mice engrafted with PBMCs from patients with SjS                  | Mice engrafted with PBMCs from SjS patients had elevated levels of cytokines, particularly IFN-γ and IL-10. Histological analysis showed signs of inflammation within the lacrimal and salivary glands of mice engrafted with SjS. These infiltrates were mostly CD4^+ and a small population of CD8^+ T cells and B cells | Young et al. (2015)                 |
| Type I diabetes  | NSG-Ab DR^+ engrafted with CD4^+ T cells pulsed with autoantigen-derived peptides | Mice injected with autoantigen-reactive CD4^+ T cells lines from diabetic donors demonstrated human T cells infiltration into mouse islets, insulin, and increased levels of demethylated β-cell–derived DNA in the bloodstream and reduced levels of insulin staining | Viehmann Milam et al. (2014)        |

*SLE* Systemic lupus erythematosus, *SjS* Sjogren’s syndrome, *CNS* central nervous system
Table 7  GvHD modelled in humanized mice

| GvHD          | Models                                                                 | Main findings                                                                                                                                                                                                 | References                  |
|---------------|------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------|
| Cardiac tissue and skin | NSG mice were engrafted with human skin and artery tissue and injected with enriched human CD34+ HSC isolated from peripheral blood of G-colony stimulated factor pre-treated adults or PBMCs autologous to CD34+ donors either separately or together | Without T cells, CD14+CD68+ macrophages infiltrate allogeneic human skin but caused minimal injury and thrombosis. However, with the adoptive transfer of T cells autologous to HSC, CD14+CD68+ macrophages infiltrated allogeneic arterial interposition grafts, induced intimal expansion and calcification | Kirkiles-Smith et al. (2009) |
| hiPSCs        | NSG mice engrafted with human fetal thymus and liver tissue under the kidney capsule and intravenously injected with autologous CD34+ human FL cells to create huBLT mice | Signs suggesting immune rejection of hiPSCs including formation of teratoma, infiltration of antigen-specific T cells and tissue necrosis were observed in these mice engrafted with autologous integration-free hiPSCs. In this study, autologous hiPSC-derived smooth muscle cells were highly immunogenic, while autologous hiPSC-derived retinal pigment epithelial cells were immune tolerated | Zhao et al. (2015)           |
| Islet         | NSG injected with human PBMCs and engrafted with human islets          | Mice demonstrated low intra- and inter-donor variability of PBMCs engraftment. When treated with streptozotocin, mice were hyperglycemic but returned to normoglycemia when transplanted with islet cells. Upon injection of HLA-mismatched human PBMCs, mice showed signs of hyperglycemia, loss of human C-peptide, and rejection of human islet grafts | King et al. (2008)           |
| PBMCs         | NSG mice injected with human PBMCs alone or incubated with MSCs or stromal cells | Effectiveness of MSC therapy was dependent on the time of administration. Mice demonstrated signs of reduced liver and gut pathology and increased survival. MSC therapy did not result in donor T cell anergy and regulatory T cells did not induce the apoptosis of PBMCs; instead, it was associated with direct inhibition of donor CD4+ T cell proliferation and reduction of human TNF-α within the serum | Tobin et al. (2013)          |

GvHD graft-versus-host disease, hiPSCs human induced pluripotent stem cells, PBMCs peripheral blood mononuclear cells, MSCs mesenchymal stem cells, HSC hematopoietic stem cell, TNF tumor necrosis factor.
| Therapeutic | Alternative names | Model | Main findings                                                                                                                                                                                                                                                                  | References |
|------------|-------------------|-------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------|
| Alemtuzumab | Campath®, Campath-1H, MabCampath and Lemtrada | NSG mice intravenously injected with human PBMCs | Similar to clinical scenarios, Alemtuzumab induced severe temperature reduction in mice and bound to CD3 and CD52 but did not induce activation of markers CD25 and CD69 | Brady et al. (2014) |
| ATG        | Thymoglobulin®    | NSG mice injected with human PBMCs | Mice that were given 150 µg of ATG intravenously became sick and were sacrificed within 1 h after treatment. Optimal dose of ATG in this study was 30 µg, where mice demonstrated mild clinical signs of drug treatment but recovered within 5 h | Brady et al. (2014) |
| Eltrombopag | Promacta®, Revolade | NOD/scid mice intravenously injected with human CD34+ UCB cells | Eltrombopag enhanced expansion and promoted multilineage hematopoiesis of HSPCs | Sun et al. (2012) |
| Ipilimumab  | Yervoy®           | Newborn NSG were intrahepatically injected with human CD34+ FL/UCB cells within 24 h of birth | Ipilimumab accelerated rejection of skin graft on humanized mice | Waldron-Lynch et al. (2012) |
| KM2760     | –                 | NOG mice were engrafted with human PBMCs and injected with Hodgkin lymphoma cell line (L-428) or cutaneous T-cell lymphoma cell line (HH) | Anti-CCR4 mAb could be used to induce anti-tumor activity by removing CCR4-expressing tumors and downregulating regulatory T cells | Ito et al. (2009) |
| Lamivudine | 3TC               | C.B-17-scid engrafted with human thymus and liver tissues under the kidney capsule (scid-hu Thy/Liv mouse) | Relative to untreated mice, intraperitoneal injection of 3TC at 30 mg/kg/day had large reductions in viral RNA from a mean of $10^4.7$ to $10^1.8$ copies per $10^6$ cells | Stoddart et al. (2014) |
| Miltefosine | Impavido          | Newborn NSG were engrafted with human CD34+ UBC cells and injected with stationary phase promastigate L. major into the footpad | Parasitic load was reduced and humanized mice demonstrated side-effects similar to clinical scenarios | Wege et al. (2011) |
| Muromonab-CD3 | Orthoclone OKT3  | NSG mice intravenously injected with human PBMCs | Administration of Muromonab-CD3, particularly intravenously resulted in cytokine storm and acute clinical symptoms such as piloerection, hypomotility and hypothermia | Brady et al. (2014) |
| Nivolumab  | Opdivo®           | RAG2−/−γc−/− mice intravenously injected with human PBMCs | In mice engrafted with human colorectal HT-29 carcinoma cells and allogeneic human PBMCs, co-administration of Nivolumab and Urelumab slowed tumor growth | Sanmamed et al. (2015) |
| Therapeutic | Alternative names | Model | Main findings | References |
|-------------|-------------------|-------|---------------|------------|
| Oseltamivir | Tamiflu®          | RAG2−/−γc−/− mice intraperitoneally injected with H7N9 | No therapeutic effects were observed when humanized mice were infected H7N9 were treated with Oseltamivir | Zheng et al. (2015) |
| Pamidronate | Arelia®           | RAG2−/−γc−/− mice intraperitoneally injected with H7N9 | Pamidronate induced controlled viral replication and suppressed H7N9 injected within humanized mice. Treating mice with Pamidronate 3 days after infection could still ameliorate the disease | Zheng et al. (2015) |
| Peg-IFNα2a  | Pegasys®          | Newborn NSG were intrahepatically injected with human CD34+ FL cells within 72 h of birth | HCV copy numbers and serum ALT levels were reduced and no leukocyte infiltrations or fibrosis were observed in HCV-infected humanized mice intramuscularly injected with Peg-IFNα2a | Keng et al. (2015) |
| PG9         |                   | C.B-17-scid engrafted with human thymus and liver tissues under the kidney capsule (scid-hu Thy/Liv mouse) | PG9 provides minimal protective functions in scid-hu Thy/Liv mice challenged with HIVNL4−3. Antibodies can penetrate tissues to prevent infection | Stoddart et al. (2014) |
| PG16        |                   | NSG-BLT mice intravenously injected with human CD34+ FL cells | Single dose of PG16 administered a day before inoculation of HIV was effective in preventing infection | Stoddart et al. (2014) |
| Regorafenib | Stivarga®         | Newborn NSG engrafted with patient primary AML cells | Regorafenib reduced the amount of engrafted human cells within the peripheral blood, extent of myeloid sarcoma and spleen size in mice injected with AML cells | Her et al. (2017) |
| Sorafenib   | Nexavar®          | Newborn NSG engrafted with patient primary AML cells | Sorafenib drastically reduced human cells in the peripheral blood, therefore, minimalising the extent of myeloid sarcoma and reducing spleen size in AML mouse model | Her et al. (2017) |
| Teplizumab  | MGA031, hOKT3y1(Ala-Ala) | Newborn NSG were intrahepatically injected with human CD34+ FL/UCB cells within 24 h of birth | Teplizumab delayed rejection of skin graft on humanized mice | Waldron-Lynch et al. (2012) |
| Theralizumab | TGN1412, CD28-SuperMAB and TAB08 | NRG mice intravenously injected with human PBMCs | Similar to clinical scenarios, humanized mice had a rapid decrease in body temperature, became sick and succumbed to TGN1412, 2–6 h after antibody administration | Weißmüller et al. (2016) |
reducing graft rejections, supporting critical immune cell subsets, and improving human-specific responses toward pathogens to maximise the potential of humanized mice as a pre-clinical platform. Despite an optimistic outlook of humanized mice, there are considerable obstacles associated with the model that has to be solved as soon as possible. This includes scarce sources of human cells and tissues, particularly obtained from fetal samples due to ethical restrictions. A solution for this limitation is underway as teams around the world perfect induced pluripotent stem cell (iPSC) technology, which enables the use of patient-specific iPSCs allowing a renewable source of autologous cells sans immune rejection (Shi et al. 2017).

In humanized mice, secondary lymphoid structures are either missing or disorganised; this curtails essential humoral responses, resulting in impairments for both class switching and affinity maturation post-immunisation. To overcome this, lymphoid tissue inducer cells should be introduced without affecting IL2rg receptors (Lim et al. 2017). Alternatively, immunodeficient mice can be engrafted with both FL and cells that support FL cell growth from the same clinical donor and supplemented with cytokines (e.g., IL-1β, IL-2, IL-7, and GM-CSF), so that differentiation and maturation of HSCs can take place to improve functional immune cells including macrophages, follicular DC, and T helper cell reconstitution (Chen et al. 2009; Lim et al. 2017; Yong et al. 2016).

An absence of essential human cytokines hinders optimal HSC engraftment, differentiation, and maturation of functional immune cells. To tackle this issue, mouse models can be hydrodynamically boosted with plasmids encoding cytokines (Chen et al. 2009). Despite this improvement, binding of human cytokines may be hindered by residual mouse cytokines or may induce mouse cells to proliferate and displace the engraftment of human cells due to the cross-reactivity between some human and mouse cytokines. Eliminating this problem entirely would require absolute depletion of murine cells or the introduction of high affinity human-specific cytokines and growth factors.

Human cell engraftment is being negatively affected by mouse cells (RBCs and innate immune cells) that were not completely depleted during the construction of immunodeficient mice. To improve this, additional gene knock-outs could be added to current strains of immunodeficient mice to further reduce mouse RBCs, granulocytes and macrophage functions (Hu et al. 2011; Hu and Yang 2012), however, because of the low human erythrocyte engraftment, excessive reduction of mouse RBCs might result in anemic mice which has short lifespans, are weak and not suitable for experiments (Rongvaux et al. 2014). A long-term solution would be to optimise and increase the engraftment rate of human RBCs in humanized mice, so that all traces of mouse RBCs can be removed (Hu and Yang 2012).
Long-termism, critical analysis, and adequate troubleshooting to solve existing problems in humanized mice would undoubtedly provide exciting opportunities for the establishment of new and improved humanized models with increased human immune cell engraftment and enhanced functionality that would greatly benefit the community.

Acknowledgements
This work was supported by the following grants: National Research Foundation Fellowship Singapore NRF-NRFFF2017-03 (Q. Chen.), Eradication of HBV TCR Program: NMRC/TCR/014-NUHS/2015, National Medical Research Council, Singapore (Q. Chen) and A*STAR graduate scholarship from Agency for Science, Technology and Research (A*STAR), Singapore (K.S.M. Yong).

Open Access
This article is distributed under the terms of the Creative Commons Attribution 4.0 International License (http://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made.

References
Akkina R (2013) New generation humanized mice for virus research: comparative aspects and future prospects. Virology 435:14–28
Ali N, Flutter B, Sanchez Rodriguez R et al (2012) Xenogeneic graft-versus-host-disease in NOD-scid IL-2Rnull mice display a T-effector memory phenotype. PLoS One 7:e44219
Amaladoss A, Chen Q, Liu M et al (2015) De novo generated human red blood cells in humanized mice support Plasmadium falciparum infection. PLoS One 10:e0129825
Ames E, Canter RJ, Grossenbacher SK et al (2015) NK cells preferentially target tumor cells with a cancer stem cell phenotype. J Immunol 195:4010–4019
Andrade D, Redecha PB, Vukelic M et al (2011) Engraftment of PBMC from SLE and APS donors into BALB-Rag2−/−IL2Rgc−/− mice: a promising model for studying human disease. Arthritis Rheum 63:2764–2779
Araínga M, Su H, Poluektova LY et al (2016) HIV-1 cellular and tissue replication patterns in infected humanized mice. Sci Rep 6:23513
Attarwala H (2010) TGN1412: From discovery to disaster. J Young Pharm 2:332–336
Bae KH, Lee F, Xu K et al (2015) Microstructured dextran hydrogels for burst-free sustained release of PEGylated protein drugs. Biomaterials 63:146–157
Balazs AB, Chen J, Hong CM et al (2011) Antibody-based protection against HIV infection by vectored immunophylaxis. Nature 481:81–84
Bankert RB, Balu-Iyer SV, Odunsi K et al (2011) Humanized mouse model of ovarian cancer recapitulates patient solid tumor progression, ascites formation, and metastasis. PLoS One 6:e24420
Bastide C, Bagnis C, Mannoni P et al (2002) A Nod Scid mouse model
B battles C, Hénig T (2015) CD28 co-stimulation in T-cell homeostasis: A recent perspective. Immunotargets Ther 4:111–122
Bility MT, Zhang L, Washburn ML et al (2012) Generation of a humanized mouse model with both human immune system and liver cells to model hepatitis C virus infection and liver immunopathogenesis. Nat Protoc 7:1608–1617
Bility MT, Cheng L, Zhang Z et al (2014) Hepatitis B virus infection and immunopathogenesis in a humanized mouse model: Induction of human-specific liver fibrosis and M2-like macrophages. PLoS Pathog 10:e1004302
Billerbeck E, Barry WT, Mu K et al (2011) Development of human CD4(+)FoxP3(+) regulatory T cells in human stem cell factor-, granulocyte-macrophage colony-stimulating factor-, and interleukin-3-expressing NOD-SCID IL2Rnull) humanized mice. Blood 117:3076–3086
Bird GA, Polsky A, Estes P et al (2014) Expansion of human and murine hematopoietic stem and progenitor cells ex vivo without genetic modification using MYC and Bcl-2 fusion proteins. PLoS One 9:e105525
Bird BH, Spengler JR, Chakrabarti AK et al (2016) Humanized mouse model of Ebola virus disease mimics the immune responses in human disease. J Infect Dis 213:703–711
Blank SV, Rubin SC, Coukos G et al (2002) Replication-selective herpes simplex virus type 1 mutant therapy of cervical cancer is enhanced by low-dose radiation. Hum Gene Ther 13:627–639
Blunt T, Gell D, Fox M et al (1996) Identification of a nonsense mutation in the carboxyl-terminal domain of DNA-dependent protein kinase catalytic subunit in the scid mouse. Proc Natl Acad Sci USA 93:10285–10290
Bosma MJ, Carroll AM (1991) The SCID mouse mutant: Definition, characterization, and potential uses. Annu Rev Immunol 9:323–350
Bosma GC, Custer RP, Bosma MJ (1983) A severe combined immunodeficiency mutation in the mouse. Nature 301:527–530
Bos RV, Nadeau PE, Abbott JR et al (2011) A Kaposi’s Sarcoma-associated herpesvirus-encoded ortholog of microRNA miR-155 induces human splenic B-cell expansion in NOD/LtSz-scid IL2Rnull mice. J Virol 85:9877–9886
Brady JL, Harrison LC, Goodman DJ et al (2014) Preclinical screening for acute toxicity of therapeutic monoclonal antibodies in a hu-SCID model. Clin Transl Immunology 3:e29
Brainard DM, Seung E, Frahm N et al (2009) Induction of robust cellular and humoral virus-specific adaptive immune responses in human immunodeficiency virus-infected humanized BLT mice. J Virol 83:7305–7321
Brehm MA, Cuthbert A, Yang C et al (2010) Parameters for establishing humanized mouse models to study human immunity: Analysis of human hematopoietic stem cell engraftment in three immunodeficient strains of mice bearing the IL2rγ(null) mutation. Clin Immunol 135:84–98
Brehm MA, Shultz LD, Luban J et al (2013) Overcoming current limitations in humanized mouse research. J Infect Dis 208(Suppl 2):S125–S130
Calderon VE, Valbuena G, Goetz Y et al (2013) A humanized mouse model of Tuberculosis. PLoS One 8:e63331
Carlton JM, Adams JH, Silva JC et al (2008) Comparative genomics of the neglected human malaria parasite Plasmodium vivax. Nature 455:757–763
Carter CC, McNamara LA, Onafuwa-Nuga A et al (2011) HIV-1 utilization of the CXCR4 chemokine receptor to infect multipotent hematopoietic stem and progenitor cells. Cell Host Microbe 9:223–234

Springer
Onoe T, Kalscheuer H, Danzl N et al (2011) Human natural regulatory T cell development, suppressive function and post-thymic maturation in a humanized mouse model. J Immunol 187:3895–3903

Oosting M, Cheng SC, Bolscher JM et al (2014) Human TLR10 is an anti-inflammatory pattern-recognition receptor. Proc Natl Acad Sci USA 111:E4478–E4484

Pain A, Böhme U, Berry AE et al (2008) The genome of the simian and human malaria parasite Plasmodium knowlesi. Nature 455:799–803

Pan CX, Shi W, Ma AH et al (2017) Humanized mice (humice) carrying patient-derived xenograft (PDX) as a platform to develop immunotherapy in bladder cancer (BCa). J Clin Oncol 35:381

Patton J, Vuyyuru R, Siglin A et al (2015) Evaluation of the efficiency of human immune system reconstitution in NSG mice and NSG mice containing a human HLA-A2 transgene using hematopoietic stem cells purified from different sources. J Immunol Methods 422:13–21

Pearson T, Greiner DL, Shultz LD (2008) Creation of “humanized” mice to study human immunity. Curr Protoc Immunol Chap 15:Unit-15.21

Phillips KA, Bales KL, Capitanio JP et al (2014) Why primate models matter. Am J Primatol 76:801–827

Ploss A, Evans MJ, Gay sinskaya VA et al (2009) Human occludin is a hepatitis C virus entry factor required for infection of mouse cells. Nature 457:882–886

Rahming S, Kronstein-Wiedemann R, Fohgrab J et al (2016) Improved human erythropoiesis and platelet formation in humanized NSGW41 mice. Stem Cell reports 7:591–601

Rehman W, Arfons LM, Lazarus HM (2011) The rise, fall and subsequent triumph of Thalidomide: Lessons learned in drug development. Ther Adv Hematol 2:291–308

Rodriguez E, Ip WH, Kolbe V et al (2017) Humanized mice reproduce acute and persistent human adenovirus infection. J Infect Dis 215:70–79

Rongvaux A, Willinger T, Takizawa H et al (2011) Human thrombopoietin knockin mice efficiently support human hematopoiesis in vivo. Proc Natl Acad Sci USA 108:2378–2383

Rongvaux A, Willinger T, Martinek J et al (2014) Development and function of human innate immune cells in a humanized mouse model. Nat Biotechnol 32:364–372

Roth MD, Harui A (2015) Human tumor infiltrating lymphocytes cooperatively regulate prostate tumor growth in a humanized mouse model. J Immunother Cancer 3:12

Sanmandi MF, Rodriguez I, Schalper KA et al (2015) Nivolumab and Urelumab enhance antitumor activity of human T lymphocytes engrafted in Rag2−/−IL-2Rγnull immunodeficient mice. Cancer Res 75:3466–3478

Sato K, Nie C, Misawa N et al (2010) Dynamics of memory and naïve CD8+ T lymphocytes in humanized NOD/SCID/IL-2γnull mice infected with CCR5-tropic HIV-1. Vaccine 28(Suppl 2):B32–B37

Sato K, Misawa N, Nie C et al (2011) A novel animal model of Epstein-Barr virus—associated hemophagocytic lymphohistiocytosis in humanized mice. Blood 117:5663–5673

Schneider M, Ekholm F, Grönvik KO (1997) Severe graft-versus-host disease in SCID mice is associated with a decrease of selective donor TCR Vβ specificities and increased expression of IFN-γ and IL-4. Scand J Immunol 46:147–158

Shanks N, Greek R, Greek J (2009) Are animal models predictive for humans? Philos Ethics Humanit Med 4:2

Sheng-Tanner X, McKerlie C, Spaner D (2000) Characterization of graft-versus-host disease in scid mice and prevention by physicochemical stressors. Transplantation 70:1683–1693

Shi Y, Inoue H, Wu JC et al (2017) Induced pluripotent stem cell technology: a decade of progress. Nat Rev Drug Discov 16:115–130

Shimizu S, Hong P, Arumugam B et al (2010) A highly efficient short hairpin RNA potently down-regulates CCR5 expression in systemic lymphoid organs in the hu-BLT mouse model. Blood 115:1534–1544

Shultz LD, Lyons BL, Burzenski LM et al (2005) Human lymphoid and myeloid cell development in NOD/LtSz-scid IL2Rγnull mice engrafted with mobilized human hematopoietic stem cells. J Immunol 174:6477–6489

Shultz LD, Pearson T, King M et al (2007) Humanized NOD/LtSz-scid IL2 receptor common gamma chain knockout mice in diabetes research. Ann NY Acad Sci 1103:77–89

Shultz LD, Saito Y, Najima Y et al (2010) Generation of functional human T-cell subsets with HLA-restricted immune responses in HLA class I expressing NOD/SCID/IL-2Rγnull humanized mice. Proc Natl Acad Sci USA 107:13022–13027

Shultz LD, Drehm MA, Garcia JV et al (2012) Humanized mice for immune system investigation: Progress, promise and challenges. Nat Rev Immunol 12:786–798

Smith MS, Goldman DC, Bailey AS et al (2010) G-CSF reactivates human cytomegalovirus in a latently infected humanized mouse model. Cell Host Microbe 8:284–291

Soullard V, Bosson-Vangha L, Lorthiois A et al (2015) Plasmid falciparum full life cycle and Plasmodium ova liver stages in humanized mice. Nat Commun 6:7690

Sridharan A, Chen Q, Tang KF et al (2013) Inhibition of megakaryocyte development in the bone marrow underlies Dengue virus-induced thrombocytopenia in humanized mice. J Virol 87:11648–11658

Stoddart CA, Galkina SA, Joshi P et al (2014) Efficacy of broadly neutralizing monoclonal antibody PG16 in HIV-infected humanized mice. Virology 462–463:115–125

Strick-Marchand H, Dusseaux M, Darche S et al (2015) A novel mouse model for stable engraftment of a human immune system and human hepatocytes. PLoS One 10:e0119820

Subramanya S, Kim S-S, Abraham S et al (2010) Targeted delivery of small interfering RNA to human dendritic cells to suppress dengue virus infection and associated proinflammatory cytokine production. J Virol 84:2490–2501

Sun Z, Denton PW, Estes JD et al (2007) Intrarectal transmission, systemic infection, and CD4(+) T cell depletion in humanized mice infected with HIV-1. J Exp Med 204:705–714

Sun H, Tsai Y, Nowak I et al (2012) Eltrombopag, a thrombopoietin receptor agonist, enhances human umbilical cord blood hematopoietic stem/primitive progenitor cell expansion and promotes multi-lineage hematopoiesis. Stem Cell Res 9:77–86

Taccioni GE, Amatucci AG, Beamish HJ et al (1998) Targeted disruption of the catalytic subunit of the DNA-PK gene in mice results in severe combined immunodeficiency and radiosensitivity. Immunity 9:355–366

Tan-Garcia A, Wai L-E, Zheng D et al (2017) Intrahepatic CD206+ macrophages contribute to inflammation in advanced viral-related liver disease. J Hepatol 67:490–500

Tary-Lehmann M, Saxon A, Lehmann PV (1995) The human immune system in hu-PBL-SCID mice. Immunol Today 16:529–533

Tezuka K, Xun R, Tei M et al (2014) An animal model of adult T-cell leukemia: Humanized mice with HTLV-1—specific immunity. Blood 123:346–355

Tobin LM, Healy ME, English K et al (2013) Human mesenchymal stem cells suppress donor CD4+ T cell proliferation and reduce pathology in a humanized mouse model of acute graft-versus-host disease. Clin Exp Immunol 172:333–348
Wege AK, Ernst W, Eckl J et al (2011) Humanized tumor mice—a new model to study and manipulate the immune response in advanced cancer therapy. Int J Cancer 129:2194–2206

Wege AK, Florian C, Ernst W et al (2012) Leishmaniasis major infection in humanized mice induces systemic infection and provokes a nonprotective human immune response. PLoS Negl Trop Dis 6:e1741

Wege AK, Schmidt M, Ueberham E et al (2014) Co-transplantation of human hematopoietic stem cells and human breast cancer cells in NSG mice: A novel approach to generate tumor cell specific human antibodies. Mabs 6:968–977

Weißmüller S, Kronhart S, Kreuz D et al (2016) TGN1412 induces lymphopenia and human cytokine release in a humanized mouse model. PLoS One 11:e0149093

Whitfield-Larry F, Young EF, Talmage G et al (2011) HLA-A2–matched peripheral blood mononuclear cells from type 1 diabetic patients, but not nondiabetic donors, transfer insulitis to NOD-scid/γc(null)/HLA-A2 transgenic mice concurrent with the expansion of islet-specific CD8(+) T cells. Diabetes 60:1726–1733

Xia Z, Taylor PR, Locklin RM et al (2006) Innate immune response to human bone marrow fibroblastic cell implantation in CB17 scid/beige mice. J Cell Biochem 98:745–765

Xu D, Nishimura T, Nishimura S et al (2014) Fialuridine induces acute liver failure in chimeric TK-NOG mice: A model for detecting hepatic drug toxicity prior to human testing. PLoS Med 11:e1001628

Yajima M, Imadome K, Nakagawa A et al (2008) 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 198:673–682

Yamada E, Yoshikawa R, Nakano Y et al (2015) Impacts of humanized mouse models on the investigation of HIV-1 infection: Illuminating the roles of viral accessory proteins in vivo. Viruses 7:1373–1390

Yao LC, Cheng M, Wang M et al (2016) Patient-derived tumor xenografts in humanized NSG-SGM3 mice: A new immuno-oncology platform. Eur J Cancer 61:203–204

Yong KSM, Keng CT, Tan SQ et al (2016) Human CD34loCD133lo fetal liver cells support the expansion of human CD34hiCD133hi hematopoietic stem cells. Cell Mol Immunol 13:605–614

Young NA, Wu LC, Bruss M et al (2015) A chimeric human–mouse model of Sjögren’s syndrome. Clin Immunol 156:1–8

Yu CI, Gallegos M, Marches F et al (2008) Broad influenza-specific CD8(+) T-cell responses in humanized mice vaccinated with influenza virus vaccines. Blood 112:3671–3678

Zayoud M, El Malki K, Frauenknecht K et al (2013) Subclinical CNS inflammation as a response to a myelin antigen in humanized mice. J Neuroimmune Pharmacol 8:1037–1047

Zhang L, Jiang Q, Li G et al (2011) Efficient infection, activation, and impairment of pDCs in the BM and peripheral lymphoid organs during early HIV-1 infection in humanized rag2(-/-) C(-/-) mice in vivo. Blood 117:6184–6192

Zhang T, Zhang L, Fan S et al (2015) Patient-derived gastric carcinoma xenograft mouse models faithfully represent human tumor molecular diversity. PLoS One 10:e0134493

Zhao T, Zhang Z-n, Westenskow PD et al (2015) Humanized mice reveal differential immunogenicity of cells derived from autologous induced pluripotent stem cells. Cell Stem Cell 17:353–359

Zheng J, Wu WL, Liu Y et al (2015) The therapeutic effect of pamidronate on lethal avian influenza A H7N9 virus infected humanized mice. PLoS One 10:e0135999

Tonomura N, Habiro K, Shimizu A et al (2008) Antigen-specific human T-cell responses and T cell-dependent production of human antibodies in a humanized mouse model. Blood 111:4293–4296

Traggiai E, Chicha L, Mazzucchelli L et al (2004) Development of a human adaptive immune system in cord blood cell-transplanted mice. Science 304:104–107

Tsoneva D, Minev B, Frentzen A et al (2017) Humanized mice with subcutaneous human solid tumors for immune response analysis of vaccinia virus-mediated oncolysis. Mol Ther Oncolytics 5:41–61

Tsujii M, Ishihara C, Arai S et al (1995) Establishment of a SCID mouse model having circulating human red blood cells and a possible growth of Plasmodium falciparum in the mouse. Vaccine 13:1389–1392

Unger WW, Pearson T, Abreu JR et al (2012) Islet-specific CTL cloned from a type 1 diabetes patient cause beta-cell destruction after engraftment into HLA-A2 transgenic NOD/SCID/IL2RG null mice. PLoS One 7:e49213

Valbuena G, Halliday H, Borisevich V et al (2014) A human lung xenograft mouse model of Nipah virus infection. PLoS Pathog 10:e1004063

Van Lent AU, Dontje W, Nagasawa M et al (2009) IL-7 enhances thymic human T cell development in “human immune system” Rag2(-/-) IL-2Rγc(-/-) mice without affecting peripheral T cell homeostasis. J Immunol 183:7645–7655

Van der Loo JC, Hanenberg H, Cooper RJ et al (1998) Nonobese diabetic/severe combined immunodeficiency (NOD/SCID) mice as a model system to study the engraftment and mobilization of human peripheral blood stem cells. Blood 92:2556–2570

Van der Worp HB, Howells DW, Sena ES et al (2010) Can animal models of disease reliably inform human studies? PLoS Med 7:e1002458

Vanden Haesevelde MM, Peeters M, Jannes G et al (1996) Sequence analysis of a highly divergent HIV-1-related lentivirus isolated from a wild captured chimpanzee. Virology 221:346–350

Vaughan AM, Mikloajczak SA, Wilson EM et al (2012) Complete Plasmodium falciparum liver-stage development in liver-chimeric mice. J Clin Invest 122:3618–3628

Viehmann Milam AA, Maher SE, Gibson JA et al (2014) A humanized mouse model of autoimmune insulinitis. Diabetes 63:1712–1724

Vuyyuru R, Liu H, Manser T et al (2011) Characteristics of Borrelia hermsii infection in human hematopoietic stem cell-engrafted mice mirror those of human relaxing fever. Proc Natl Acad Sci USA 108:20707–20712

Waldron-Lynch F, Deng S, Preston-Hurtburt P et al (2012) Analysis of human biologics with a mouse skin transplant model in humanized mice. Am J Transplant 12:2652–2662

Wang X, Berger C, Wong CW et al (2011) Engraftment of human central memory-derived effector CD8(+) T cells in immunodeficient mice. Blood 117:1888–1898

Wang LX, Kang G, Kumar P et al (2014) Humanized-BLT mouse model of Kaposi’s sarcoma-associated herpesvirus infection. Proc Natl Acad Sci USA 111:3146–3151

Washburn ML, Bility MT, Zhang L et al (2011) A humanized mouse model to study hepatitis C virus infection, immune response, and liver disease. Gastroenterol 140:1334–1344

Watanabe S, Ohta S, Yajima M et al (2007) Humanized NOD/SCID/IL2Rγnull mice transplanted with hematopoietic stem cells under nonmyeloablative conditions show prolonged life spans and allow detailed analysis of human immunodeficiency virus type 1 pathogenesis. J Virol 81:13259–13264

Watanabe Y, Takahashi T, Okajima A et al (2009) The analysis of the functions of human B and T cells in humanized NOD/scid/γcnull (NOG) mice (hu-HSC NOG mice). Int Immunol 21:843–858