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Advances and applications of genome-edited animal models for severe combined immunodeficiency

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ABSTRACT

Severe combined immunodeficiency disease (SCID), characterized by profound immune system dysfunction, can lead to life-threatening infections and death. Animal models play a pivotal role in elucidating biological processes and advancing therapeutic strategies. Recent advances in gene-editing technologies, including zinc-finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs), CRISPR/Cas9, and base editing, have significantly enhanced the generation of SCID models. These models have not only deepened our understanding of disease pathophysiology but have also driven progress in cancer therapy, stem cell transplantation, organ transplantation, and infectious disease management. This review provides a comprehensive overview of current SCID models generated using novel gene-editing approaches, highlighting their potential applications in translational medicine and their role in advancing biomedical research.

Keywords: Gene-editing technology; Animal model; Translational biomedicine; Severe combined immunodeficiency disease

INTRODUCTION

Severe combined immunodeficiency disease (SCID) is a hereditary disease marked by profound immune system dysfunction (Cirillo et al., 2015). The estimated prevalence of SCID ranges from 1 to 2 in 100 000 live births (Zhang et al., 2023). Early clinical manifestations typically include recurrent and severe infections, dysplasia, pneumonia, otitis media, skin infections, and disseminated Bacillus Calmette-Guerin infection. A hallmark feature of SCID is the absence of T lymphocytes in the peripheral blood and lymphoid tissues (Chien et al., 2015). SCID can be broadly classified into two subtypes: those with or without B lymphocytes. Approximately 23%–30% of SCID patients have detectable levels of natural killer (NK) cells but no B and T lymphocytes. The absence of functional T, B, and NK cells severely compromises immune responses, necessitating robust animal models to investigate disease mechanisms and therapeutic approaches (Kim et al., 2021).

Immunodeficient animal models, which either harbor congenital genetic mutations or exhibit immune deficiencies induced through biotechnological methods, serve as indispensable tools in biomedical research. These models are widely applied in oncology, stem cell therapy, immune system, and infectious disease studies (Kim et al., 2021). The emergence of genome-editing technologies, including zinc finger nucleases (ZFN), transcription activator-like effector nucleases (TALEN), and clustered regularly interspaced short palindromic repeats associated with protein-9 nuclease (CRISPR/Cas9), has revolutionized the development of SCID animal models by providing precise and efficient tools for targeted genetic modification (Wang & Doudna, 2023; Wood et al., 2011). Several SCID animal models have been developed using mice (*Mus musculus*), rats (*Rattus*), and pigs (*Sus scrofa*) (Boettcher et al., 2020; He et al., 2019; Mombaerts et al., 1992; Suzuki et al., 2012). Humanized derivatives of these models, which incorporate human immune components, have gained popularity in both academic and

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commercial settings. Despite these advancements, the selection of appropriate SCID models for specific research applications remains a complex and critical challenge.

This review presents a comprehensive analysis of the development and application of SCID animal models generated using advanced gene-editing technologies based on DNA nucleases. It examines the characteristics of SCID models created using different gene-editing approaches and explores their potential future applications as experimental models. Overall, the generation of SCID animal models represents a critical resource for advancing disease research.

PATHOLOGICAL AND IMMUNOLOGICAL CHARACTERISTICS OF SCID MODEL

SCID is characterized by a range of clinical features, including persistent diarrhea, persistent oral thrush, recurrent, severe, or opportunistic infections, lymphadenopathy, hepatosplenomegaly, erythrodermic rash (Omenn's syndrome), and lymphopenia (Rivers & Gaspar, 2015). A key pathological hallmark of SCID, according to the 2022 diagnostic and classification criteria, is the significant reduction or absence of autologous T cells. Genetic analyses have identified pathogenic variants of SCID-associated genes in 93% of patients, with seven genes (*IL2RG*, *RAG1*, *ADA*, *IL7R*, *DCLRE1C*, *JAK3*, and *RAG2*) accounting for 89% of typical SCID cases. Additionally, three genotypes (*RAG1*, *ADA*, and *RMRP*) are implicated in 57% of leakage or atypical SCID cases (Dvorak et al., 2023). The underlying pathogenesis of SCID involves various genetic defects that disrupt critical immune functions (Table 1), including anomalies in cytokine signaling (*IL2RG*, *JAK3*, and *IL7RA*), defects in V(D)J recombination, T cell receptor (TCR) assembly (*RAG1/RAG2*, *DCLRE1C*, *NHEJ1*, *LIG4*, and *PRKDC*), impaired survival of hematopoietic precursors (*AK2*), accumulation of toxic metabolites (*ADA*), abnormalities in TCR signaling (*CD45*, *CD3D*, *CD3Z*, *CD3δ*, *CD3ε*, and *CORO1A*), and thymic abnormalities (*FOXN1*) (Kumrah et al., 2020).

NOVEL GENOME-EDITING TECHNIQUES FOR GENERATING SCID ANIMAL MODELS

Gene-editing technologies have revolutionized the development of gene therapies by enabling precise modifications of DNA sequences and gene functions, facilitating the treatment of various diseases (Gaj et al., 2016). Various gene-modified animal models, including transgenic, gene knockout (KO), and gene insertion models, have been constructed using genetic manipulation methods, such as lentiviral vectors, transposons, embryonic stem cells, and targeted nucleases (Doyle et al., 2012). However, the application of traditional approaches to establish gene-edited large animal models remains underexplored (Volobueva et al., 2019). This gap highlights the critical importance of employing gene-editing technologies to generate SCID animal models (Figure 1).

DNA nuclease-based gene-editing techniques, such as ZFN, TALEN, CRISPR/Cas9, base editing, and prime editing, represent the forefront of genomic engineering (Newby & Liu, 2021). These technologies continue to advance, offering increased efficiency, reduced costs, and an ever-expanding range of applications. While base and prime editing operate through distinct mechanisms, the other three technologies rely on the repair of DNA double-strand breaks (DSBs) via homologous recombination or non-homologous end-joining (Zhou et al., 2022). The following sections review SCID models constructed using specific gene-editing techniques (Table 2).

SCID models constructed using ZFN

Before the emergence of ZFN technology, targeted genetic engineering was highly challenging in both animal models and human tissue culture systems (Palpant & Dudzinski, 2013). In 2010, researchers reported the first successful ZFN-based construction of an X-linked SCID rat model via *IL2RG* gene knockout (Mashimo et al., 2010). These rats exhibited abnormal thymus and spleen development, significantly reduced lymphocyte populations, and susceptibility to human tumor cell xenotransplantation, leading to tumor formation. Subsequent advancements included the generation of *RAG1*-KO rats, which displayed a near-complete absence of lymphocytes in the spleens and lymph nodes, and the simultaneous knockout of *PRKDC* and *IL2RG*, resulting in FSG rats with an immunodeficient phenotype.

ZFN technology has also demonstrated versatility in large animal models. Notably, Yang et al. (2011) successfully knocked out *PPARG* in pigs using ZFN, although the resulting phenotypes were not described. Similarly, Watanabe et al.

Table 1 SCID-related genes and their phenotypic changes

Pathogenesis	Gene name	Phenotypic changes
Thymic abnormalities	*FOXN1*	No hair, no thymus Lymphocyte reduction
Cytokine signaling anomalies	*IL2RG* *JAK3* *IL7RA*	Lack of T and NK cells, dysfunction of B lymphocytes
V(D)J recombination and T-cell receptor defects	*RAG1/RAG2* *DCLRE1C* *NHEJ1* *LIG4* *PRKDC*	T and B cell deficiency
Defective survival of hematopoietic precursors	*AK2*	T, B, and NK cell deficiency
Toxic metabolite accumulation	*ADA*	T, B, and NK cell deficiency
TCR abnormalities	*CD45* *CD3* *CORO1A*	T cell deficiency
Others	*B2M* *PRF1*	T and NK cell deficiency

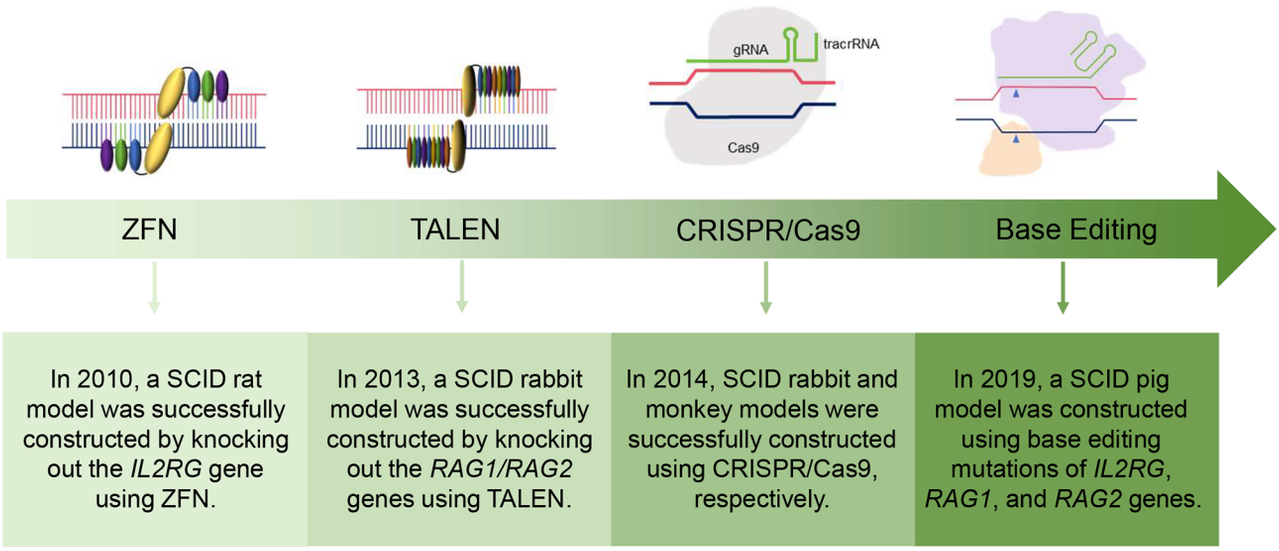

Figure 1 SCID animal models generated based on different DNA gene-editing techniques

Table 2 SCID animal models generated using different gene-editing techniques

Gene-editing technology	Species	Targeted genes	References
ZFNs	Rat (*Rattus*)	*IL2RG*	Mashimo et al., 2010
	Rat (*Rattus*)	*RAG1*	Zschemisch et al., 2012
	Rat (*Rattus*)	*PPARG*, *IL2RG*	Mashimo et al., 2012
	Pig (*Sus scrofa*)	*PPARG*	Yang et al., 2011
	Pig (*Sus scrofa*)	*IL2RG*	Watanabe et al., 2013
	Monkey (*Callithrix jacchus*)	*IL2RG*	Sato et al., 2016
TALENs	Mouse (*Mus musculus*)	*IL2RG*	Xiao et al., 2015
	Rat (*Rattus*)	*IL2RG*	Ménoret et al., 2018
	Rat (*Rattus*)	*RAG2*	Liu et al., 2015; Noto et al., 2018
	Rabbit (*Oryctolagus cuniculus*)	*RAG1/ RAG2*	Song et al., 2013
	Pig (*Sus scrofa*)	*RAG2*	Lee et al., 2014
	Pig (*Sus scrofa*)	*RAG1/ RAG2*	Huang et al., 2014
	Monkey (*Callithrix jacchus*)	*IL2RG*	Sato et al., 2016
CRISPR/Cas9	Mouse (*Mus musculus*)	*FOXN1*	Wei et al., 2017
	Rat (*Rattus*)	*IL2RG*, *RAG2*	Miyasaka et al., 2022
	Rabbit (*Oryctolagus cuniculus*)	*RAG1*, *RAG2*, *IL2RG*	Yan et al., 2014
	Rabbit (*Oryctolagus cuniculus*)	*FOXN1*	Song et al., 2021
	Rabbit (*Oryctolagus cuniculus*)	*FOXN1*, *RAG1*, *RAG2*, *IL2RG*, *PRKDC*	Song et al., 2017, 2018
	Rabbit (*Oryctolagus cuniculus*)	*IL2RG*	Hashikawa et al., 2020
	Pig (*Sus scrofa*)	*IL2RG*	Kang et al., 2016; Ren et al., 2020
	Pig (*Sus scrofa*)	*DCLRE1C*, *IL2RG*	Boettcher et al., 2020
	Monkey (*Macaca fascicularis*)	*PPARG*, *IL2RG*	Niu et al., 2014
Base Editor	Pig (*Sus scrofa*)	*IL2RG*, *RAG1*, *RAG2*	Xie et al., 2019
	Monkey (*Macaca fascicularis*)	*IL2RG*, *RAG1*	Zheng et al., 2023

(2013) established *IL2RG*-KO pigs, characterized by a complete absence of thymus, T cells, and NK cells, closely resembling the immunological profile of patients with X-linked SCID. Sato et al. (2016) generated a marmoset model with SCID by efficiently editing the *IL2RG* gene to produce an immunodeficiency phenotype, providing insights into thymocyte development in primates and novel therapeutics for human X-linked SCID. However, despite its relatively high editing efficiency, ZFN technology has several limitations that constrain its broader application, such as off-target effects, potential for carcinogenesis, and cumbersome operation.

SCID models constructed using TALEN

TALEN technology offers several advantages over ZFN, including easier design and construction and the ability to target any genomic site with high precision and efficiency. TALEN technology was first employed in 2013 to generate an immunodeficient rabbit model via *RAG1* and *RAG2* knockout (Song et al., 2013). Since then, TALEN has been utilized across a wide range of applications, from creating genetically modified crops and livestock to developing experimental models in diverse organisms. TALEN became the first genome-editing tool to achieve clinical success, curing relapsed refractory CD19-positive acute lymphoblastic leukemia, and further demonstrated its versatility with the commercialization of the first genome-edited crop (Becker & Boch, 2021; Menz et al., 2020; Qasim et al., 2017).

TALEN has played a pivotal role in constructing SCID

mouse models with distinct immunodeficiency phenotypes based on *IL2RG* gene editing. For example, Xiao et al. (2015) utilized these models to validate ANGPTL7 as a regulator of human hematopoietic stem and progenitor cell expansion and regeneration. Ménoret et al. (2018) generated *IL2RG*-deficient rats using TALEN, which exhibited more severe immunodeficiencies compared to *RAG1*-deficient rats, characterized by the partial loss of T, B, and NK cells. When these *IL2RG*-deficient rats were crossed with *RAG1*-deficient rats, the resulting offspring displayed severe immunosuppression, with undetectable levels of T, B, and NK cells (Ménoret et al., 2018). Previous studies have also developed *RAG2*-KO rats using TALEN technology, which demonstrated a deficiency in mature T and B cells, along with a compensatory increase in NK cells, making them highly susceptible to infections such as cowpox virus and xenotransplantation of human tumor cells (Liu et al., 2015; Noto et al., 2018). Song et al. (2013) successfully obtained *RAG*-KO rabbits via microinjection of TALEN mRNA into embryos, resulting in underdeveloped lymphoid organs, early obstruction of T and B cell development, and obstacles in V(D)J recombination.

TALEN technology has further been employed in large animal models. For example, Huang et al. (2014) developed *RAG*-KO pigs, which exhibited immune organ hypoplasia, an inability to undergo V(D)J rearrangement, and a loss of mature B and T cells. By targeting the *RAG2* gene in pigs using TALEN, Lee et al. (2014) produced an SCID model lacking both T and B cells, with subsequent injection of human-induced pluripotent cells inducing teratoma formation that recapitulated various human tissues. Furthermore, Sato et al. (2016) developed an immunodeficient marmoset model using both ZFN and TALEN vectors with distinct DSB activities, which improved editing efficiency and reduced the risk of chimerism but also posed challenges due to its technical complexity.

SCID models constructed using CRISPR/Cas9

CRISPR/Cas9 has become a widely used gene-editing approach due to its high efficiency, single-guide RNA (sgRNA) design, high specificity, and low off-target effects (Hochheiser et al., 2018). Using this approach, Wei et al. (2017) generated a novel nude NOD/SCID/*IL2rg*$^{-/-}$ (NSIN) mouse strain by knocking out *Foxn1* in NOD/SCID/*IL2rg*$^{-/-}$ (NSI) mice. The absence of B, T, and NK cells in these mice impaired T cell reconstruction and thymus regeneration ability following allogeneic bone marrow nucleated cell transplantation while enhancing engraftment of leukemia and solid tumor cells. Similarly, Miyasaka et al. (2022) used CRISPR/Cas9 to successfully edit *IL2RG* and *RAG2* in rats, generating SRG models that lacked mature T, B, and circulating NK cells. Yan et al. (2014) also applied this technology to simultaneously knockout *RAG1*, *RAG2*, and *IL2RG* in rabbits, although the production of live immunodeficient animals was not reported. Subsequent efforts have successfully produced immunodeficient rabbits with mutations in *FOXN1*, *RAG1*, *RAG2*, *IL2RG*, and *PRKDC*, which demonstrated reproductive transmission and yielded F1 generation offspring (Song et al., 2017, 2018). Notably, X-SCID rabbits generated by targeting *IL2RG* alone showed T and B cell loss, thymic hypoplasia, and improved outcomes in allogeneic skin tissue transplantation (Hashikawa et al., 2020). With CRISPR/Cas9 tools, Song et al. (2021) edited *FOXN1* in rabbits, producing hairless, thymus-free animals with severe immunodeficiency, suitable for human stem cell xenotransplantation.

CRISPR/Cas9 has also shown remarkable utility in large animal models. Notably, Boettcher et al. (2020) performed site-specific CRISPR/Cas9 mutations in *IL2RG* from pigs with an *ART*$^{-/-}$ genetic background, generating T^{-}B^{-}NK^{-}SCID pigs lacking T, B, and NK cells in their peripheral blood and lymphoid tissues. Similarly, *IL2RG*-KO pigs generated using CRISPR/Cas9 technology have been shown to exhibit a SCID phenotype (Kang et al., 2016) and support the growth of human melanoma-derived tumors (Ren et al., 2020). Niu et al. (2014) employed CRISPR/Cas9 technology to precisely target the *PPAR-γ* and *RAG1* genes in crab-eating monkeys. Ongoing efforts to optimize CRISPR/Cas9 for SCID model development include the use of engineered Cas9 variants and the identification of immune orthogonal orthologs to minimize adverse events and improve editing outcomes (Uddin et al., 2020).

SCID models constructed using base editing

Base editing systems, including cytosine base editors (CBE), adenine base editors (ABEs), and guanine editors, enable precise genetic modifications without causing DNA DSBs, thereby avoiding activation of the DNA double-strand repair mechanisms. Xie et al. (2019) first used base editing to mutate *IL2RG*, *RAG1*, and *RAG2* in pigs, generating an immunodeficient model that exhibited severe infections and high mortality. Similarly, Zheng et al. (2023) employed the CBE4max system to inactivate *IL2RG* and *RAG1* in monkeys, establishing an immunodeficient model characterized by lymphopenia, lymphoid organ atrophy, and a lack of mature T cells. These base-edited monkeys supported the engraftment and growth of human breast cancer cells, leading to tumor formation.

Although base editing has shown promise, challenges remain regarding off-target effects associated with CRISPR/Cas9-based systems, including base and prime editing technologies. These limitations continue to hinder the therapeutic and clinical translation of CRISPR/Cas9-based systems for treating diseases. Furthermore, the use of gene-editing technology to generate SCID animal models requires ongoing refinement to enhance their precision and applicability.

COMPARISON OF GENE-EDITING TECHNIQUES AND SPECIES IN GENERATING SCID MODELS

Comparison of gene-editing technologies

ZFN technology, despite being one of the earliest genome-editing tools, faces significant challenges due to off-target effects and associated cytotoxicity (Carroll, 2011). Furthermore, the immune responses triggered by the microinjection of mRNA encoding ZFNs, along with inefficiencies in the process and the generation of mutant chimeras during SCID model construction, significantly limit the applicability of this technology (Li et al., 2014; Mashimo, 2014). Additional factors such as strain variability, species differences, chromatin structure, and technical manipulation also impact its efficiency in genetic modification.

In contrast, TALEN technology employs a DNA binding module that recognizes single nucleotides (Tsai et al., 2022), offering greater precision and ease in targeting specific genomic loci. TALEN was the first gene-editing tool that could

be designed and constructed relatively easily for precise genome editing. However, cytotoxicity and off-target effects remain concerns, and the extensive molecular cloning and sequencing required for constructing SCID models using TALEN present certain limitations in terms of technical manipulation and model construction cycle.

CRISPR/Cas9 has gained prominence due to its simplicity, requiring only the design and synthesis of gRNAs targeting specific genes (Bhatia et al., 2023). However, its reliance on DSBs introduces risks, such as DNA damage (Haapaniemi et al., 2018), chromosomal fragmentation and instability (Wang et al., 2019), off-target effects (Zhang et al., 2015b), and immunogenicity associated with Cas9 nucleases (Crudele & Chamberlain, 2018; Mehta & Merkel, 2020). Thus, when constructing SCID models with CRISPR/Cas9, considerations extend beyond gene-editing efficiency to include the evaluation of pathological phenotypes, immunological changes, off-target effects, and cytotoxicity.

Base editing has the advantages of programmability and flexibility, overcoming the limitations of traditional Cas9 nuclease gene editing (Porto et al., 2020). Optimized base editing techniques allow precise targeting of immunodeficiency genes, improving gene-editing efficiency. However, comprehensive safety evaluations remain essential to address potential off-target effects. Advances in sequencing technologies and bioinformatics analysis are critical for achieving the precision needed to assess and mitigate these risks (Blattner et al., 2020).

Prime editing, although still in its infancy, represents a promising development in gene editing. It has been successfully applied in cell lines, post-mitotic neurons, mice, organoids, and plants (Gao et al., 2021; Schene et al., 2020; Xu et al., 2020). Despite these advances, prime editing faces many obstacles, including off-target effects, delivery selection, and immunogenicity. Notably, SCID models generated using prime editing have not yet been reported.

SCID models generated using these technologies have successfully replicated the phenotypes of immunodeficiency diseases. As gene-editing methods continue to advance, improvements in efficiency, ease of operation, and safety will also occur. While CRISPR/Cas9 technology is currently the most widely used due to its versatility, the appropriate gene-editing technology should be selected according to the target organism and specific gene to be edited. Simultaneously, the exploration and development of alternative methods like base and prime editing should not be overlooked. To address possible off-target effects, robust evaluations are required, including quantification of input DNA, unbiased bioinformatics analysis, traceable validation methods, and sensitivity assessments. In addition, while comparing different gene-editing techniques for SCID model construction, the appropriateness of the technical conditions during operation and possible differences between species should be considered.

Comparisons across species

Recent advancements in gene-editing techniques have enabled the development of a diverse range of SCID animal models, encompassing both large and small species. These models are widely used in biomedicine, each offering unique advantages, with a primary focus on mice, rats, rabbits, pigs, and non-human primates (NHPs).

SCID mice: Small immunodeficient animal models, particularly mice, are extensively utilized due to their short reproductive cycles, high fertility rates, ease of husbandry, and well-characterized genomes. The availability of mature gene modification technologies further enhances their applicability. Common SCID mouse models include SCID, NOD/SCID, NOD-SCID-Il2rgnull, NRG, and BRG mice (Shultz et al., 1995, 2007). The development of humanized mouse models, achieved through homozygous mutations in *PRKDC*-SCID or defects in *RAG1/RAG2*, has facilitated the effective implantation of human immune system components and xenografts. These humanized mouse models represent innovative platforms for human tumor immunology research, enabling study of the interaction between human tumors and the immune system and evaluation of immunotherapy efficacy (Cogels et al., 2021).

Despite significant advances in the translational potential of humanized mice in clinical oncology, no mouse model can fully replicate all aspects of human biology (Chuprin et al., 2023). Additionally, some SCID mice develop immune leakage as they age, although the underlying molecular mechanisms remain unclear. Consequently, there is an urgent need to develop immunodeficient models that more closely resemble human physiology to address the limitations of existing systems and improve translational research outcomes.

SCID rats: Before the advent of gene-editing technology, progress in developing immunodeficient rat models was slow. The emergence of these technologies has facilitated the generation of many immunodeficient rat strains, providing a valuable complement to mouse models. Existing immunodeficient rat strains include X-SCID (*IL2RG*), RAG1-KO (*RAG1*), SDR (*RAG2*), SCID (*PRKDC*), FSG (*PRKDC*, *IL2RG*), NSGL (*PRKDC*, *IL2RG*, *hSIRPα*), SD-RG (*RAG1*, *RAG2*, *IL2RG*), and SRG (*RAG2*, *IL2RG*) (Liu et al., 2015; Mashimo et al., 2010, 2012; Ménoret et al., 2020; Noto et al., 2020; Yang et al., 2018; Zschemisch et al., 2012).

SCID rats offer several advantages over mouse models. Notably, they support the engraftment of a broader range of human cells and tissues and have a longer lifespan, enabling extended studies. Their larger size facilitates surgical procedures and allows the collection of more experimental samples, addressing several shortcomings of mice. In addition, rats have been widely used in pharmacology and toxicology experiments, enhancing their utility in translational studies.

However, SCID rats have certain limitations. For instance, human $CD34^+$ hematopoietic stem cells cannot be successfully transplanted into FSG rats, hindering their use in developing humanized immune systems. Furthermore, *PRKDC*-KO rats exhibit growth inhibition (Mashimo et al., 2012), which may affect experimental outcomes and limit their application.

SCID rabbits: Genome-edited rabbit models hold significant promise for advancing our understanding of disease mechanisms and facilitating the development of novel gene therapies (Hornyik et al., 2022). Rabbits possess a unique immune system that makes them valuable for immunological and microbiological studies. Their extensive B cell repertoire enables the production of a diverse array of antibodies, and their affinity optimization mechanisms are more effective than those of rodents (Weber et al., 2017), making them particularly useful for antibody production studies. Additionally, rabbits are highly sensitive to a range of viruses and pathogenic bacteria, making SCID rabbit models well-suited for studying human

microbial infections and their associated diseases.

SCID pigs: Minipigs, widely used as non-rodent large laboratory animals in biomedical research, possess gastrointestinal, digestive, renal, and immune systems similar to humans, making them valuable models for studying human diseases. In addition, minipigs offer practical advantages over NHPs, including easier handling, husbandry, sampling, and drug administration. They are also free from zoonotic diseases commonly associated with NHPs and pose fewer ethical and conservation concerns. The availability of sexually mature animals and ease of transportation further enhance their utility.

Miniature pigs are widely used in biomedicine, not only as an ideal model for human diseases but also for organ xenotransplantation, cardiovascular research, and neurological studies. Compared with small animal models, pigs exhibit higher sequence similarity with human heterologous receptors, allowing for more accurate predictions of pharmacological and pharmacokinetic responses. Using advanced gene-editing techniques, researchers have developed various immunodeficient pig models, which are increasingly recognized as indispensable tools in translational and preclinical research.

Non-human primates SCID models: The availability of complete genome sequences has significantly advanced our understanding of biological processes at the molecular level. This has fueled the development of SCID models in NHPs, which provide critical insights from a molecular and developmental biology perspective (Nakamura et al., 2021). Although NHP-SCID models are promising, they face certain limitations, such as higher early infection mortality. This challenge necessitates anti-infection measures to ensure animal survival for meaningful studies. In addition, the problem of chimerism in NHP-SCID models presents a significant barrier to their use in pathophysiological studies.

In summary, SCID models derived from different species exhibit distinct disease phenotypes due to variations in anatomical, physiological, and genetic characteristics. While humanized mouse models are the most widely used for studying the human immune system, large-animal models, such as pigs and NHPs, more closely replicate human biological systems (Li & Lai, 2024; Rahman et al., 2023; Zhao et al., 2019), offering higher translational relevance. However, small animal models offer advantages such as shorter reproduction cycles and lower costs, characteristics that somewhat limit the widespread use of large animals. In addition, large-animal models are constrained by higher ethical and logistical considerations, including societal acceptance and regulatory restrictions. The optimal approach involves the joint use of different species to address specific research goals. Selecting the appropriate species for SCID model development should be guided by the experimental design, research objectives, and practical considerations such as cost and ethical implications.

SCID ANIMAL MODEL APPLICATIONS

The continuous development of targeted gene-editing techniques has transformed the generation of SCID animal models, expanding their applications in tumor xenotransplantation, immunology, stem cell therapy, and infectious disease treatment. These models provide invaluable insights into SCID-related diseases and facilitate translational biomedical research (Figure 2).

Tumor xenotransplantation

SCID animal models are essential for studying tumor growth, metastasis, and recurrence, as well as for evaluating antitumor drug screening. Cell-derived xenograft models, created by implanting tumor cell lines subcutaneously, intravenously, or *in situ* into immunodeficient or humanized animals, enable the study of subcutaneous or *in situ* tumor growth (Tsumura et al., 2020). While traditional *in vitro* cell lines lack the complexity of primary tumor microenvironments, patient-derived xenograft (PDX) models have addressed this limitation by transferring tumor tissues from patients into immunodeficient animals, paving the way for personalized therapeutic strategies (Yin et al., 2021). For example, *IL2RG*-deficient rats can serve as hosts for human ovarian cancer xenografts, with X-SCID rats developing tumors within 14 days of cell injection (Mashimo et al., 2010). Similarly, SCID mice subcutaneously inoculated with human medulloblastoma cells have provided insights into the protective effects of human protein analogs against TMZ-induced apoptosis in male germ cells (Jia et al., 2019). Non-small cell lung cancer models have also been developed by implanting *in vitro* tumor cells into SCID mice, enabling researchers to evaluate disease outcomes (Anderson et al., 2003). In addition, SCID mice have been used to construct skin xenograft models, facilitating the study of long-term ultraviolet irradiation effects (Hachiya et al., 2009).

The continuous optimization of SCID models and the anatomical limitations of small-animal systems have driven the development of SCID models in larger species, such as rabbits, pigs, and monkeys, using advanced gene-editing techniques and enabling comprehensive evaluation through tumor xenografts. For example, the breast cancer cell line MDA-MB-231 was successfully transplanted into a CRISPR/Cas9-generated immunodeficient monkey model (Zheng et al., 2023). Similarly, *FOXN1*-KO naked rabbits generated using CRISPR/Cas9-mediated editing have been applied for teratoma detection, tissue engineering, and vascular transplantation, while NOD/SCID/*IL2rg*$^{-/-}$ mice have facilitated *in vivo* monitoring and imaging of solid tumors (Wei et al., 2017). Moreover, *IL2RG*$^{-/Y}$ pigs created with CRISPR/Cas9 support human tumorigenesis, forming teratomas representing various human tissues when injected with human-induced pluripotent stem cells (Lee et al., 2014). Therefore, SCID models have significantly advanced our understanding of tumorigenesis and the complex interactions between tumors and host environments.

Immunotherapy

The successful transplantation of human tumors and active human immune cells into SCID mice has been a cornerstone for the development and use of humanized mouse models to evaluate cancer therapy efficacy (Bankert et al., 2001). The clinical success of immunotherapy has spurred interest from academic researchers, as well as biotechnology and pharmaceutical industries, in its potential role in cancer treatment. However, given the high cost of clinical development and the risk of inflammatory toxicity, robust model systems are essential for preclinical research. These systems include transplantable tumor models, transgenic or gene knockout tumor-generating models, and humanized mouse models inoculated with human tumor xenografts (Dranoff, 2011). Selecting the most appropriate animal models is critical for conducting systematic preclinical cancer

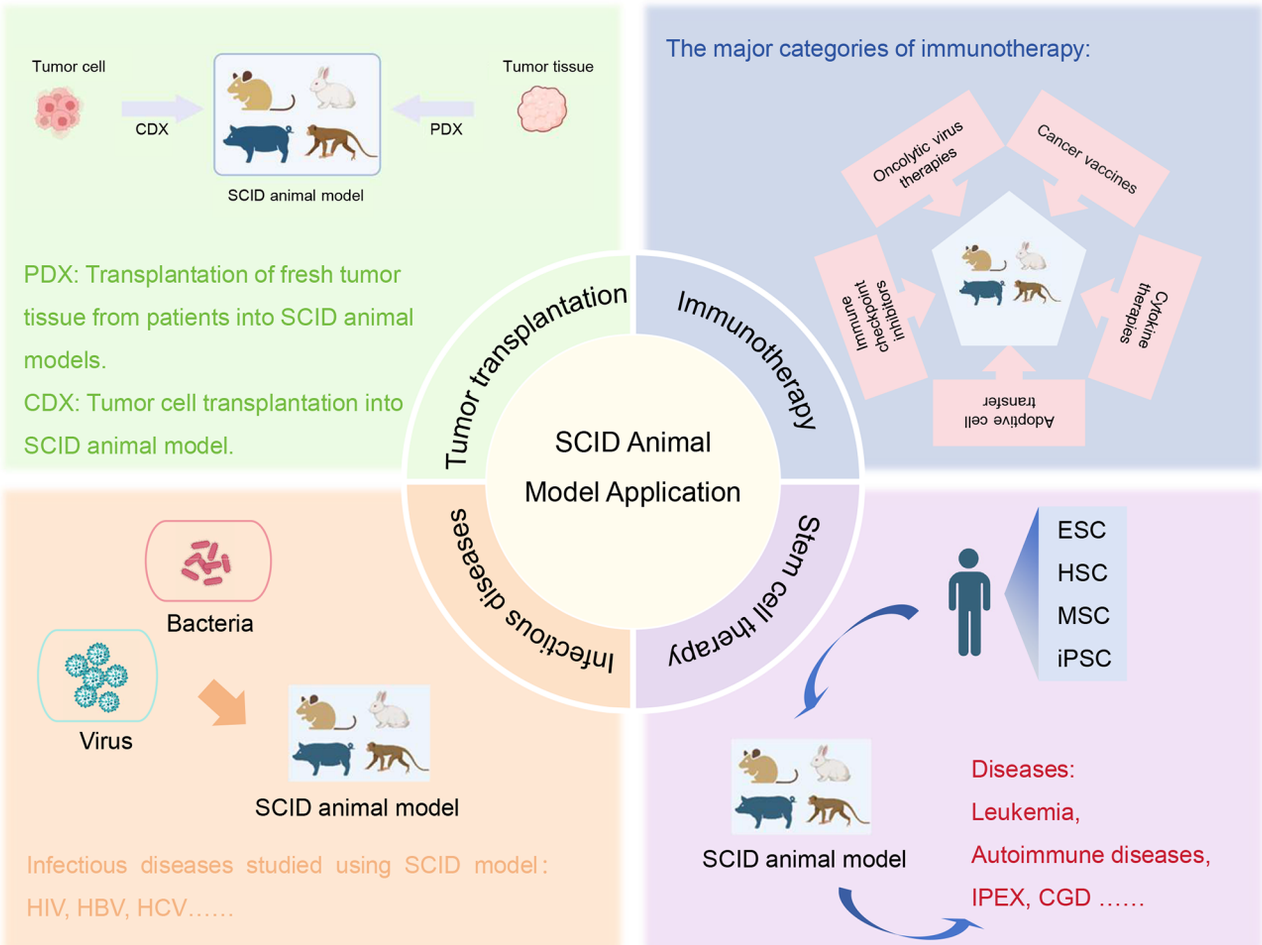

Figure 2 SCID animal model applications

Created using BioRender.com. CDX: Cell line-derived xenograft. PDX: Patient-derived xenografts. IPEX: Immune dysregulation, polyendocrinopathy, enteropathy, X-linked. CGD: X-linked chronic granulomatous disease.

immunotherapy studies.

Currently, cancer immunotherapies encompass oncolytic viral therapies, cancer vaccines, cytokine therapies, adoptive cell transplantation, and immune checkpoint inhibitors, all of which have shown promise in clinical practice (Zhang & Zhang, 2020). SCID models have been instrumental in advancing basic and translational research in these areas. For example, preclinical studies using NSG mice carrying human tumors and treated with specific antibodies against human CD47 have demonstrated effective tumor cell phagocytosis and elimination, reinforcing CD47 as an effective target for cancer treatment (Willingham et al., 2012). Similarly, the efficacy of CAR-T cell therapy for leukemia has been evaluated using NSG mice engineered with T cells expressing CD33-specific antigen receptors (Kenderian et al., 2015). In addition, humanized peripheral blood mononuclear cell (PBMC)-transfected MHC class I/II-deficient NOG mice have proven valuable for assessing human immune responses (Yaguchi et al., 2018), providing a platform for testing cancer immunotherapy drugs (Morillon et al., 2020). Therefore, the generation of novel humanized SCID animal models with reconstructed immune systems represents a significant advancement in preclinical immunotherapy research, allowing for comprehensive assessment of potential risks, such as cytokine storms and localized effects of CAR-T cells on tissues.

Stem cell therapy

SCID models, characterized by their immunodeficient status, are ideal for studying stem cell therapy as they can receive human cell transplants without triggering the immune system. For example, NOD/SCID and NOD-SCID-IL2Rγnull (NSG) mouse models are commonly used to determine the multispectral implantation potential and safety of candidate classes of hematopoietic stem cells (HSCs) from different sources. These models also serve as platforms for evaluating gene-edited HSCs as potential therapies for human immunodeficiency virus (HIV) (Holt et al., 2010; Li et al., 2013; Lux et al., 2019; Watanabe et al., 2007). Similarly, SCID rats have been used in preclinical studies involving human-induced pluripotent stem cell-derived neural precursor cell transplantation to model neonatal hypoxic-ischemic brain injury (Beldick et al., 2018). X-SCID rats further demonstrate their utility in stem cell research by tolerating transplants of human dopaminergic neurons derived from artificial blood stem cells without initiating an immune response, enabling evaluation of human dopaminergic neuronal function *in vivo* and providing a theoretical basis for stem cell therapies targeting neurological diseases (Samata et al., 2015).

Beyond rodents, SCID models in larger species have expanded the scope of stem cell research. In X-SCID dogs, $CD34^+$ bone marrow cells have been shown to re-establish normal B and T cell function (Bauer et al., 2013). SCID pig

models have also been instrumental in advancing stem cell research, ranging from HSC transplantation to regenerative medicine (Boettcher et al., 2019). Humanized SCID models are particularly valuable for studying rare genetic immune system disorders, such as immune dysregulation, polyendocrinopathy, enteropathy, X-linked syndrome (IPEX) (Goettel et al., 2015), inflammatory bowel disease (Goettel et al., 2019), and X-linked chronic granulomatous disease (CGD) (Naumann et al., 2007). These models enable preclinical evaluation of HSC-based gene therapies or *in situ* gene correction studies, offering a safer alternative to direct patient trials. Overall, humanized SCID animal models provide a critical platform for preclinical studies of stem cell gene therapies and *in situ* gene correction, while reducing risks to patients.

Infectious diseases

Animal models are invaluable for testing the efficacy and safety of experimental vaccines and therapies prior to human studies (Sarkar & Heise, 2019). The heightened sensitivity of SCID animals to environmental bacteria and viruses makes them ideal for studying microbial infections and host-pathogen interactions. For example, the development of a humanized mouse model based on TK-NOG has advanced research into hepatitis B virus (HBV) and hepatitis C virus (HCV) infections (Kosaka et al., 2013). Likewise, SCID rats have been utilized to monitor and manage symbiotic multi-tumor viruses through T cell monitoring strategies (Besch-Williford et al., 2017; Rigatti et al., 2016; Tanaka et al., 2018). X-linked SCID dogs have been developed to analyze the progression of metastatic squamous cell carcinoma (SCC) during advanced oncoviral infection (Goldschmidt et al., 2006). SCID pigs have also been used to study the replication dynamics of porcine reproductive and respiratory syndrome virus (Chen et al., 2015). Furthermore, SCID rats have been used to explore the role of host immunity in regulating the carrying capacity of *Hymenolepis diminuta* within the intestinal tract (Ohno et al., 2018), with antibiotic interventions found to be useful for managing *Campylobacter bovis*-associated clinical disease (Pires et al., 2023). Additionally, SCID mouse models engrafted with human lung tissue have been used to investigate SARS-CoV-2 infection (Fu et al., 2021).

While SCID models are useful for infectious disease research, their inability to fully replicate the complexity of the human immune response limits their utility in studying emerging infectious diseases. The prevention and control of infectious diseases require multifaceted approaches, and SCID models should be complemented with other research systems to gain insights into complex disease mechanisms and immune interactions.

SUMMARY AND FUTURE PERSPECTIVES

The continuous emergence of novel SCID models has driven advancements in biomedical research. This review systematically examined SCID animal models, including mice, rats, rabbits, pigs, and monkeys, constructed using CRISPR/Cas9 and other gene-editing technologies, highlighting their potential applications and contributions.

CRISPR-based genome editing technologies include CRISPR/Cas9, CRISPR-Cas12a, base editing, prime editing, transcriptional regulation, and RNA editing, which provide targeted and precise genome modification capabilities (Pacesa et al., 2024). Compared to the more complex and off-target effects of ZFN and TALEN, CRISPR offers a simpler yet highly versatile platform. However, limitations persist, particularly regarding editing activity, specificity, and delivery. Despite these challenges, the refinement of CRISPR-based technologies has significantly advanced SCID model development and disease research.

Currently, HSC transplantation remains the primary treatment for patients with SCID. Recent progress in CRISPR genome editing has shifted from preclinical studies based on cellular and animal models to human clinical trials. Therefore, the use of gene correction strategies has brought SCID gene therapy closer to reality (Iancu et al., 2023), consisting of the extraction of leukocytes from the bone marrow of an affected child, the correction of genetic defects via gene editing, and the reinfusion of modified cells into the patient. These developments emphasize the expanding utility of SCID models in both therapeutic innovation and fundamental disease research.

Species-specific differences further underscore the importance of selecting appropriate animal models for SCID research. For instance, in the development of STING agonist-based anticancer drugs, DMXAA and CMA demonstrated robust antitumor activity in preclinical mouse and rat models but failed in clinical trials due to their inability to effectively activate human STING (Cavlar et al., 2013; Zhang et al., 2015a).

These findings highlight the critical need for models that closely mimic human genetic and immunological responses. Large SCID animal models have become indispensable for translational research due to their genetic, anatomical, physiological, and phylogenetic similarities to humans. Microinjection and somatic cell nuclear transfer (SCNT) are the main methods for preparing gene-edited animal models (Matoba & Zhang, 2018; Shakweer et al., 2023). Microinjection, commonly used for small animal models such as mice, rats, and rabbits, presents challenges, such as chimeric outcomes and the inability to genotype animals until after birth, making it less cost-effective for large animal models. In contrast, SCNT permits genotype identification of nuclear donor cell clones before birth, thereby reducing costs and increasing efficiency. This method has been successfully employed in generating gene-edited pigs, although it requires a high level of technical expertise.

Pigs have emerged as highly suitable animal models for studying human SCID (Iqbal et al., 2019). In addition to their application in SCID research, pigs have garnered significant attention in organ transplantation and clinical applications. Recent breakthroughs include successful pig-to-human transplants of the heart (Griffith et al., 2022; Mohiuddin et al., 2023), kidney (Stone, 2023), and liver (Mallapaty, 2024). Notably, the first living pig kidney transplantation was performed at Massachusetts General Hospital in 2024, with the patient demonstrating positive recovery (Mallapaty & Kozlov, 2024).

Given their phylogenetic relationship with humans, NHPs provide another critical platform for SCID research, with gene-editing technologies, such as CRISPR/Cas9 editing and single nucleotide editing, significantly advancing the development of SCID monkey models. Researchers have successfully produced homozygous NHP models, incorporating reproductive techniques and F1 generation semen to establish immunodeficient monkeys within a reasonable timeframe. Furthermore, the ability to generate monkey embryos and

offspring with specific gene mutations or insertions with high efficiency has expanded the scope of NHP-based studies (Lu et al., 2022; Qiu et al., 2019). Innovative techniques, such as xenotransplantation of testicular tissue, have accelerated sperm maturation, thereby shortening cycle time and improving the utility of NHPs for both basic and biomedical research (Liu et al., 2016; Yao et al., 2018). Additionally, SCNT-based methods have proven invaluable for constructing genetically modified NHP models (Liu et al., 2018, 2019), including SCID monkeys. Despite the remarkable progress in SCID animal model development, the effectiveness of genome editing in such models must be further validated to ensure reproducibility and reliability. Additionally, the ethical concerns, high costs, and supply issues associated with large animal models should be addressed to ensure broader application.

Although SCID models have become an indispensable tool in biomedical research, their continued refinement is essential to better simulate human diseases and evaluate treatments. Enhancements, such as targeting multiple immunodeficiency-related genes, could further amplify the immunodeficiency phenotype, improving model fidelity for translational studies. Large-animal SCID models hold particular promise for future stem cell therapies, regenerative medicine, transplantation studies, infectious diseases, immunology, and cancer therapy. Nevertheless, challenges persist, including limited model availability, high costs, the need for standardized protocols, ethical concerns, improving the accuracy of disease representation, and optimizing gene-editing and humanization techniques for SCID models.

CONCLUSIONS

SCID animal models have become invaluable tools for advancing our understanding of hematopoietic and immune system regulation, offering insights into the cellular and molecular mechanisms underlying various human diseases, with unique applications in virology, regenerative medicine, hematology, cancer, immunology, and immunodeficiency. Compared to traditional models, SCID animal models generated by gene editing more accurately replicate the complex phenotypes associated with immunodeficiency and provide a robust platform for studying disease pathophysiology and translational biomedicine.

COMPETING INTERESTS

The authors declare that they have no competing interests.

AUTHORS' CONTRIBUTIONS

X.Z. wrote the original draft. C.H.H., S.Y., and M.D.R. revised the manuscript. All authors read and approved the final version of the manuscript.

REFERENCES

Anderson TM, Hess SD, Egilmez NK, et al. 2003. Comparison of human lung cancer/SCID mouse tumor xenografts and cell culture growth with patient clinical outcomes. *Journal of Cancer Research and Clinical Oncology*, **129**(10): 565–568.

Bankert RB, Egilmez NK, Hess SD. 2001. Human-SCID mouse chimeric models for the evaluation of anti-cancer therapies. *Trends in Immunology*, **22**(7): 386–393.

Bauer TR, Tuschong LM, Calvo KR, et al. 2013. Long-term follow-up of foamy viral vector-mediated gene therapy for canine leukocyte adhesion deficiency. *Molecular Therapy*, **21**(5): 964–972.

Becker S, Boch J. 2021. TALE and TALEN genome editing technologies. *Gene and Genome Editing*, **2**: 100007.

Beldick SR, Hong J, Altamentova S, et al. 2018. Severe-combined immunodeficient rats can be used to generate a model of perinatal hypoxic-ischemic brain injury to facilitate studies of engrafted human neural stem cells. *PLoS One*, **13**(11): e0208105.

Besch-Williford C, Pesavento P, Hamilton S, et al. 2017. A naturally transmitted epitheliotropic polyomavirus pathogenic in immunodeficient rats: characterization, transmission, and preliminary epidemiologic studies. *Toxicologic Pathology*, **45**(5): 593–603.

Bhatia S, Pooja, Yadav SK. 2023. CRISPR-Cas for genome editing: classification, mechanism, designing and applications. *International Journal of Biological Macromolecules*, **238**: 124054.

Blattner G, Cavazza A, Thrasher AJ, et al. 2020. Gene editing and genotoxicity: targeting the off-targets. *Frontiers in Genome Editing*, **2**: 613252.

Boettcher AN, Cunnick JE, Powell EJ, et al. 2019. Porcine signal regulatory protein alpha binds to human CD47 to inhibit phagocytosis: implications for human hematopoietic stem cell transplantation into severe combined immunodeficient pigs. *Xenotransplantation*, **26**(2): e12466.

Boettcher AN, Li YS, Ahrens AP, et al. 2020. Novel engraftment and T cell differentiation of human hematopoietic cells in ART$^{-/-}$ IL2RG$^{-/Y}$ SCID Pigs. *Frontiers in Immunology*, **11**: 100.

Carroll D. 2011. Genome engineering with zinc-finger nucleases. *Genetics*, **188**(4): 773–782.

Cavlar T, Deimling T, Ablasser A, et al. 2013. Species-specific detection of the antiviral small-molecule compound CMA by STING. *The EMBO Journal*, **32**(10): 1440–1450.

Chen NH, Dekkers JCM, Ewen CL, et al. 2015. Porcine reproductive and respiratory syndrome virus replication and quasispecies evolution in pigs that lack adaptive immunity. *Virus Research*, **195**: 246–249.

Chien YH, Chiang SC, Chang KL, et al. 2015. Incidence of severe combined immunodeficiency through newborn screening in a Chinese population. *Journal of the Formosan Medical Association*, **114**(1): 12–16.

Chuprin J, Buettner H, Seedhom MO, et al. 2023. Humanized mouse models for immuno-oncology research. *Nature Reviews Clinical Oncology*, **20**(3): 192–206.

Cirillo E, Giardino G, Gallo V, et al. 2015. Severe combined immunodeficiency--an update. *Annals of the New York Academy of Sciences*, **1356**(1): 90–106.

Cogels MM, Rouas R, Ghanem GE, et al. 2021. Humanized mice as a valuable pre-clinical model for cancer immunotherapy research. *Frontiers in Oncology*, **11**: 784947.

Crudele JM, Chamberlain JS. 2018. Cas9 immunity creates challenges for CRISPR gene editing therapies. *Nature Communications*, **9**(1): 3497.

Doyle A, Mcgarry MP, Lee NA, et al. 2012. The construction of transgenic and gene knockout/knockin mouse models of human disease. *Transgenic Research*, **21**(2): 327–349.

Dranoff G. 2011. Experimental mouse tumour models: what can be learnt about human cancer immunology?. *Nature Reviews Immunology*, **12**(1): 61–66.

Dvorak CC, Haddad E, Heimall J, et al. 2023. The diagnosis of severe combined immunodeficiency: Implementation of the PIDTC 2022 Definitions. *Journal of Allergy and Clinical Immunology*, **151**(2): 547–555. e5.

Fu WK, Wang W, Yuan LZ, et al. 2021. A SCID mouse-human lung xenograft model of SARS-CoV-2 infection. *Theranostics*, **11**(13): 6607–6615.

Gaj T, Sirk SJ, Shui SL, et al. 2016. Genome-editing technologies: principles and applications. *Cold Spring Harbor Perspectives in Biology*, **8**(12): a023754.

Gao P, Lyu Q, Ghanam AR, et al. 2021. Prime editing in mice reveals the

essentiality of a single base in driving tissue-specific gene expression. *Genome Biology*, **22**(1): 83.

Goettel JA, Biswas S, Lexmond WS, et al. 2015. Fatal autoimmunity in mice reconstituted with human hematopoietic stem cells encoding defective FOXP3. *Blood*, **125**(25): 3886–3895.

Goettel JA, Kotlarz D, Emani R, et al. 2019. Low-dose interleukin-2 ameliorates colitis in a preclinical humanized mouse model. *Cellular and Molecular Gastroenterology and Hepatology*, **8**(2): 193–195.

Goldschmidt MH, Kennedy JS, Kennedy DR, et al. 2006. Severe papillomavirus infection progressing to metastatic squamous cell carcinoma in bone marrow-transplanted X-linked SCID dogs. *Journal of Virology* **80**(13): 6621–6628.

Griffith BP, Goerlich CE, Singh AK, et al. 2022. Genetically modified porcine-to-human cardiac xenotransplantation. *New England Journal of Medicine*, **387**(1): 35–44.

Haapaniemi E, Botla S, Persson J, et al. 2018. CRISPR-Cas9 genome editing induces a p53-mediated DNA damage response. *Nature Medicine*, **24**(7): 927–930.

Hachiya A, Sriwiriyanont P, Fujimura T, et al. 2009. Mechanistic effects of long-term ultraviolet B irradiation induce epidermal and dermal changes in human skin xenografts. *The American Journal of Pathology* **174**(2): 401–413.

Hashikawa Y, Hayashi R, Tajima M, et al. 2020. Generation of knockout rabbits with X-linked severe combined immunodeficiency (X-SCID) using CRISPR/Cas9. *Scientific Reports*, **10**(1): 9957.

He D, Zhang JH, Wu WW, et al. 2019. A novel immunodeficient rat model supports human lung cancer xenografts. *FASEB Journal* **33**(1): 140–150.

Hochheiser K, Kueh AJ, Gebhardt T, et al. 2018. CRISPR/Cas9: a tool for immunological research. *European Journal of Immunology*, **48**(4): 576–583.

Holt N, Wang JB, Kim K, et al. 2010. Human hematopoietic stem/progenitor cells modified by zinc-finger nucleases targeted to *CCR5* control HIV-1 *in vivo*. *Nature Biotechnology* **28**(8): 839–847.

Hornyik T, Rieder M, Castiglione A, et al. 2022. Transgenic rabbit models for cardiac disease research. *British Journal of Pharmacology*, **179**(5): 938–957.

Huang J, Guo XG, Fan NN, et al. 2014. RAG1/2 knockout pigs with severe combined immunodeficiency. *The Journal of Immunology*, **193**(3): 1496–1503.

Iancu O, Allen D, Knop O, et al. 2023. Multiplex HDR for disease and correction modeling of SCID by CRISPR genome editing in human HSPCs. *Molecular Therapy-Nucleic Acids*, **31**: 105–121.

Iqbal MA, Hong K, Kim JH, et al. 2019. Severe combined immunodeficiency pig as an emerging animal model for human diseases and regenerative medicines. *BMB Reports*, **52**(11): 625–634.

Jia Y, Lue Y, Swerdloff RS, et al. 2019. The humanin analogue (HNG) prevents temozolomide-induced male germ cell apoptosis and other adverse effects in severe combined immuno-deficiency (SCID) mice bearing human medulloblastoma. *Experimental and Molecular Pathology*, **109**: 42–50.

Kang JT, Cho B, Ryu J, et al. 2016. Biallelic modification of *IL2RG* leads to severe combined immunodeficiency in pigs. *Reproductive Biology and Endocrinology*, **14**(1): 74.

Kenderian SS, Ruella M, Shestova O, et al. 2015. CD33-specific chimeric antigen receptor T cells exhibit potent preclinical activity against human acute myeloid leukemia. *Leukemia*, **29**(8): 1637–1647.

Kim YY, Yun JW, Kim JS, et al. 2021. Comparison of genetically engineered immunodeficient animal models for nonclinical testing of stem cell therapies. *Pharmaceutics*, **13**(2): 130.

Kosaka K, Hiraga N, Imamura M, et al. 2013. A novel TK-NOG based humanized mouse model for the study of HBV and HCV infections. *Biochemical and Biophysical Research Communications*, **441**(1): 230–235.

Kumrah R, Vignesh P, Patra P, et al. 2020. Genetics of severe combined immunodeficiency. *Genes & Diseases*, **7**(1): 52–61.

Lee K, Kwon DN, Ezashi T, et al. 2014. Engraftment of human iPS cells and allogeneic porcine cells into pigs with inactivated *RAG2* and accompanying severe combined immunodeficiency. *Proceedings of the National Academy of Sciences of the United States of America*, **111**(20): 7260–7265.

Li HL, Nakano T, Hotta A. 2014. Genetic correction using engineered nucleases for gene therapy applications. *Development, Growth & Differentiation*, **56**(1): 63–77.

Li LJ, Krymskaya L, Wang JB, et al. 2013. Genomic editing of the HIV-1 coreceptor CCR5 in adult hematopoietic stem and progenitor cells using zinc finger nucleases. *Molecular Therapy*, **21**(6): 1259–1269.

Li XJ, Lai LX. 2024. A booming field of large animal model research. *Zoological Research*, **45**(2): 311–313.

Liu Q, Fan CF, Zhou SY, et al. 2015. Bioluminescent imaging of vaccinia virus infection in immunocompetent and immunodeficient rats as a model for human smallpox. *Scientific Reports*, **5**: 11397.

Liu Z, Cai YJ, Liao ZD, et al. 2019. Cloning of a gene-edited macaque monkey by somatic cell nuclear transfer. *National Science Review*, **6**(1): 101–108.

Liu Z, Cai YJ, Wang Y, et al. 2018. Cloning of macaque monkeys by somatic cell nuclear transfer. *Cell*, **172**(4): 881–887. e7.

Liu Z, Nie YH, Zhang CC, et al. 2016. Generation of macaques with sperm derived from juvenile monkey testicular xenografts. *Cell Research*, **26**(1): 139–142.

Lu ZY, He ST, Jiang J, et al. 2022. Base-edited cynomolgus monkeys mimic core symptoms of STXBP1 encephalopathy. *Molecular Therapy*, **30**(6): 2163–2175.

Lux CT, Pattabhi S, Berger M, et al. 2019. TALEN-Mediated Gene Editing of HBG in Human Hematopoietic Stem Cells Leads to Therapeutic Fetal Hemoglobin Induction. *Molecular Therapy-Methods & Clinical Development*, **12**: 175–183.

Mallapaty S. 2024. First pig liver transplanted into a person lasts for 10 days. *Nature*, **627**(8005): 710–711.

Mallapaty S, Kozlov M. 2024. First pig kidney transplant in a person: what it means for the future. *Nature*, **628**(8006): 13–14.

Mashimo T. 2014. Gene targeting technologies in rats: zinc finger nucleases, transcription activator-like effector nucleases, and clustered regularly interspaced short palindromic repeats. *Development, Growth & Differentiation*, **56**(1): 46–52.

Mashimo T, Takizawa A, Kobayashi J, et al. 2012. Generation and characterization of severe combined immunodeficiency rats. *Cell Reports* **2**(3): 685–694.

Mashimo T, Takizawa A, Voigt B, et al. 2010. Generation of knockout rats with X-linked severe combined immunodeficiency (X-SCID) using zinc-finger nucleases. *PLoS One*, **5**(1): e8870.

Matoba S, Zhang Y. 2018. Somatic cell nuclear transfer reprogramming: mechanisms and applications. *Cell Stem Cell*, **23**(4): 471–485.

Mehta A, Merkel OM. 2020. Immunogenicity of Cas9 protein. *Journal of Pharmaceutical Sciences*, **109**(1): 62–67.

Ménoret S, Ouisse LH, Tesson L, et al. 2018. Generation of immunodeficient rats with *Rag1* and *Il2rg* gene deletions and human tissue grafting models. *Transplantation*, **102**(8): 1271–1278.

Ménoret S, Ouisse LH, Tesson L, et al. 2020. In vivo analysis of human immune responses in immunodeficient rats. *Transplantation*, **104**(4): 715–723.

Menz J, Modrzejewski D, Hartung F, et al. 2020. Genome edited crops touch the market: a view on the global development and regulatory environment. *Frontiers in Plant Science*, **11**: 586027.

Miyasaka Y, Wang JX, Hattori K, et al. 2022. A high-quality severe combined immunodeficiency (SCID) rat bioresource. *PLoS One*, **17**(8):

e0272950.
Mohiuddin MM, Singh AK, Scobie L, et al. 2023. Graft dysfunction in compassionate use of genetically engineered pig-to-human cardiac xenotransplantation: a case report. *The Lancet*, **402**(10399): 397–410.
Mombaerts P, Iacomini J, Johnson R S, et al. 1992. RAG-1-deficient mice have no mature B and T lymphocytes. *Cell*, **68**(5): 869–877.
Morillon YM, Sabzevari A, Schlom J, et al. 2020. The development of next-generation PBMC humanized mice for preclinical investigation of cancer immunotherapeutic agents. *Anticancer Research*, **40**(10): 5329–5341.
Nakamura T, Fujiwara K, Saitou M, et al. 2021. Non-human primates as a model for human development. *Stem Cell Reports*, **16**(5): 1093–1103.
Naumann N, De Ravin SS, Choi U, et al. 2007. Simian immunodeficiency virus lentivector corrects human X-linked chronic granulomatous disease in the NOD/SCID mouse xenograft. *Gene Therapy*, **14**(21): 1513–1524.
Newby GA, Liu DR. 2021. *In vivo* somatic cell base editing and prime editing. *Molecular Therapy*, **29**(11): 3107–3124.
Niu YY, Shen B, Cui YQ, et al. 2014. Generation of gene-modified cynomolgus monkey via Cas9/RNA-mediated gene targeting in one-cell embryos. *Cell*, **156**(4): 836–843.
Noto FK, Adjan-Steffey V, Tong M, et al. 2018. Sprague dawley *Rag2*-null rats created from engineered spermatogonial stem cells are immunodeficient and permissive to human xenografts. *Molecular Cancer Therapeutics*, **17**(11): 2481–2489.
Noto FK, Sangodkar J, Adedeji BT, et al. 2020. The SRG rat, a sprague-dawley Rag2/Il2rg double-knockout validated for human tumor oncology studies. *PLoS One*, **15**(10): e0240169.
Ohno T, Kai T, Miyasaka Y, et al. 2018. Intestinal immunity suppresses carrying capacity of rats for the model tapeworm, Hymenolepis diminuta. *Parasitology International*, **67**(4): 357–361.
Pacesa M, Pelea O, Jinek M. 2024. Past, present, and future of CRISPR genome editing technologies. *Cell*, **187**(5): 1076–1100.
Palpant NJ, Dudzinski D. 2013. Zinc finger nucleases: looking toward translation. *Gene Therapy*, **20**(2): 121–127.
Pires EM, Pugazhenthi U, Fink MK, et al. 2023. Antibiotic treatment of *Corynebacterium bovis*-associated clinical disease in NSG mice. *Comparative Medicine*, **73**(6): 461–465.
Porto EM, Komor AC, Slaymaker IM, et al. 2020. Base editing: advances and therapeutic opportunities. *Nature Reviews Drug Discovery*, **19**(12): 839–859.
Qasim W, Zhan H, Samarasinghe S, et al. 2017. Molecular remission of infant B-ALL after infusion of universal TALEN gene-edited CAR T cells. *Science Translational Medicine*, **9**(374): eaaj2013.
Qiu PY, Jiang J, Liu Z, et al. 2019. BMAL1 knockout macaque monkeys display reduced sleep and psychiatric disorders. *National Science Review*, **6**(1): 87–100.
Rahman A, Li YH, Chan TK, et al. 2023. Large animal models of cardiac ischemia-reperfusion injury: Where are we now?. *Zoological Research*, **44**(3): 591–603.
Ren JL, Yu DW, Fu R, et al. 2020. *IL2RG*-deficient minipigs generated via CRISPR/Cas9 technology support the growth of human melanoma-derived tumours. *Cell Proliferation*, **53**(10): e12863.
Rigatti LH, Toptan T, Newsome JT, et al. 2016. Identification and characterization of novel rat polyomavirus 2 in a colony of X-SCID rats by P-PIT assay. *mSphere*, **1**(6): e00334–16.
Rivers L, Gaspar HB. 2015. Severe combined immunodeficiency: recent developments and guidance on clinical management. *Archives of Disease in Childhood*, **100**(7): 667–672.
Samata B, Kikuchi T, Miyawaki Y, et al. 2015. X-linked severe combined immunodeficiency (X-SCID) rats for xeno-transplantation and behavioral evaluation. *Journal of Neuroscience Methods*, **243**: 68–77.
Sarkar S, Heise MT. 2019. Mouse models as resources for studying infectious diseases. *Clinical Therapeutics*, **41**(10): 1912–1922.
Sato K, Oiwa R, Kumita W, et al. 2016. Generation of a nonhuman primate model of severe combined immunodeficiency using highly efficient genome editing. *Cell Stem Cell*, **19**(1): 127–138.
Schene IF, Joore IP, Oka R, et al. 2020. Prime editing for functional repair in patient-derived disease models. *Nature Communications*, **11**(1): 5352.
Shakweer WME, Krivoruchko AY, Dessouki SM, et al. 2023. A review of transgenic animal techniques and their applications. *Journal of Genetic Engineering and Biotechnology*, **21**(1): 55.
Shultz LD, Ishikawa F, Greiner DL. 2007. Humanized mice in translational biomedical research. *Nature Reviews Immunology* **7**(2): 118–130.
Shultz LD, Schweitzer PA, Christianson SW, et al. 1995. Multiple defects in innate and adaptive immunologic function in NOD/LtSz-scid mice. *The Journal of Immunology*, **154**(1): 180–191.
Song J, Hoenerhoff M, Yang DS, et al. 2021. Development of the nude rabbit model. *Stem Cell Reports*, **16**(3): 656–665.
Song J, Wang GS, Hoenerhoff MJ, et al. 2018. Bacterial and *Pneumocystis* infections in the lungs of gene-knockout rabbits with severe combined immunodeficiency. *Frontiers in Immunology*, **9**: 429.
Song J, Yang DS, Ruan JX, et al. 2017. Production of immunodeficient rabbits by multiplex embryo transfer and multiplex gene targeting. *Scientific Reports*, **7**(1): 12202.
Song J, Zhong J, Guo XG, et al. 2013. Generation of *RAG*1- and 2-deficient rabbits by embryo microinjection of TALENs. *Cell Research*, **23**(8): 1059–1062.
Stone L. 2023. Kidney xenotransplantation. *Nature Reviews Urology*, **20**(11): 641.
Suzuki S, Iwamoto M, Saito Y, et al. 2012. *Il2rg* gene-targeted severe combined immunodeficiency pigs. *Cell Stem Cell*, **10**(6): 753–758.
Tanaka M, Kuramochi M, Nakanishi S, et al. 2018. Rat polyomavirus 2 infection in a colony of X-linked severe combined immunodeficiency rats in Japan. *Journal of Veterinary Medical Science*, **80**(9): 1400–1406.
Tsai HC, Pietrobon V, Peng MY, et al. 2022. Current strategies employed in the manipulation of gene expression for clinical purposes. *Journal of Translational Medicine*, **20**(1): 535.
Tsumura R, Koga Y, Hamada A, et al. 2020. Report of the use of patient‐derived xenograft models in the development of anticancer drugs in Japan. *Cancer Science*, **111**(9): 3386–3394.
Uddin F, Rudin CM, Sen T. 2020. CRISPR gene therapy: applications, limitations, and implications for the future. *Frontiers in Oncology*, **10**: 1387.
Volobueva AS, Orekhov AN, Deykin AV. 2019. An update on the tools for creating transgenic animal models of human diseases - focus on atherosclerosis. *Brazilian Journal of Medical and Biological Research*, **52**(5): e8108.
Wang HF, Nakamura M, Abbott TR, et al. 2019. CRISPR-mediated live imaging of genome editing and transcription. *Science*, **365**(6459): 1301–1305.
Wang JY, Doudna JA. 2023. CRISPR technology: a decade of genome editing is only the beginning. *Science*, **379**(6629): eadd8643.
Watanabe M, Nakano K, Matsunari H, et al. 2013. Generation of interleukin-2 receptor gamma gene knockout pigs from somatic cells genetically modified by zinc finger nuclease-encoding mRNA. *PLoS One*, **8**(10): e76478.
Watanabe S, Ohta S, Yajima M, et al. 2007. Humanized NOD/SCID/IL2Rgamma(null) mice transplanted with hematopoietic stem cells under nonmyeloablative conditions show prolonged life spans and allow detailed analysis of human immunodeficiency virus type 1 pathogenesis. *Journal of Virology* **81**(23): 13259–13264.
Weber J, Peng HY, Rader C. 2017. From rabbit antibody repertoires to rabbit monoclonal antibodies. *Experimental & Molecular Medicine*, **49**(3): e305.

Wei XR, Lai YX, Li BH, et al. 2017. CRISPR/Cas9-mediated deletion of *Foxn1* in NOD/SCID/IL2rg$^{-/-}$ mice results in severe immunodeficiency. *Scientific Reports*, **7**(1): 7720.

Willingham SB, Volkmer JP, Gentles AJ, et al. 2012. The CD47-signal regulatory protein alpha (SIRPa) interaction is a therapeutic target for human solid tumors. *Proceedings of the National Academy of Sciences of the United States of America*, **109**(17): 6662–6667.

Wood AJ, Lo TW, Zeitler B, et al. 2011. Targeted genome editing across species using ZFNs and TALENs. *Science*, **333**(6040): 307–307.

Xiao YR, Jiang ZW, Li Y, et al. 2015. ANGPTL7 regulates the expansion and repopulation of human hematopoietic stem and progenitor cells. *Haematologica*, **100**(5): 585–594.

Xie JK, Ge WK, Li N, et al. 2019. Efficient base editing for multiple genes and loci in pigs using base editors. *Nature Communications*, **10**(1): 2852.

Xu RF, Li J, Liu XS, et al. 2020. Development of plant prime-editing systems for precise genome editing. *Plant Communications*, **1**(3): 100043.

Yaguchi T, Kobayashi A, Inozume T, et al. 2018. Human PBMC-transferred murine MHC class I/II-deficient NOG mice enable long-term evaluation of human immune responses. *Cellular & Molecular Immunology*, **15**(11): 953–962.

Yan QM, Zhang QJ, Yang HQ, et al. 2014. Generation of multi-gene knockout rabbits using the Cas9/gRNA system. *Cell Regeneration*, **3**(1): 12.

Yang DS, Yang HQ, Li W, et al. 2011. Generation of PPARγ mono-allelic knockout pigs via zinc-finger nucleases and nuclear transfer cloning. *Cell Research*, **21**(6): 979–982.

Yang XL, Zhou JL, He JJ, et al. 2018. An immune system-modified rat model for human stem cell transplantation research. *Stem Cell Reports*, **11**(2): 514–521.

Yao X, Liu Z, Wang X, et al. 2018. Generation of knock-in cynomolgus monkey via CRISPR/Cas9 editing. *Cell Research*, **28**(3): 379–382.

Yin ZY, Maswikiti EP, Liu Q, et al. 2021. Current research developments of patient-derived tumour xenograft models (review). *Experimental and Therapeutic Medicine*, **22**(5): 1206.

Zhang H, Han MJ, Tao JL, et al. 2015a. Rat and human STINGs profile similarly towards anticancer/antiviral compounds. *Scientific Reports*, **5**: 18035.

Zhang XH, Tee LY, Wang XG, et al. 2015b. Off-target effects in CRISPR/Cas9-mediated genome engineering. *Molecular Therapy-Nucleic Acids*, **4**: e264.

Zhang XP, Kang XY, Yang MY, et al. 2023. A variant of *RAG1* gene identified in severe combined immunodeficiency: a case report. *BMC Pediatrics*, **23**(1): 56.

Zhang YY, Zhang ZM. 2020. The history and advances in cancer immunotherapy: understanding the characteristics of tumor-infiltrating immune cells and their therapeutic implications. *Cellular & Molecular Immunology*, **17**(8): 807–821.

Zhao JG, Lai LX, Ji WZ, et al. 2019. Genome editing in large animals: current status and future prospects. *National Science Review*, **6**(3): 402–420.

Zheng X, Huang CH, Lin YQ, et al. 2023. Generation of inactivated IL2RG and RAG1 monkeys with severe combined immunodeficiency using base editing. *Signal Transduction and Targeted Therapy*, **8**(1): 327.

Zhou WL, Yang JR, Zhang YL, et al. 2022. Current landscape of gene-editing technology in biomedicine: applications, advantages, challenges, and perspectives. *Medcomm*, **3**(3): e155.

Zschemisch NH, Glage S, Wedekind D, et al. 2012. Zinc-finger nuclease mediated disruption of *Rag1* in the LEW/Ztm rat. *BMC Immunology*, **13**: 60.