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REVIEW

Humanized immune system animal models and their recent applications

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Abstract

Human or humanized immune system (HIS) animal models have emerged as indispensable tools for studying human biology and disease in vivo. By engrafting human hematopoietic and hematopoietic stem cells (HSC) into immunodeficient hosts, these models have enabled the development of a functional HIS, allowing the study of immune responses, disease mechanisms, and therapeutic interventions in a physiologically relevant setting. HIS models have broad applications across cancer research, infectious disease, regenerative medicine, and immunotherapy development. This review provides a comprehensive overview of the current landscape of HIS model generation, including HSC-based approaches, host strain selection, and recent advances involving genetically engineered mouse models expressing human cytokines and human leukocyte antigen molecules. We evaluated the strengths and limitations of these models, including issues with incomplete immune reconstitution and species-specific incompatibilities. We also discuss their increasing role in preclinical drug development and explore emerging innovations such as multitissue humanization and genome-editing strategies. As HIS models continue to evolve, they provide strong opportunities to bridge basic research and clinical translation.

KEYWORDS
animal science, humanized mice, immunotherapy, patient-derived xenografts, vaccines

1 | INTRODUCTION

In recent years, human or humanized immune system (HIS) animal models have become critical tools for advancing both basic and translational research across a broad range of biomedical disciplines.[1,2] The growing utility of HIS models stems from their ability to more accurately recapitulate aspects of human physiology within a controlled animal system and provides insights not achievable using traditional murine models or in vitro systems alone (Figure 1). Severely immunodeficient mouse strains, described later, are currently the predominant hosts used in humanization studies due to their limited ability to reject human cells. However, recent efforts have expanded the scope of host systems beyond mice to include other species (e.g., rats), and genetically engineered mouse models (GEMM), which provide enhanced support for human cell engraftment.[3] These GEMMs are specifically modified to express human genes, such as human cytokines, human leukocyte antigen (HLA) molecules, or growth factors that better support the human immune cells within the murine environment. In this review, we provide an updated overview of the current

state of humanized animal research. We examine the variety of techniques used to develop humanized mice, including their respective strengths and weaknesses (Figure 1). We also describe the limitations of HIS models and highlight the applications and future directions in humanized animal research (Figure 2). As the field continues to grow, humanized animal models will play an increasingly central role in bridging experimental science and clinical application.

2 | HIS ANIMAL MODELS

The term "humanized animal model" refers to a range of experimental models. Generally, it refers to a murine model that harbors engrafted human tissues or cells, such as components of the HIS or tumor tissue, or expresses human genes introduced through transgenic methods. These methods include random integration of transgenes, targeted knock-in mutations, or exon swapping. In immunology and translational research, humanized mice usually refer to immunodeficient mice engrafted with human $CD34^+$ hematopoietic stem cells (HSC). After transplantation, these HSCs home to the murine bone marrow niche, where they undergo multilineage differentiation and produce major immune cell types, thereby establishing a human-like immune system within a murine host. The development and use of humanized mice for comparative modeling were made possible through the foundational development of transgenic mouse models, such as severe combined immunodeficiency (SCID), nonobese diabetic (NOD), and recombination-activating gene knockouts.[4,5] By combining these immunodeficient strains, researchers developed host mice that could facilitate HSC engraftment with minimal rejection.[6] Importantly, such models support the development of human immune populations, including T, B, and natural killer (NK) cells, and antigen-presenting cells. As a result, HIS animal models now serve

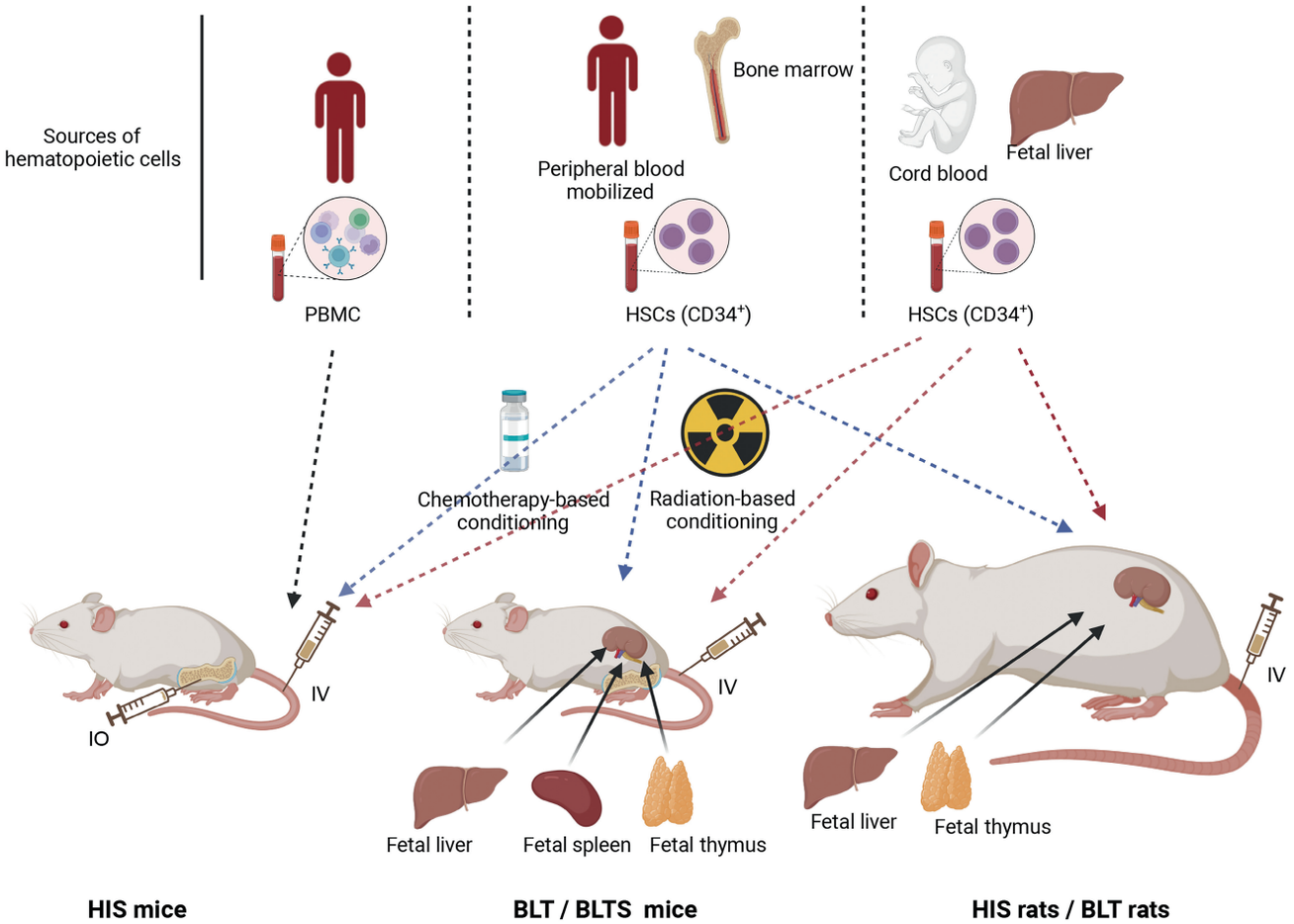

FIGURE 1 Development of humanized animal models with tissue sources and delivery methods. Illustration aims to provide a simple overview of the development of the various human immune system models in mice and rats. There are multiple types of tissues/cells of origin (PBMC, blood, bone marrow, cord blood, or fetal liver), and injection of hematopoietic cells can be combined with transplantation of hematopoietic organs/tissues to improve human reconstitution within the host. Of note, IO injections can significantly reduce the number of HSCs required for engraftment compared to IV injections (up to 1-log). BLT, bone marrow, liver, thymus; BLTS, bone marrow, liver, thymus, spleen; HIS, human or humanized immune system; HSC, hematopoietic stem cell; IO, intraosseous; IV, intravenous; PBMC, peripheral blood mononuclear cell. Figure was created using BioRender.com.

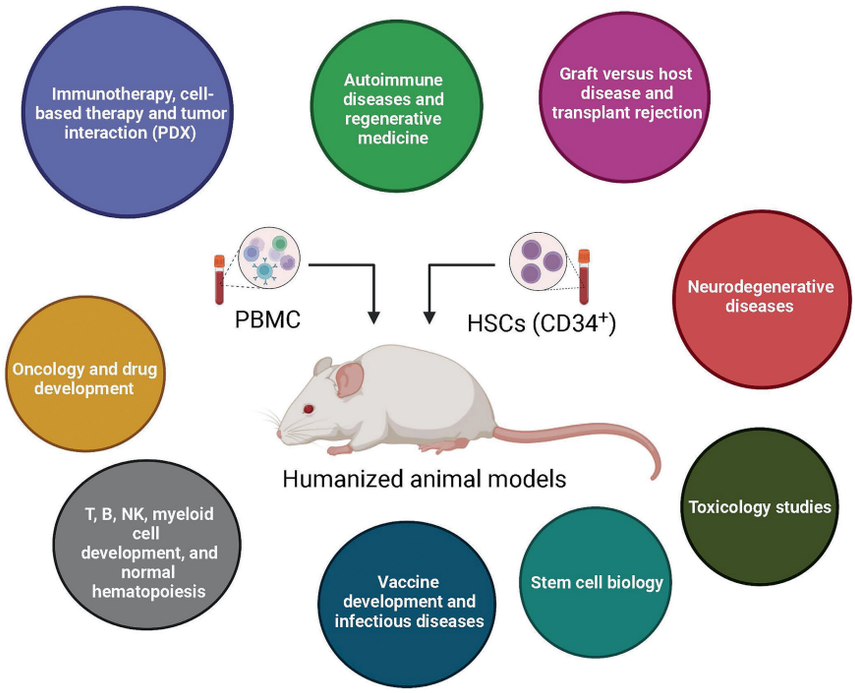

FIGURE 2 Human immune system animal models and applications. Schematic representation highlighting the various uses and applications of humanized animal models. HIS models share many similarities with humans in terms of their immune system functions making them a valuable tool for studying human diseases. Specifically, they can promote basic and translational research in the areas of hematology, cancer and stem cell biology, immunotherapy, infectious disease, neurodegenerative disease, or regenerative medicine. HIS, human or humanized immune system; HSC, hematopoietic stem cell; PBMC, peripheral blood mononuclear cell. Figure was created using BioRender.com.

as platforms for investigating human immune development, tolerance, autoimmunity, infectious disease, and immuno-oncology.[1,7]

3 | HEMATOPOIETIC CELL SOURCES

Humanization of immunodeficient mouse models is commonly achieved through the engraftment of peripheral blood mononuclear cells (PBMC) or CD34-positive ($CD34^+$) HSCs derived from bone marrow, umbilical cord blood, or fetal liver, often co-injected with fragments of human thymus (Figure 1; Table 1).[6,8] Typically, human cells are transplanted after host myeloablation via sublethal irradiation or chemotherapy (e.g., busulfan) conditioning to facilitate niche access (Figure 1). Human HSC injection is performed through intravenous (tail vein, retro-orbital in venous sinus) or intraosseous (femur) routes in adult animals and can also be performed through intrahepatic routes in newborn pups.[6] Of note, intraosseous injections significantly reduce (by 1-log) the number of HSCs required for engraftment.[9] For PBMCs, intrasplenic, intraperitoneal, or intravenous injection can be performed. The choice of the hematopoietic source is a central determinant of reconstitution efficiency, immune cell repertoire, and experimental utility, with each approach offering distinct advantages and limitations.

Common drawbacks remain across all hematopoietic sources and include incomplete immune system reconstitution and eventual development of graft-versus-host disease (GvHD).[10] GvHD results from a mismatch between the murine major histocompatibility complex (MHC) and the injected HLAs and is driven primarily by $CD8^+$ T cells.[11] Human T cells will attack mouse tissues leading to severe consequences for the animal, such as hair loss, lethargy, hunched posture, or weight loss. Systematic discussion and consideration of GvHD is important as it introduces variability and concerns from an animal welfare perspective. This will also affect study design, survival, and interpretation of readouts. Common strategies to mitigate GvHD include using HSCs instead of PBMCs and selecting mouse strains with reduced xenoreactivity (as described in Section 4). Careful animal monitoring for clinical signs is required throughout the experiment to reduce welfare concerns and allow optimal readouts.

3.1 | Bone marrow

Bone marrow (BM)–derived $CD34^+$ HSCs provide robust, multilineage hematopoietic development, including myeloid cells, and functional T and B cells. Injecting large numbers of cells typically produces robust levels of human chimerism in BM and peripheral blood (PB) of the recipient mice, usually after 10–12 weeks. It also allows for modeling of host-specific tolerance and long-term studies.[12] It is compatible with patient-derived xenograft (PDX) studies, which preserve disease

TABLE 1 Sources of human hematopoietic cells and hematopoietic stem cells for engraftment in animal models.

Source	Advantages	Disadvantages
BM HSCs	Easy access Long experimental window Development of various hematopoietic lineages	Expensive Limited HSC yield Lengthy Lack of T-cell education Late GvHD Slower engraftment versus FL and CB
CB HSCs	Long experimental window Development of various hematopoietic lineages Good HSC yield	Limited access Lengthy Expensive Lack of T-cell education Late GvHD Slower engraftment versus FL
FL HSCs	Long experimental window Development of various hematopoietic lineages Faster engraftment versus BM and CB Best HSC yield	Restricted access Expensive Lengthy Lack of T-cell education Late GvHD
Mobilized peripheral blood HSCs	Easy access Long experimental window Development of various hematopoietic lineages Faster engraftment versus BM and CB	Lengthy Lack of T-cell education Late but higher risk of severe GvHD
Peripheral blood mononuclear cells	Easy to establish and quick Inexpensive Immediate engraftment—T-cell engraftment	Short experimental window Low myeloid engraftment Early GvHD

Abbreviations: BM, bone marrow; CB, cord blood; FL, fetal liver; GvHD, graft-versus-host disease; HSC, hematopoietic stem cell.

heterogeneity. In addition to HSCs, recent PDX protocols have been using BM niche-forming cells.[13] This has improved preservation of clonal heterogeneity and leukemic stem-cell self-renewal. However, BM collection is invasive and expensive, and donor variability can impact engraftment outcomes and lineage differentiation. Particularly, donor variability can be overcome by pooling several donors. Compared to cord blood or fetal liver sources, BM HSCs contain fewer long-term HSCs, which may restrict the duration of reconstitution.

3.2 | Umbilical cord blood

Cord blood is a widely used source of HSCs due to its relative availability and higher proportions of long-term HSCs.[14] The limited number of cells recovered per donor might require the pooling of donors. Engraftment typically takes slightly longer (12–16 weeks) compared to adult BM HSCs because of fewer committed progenitors. However, it leads to more durable multilineage reconstitution.[15] Furthermore, the use of immature cord blood cells can reduce alloreactivity and the risk of early GvHD compared to adult BM cells.

3.3 | Fetal liver tissue

Fetal liver is the most enriched source of primitive HSCs and provides highly efficient engraftment across multiple lineages, usually higher than BM or cord blood products.[16,17] Isolated HSCs are often used in combination with fetal thymus and spleen transplantations in BLT/BLTS (bone marrow, liver thymus/ bone marrow, liver, thymus, spleen) models, as described later. Although this adds a layer of complexity to the model, it supports T-cell selection and B-cell development and is useful to study HLA-specific responses or human immunodeficiency virus (HIV) mucosal transmission. However, fetal tissue is difficult to obtain in many regions due to ethical and governmental restrictions. The regulatory burden and the technical complexity tend to restrict a broader use.

3.4 | Human mobilized PB

Mobilized PB is another clinically relevant source of $CD34^+$ HSCs, obtained by treating adult donors with cytokines, such as granulocyte colony-stimulating factor to induce the release of HSCs from the BM into circulation in PB. These cells can then be collected through a simple blood draw. Compared to regular PB, mobilized PB provides significantly higher yields of $CD34^+$ cells. It allows for efficient engraftment, particularly in newborn mice, with rapid peripheral reconstitution observed in 8–10 weeks, though PB engraftment can remain modest.[14,16] However, mobilized PB is less enriched in long-term HSCs than cord blood or fetal liver, leading to variable long-term engraftment efficiency. There is also more variability between donors depending on the efficiency of the mobilization, which requires pharmacological intervention and can be expensive to perform. This remains an option, particularly for

tertiary care centers performing autologous and allogeneic stem-cell transplants, where residual clinical cells could be donated for research purposes.

3.5 | Peripheral blood mononuclear cells

Use of PBMCs is the most straightforward and fastest approach to engraft animal models.[14,18] Blood is easily collected with minimally invasive procedures. It is cost effective, and large numbers of cells can be recovered. Compared to HSC transplant, PBMC injection requires millions of cells. Human T-cell expansion is detectable as early as 2 weeks posttransplant. These models are particularly useful for short-term studies in immuno-oncology, HIV studies, or rapidly growing PDXs.[19] However, PBMC models are limited by the lack of multilineage reconstitution (poor myeloid and B-cell development) and the rapid appearance of xenogeneic GvHD within 3–5 weeks, which impacts the experimental window.[20] As discussed later, specific murine models have been developed to circumvent the quick GvHD phenomenon. Finally, the T cells are restricted to the donor HLA, which is another limitation in the flexibility of the model.

3.6 | General thoughts

Choosing the appropriate source of hematopoietic cells is critical and depends on the research goals to achieve as each source has trade-offs between engraftment efficiency, experimental duration, and translational relevance. In addition, the degree of chimerism, lineage balance, and experimental longevity depend not only on the hematopoietic source but also on animal backgrounds (Figure 3; Table 2).

4 | THE RECIPIENTS

The hematopoietic cells sourced from various human sources described earlier are implanted into immunodeficient recipient animals to allow engraftment of a HIS. Various models and iterations have been developed, and we are reviewing the main ones here (Table 2).

4.1 | NSG - NOD.Cg-*Prkdc*^{scid} *Il2rg*^{tm1Wjl}/SzJ

The NOD.Cg-*Prkdc*^{scid} *Il2rg*^{tm1Wjl}/SzJ (NSG) mice are a highly immunodeficient mouse strain developed by the Jacbkson Laboratory.[12,21] These mice carry mutations that ablate the *Prkdc* gene and the *X-linked IL2rg* gene, impairing the development of T, B, and NK lymphocytes. NSG mice were produced by breeding NOD-SCID females with *IL2rg*-null males and backcrossing the male offspring with NOD-SCID females for eight generations, followed by a final cross between a NOD-SCID female heterozygous for the *IL2rg*-null mutation and an *IL2rg*-null male. Compared to older SCID models, NSG mice exhibit a "nonleaky" phenotype, with no detectable mouse immunoglobulin often past 1 year of age, a sixfold increase in CD45$^+$ cell engraftment in BM, and an extended lifespan.[21] Although NSG mice provide a robust platform for human hematopoietic cell engraftment,[22] they are susceptible to GvHD after CD34$^+$ HSC transplantation. Although the development of the NSG mice has been a critical step for HIS models, new models are becoming more suitable for long-term studies.

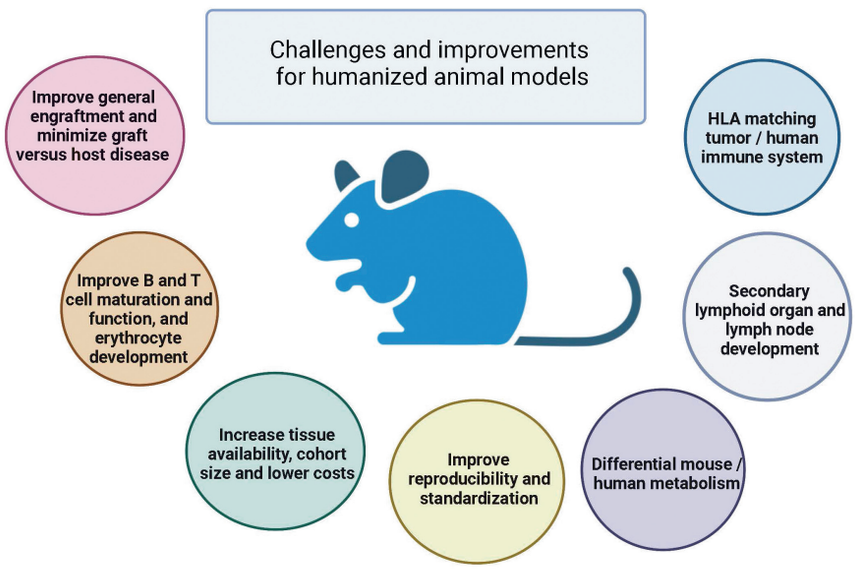

FIGURE 3 Human immune system (HIS) animal models, challenges, and improvements. Schematic exhibiting the ongoing challenges and future of the HIS animal models. Despite being cutting-edge and valuable tools, these models still require improvements and present issues such as species differences between mice and humans as well as limitations in our current understanding of the immune system and its complexity. HLA, human leukocyte antigen. Figure was created using BioRender.com.

TABLE 2 Main humanized animal murine models and their characteristics and applications.

Strain name	Supplier	Genetic background	Deficiency	Specificity	Applications
NSG	Jackson Laboratory Taconic	NOD.Cg-*Prkdc*^{scid} *Il2rg*^{tm1Wjl}/SzJ	Lack T, B, and NK cells Loss of C5 complement Defective macrophages and dendritic cells Active neutrophils	Low radiation tolerance	Broad platform for immuno-oncology and infectious disease research, HIS engraftment, PDX, CAR-T studies
NRG	Jackson Laboratory	NOD.Cg-*Rag1*^{tm1Mom} *Il2rg*^{tm1Wjl}/SzJ	Same as NSG	Higher radiation tolerance	Same as NSG with improved suitability for protocols involving irradiation
NOG	Taconic	NOD/Shi-*scid IL2rg*$^{-/-}$	Same as NSG	Low radiation tolerance	Same as NSG
NCG	Charles River	NOD.*Prkdc*^{em26Cd52}*Il2rg*^{em26Cd22}/NjuCrl-*Prkdc*^{scid} *Il2rg*^{tm1Wjl}	Lacks mature T, B, and NK cells Reduced macrophage and dendritic function	Low radiation tolerance	Same as NSG
NSG-SGM3 NSGS	Jackson Laboratory	NOD.Cg-*Prkdc*^{scid} *Il2rg*^{tm1Wjl} Tg(CMV-IL3,CSF2,KITLG)1Eav/MloySzJ	Similar to NSG (T-, B-, and NK-cell deficiency + cytokines to improve engraftment of human myeloid and T-cell populations)	Expresses human GM-CSF, IL-3, and SCF Low radiation tolerance	Modeling of myeloid malignancies (leukemia), cytokine-driven disorders, and evaluation of immunotherapies requiring myeloid cells
NOG-EXL	Taconic	NOD.Cg-*Prkdc*^{scid} *Il2rg*^{tm1Sug} Tg(SV40/HTLV-IL3,CSF2)10-7Jic/JicTac	Similar to NOG (T-, B-, and NK-cell deficiency + low levels of cytokines to increase engraftment by reducing exhaustion of stem cell pool)	Expresses human GM-CSF, IL-3 Low radiation tolerance	Suitable for myeloid malignancies (leukemia), infection studies, and evaluation of immunotherapies requiring myeloid cells
NSG-IL15	Jackson Laboratory	NOD.Cg-*Prkdc*^{scid} *Il2rg*^{tm1Wjl} Tg(IL15)1Sz/SzJ	Similar to NSG (T-, B-, and NK-cell deficiency + enhanced ability to develop human NK cells)	Expresses human IL-15 Low radiation tolerance	Study of NK cell biology, antibody-dependent cellular cytotoxicity, NK-based therapies
NSG-IL6	Jackson Laboratory	BAC with human IL-6 region	Similar to NSG (T-, B-, and NK-cell deficiency + cytokines)	Expresses human IL-6 Heightened growth of U2932 cells than NSG-IL6/SGM3 mice and longer median survival Can engraft plasma cells Low radiation tolerance	Modeling of plasma cell disorders (multiple myeloma), chronic inflammation, and IL6-dependent malignancies
NSG-IL2/ NOG-IL2	Jackson Laboratory Taconic	NSG or NOG background, expressing human IL-2	Same as NSG/NOG	Promotes functional human NK cells with receptors and granzyme A and perforin production up stimulation Low radiation tolerance	Study of NK cell biology, NK-based therapies, and IL2-dependent immune regulation
NSG-MHCI/II KO	Jackson Laboratory	NOD.Cg-*Prkdc*^{scid} *H2-K1*^{tm1Bpe} *H2-Ab1*^{em1Mvw} *H2-D1*^{tm1Bpe} *Il2rg*^{tm1Wjl}/SzJ	Lacks T, B, and NK cells MHC class I molecule deficiency (H2-K and D) MHC class II molecule deficiency (IA)	Significant delay in onset of GvHD that is dependent on irradiation and cell doses	Delayed GvHD models for prolonged PBMC/HSC studies Improved window for T-cell therapies and xenogeneic immune interaction studies

(Continues)

TABLE 2 (Continued)

Strain name	Supplier	Genetic background	Deficiency	Specificity	Applications
NOG-B2M KO	Taconic	NOD.Cg-*B2m*^{em1Tac} *Prkdc*^{scid} *Il2rgtm1Sug*/JicTac	Lacks T, B, and NK cells Reduced complement activity Defective macrophages and dendritic cells MHC class I antigen disruption	Significant delay in onset of GvHD Reduced PBMC engraftment efficiency $CD4^+$ bias in engrafted PB T cells	Delayed GvHD models for prolonged PBMC/HSC studies Suitable for CD4-biased immune response analyses
NBSGW	Jackson Laboratory	NOD.Cg-*Kit*$^{W-41J}$ *Tyr*$^+$*Prkdc*^{scid} *Il2rg*^{tm1Wjl}/ThomJ	Deficient for T, B, and NK cells	Support HSC engraftment without radiation Increased human erythroid cell development	Conditioning-free (radiation/chemo) HSC engraftment to study hematopoiesis, anemia, and bone marrow disorders

Abbreviations: BAC, bacterial artificial chromosome; CAR, chimeric antigen receptor; GM-CSF, granulocyte-macrophage colony-stimulating factor; GvHD, graft-versus-host disease; HIS, human or humanized immune system; HSC, hematopoietic stem cell; IA, Immune response region A; IL-3, interleukin-3; KO, knockout; MHC, major histocompatibility complex; NBSGW, NOD.Cg-KitW-41J Tyr+ Prkdcscid Il2rgtm1Wjl/ThomJ; NCG, NOD-*Prkdc*^{em26Cd52}*Il2rg*^{em26Cd22}/NjuCrl; NOD, nonobese diabetic; NOG, NOD.Cg-*Prkdc*^{scid} *Il2rg*^{tm1Sug}/JicTac; NRG, NOD.Cg-*Rag1*^{tm1Mom} *Il2rg*^{tm1Wjl}/SzJ; NSG, NOD.Cg-*Prkdc*^{scid} *Il2rg*^{tm1Wjl}/SzJ; PB, peripheral blood; PBMC, peripheral blood mononuclear cell; PDX, patient-derived xenograft; SCF, stem cell factor; SCID, severe combined immunodeficiency; SGM3, stem cell factor/GM-CSF/IL-3.

4.2 | NRG - NOD.Cg-*Rag1*^{tm1Mom} *Il2rg*^{tm1Wjl}/SzJ

The NOD.Cg-*Rag1*^{tm1Mom} *Il2rg*^{tm1Wjl}/SzJ (NRG) mice are another strain of severely immunodeficient mice developed by the Jackson Laboratory. These mice have a knockout mutation in the recombination activation gene 1 (*Rag1*) and the *IL2rg* gene. These mutations impair B- and T-cell development and cytokine signaling (leading to deficient NK cell function).[23] NRG mice were produced by crossing NOD-*Rag1*^{null} mice with NOD-*scid IL2r* γ^{null} mice and producing mice homozygous for both mutations through further crosses.[24] NRG mice have similar engraftment of a HIS as NSG mice with the added benefit of an increased tolerance of irradiation and chemotherapy conditioning.[23]

4.3 | NCG - NOD-*Prkdc*^{em26Cd52}*Il2rg*^{em26Cd22}/NjuCrl

The Charles River NOD-*Prkdc*^{em26Cd52}*Il2rg*^{em26Cd22}/NjuCrl (NCG) mouse model was developed by the Nanjing Biomedical Research Institute of Nanjing University and Nanjing Galaxy Biopharma through CRISPR-Cas9 editing of the *Prkdc* and *Il2rg* genes in NOD/Nju mice, which already have a mutation in the Sirp*SIRPA* gene impairing macrophage function. This results in a triple immunodeficient mouse model, which allows for greater success in engraftment of human HSCs. The gene edits result in T-, B-, and NK-cell function impairment, similar to NSG and NOD.Cg-*Prkdc*^{scid} *Il2rg*^{tm1Sug}/JicTac (NOG) mice.[25] Additionally, NCG mice tolerate irradiation and chemotherapy (e.g., busulfan) conditioning necessary to prepare the mice for engraftment.[25] NCG mice humanized with either $CD34^+$ HSCs (HuCD34$^+$ NCG) or PBMCs (HuPBMC NCG) are also available for long-term and short-term studies, respectively. HuCD34$^+$ NCG mice exhibit engraftment across multiple hematopoietic lineages, whereas HuPBMC NCG mice exhibit primarily T-cell engraftment.[25,26]

4.4 | NOG - NOD.Cg-*Prkdc*^{scid} *Il2rg*^{tm1Sug}/JicTac

NOG mice are another immunodeficient mouse model developed for human xenografts by Mamoru Ito from the Central Institute of Experimental Animals.[12] NOG mice were developed through eight backcrosses between C57BL/6J-gamma(c)(null) mice and NOD/Shi-SCID mice. These mice are similar to NSG mice; however, the *IL2rg* mutation is truncated in the intracellular signaling domain rather than completely null, allowing for cytokines to bind to the receptor but not activate it.[12] NOG mice exhibit impaired T-, B-, and NK-cell function and cytokine production and reduced macrophage, dendritic cell, and complement functions.[27] This mouse model may result in improved PB engraftment.[27] However, these mice, like NSG mice, are susceptible to GvHD, even with only a small number of human hematopoietic cells engrafted.[28] Taconic Biosciences, which currently sells NOG mice, also offers a huNOG mouse model that exhibits stable engraftment and hematopoiesis for over a year.[28,29]

5 | GENETICALLY ENGINEERED MOUSE MODELS

New mouse strains expressing human hematopoietic growth factors on the NSG, NOG, or NCG background have been developed to enhance human myeloid and immune cell development after HSC engraftment. Knock-in approaches allow for physiological cytokine levels, avoiding some of the drawbacks seen with transgenic overexpression. Ongoing advances, including MHC-deficient and HLA-transgenic models, aim to further reduce GvHD and improve the development, functionality, and specificity of human immune cells in these systems.[2,10,30] Here, we describe some of the most used models (Table 2).

5.1 | NSG-SGM3

NSG-SGM3 or NSGS mice are produced from NSG mice, genetically engineered to express three human cytokines, namely stem-cell factor/KITLG, granulocyte-macrophage colony-stimulating factor (GM-CSF/CSF2), and interleukin-3 (IL-3), under a CMV promoter. These cytokines were selected to support enhanced human myeloid differentiation and overall hematopoiesis. The expression of these cytokines enables improved engraftment, expansion, and differentiation of human hematopoietic lineages, particularly myeloid cells, compared to nontransgenic NSG strains. In comparative studies, NSG-SGM3 mice outperformed standard NSG mice in supporting human neutrophil and monocyte development.[31] NSG-SGM3 mice have also demonstrated accelerated human B-cell maturation, enhanced myeloid reconstitution, and a reduced time to engraftment compared to conventional NSG and BLT models.[32] Recent studies have highlighted their utility as a robust preclinical platform.[33] However, the NSG-SGM3 mice can also drive adverse outcomes. These mice are prone to developing mast cell and eosinophil hyperplasia, and Hemophagocytic Lymphohistiocytosis/Macrophage Activation Syndrome (HLH/MAS)-like syndromes after engraftment with human $CD34^+$ cells or leukemia xenografts.[34,35] These pathologies are driven by the transgenic cytokines and can confound experimental outcomes, highlighting the need for caution when utilizing this model.

5.2 | NOG-EXL

NOG-EXL, a derivative of NOG mice, expresses human GM-CSF and IL-3 under the SV40 promoter. Unlike NSG-SGM3, which uses the CMV promoter, NOG-EXL maintains more physiological cytokine levels and supports long-term engraftment without stem-cell exhaustion. In humanization studies, NOG-EXL mice demonstrated superior reconstitution of diverse human immune lineages, including plasmacytoid dendritic cells, NK cells, B cells, and myeloid subsets.[36,37] They also exhibited physiological production of human immunoglobulin G (IgG) in both blood and tumor tissues.[38] They are less prone to HLH/MAS-like syndromes.

5.3 | hIL15-NSG, hIL15-NOG, and hIL15-NCG

Human IL-15-expressing mice have been shown to sustain differentiation, survival, and expansion of NK cells, which are poorly maintained in conventional HIS models.[39] hIL15-NSG or NSG-IL15 (JAX), hIL15-NOG or NOG-IL15 (Taconic), and hIL15-NCG or NCG-IL15 (Charles River) were produced by introducing a human *Il15* transgene through microinjection and backcrossing onto the NSG, NOG, or NCG background. Despite enhanced NK reconstitution, humanization of these *Il15* transgenic strains with PBMCs or HSCs is generally challenging, as it can shorten the lifespan due to increased immune activation and GvHD-like pathology.[39,40]

5.4 | hIL6-NSG

Human IL-6-expressing mice have been shown to support enhanced differentiation of human monocytes/macrophages and other myeloid subsets.[41,42] hIL6-NSG or NSG-IL6 mice were originally produced by inserting a bacterial artificial chromosome encoding the human *Il6* gene into fertilized NSG mouse embryos to achieve physiologically relevant expression levels. These mice produced measurable levels of circulating human IL-6, typically ranging from 200 to 300 pg/mL, which is significantly higher than normal human serum but consistent with levels found in plasma cell disorders. When engrafted with patient-derived malignant and premalignant plasma cells, hIL6-NSG mice reliably developed key clinical features of human disease such as serum M-protein spikes, anemia, hypercalcemia, and bone lesions. Importantly, the model is used as predictive for chimeric antigen receptor (CAR)-T therapeutic response.[42] Collectively, these features make hIL-6 mice a powerful platform for studying myeloma engraftment and biology.

5.5 | hIL2-NOG

The hIL-2 NOG or NOG-IL2 mouse expresses human interleukin-2 and enables enhanced human $CD56^{Cd}$ NK cell development (10-fold compared to NOG strain) after $CD34^+$ HSC engraftment.[43] It allows for NK cell functional maturation through granzyme A, perforin production, and receptor diversity. In melanoma PDX models using hIL-2 NOG mice, adoptive tumor-infiltrating lymphocytes (TIL) or CAR-T cell therapies produced significant tumor regression.[44] This model was the first to recapitulate patient-specific clinical responses using autologous tumor and TIL transfer in vivo,[11] representing a good translational platform for evaluating human immune–based therapies.

5.6 | MHCI/II-deficient mice

The development of MHC class I/II double-knockout NSG (NSG-MHCI/II KO) mice is important in the refinement of HIS models. These mice combine SCID with targeted deletions in murine MHC class I and II molecules, produced using TALEN-mediated nonhomologous end joining to disrupt exon 2 of the MHC class II allele, followed by intercrossing to obtain homozygous offspring (JAX strain 025216).[20] The absence of mouse MHC complexes prevents recognition by human T cells, thereby significantly delaying the onset of GvHD compared to conventional NSG mice. This enables long-term engraftment of human PBMCs, with survival exceeding 100 days, and allows extended experimental windows.[20,45] PBMC-engrafted MHCI/II–deficient mice inoculated with influenza exhibited elevated and persistent hemagglutinin (HA) NOD.Cg-KitW-41J Tyr+ Prkdcscid Il2rgtm1Wjl/ThomJ (NBSGW)-specific IgG responses, underscoring their potential for vaccine and infectious disease research.[45] Furthermore, differences in circulating T-cell subsets across knockout backgrounds, such as persistent $CD8^+$ cells in the double knockout, suggest that $CD4^+$ T cells may play a central role in GvHD pathogenesis. Together, these findings establish MHCI/II double-knockout models as promising platforms for investigating human adaptive immune function while mitigating the limitations imposed by acute GvHD.

5.7 | β2-microglobulin-deficient NOG

The β2-microglobulin (B2M) knockout NOG (NOG-B2M KO) mouse, similar to the MHC class I/II double-knockout NSG mouse, allows extended engraftment windows after human PBMC or tumor transplantation. In these mice, CRISPR/Cas9 was employed to introduce a nonsense mutation in exon 1 of the *B2M* gene, eliminating murine MHC class I antigen presentation and thus reducing recognition by human $CD8^+$ T cells. Importantly, these mice exhibit both improved engraftment of human PBMCs and better tumor xenograft take,[14,46] making them particularly useful for immuno-oncology and adoptive T-cell therapy research. Comparative studies indicate that B2M-deficient hosts support prolonged survival and more stable human T-cell populations than conventional NOG hosts, though engraftment dynamics and the balance between $CD4^+$ and $CD8^+$ T cells can shift over time.[2,20] Despite their utility, the lack of MHC I expression may skew $CD4^+$ T-cell predominance and potentially affect $CD8^+$ T-cell-mediated immune responses.

5.8 | NBSGW

The NOD.Cg-KitW-41J Tyr+ Prkdcscid Il2rgtm1Wjl/ThomJ (NBSGW) mouse model from the Jackson Laboratory, developed through multiple generations of genetic crosses to combine mutations in *c-Kit*, *Prkdc*, *IL2rg*, and *Sirpa*, enables robust human HSC engraftment without the need for irradiation, using a hypermorphic *c-Kit* (W41) mutation.[17,47] This model achieves hematopoietic chimerism levels comparable to irradiated NSG mice but avoids the tissue damage and inflammation associated with conditioning regimens. Studies using NBSGW mice demonstrate that HSC source strongly influences immune lineage outcomes and that low-dose busulfan can further enhance engraftment efficiency while reducing the number of $CD34^+$ cells required.[48] Additionally, combining *c-Kit* mutations with immunodeficiency supports more robust human erythropoiesis.[17,47] Together, these findings highlight NBSGW mice as a powerful tool for modeling human hematopoiesis in vivo, with advantages in safety, reproducibility, and lineage-specific human cell development.

5.9 | BLT mouse models

The BLT mouse model and its derivatives (BLTS [spleen] and pro-BLT) represent some of the most physiologically advanced and relevant HIS platforms. They are beneficial in studying certain diseases, such as HIV, cancer, and immunodeficiency. In the classic BLT approach, human fetal liver and thymus fragments are implanted under the renal capsule of immunodeficient mice (typically NSG or NOG strains), followed by intravenous injection of $CD34^+$ HSCs. This design allows for T-cell development and education within a human thymic environment, resulting in HLA-restricted, antigen-specific adaptive immunity. BLTS mice extend this model by incorporating human spleen tissue alongside fetal liver and thymus, thereby enhancing lymphoid organ architecture and enabling studies of processes such as lymphoid fibrosis, a key feature of HIV infection even under antiretroviral therapy.[49] More recently, the pro-BLT model has emerged as a scalable alternative, using secondary transfer of BM cells and thymus implants from existing BLT mice into naive NSG mice, thereby overcoming reliance on limited fetal tissue while maintaining levels of human immune reconstitution comparable to primary BLT mice.[50] In HIV research, BLT models are particularly effective due to their high T-cell reconstitution and ability to replicate a primary immune response against the infection. This makes them a valuable platform for testing long-term therapy and strategies to prevent secondary infection.[51,52] Additionally, anti-(PD1) programmed cell death protein 1 therapy, a clinically effective checkpoint inhibitor, can cause immune-mediated adverse events in humans that are often not predicted by non-clinical studies. BLT mice can model these toxicities, including pneumonitis, hepatitis, nephritis, and dermatitis.[53] Finally, transgenic modifications such as IL-34 expression further expand BLT utility by promoting human microglia development, enabling modeling of the central nervous system (CNS) reservoir of HIV.[2] Despite their translational advantages, BLT and derivative models remain technically demanding, resource intensive, and subject to donor-to-donor variability. They are also susceptible to xenogeneic complications, such as GvHD.

5.10 | Rat models

Successful engraftment of human hematopoietic cells into rats requires the host to be deficient in B, T, and NK cells. Typically, the rat model is SCID based with a mutation in *IL2rg*. There are also models with engraftment of human PBMCs and implantation of fetal liver and thymus under the renal capsule. This method of implantation was utilized for the development of human skin and immune system rodents. These rodents were modeled after NOD-SCID *IL2rg* and Sprague–Dawley *Rag2 IL2rg* rat models, with the addition of human skin engraftment. Introducing human skin to humanized rodent models provides the opportunity to study human pathogen transmission through the skin barrier, which is the first line of protection against external injuries. For example, co-engraftment of human full-thickness skin, autologous lymphoid tissues, and autologous immune cells supports human skin infection after *Staphylococcus aureus* inoculation.[54] Very recently, Ménoret et al.[55] and Jung et al.[56] from Nantes Université in France have developed the Rag1-deficient/Il2rg-deficient/human SIRPA (RRGS) rodent model by crossing *Rag1*-deficient rodents with *Il2rg*-deficient rodents across eight generations and inserting a human *Sirpa* gene through *piggyBac* transposition. These RRGS rodents have been used to successfully engraft a HIS through intravenous injection of human PBMCs. High levels of human $CD45^+$ cells and cells from multiple lineages were found in the rats. Lymphoid cell distribution was also found to mirror humans, with T-cell counts predominating over B-cell counts. RRGS rodents had a functioning HIS after engraftment with human immunoglobulin M and IgG detected in serum, and T and B cells being functionally responsive to Orthoclone-muromonab-CD3 (OKT3) and cytokines. Additionally, these rodents never developed GvHD through 40 weeks of human hematopoietic cell engraftment, presenting an advantage over NSG/NOG mice.[10] The development of RRGS rodents and the demonstration of successful HIS engraftment with no GvHD development suggest that this model could be a valuable tool in performing long-term disease studies in a larger animal model.

6 | APPLICATIONS

HIS animal models have become indispensable tools in cancer research for modeling human immune–tumor interactions (Figure 2). HIS mice enable partial reconstitution of human immune compartments within the murine host, and co-engraftment of PDXs (HIS-PDX) enables the study of tumor evasion, resistance, and immunotherapy responses in a physiologically relevant context.[46,57] Despite significant limitations, including suboptimal lymphoid architecture, HLA mismatch, and incomplete myeloid maturation, HIS models remain the gold standard for in vivo human immune studies (Table 2).

6.1 | HIS models for cancer and immunotherapy

Pre-2020 studies demonstrated feasibility across highly immunogenic cancers such as melanoma and triple-negative breast cancer, as well as immune-cold tumors like pancreatic ductal adenocarcinoma and prostate cancer.[7,58–60] In lung cancer, HIS-PDX models have been used to study checkpoint blockade and resistance pathways,[61,62] whereas colorectal cancer models have enabled comparison of microsatellite instability high versus microsatellite stable immune responses.[63] Hematologic malignancies, including acute myeloid leukemia and diffuse large B-cell lymphoma, have leveraged HIS-PDX for CAR-T, bispecific antibody, and antibody-based therapy evaluation.[59] More recently, in glioblastoma (GBM), researchers have implanted GBM tumors into HIS-NSG mice.[64] Then single-cell RNA sequencing was performed on the tumors; results revealed that a broad spectrum of human immune cells (T, NK cells, and myeloid lineages) effectively infiltrated the tumor microenvironment mirroring what is observed in human GBM. In liposarcoma, a study demonstrated the potential of HIS models for preclinical evaluation of checkpoint inhibitors by investigating the efficacy of pembrolizumab in humanized NSG mice engrafted with dedifferentiated liposarcoma.[65] These mice were reconstituted with human immune cells and treated with pembrolizumab, resulting in significant tumor regression, increased infiltration of human $CD8^+$ T cells, and elevated expression of activation markers such as interferon γ.[65] Additionally, the study linked the molecular features of patient tumors, such as immune-related gene expression, to response outcomes, enhancing translational relevance. More recently, a group has established a nasopharyngeal carcinoma (NPC) humanized-PDX model by engrafting HSCs into NSG mice, followed by transplantation of NPC tumor tissue. The model recapitulated the key features of human NPC, including immune infiltration and expression of Epstein–Barr virus antigens.[66] In another study, Zhao et al.[67] discovered that implanting HLA1-matched liver cancer-PDX tumors into NSG mice produced a model that mirrored clinical outcomes, with the tumor inducing the immune system to exhibit exhaustion phenotypes. This model also mirrored the clinical therapeutic and adverse effects of pembrolizumab and ipilimumab, reinforcing the fact that results from HIS-PDX models can correlate with those from clinical trials.[68]

6.2 | HIS models for cell therapy

HIS mice have become indispensable for preclinical evaluation of CAR-T and CAR-NK cell therapies. These models, particularly those engrafted with human HSCs, provide a more accurate representation of human immune responses compared to traditional murine models.[59,69] By supporting the development of functional human T and NK cells, humanized mice enable the assessment of the efficacy, persistence, safety, and potential toxicities of CAR-modified immune cells in a human-like environment.[70] Studies have demonstrated that HIS mice effectively model B-cell malignancies, solid tumors, and leukemia, offering critical insights into CAR-T and CAR-NK therapy performance. For example, NSG mice reconstituted with HSCs have been used to evaluate anti-CD19 CAR-T cells in B-cell acute lymphoblastic leukemia, providing data on treatment efficacy, persistence, and adverse effects such as cytokine release syndrome (CRS).[69,71] Moreover, these models facilitate investigation of combination therapies, including CAR-T cells

paired with immune checkpoint inhibitors, as well as testing novel CAR constructs or gene-editing approaches to optimize immune cell function. These models critically contribute to the development of safer and more effective CAR-T and CAR-NK cell therapies.[30,70,72]

6.3 | HIS models for human viral pathogenesis and neurological disorders

HIS models have also become important in the study of viral infection and human-specific pathogens in vivo.[4] As described earlier, each model provides distinct advantages: HSC-engrafted mice support multilineage hematopoiesis and T- and B-cell development. BLT mice allow more complete T-cell maturation and HLA restriction. PBMC-transplanted mice enable rapid establishment of human T-cell populations, though at the expense of GvHD.[6,30,59] A major area of application has been HIV research, in which HIS mice permit persistent viral replication, enable the study of mucosal transmission, and provide mechanistic insights into latency and immune evasion.[51,52] Beyond HIV, HIS mice have been useful in herpes and variola virus vaccine development.[73,74] More recently, the COVID-19 pandemic highlighted their translational versatility. Humanized angiotensin-converting enzyme 2 (ACE2)-expressing and $CD34^+$-engrafted mice have been used to model severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection dynamics, investigate antiviral immunity, and uncover tissue-specific immune signatures.[75–78]

Although traditionally applied to infectious disease, HIS models have also been extended to neurological disorders by enabling human-specific immune and neural interactions in vivo. As murine immune and neuronal cells differ substantially from human cells, they allow mechanistic exploration of how innate and adaptive immunity contributes to CNS pathology and behavior.[79] Co-transplantation of induced pluripotent stem cell–derived neural progenitors with HSCs, together with human IL-34 supplementation, enhances human glial and macrophage development, allowing partial reconstruction of the brain's immune environment.[80,81] More recently, researchers have used the humanized HLA-DR4-transgenic/Rag1-deficient/Il2rg-deficient and DRAG/HLA-A2-transgenic (DRAG and DRAGA) models to study pathological neurological conditions, including neurodegeneration, tumors, and traumatic injury, and to evaluate potential therapeutic interventions in these contexts. DRAG and DRAGA models are NRG mice that express HLA-DR4 and both HLA-DR4 and HLA-2, respectively, enabling improved human T-cell reconstitution, function, and B-cell IgG class switching. These mice have previously been used to develop vaccinations and treatments against various infectious diseases, such as HIV, influenza, Zika virus, and COVID-19.[82–84] In neuroinflammatory and neurodegenerative contexts, humanized DRAGA mice with cord blood $CD34^+$ HSCs have demonstrated the capacity to reconstitute human microglia and T cells within the host brain.[85] Humanized DRAG mice have also proven useful in neuro-oncological research, with successful engraftment of GBM and immunological responses to existing immunotherapies.[86] Altogether, these models support studies of HIV-associated neurocognitive disorders,[81,82] Parkinson's disease, and Alzheimer's disease, revealing human-specific microglial and macrophage transcriptional responses to neuronal injury and amyloid-β pathology.[79,87,88] Despite these improvements, these current HIS platforms retain the aforementioned limitations such as incomplete immune reconstitution and maturation, and GvHD risk which limits the experimental window in some models.[2,30] Ongoing refinements (e.g., knock-in of human cytokine genes, development of niche-forming PDXs) are essential to expand HIS model utility in vaccine testing and modeling of viral infections and neurological disorders (Figure 3).[1,89]

7 | CONCLUDING REMARKS

HIS animal models have become important tools for many research applications, including human immune function, infectious diseases, autoimmune diseases, cancer, and organ or tissue transplantation (Figure 2; Table 2).[1,30] HIS models enable specific in vivo analysis of human immune responses that are not possible in traditional murine systems, such as T- and B-cell development, NK cell functions, and cytokine signaling. They also provide a platform for assessing the safety and efficacy of immunotherapies and for infection studies involving human pathogens such as HIV, hepatitis B virus, or SARS-CoV-2.[4,90] More recent works really emphasize HIS model use in liquid and solid tumor xenografts to investigate the tumor–immune interactions and tumor resistance to treatment.[58,68]

Despite their value, these models come with significant challenges (Figure 3).[2,30,91] They are expensive to produce and to maintain. First, HIS models require specialized facilities to house severely immunodeficient animals, specific reagents, and sterile conditions. Second, they require expertise and knowledge of irradiation, HSC biology and handling, surgical implantation, and validation of immune engraftment, which impact the scalability. Third, many models still fail to fully replicate the diversity and function of the HIS, particularly the myeloid compartment, lymph node structure, and antigen-specific responses.[92] Indeed, secondary lymphoid organs (SLO—lymph nodes and spleen) present structural deficiencies in HIS mice. SLOs often lack properly organized human follicular structures and canonical germinal centers, which are essential for lymphocyte interaction or maturation of high-affinity antibodies.[93] Reduced B-cell differentiation, reduced class switching,[94] and reduced antibody maturation after vaccination or immunization limit the use of HIS models for research related to modeling human antibody responses and vaccine development.

The differences between human and murine cytokines, adhesion molecules, and stromal environments add another level of complexity and limitations. Several models also present a high risk of GvHD,[10] which restricts the experimental windows. Finally, the use of fetal tissue and cord blood could raise ethical concerns and may soon face regulatory barriers, which could halt the development of HIS

models. Nevertheless, ongoing research and innovations, including gene-edited host strains, multitissue humanization strategies, and improved cytokine support, should address some of these issues. As discussed earlier, murine models expressing cytokines exist to enhance immune reconstitution and development of specific lineages such as myeloid cells, Tregs, or NK cells. CRISPR/Cas9 gene-editing technology enables precise knock-in or knockout of human genes in host animals allowing further development of these models.[22,56] Using this technology to reduce the risk of GvHD will allow for longer-term and more stable engraftment. In addition to mice, humanized rat models are gaining traction due to their larger size, which allows locoregional procedures, such as transarterial embolization or chemoembolization and repeated blood or tissue sampling, enhancing their translational relevance, particularly in liver cancer research.[95] Another area of active research is exploring multihumanization and autologous transplantation strategies, where hematopoietic or immune cells, as well as other cell types (e.g., stromal cells, hepatocytes) and/or cancer cells (e.g., PDX) from the same patient, are introduced into one animal model, creating new avenues for personalized medicine and individualized therapy testing.[28] In conclusion, the field of HIS models has considerably grown in the recent years. HIS models have become powerful tools as they offer novel and nuanced approaches to improve preclinical models, drug development pipelines, and personalized therapeutic strategies.

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