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Review

Humanized mouse models of genetic immune disorders and hematological malignancies

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ABSTRACT

The immune system is quite remarkable having both the ability to tolerate innocuous and self-antigens while possessing a robust capacity to recognize and eradicate infectious pathogens and foreign entities. The genetics that encode this delicate balancing act include multiple genes and specialized cell types. Over the past several years, whole exome and whole genome sequencing has uncovered the genetics driving many human immune-mediated diseases including monogenic disorders and hematological malignancies. With the advent of genome editing technologies, the ability to correct genetic immune defects in autologous cells holds great promise for a number of conditions. Since assessment of novel therapeutic strategies have been difficult in mice, in recent years, immunodeficient mice capable of engrafting human cells and tissue have been developed and utilized for a variety of research applications. In this review, we discuss immune-humanized mice as a research tool to study human immunobiology and genetic immune disorders *in vivo* and the promise of future applications.

1. Introduction

Although genetic information between mice and humans is highly similar, comparisons described under the ENCyclopedia Of DNA Elements (ENCODE) project identified many differences in gene regulation, particularly those associated with immune and metabolic processes in cells and tissues [1,2]. For these reasons, it can be challenging to extrapolate data stemming from investigations into complex biological processes of the immune system using traditional inbred mice [3–6]. The introduction of human cells and tissues into specific strains of immunodeficient mice to generate mouse-human chimeras is one strategy that has enabled investigations pertaining to human biology within a tractable host without placing individuals at risk. This review focuses on the generation of human immune system (HIS)-mice (also referred to as humanized mice) as a tool for pre-clinical testing and investigations of immune disorders driven by genetic defects.

1.1. Immunodeficient mice and initial progress

In 1983, a spontaneous autosomal recessive mutation arose in a C.B-17 mouse strain at Fox Chase that was described as having a severe combined immunodeficiency (SCID) similar to what had been also observed in humans [7,8], with the causal mutation in mice later identified in the gene encoding Protein Kinase, DNA-Activated, Catalytic Subunit (*Prkdc*) [9–13]. Being devoid of mature B and T cells, SCID mice are non-responsive towards B and T cell antigens and therefore their immune system does not reject xenogeneic cells or tissues [14–16]. Soon after, investigators began utilizing this strain to engraft human peripheral blood mononuclear cells (PBMCs) that enabled the study of antigen-specific acquired immune response (donor primed) in

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Prkdc^{scid} mice repopulated with human PBMCs [17,18]. Subsequent uses of SCID mice included the transplantation of primary human tissues to study various pathologies affecting skin, vaginal, retinal, ovarian, renal, splenic, and intestinal tissues [19–26]. A significant advancement in translational biomedical research was the demonstration that SCID mice engrafted with autologous human fetal thymus and human liver tissue fragments underwent long-term hematopoiesis permitting human B and T cell development within a murine host [27,28]. The fetal liver tissue provided the niche for hematopoietic stem cells and the thymic tissue provided the necessary structure to facilitate proper T cell education. Collectively, these seminal findings paved the way for the development of an *in vivo* platform to study human immunobiology.

1.2. Development and characterization of immunodeficient strains

Like the C.B-17 inbred strain, several other inbred strains have been widely utilized over the years. Backcrossing the *Prkdc*^{scid} mutation onto various inbred strains revealed that non-obese diabetic (NOD).*Prkdc*^{scid} mice support higher levels of human PBMC engraftment than any other strain tested [29]. This is largely due to NOD mice having impaired development and function of macrophages, natural killer (NK), NKT cells, and regulatory T cells (although not relevant for mice homozygous for *Prkdc*^{scid}) [30–35]. Immune cell defects in the NOD strain have largely been mapped to two loci designated insulin-dependent diabetes susceptibility 3 (*Idd3*) and *Idd5,* which contain genes encoding interleukin-2 (*Il2*), *Il21*, and *Ctla4* (as reviewed in [36]). In the early 2000s, another advancement in strain development occurred when targeted null mutations of the IL-2 receptor gamma chain (*Il2rg*) [37,38] were combined with mice harboring *Prkdc*^{scid} or null mutations in recombination activating genes (*Rag1* or *Rag2*), which also results in lymphopenia. This addition further improved human immune cell engraftment and development in various strains including NOD.*Prkdc*^{scid}*Il2rg*^{tm1Wjl}/SzJ (NSG), NOD.*Prkdc*^{scid}*Il2rg*^{tm1Sug} (NOG), NOD.*Rag1*^{tm1Mom}*Il2rg*^{tm1Wjl} (NRG), and BALB/c. *Rag2*^{tmFwa1}*Il2rg*^{tm1Sug} (BRG) [39–42]. Furthermore, the introduction of the *Il2rg* mutation improved human thymocyte development in the murine thymus of mice reconstituted with fetal or cord blood sourced CD34$^+$ HSCs [43]. Surprisingly, NSG and NRG strains supported greater engraftment of human cells than that observed in BRG mice [44]. The difference observed in the efficacy of human cell engraftment was postulated to be due to a polymorphism in the signal regulatory protein alpha (*Sirpa*) locus of NOD mice that was similar to human SIRPA. SIRPα binds to CD47 and is expressed on most hematopoietic and non-hematopoietic cells and effective interaction between SIRPα and CD47 communicates a “do not eat me” signal to macrophages [45]. This role for SIRPA in permitting more robust human cell engraftment and development in NOD mice was confirmed using a NOD-*Sirpa* congenic and a human *SIRPA* transgenic BRG (BRGS) mouse strain [46–49]. Over the years, many immunodeficient murine strains have been developed that improve human immune cell engraftment, development, and/or function (summarized in Table 1).

2. Genetics driving immune dysregulation

2.1. Monogenic disorders of the immune system

Genome-wide association studies (GWAS) have linked specific genetic polymorphisms to a variety of chronic inflammatory diseases. Although GWAS implicates genes involved in disease susceptibility, most immune disorders and diseases are not monogenic. The penetrance of inheritable autosomal dominant/recessive and X-linked disorders define causal loci for a number of monogenic immune-mediated diseases. Diagnosis of monogenic immune-mediated diseases typically occurs following a suspected underlying immune deficiency leading to aberrant responses to pathogens presenting early in life. The World Health Organization currently estimates a global prevalence of monogenic disease at 1/100 births with novel causative variants for many inflammatory disorders still being defined. While immune monogenic diseases such as sickle cell anemia are more common, conditions such as hemophilia type A in males and severe combined immunodeficiency affect 1:5000 and 1:58,000 live births, respectively are rare. Far more rare are immunological diseases such as immunodysregulation polyendocrinopathy enteropathy X-linked (IPEX) syndrome, interleukin-10 receptor (*IL10R*) deficiency, chronic granulomatous disease (CGD), and others (Table 2) that often present early in life. Collectively these conditions pose a significant increase in mortality rate and limited treatment options carry a high economic burden. In theory, most of these conditions are curable with hematopoietic stem cell transplantation (HSCT). Nevertheless, identification of a suitable allogeneic donor or health status of the patient confound treatment options. Those patients who do receive allogeneic HSCT require systemic immunosuppression, which is not without additional risk.

Since the presentation and genetic causes of monogenic diseases can be identified early in life, gene therapy or *in situ* gene correction with emerging technologies like clustered regularly interspaced short palindromic repeats (CRISPR) with its CRISPR-associated system 9 (Cas9) permit the use of autologous HSC that would eliminate long-term immune suppression, complications from graft vs. host disease, or reduce graft rejection. Both gene therapy and CRISPR-mediated gene editing is promising, but not without complications and controversy. Nevertheless, pre-clinical investigations using immune-humanized mice may mitigate risk in efforts employed to cure these patients.

2.2. Humanized mice to study monogenic immune disorders

The opportunity to study immune cells from patients with rare diseases *in vivo* without putting them at risk would be an ideal setting for characterization and pre-clinical therapeutic applications. Since many patients with these rare diseases are often diagnosed early in life, acquisition of patient samples for research purposes can be difficult. Furthermore, immune cell characterization is often restricted to

Table 1
Immunodeficient strains commonly used to generate immune humanized mice.

Common nomenclature	Strain background	Targeted/mutated murine loci	Human gene(s) present
NS	NOD	*Prkdc*^{scid}	–
NSG	NOD	*Prkdc*^{scid}, *Il2rg*	–
NSGAb^{o}DR1	NOD	*Prkdc*^{scid}, *Il2rg*, *H2-Ab1*	Tg(HLA-DRA*0101,HLA-DRB1*0101)
NSGMHCIIDQ8	NOD	*Prkdc*^{scid}, *Il2rg*, *H2*$^{dlAb1\text{-}Ea}$	Tg(HLA-DQA1,HLA-DQB1)
NSG-SGM3	NOD	*Prkdc*^{scid}, *Il2rg*	Tg(*KITLG*, *GM-CSF*, *IL3*)
NOG	NOD	*Prkdc*^{scid}, *Il2rg** (truncation)	–
NRG	NOD	*Rag1*, *Il2rg*	–
BRG	BALB/c	*Rag2*, *Il2rg*	–
BRGS	BALB/c	*Rag2*, *Il2rg*, congenic(NOD.*Sirpa*)	–
MITRG	BALB/c	*Rag2*, *Il2rg*	*CSF1*, *CSF2*, *IL3*, *TPO*
MISTRG	BALB/c	*Rag2*, *Il2rg*	*CSF1*, *CSF2*, *IL3*, *TPO*, Tg(SIRPA)

Table 2
Examples of monogenic disorders of the immune system.

Immune monogenic diseases	Genes	Manifestation	Ref.
IPEX, IPEX-like syndrome	*FOXP3, CD25, CTLA4*	Enteropathy, dermatitis, endocrinopathy (T1DM, thyroiditis)	[50–54]
Infantile inflammatory bowel disease	*IL10, IL10RA, IL10RB, TTC7, ADAM17,*	Colonic inflammation	[55–58]
Omenn syndrome	*RAG1, RAG2, JAK3, ADA, IL2RG*	Erythoderma, desquamation, alopecia, chronic diarrhea, lymphadenopathy, hepatosplenomegaly	[59–62]
Chronic granulomatous disease	*CYBA, CYBB, NCF1, NCF2, NCF4, CYBC1*	Infections, abnormal wound healing, granulomatous dermatitis, intestinal inflammation	[63–65]
Hyper IgE syndrome	*STAT3*	Infections, dermatitis, elevated serum IgE	[66,67]
Hemophagocytic lymphohistocytosis	*PRF1, STXBP2, STX11*	Febrile illness with multiple organ involvement	[68,69]
Complement deficiency	*C1QA, C1QB, C1QC, C4A, C4B, C2*	Infections with encapsulated bacteria, systemic lupus erythematosus like	[70,71]
Wiskott-Aldrich syndrome	*WAS*	Thrombocytopenia, eczema, neutropenia	[72,73]

peripheral blood, which only provides limited assessment into disease pathophysiology. For patients with IPEX, *IL10R* deficiency, or Wiskott-Aldrich syndrome (WAS), allogeneic HSCT is curative and, in preparation for transplant, can provide an opportunity to obtain patient HSCs for experimental purposes. During anesthesia for central line placement, we obtained bone marrow CD34$^+$ HSCs from a patient with IPEX syndrome prior to transplantation. The isolated IPEX CD34$^+$ HSCs were injected into lightly irradiated 1-day old NSG mice or NSG mice deficient for murine major histocompatibility complex class II (MHCII) (referred to as Ab^o) and instead express a human transgene encoding human leukocyte antigen-DR1 (HLA-DR1) respectively. Interestingly, the NSGAb^{o}DR1-IPEX mice had a high mortality rate, developed lung and liver inflammation, and generalized autoantibody production similar to what is observed in mice and humans with defective FOXP3 [74]. This phenotype was not observed in NSG-IPEX mice. Of note, the NSGAb^{o}DR1-IPEX mice did not develop insulitis and had limited small bowel inflammation which is not consistent with classic IPEX syndrome and the reasons for this discrepancy remains unclear. Consistent with our findings, Keven Herold's group inhibited human Treg function in humanized mice using a monoclonal blocking antibody directed against CTLA-4 and documented hepatitis, autoantibody production, and a high mortality rate but did not report small bowel inflammation or insulitis and similar to what we observed in NSGAb^{o}DR1-IPEX mice [75].

In addition to assessing HSCs with mutations in *FOXP3*, our group also obtained bone-marrow derived CD34$^+$ HSCs from a patient with an *IL10RA* loss-of-function mutation who presented with severe medical-refractory infantile-onset inflammatory bowel disease (IBD). These cells were injected into a similar strain of NSG mice lacking murine MHCII and expressing HLA-DQ8 as recently described [76]. Surprisingly, the immune reconstituted mice did not develop spontaneous intestinal inflammation as occurs in mice and humans with mutations in *IL10* or *IL10R* genes [56]. Analysis of peripheral lymphoid cells showed an increase in the frequency of CD19$^+$ B cells in the spleen and mesenteric lymph node over control (Fig. 1A). This observation is intriguing given that some patients with deleterious *IL10R* mutations develop B cell lymphoma, suggesting a potential role for this pathway in regulating B cell development [77,78]. Human immune cells recovered from these mice were non-responsive to exogenous IL-10 treatment (Fig. 1B, C), consistent with results obtained using primary PBMCs obtained from the patient. Interestingly, NSG mice harboring transgenes encoding human *KITLG*, *GM-CSF*, and *IL3* (NSG-SGM3) injected with IL-10R1-deficient PBMCs were more susceptible to DSS-induced colitis compared to mice receiving PBMCs from healthy control, which may enable assessment of therapeutics for patients with *IL10R* mutations (Fig. 2). Nevertheless, full immune reconstitution using CD34$^+$ HSCS from patients with *IL10R* mutations, while unsuitable for assessing therapeutics for spontaneous intestinal inflammation, would be appropriate to screen gene therapy-based approaches designed to restore IL-10R signaling. This approach would yield information on vector insertion, selective advantage/disadvantage in targeted HSCs, and potentially predict efficacy and perhaps safety.

To our knowledge, other monogenic disorders such as WAS have not yet been assessed. The disease manifestation of WAS is predominately thrombocytopenia and although megakaryocytes are detected in the bone marrow of humanized mice, platelets are scarce due to rapid clearance by murine macrophage [79]. As *in situ* gene correction using genome editing technologies such as CRISPR/Cas9 or next generation viral vectors for gene therapy-based approaches mature, other monogenic disorders including Omen syndrome and chronic granulomatous disease will find value in humanized mice reconstituted with patient cells as a first step towards developing effective targeting vectors and good manufacturing practices prior to clinical application [80]. Thus, using humanized mice to study monogenic immune disorders depends on the cell type(s) affected by the particular genetic mutation with understanding that the complete phenotype of the patient may not be recapitulated in current strains used to generate humanized mice.

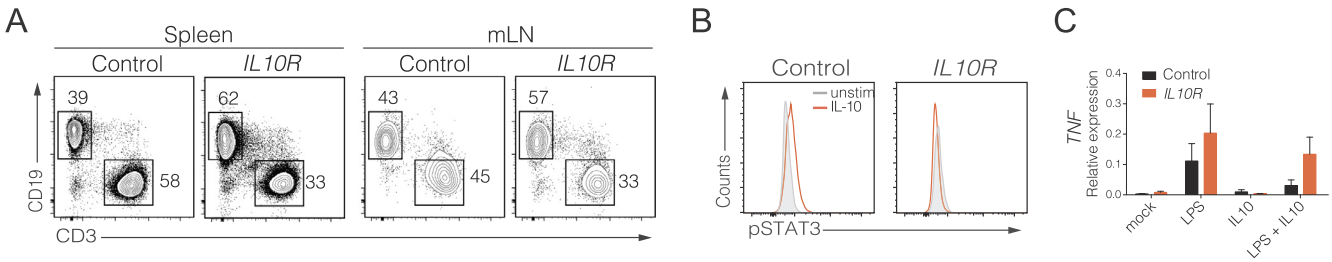

Fig. 1. NSGMHCIIDQ8 mice reconstituted with IL-10R2-deficient HSCs exhibit defective IL-10R signaling. A) Representative flow cytometry dot plot depicting human lymphocytes previously gated on live, human CD45$^+$ cells isolated from the spleen and mesenteric lymph node (mLN) of NSGMHCIIDQ8 mice reconstituted using HSCs isolated from healthy control or IL-10R2-deficient patient. B) Splenic cells stimulated with 20 ngml^{-1} IL-10 for 15 min. Intracellular staining for phospho-STAT3 was determined on human CD3$^+$ T cells gated on human CD45. C) Bone marrow-derived macrophage were generated from *IL10RB* or Control reconstituted NSGMHCIIDQ8 mice and stimulated for 4 h with LPS (10 ngml^{-1}), IL-10 (20 ngml^{-1}), or LPS following a 1 hr IL-10 pre-treatment. *TNF* was quantified by qPCR.

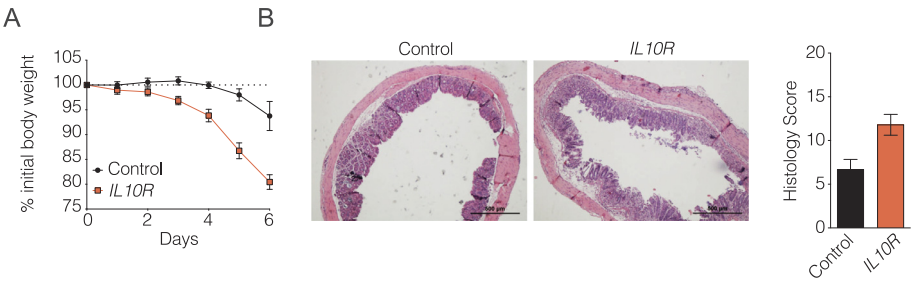

Fig. 2. IL-10R1-deficient PBMC reconstituted humanized mice are more susceptible to DSS-induced colitis. NSG-SGM3 mice were injected with $1x10^7$ PBMCs isolated from healthy control or an IL-10R1-deficient patient, and on the next day administered 1.75% dextran sulfate sodium (DSS) in the drinking for water for 6 days and weighed daily. A) Percent weight loss of both groups (n = 12–13 mice per group). B) Hematoxylin & Eosin stained colonic tissue sections from formalin-fixed and paraffin-embedded colonic blocks 6 days following DSS treatment (left) with intestinal inflammation quantified (right).

3. Malignancies driven by mutations in immune cells

3.1. Hematological malignancies

Cancers of the hematopoietic system can occur sporadically or inherited in rare cases such as *CEBPA*-associated familial acute myeloid leukemia (AML) or mutations in *KDM1A*, which increases risk for multiple myeloma. Somatic mutations in tumor suppressor genes give rise to multiple cytogenic alterations or translocations and have been linked to a variety of malignancies including lymphoma, lymphoid and myeloid leukemias, myelodysplastic syndromes, myeloproliferative disorders, and multiple myeloma. Over the last few decades, survival rates for many types of leukemias and lymphomas have improved due to early detection and therapeutic intervention. Nonetheless, some patients do not respond to current treatment regiments and the ability to assess novel therapeutics or combination therapies in humanized murine systems may enable personalized approaches to achieve remission and cure. In recent years, investigators have demonstrated utility of immune humanized mice to study hematological malignancies. Askmyr and colleagues generated NSG humanized mice using $CD34^+$ HSCs transduced to express the BCR-ABL1 fusion, a gene encoding a constitutively active tyrosine kinase, that resulted in a block at the pre-B-cell stage similar to what was found in patients with chronic myeloid leukemia [81]. Similarly, the Ren lab generated humanized mice by injecting HSCs transduced with DEK-NUP214, a chimeric product of a t(6;9)(p22;q34) chromosome rearrangement found in a subset of AML patients, which phenocopied many aspects of the human condition [82]. In some instances, the model is not an ideal surrogate as the t(4;11)(q21;q23) translocation resulting in AF4-MLL fusion is not sufficient to cause leukemia when transduced $CD34^+$ HSCs are transplanted into NSG mice [83]. It is suggested that intrinsic determinants may underlie different outcomes observed in humanized mice.

3.2. A platform for immunotherapy

Harnessing the power of immune surveillance for targeted killing of malignant cells has become one of the most exciting developments in cancer research. Since the initial description of cell surface antigens in defining cells of different lineages [84], classification of various "clusters of differentiation" markers provide a potential target in which to direct immunogenicity. The seminal finding by Miller and colleagues describing monoclonal antibodies directed against a patients idiotype of a B cell lymphoma was able to induce remission was remarkable [85]. This finding led to the approval and commercialization in 1997 of the first monoclonal antibody (Rituximab) to treat relapsed or refractory CD20 positive low-grade or follicular B cell non-Hodgkin lymphoma and led to several additional antibody-based therapeutics coming to market in the years to come. Building upon this work, a combination of techniques converged using genetically modify T cells engineered to recognize and target specific antigens to treat patients with melanoma and lymphoma respectively [86,87]. For these therapeutic strategies, humanized mice proved to be ideal models for both immunoglobulin depletion of B cells [88], as well as chimeric antigen receptor (CAR) T cells directed at epitopes expressed on malignant cells [89,90].

In addition to immune-oncology, others have exploited genome editing of HSCs to demonstrate novel therapeutic strategies using humanized mice. Although antiretroviral therapy (ART) is effective in reducing viral titers in patients infected with human immunodeficiency virus (HIV), latent viral reservoirs necessitate continuous ART. Targeting the co-receptor that mediates HIV entry into $CD4^+$ T cells using CRISPR/Cas9 in $CD34^+$ HSCs, Mandal *et al.* demonstrated effective targeting of *CCR5* that did not disrupt HSC pluripotent potential [91]. A sophisticated approach by Khamaikawin and colleagues transduced $CD34^+$ HSCs with vectors expressing short hairpin RNAs against *CCR5* and the long terminal repeat sequence of HIV and therapeutically administered these transduced HSCs to humanized mice previously infected with HIV. Downregulation of CCR5 was achieved and interestingly there was a selective advantage of gene-modified HSCs in HIV infected mice [91,92]. Thus, these approaches in autologous HSCs may be an option to deplete latent reservoirs in HIV infected patients and has been a recent topic of debate using this technology in humans [93,94].

Collectively, immune humanized mice have shown to recapitulate many features of human immunobiology. The opportunity to investigate novel approaches for immune oncology or other conditions including chronic infection in a tractable model system can help assess human target engagement and even efficacy without risk to human subjects is of great clinical significance.

4. Considerations using HIS-mice and future developments

There are multiple cell types and methods that have been used to generate HIS mice, which are summarized in Table 3. The simplest method to reconstitute a human immune system in immunocompromised mice is via injection of human PBMCs. In this model, transplanted human lymphocytes rapidly expand with few cells

Table 3
Cell types and methods used to generate immune humanized mice.

Human cells	Injection route	Recipient	Conditioning
PBMCs	Intravenous, Intraperitoneal	Adult	–
$CD4^+$	Intravenous, Intraperitoneal	Adult	–
$CD34^+$	Intrahepatic, facial vein, Intracardiac	1 day-old pup	Sublethal irradiation
	Intravenous	Adult	Sublethal irradiation
BLT	Implantation of fetal liver & thymus under renal capsule, intravenous injection of autologous HSCs	Adult	Sublethal irradiation

of the myeloid lineage detected after a few days/weeks [95,96]. The engrafted human T cells will become xeno-reactive and initiate a lethal xeno-graft-versus-host-disease (x-GVHD) within a few weeks but these mice can be valuable for studies investigating immunosuppressive therapies targeting human T cells [97]. Transplantation and repopulation using human CD34$^+$ HSCs obtained from G-CSF mobilized blood, bone marrow, fetal liver, or umbilical cord blood all promote varying degrees of human hematopoiesis, but engraftment is generally more robust when mice are reconstituted using cord blood or fetal liver sourced CD34$^+$ cells [3,98]. The bone marrow, liver, and thymus (BLT) system, in which autologous fetal tissue is used, yields improved adaptive immune responses when compared to other models. However, complications from xGVHD as well as acquisition of materials and funding of fetal tissue research introduces additional logistical and ethical considerations. Furthermore, while both BLT and CD34$^+$ HSC models exhibit robust human immune cell chimerism after 16 weeks, both models are relatively poor in myeloid cell development (due to limited cross-reactivity of key cytokines and/or growth factors) and circulation of human red blood cells (RBCs) and platelets due to clearance by murine cells [79,99–101]. Finally, the source of CD34$^+$ cells used to generate HIS mice has distinct advantages and disadvantages depending on the questions being addressed (Fig. 3).

Effective strategies have been employed to address these limitations including exogenous administration of human cytokines [102], human cytokine-encoding plasmids [103,104], or transgenic expression of human cytokines [105–107]. However, these methods may results in supraphysiological cytokine concentrations producing unwanted and artifactual effects as well as altered immune cell frequencies [105]. Elegant work from Richard Flavell's group has used a knock-in approach whereby human cytokines/growth factors are targeted to the endogenous murine locus, ensuring faithful transcriptional regulation leading to improved HSC, myeloid, B cell, and NK cell development [101,108–112]. While the combination of multiple human cytokine "knock-ins" showed improved engraftment and human myeloid cell development, the lifespan of engrafted mice is shortened due to anemia resulting from human HSCs outcompeting murine HSC that are required for RBC production [108]. Strategies that enable sufficient human RBC development, possibly by expression of human erythropoietin, may overcome this limitation in the future. Other efforts include methods to improve human T cell responses. Our group combined transgenic expression of HLA molecules in NSG mice deficient for murine MHC class I/II, which was required in order to recapitulate a T cell-mediated immunopathology [74,113]. Similar approaches documented for NRG mice expressing HLA-DR4 also show evidence of

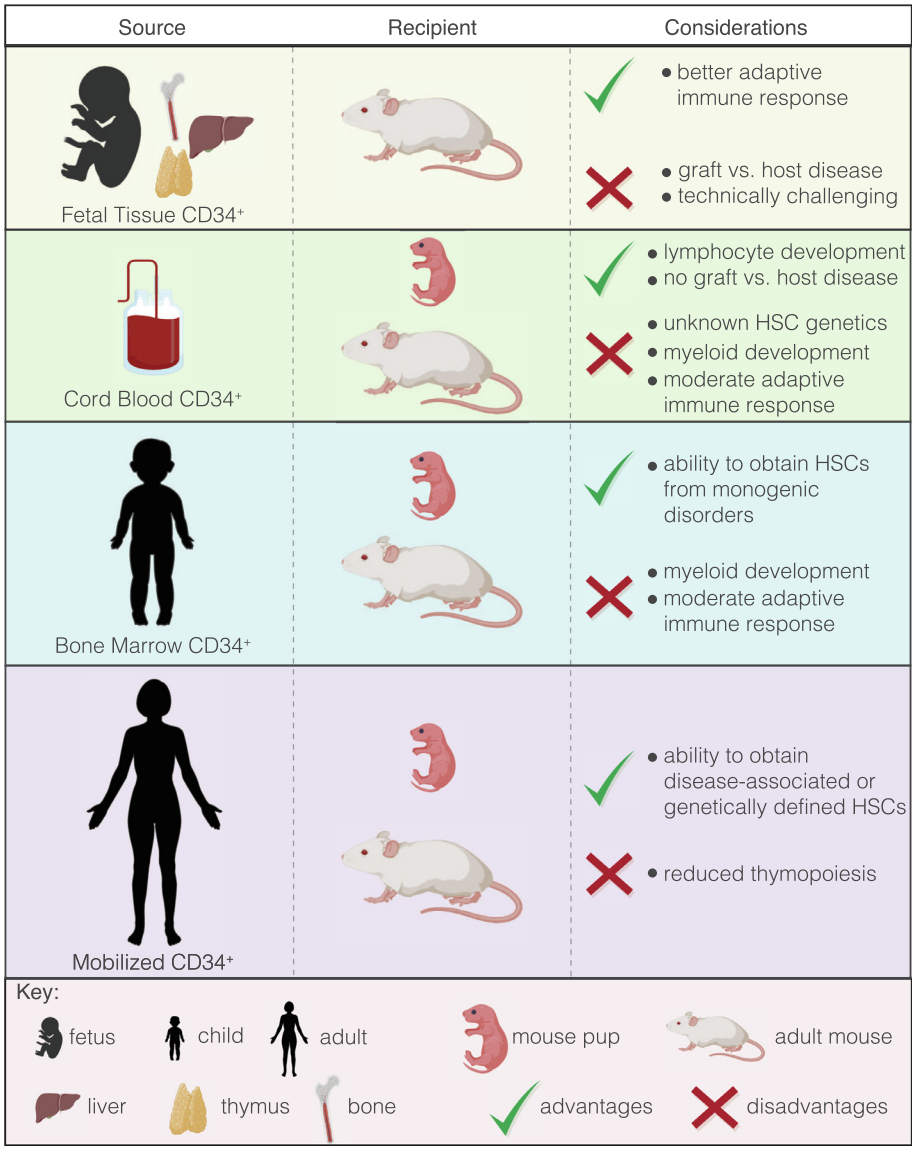

Fig. 3. Source of CD34$^+$ HSCs and considerations in their use for generating immune-humanized mice. Image generated using *BioRender*.

improved T cell responses including immunoglobulin class switch recombination [114]. We have been curious if improved myeloid cell development in combination with T cell selection on human HLA molecules in the murine thymus would further improve adaptive immunity in humanized mice. Our group has backcrossed the human *CSF1* knock-in with NSG mice deficient for MHC molecules and instead express HLA-DQ8 and HLA-A2 and we will be assessing immune responses in reconstituted mice in the near future.

Finally, it is rewarding to see that efforts put forth by many investigators to develop and improve immune humanized murine systems has yielded insights into human immunobiology that can ultimately inform clinical approaches to improve patient care. These xenobiotic platforms will only increase in utility and value for pre-clinical assessment of novel therapeutics directed at immune regulation, patient derived xenografts for solid tumors, as well as cancer immunotherapy in the coming years.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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