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Review Article

Humanized mouse model: a review on preclinical applications for cancer immunotherapy

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Abstract: Due to the refractory and partial sensitive treatments to malignant cancers, immunotherapy has increasingly become a hotspot in effective anti-tumor research. However, at present, existing animal models could not accurately describe the interaction between human tissue and tumor cells for preclinical trials. Furthermore, it is a tough obstacle to reconstitute the immune system and microenvironment in a mouse model identical to humans due to species differences. In the establishment of the humanized mouse model, the co-transplantation of human immunocytes with/without tissues and tumor cells is the key breakthrough to solve this problem. The compelling progress has been investigated in the preclinical drug test for diverse tumor types. This review mainly summarized the development of immunodeficient mice, and the construction and practicability of the humanized mouse model. Furthermore, the investigators also highlight the pros and cons, and recent progress in immunotherapy research for advanced utility of human cancer diseases.

Keywords: Immunodeficient mice, humanized mouse model, cancer, immunotherapy

Introduction

With the aging of the population, the morbidity of malignant tumor increases with the global increase in mortality [1]. Even though chemotherapy, radiotherapy and ectomy are the most effective methods for treatment, the postoperative five-year survival rate remains unsatisfactory. As a remarkable leap forward in the development of onco-immunology, the immunotherapy attacks tumor cells by potentiating functional lymphocytes, rather than direct killing, and this has become one of the hot position fields in cancer treatment [2-4]. However, the development of its preclinical therapeutic evaluation in animal experiments has always been plagued by the species diversity between mice and humans [5]. Although the mice experiment is advantageous for specific questions, the curative effect of immunotherapy in clinical trials could not be accurately predicted due to the discrepancy in immune system activation and responses. Therefore, there is an urgent demand for novel preclinical models that could help bridge this gap with an appropriate immune microenvironment.

The humanized mouse model, as a novel experimental animal in biomedical research, was established by the transplantation of human-derived peripheral blood mononuclear cells (PBMCs) or hematopoietic stem cells (HSCs), and this could be identified as foreign tissues by murine innate and adaptive immune systems [6-8]. In order to optimize this effect, the modification of specific genes in animals could make these immunodeficient, and completely lack of T, B and NK cells, leading to a highly efficient approach to solve the challenge of immune rejection. Furthermore, humanized mice could accept the xenograft steady growth with the mimic human immune system, and recent studies have investigated the comparability of attendant tumor biological reaction and those in cancer patients [9]. As a result, the establishment of cell-line-derived xenograft (CDX) and patient-derived xenograft (PDX) into humanized mouse models is a remarkable stride for facili-

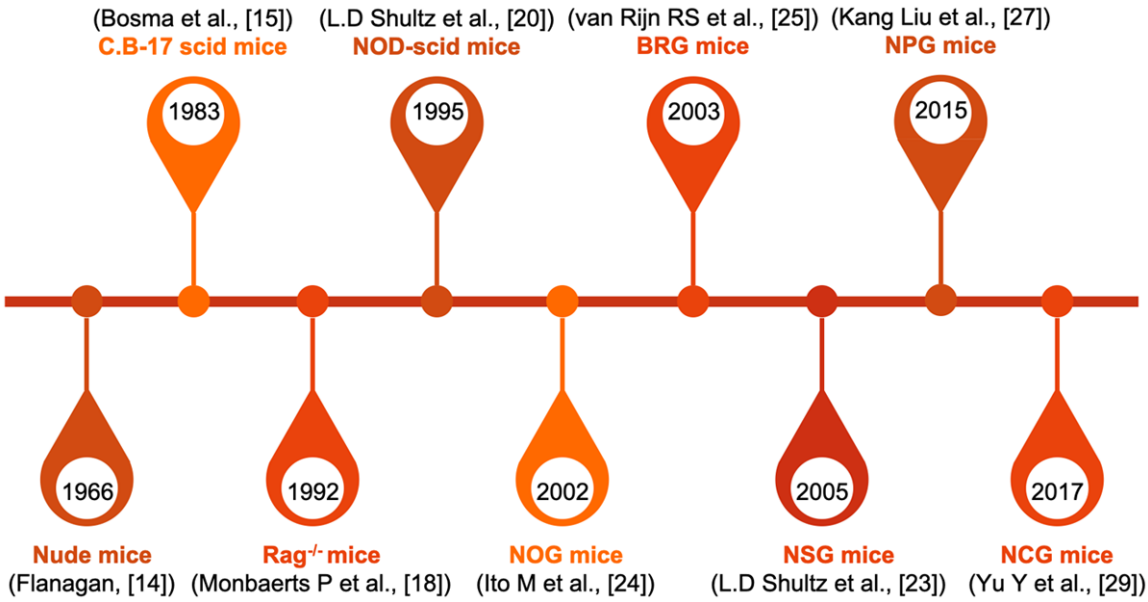

Figure 1. Advances in immunodeficient mice strain.

tating diverse applications in the exploration of cancer pathogenesis, and the evaluation of therapeutic effects [10-12].

This study provides an overview of the recent advances in humanized mouse models, and highlights the pros and cons of different ways of modeling, in order to gain a promising perspective of different onco-immunology applications.

The brief history of immunodeficient mice strain

In order to establish humanized mice models, constant pursuits in specific genes deletion could become indispensable parts of engineering processes (**Figure 1**). That is, the aim of the purposeful mutation was to reduce murine cells, allowing severely immunodeficient mice to accept the transplanted human-derived immune cells or HSCs.

Nude mice, which lack of T lymphocytes, have been considered as the first models for malignant disease research, in which human tumor cells could steadily grow. However, the presence of B cells and innate immune cells led to severe rejection in the reconstruction of the human immune system [13, 14]. As the root of the development of humanized mice, the C.B-17-Prkdc^{scid} (C.B-17 scid) mice strain originated from the spontaneous subgene-mutation of catalytic polypeptide of DNA-activated protein kinase (Prkdc) [15]. The non-expression of Prkdc led to the lack of T and B lymphocytes, which is known as the symptom of severe combined immune deficiency (scid) syndrome [16]. Resulting in the avoidance of xenoreactivity, C.B-17 scid mice was made for the successful engraftments of human normal cells. Alternatively, the knockout of recombination activating gene (Rag) 1 or 2 (Rag1^{null} or Rag2^{null}) could cause the disruption of the V(D)J recombination. This might be a useful candidate for immunodeficiency [17-19]. Since these mice were developed and applied in scientific research, genetic engineering has become an important research field in human immune system reconstruction.

A significant breakthrough came with the introduction of scid mutation to the background of non-obese diabetic (NOD) mice, triggering a neoteric NOD-scid mice strain. These mice could remarkably improve the compatibility of the human immune system, which was driven by the reduction of innate immunity through the defective levels of NK and myeloid cell function [20-22]. However, mice with poor engraftment of human HSCs still remained to have a low utilization rate, which was probably due to the leakiness of murine T and B cells, and the remaining activity of NK cells.

In the early 2000, a major achievement in the history of humanized mice might be regarded as the knockout mutation that targeted the interleukin-2 receptor common γ-chain ($IL2rg^{null}$), and this played an essential role in the murine cytokine expression, including IL-2, 4, 7, 9, 15 and 21 [23-25]. The introduction of $IL2rg^{null}$ mutation combined with scid mutation or Rag knockout generated three severely immunodeficient mice strains, namely, NSG (NOD.Cg-$Prkdc^{scid}IL2rg^{tm1Wjl}$), NOG (NOD.Cg-$Prkdc^{scid}IL2rg^{tm1Sug}$), and BRG (Balb/c $Rag2^{-/-}IL2rg^{-/-}$) [26]. Recently, some emerging strains have rapidly been developed, and have been internationally recognized as the family of the highest immunodeficient models, such as NPG (NOD-$Prkdc^{scid}IL2rg^{null}$) and NCG (NOD-$Prkdc^{em26}IL2rg^{em26}$Nju) [27-29]. These new mice generations have exhibited a dramatic improvement in the rate of human engraftment, and have been recognized as the most frequently used models in the recapitulation of the human immune system.

Establishment of the humanized immune mouse model

As one of the apparently different aspects between humans and mice, the innate immune system is the main reason for the mutual repulsion, which can be probably explained by the divergent evolution of these two species. The humanized immune mouse model, with severe combined immune deficiency as the background, has provided the possibility to overcome this problem. The principal standard approaches of humanization are summarized, as follows: (i) Hu-PBL, (ii) Hu-SRC, and (iii) Hu-BLT. The respective characteristics are described in detail below:

Hu-PBL mouse model

As widely recognized as the most cost-effective pattern, the hu-PBL mouse model has the simplest establishment process and a low expenditure. The transplantation of human PBMCs into immunodeficient mice by intravenous injection (I.V.) resulted in the majority of engraftment that comprised of T cells [7]. Researchers have argued that there is a lack of specific cytokines necessary for survival, which prevents B and NK cells from proliferating in vivo, but this could barely affect the microenvironment for the growth of T cells [30]. Compared with transplantation of CD34+ HSCs (explained below), the hu-PBL mouse model could reconstruct higher levels of human mature T cells, as well as the acquisition of CD3+, at approximately four weeks in advance [6, 31]. The administration of IL-18 enhanced the grafting of human CD4+ and CD8+ T cells, as well as the observation of immunoglobulin A (IgA) deposits, impelling the development of an ideal humanized mouse model for IgA nephropathy [32]. In another combination with donor-matched dendritic cells (DCs), Harui A et al. reported that this represents a remarkable improvement in antigen responsiveness, breaking through the constraint of the absence of antigen-presenting cells [33]. Furthermore, these findings revealed that recombinant human prolactin (rhPRL) stimulation could dramatically promote the amount of human T cells engrafted into the thymus, lymph nodes and spleens, which is prone to the reconstitution of the human immune system in hu-PBL mice [34].

Although this made hu-PBL mice the best model for T-cell-related immune research, the invariability of the development of severe graft-versus-host disease (GVHD) has always led to limited experimental windows (usually a couple of weeks after PBMC injection, and depends on the quantity of the cell implantation) [35]. Recent reports have investigated that the predepletion of CD4+ T cells could yield to the alleviation of GVHD symptoms, even with the reduction in application scope [36]. For the alleviation of interracial immune rejection, a more common approach is the introduction of the genetic knock-out of the murine major histocompatibility complex (MHC) to prolong the survival period [37].

Hu-SRC mouse model

With a more complete immune reconstitution, the Hu-SRC mouse model, which is desired by the better recapitulation of human diseases, has also been commonly used. The approach of its establishment is to transfer the CD34+ HSCs obtained from the human bone marrow, umbilical cord blood (UCB), fetal liver (FL), or granulocyte colony-stimulating factor (G-CSF)-mobilized peripheral blood (MPB), presenting a multilineage development of hematopoietic cells [38]. In general, the criterion for its successful reconstruction is the existence of more

than 25% of human CD45+ T cells in peripheral blood [8]. With the re-education of immunocytes generated from human HSCs in mice, the host rarely produces immunological rejection, thereby preserving the stabilization for up to 12 weeks [39]. The factors that affect the differentiation and maturation of immunocytes are mainly summarized as the HSC source, route of injection, and age of the recipient. Previously, Lepus et al. reported that FL or UCB-derived HSCs are superior to be colonized in vivo, when compared to the source of bone marrow and MPB, in terms of the significant proliferation of human CD45+ cells [40]. Newborn immunodeficient mice implanted with HSCs through intrahepatic injection within 72 hours of birth exhibited an accelerated T-cell maturation and better transplantation effect, when compared to adult mice [41]. However, this mouse model is hampered by the maturation of human T cells in the murine thymus with murine MHC, leading to the development of H2-restricted T cells, but these were not human leukocyte antigen (HLA)-restricted [38]. In addition, pre-experimental sub-lethal γ-irradiation is an indispensable step to emphasize the facilitation of human HSC engraftment due to the consequence of murine HSC depletion [42].

Hu-BLT mouse model

With regard to the distinction of MHCs, the advancement of the hu-BLT (bone marrow, liver and thymus) mouse model has strongly promoted the T-cell maturation in an autologous human thymus, getting rid of the murine H2 restriction. This provides T-cell differentiation a condition with human HLA restriction, and circumvents the critical unfeasibility of antigen-specific responses [43]. The latest protocol was described as the simultaneous implantation of human fetal thymus (FT) and FL pieces into the sub-renal capsule, and the injection of autologous CD34+ HSCs (from the same FL) [44]. Compared to the group without hepatocytes, Jinglong Guo et al. reported that the autologous hepatocyte engraftment was able to better reconstitute the immunocytes and chemokines in the liver, which is a potential for studies on immunopathogenesis and hepatotropic virus infections [45]. Using this strategy, this not only presented with the highest level of immune reconstitution with the complete immune components of B and T lymphocytes, macrophages, and dendritic cells, but also triggered a revolutionary leap to the repopulation of the human mucosal system for further studying mucosa-associated diseases, such as human immunodeficiency virus (HIV) and Ebola virus infection [46]. For this reason, the hu-BLT mouse model was applied to many aspects in HIV, including epidemic prevention, viral latency, and innate and adaptive immunity [47]. Importantly, the long-term maintenance of fully functional immunocytes in vivo drove its potential for an ideal model in immunology research, playing a specialty in tumor immunotherapy, chemotherapeutic drug response, and the prediction of cytokine release [48-50]. However, even though its mature human T cells are HLA-restricted, the experiment window was still greatly limited by the vulnerability to GVHD, and the symptoms revealed that this was significantly lighter than that of hu-PBL mice [51]. This might be attributed to the participation of murine DCs in the negative and positive selection process of human T-cell development. Kerry J Lavender et al. further revealed another strain with the genetic inactivation of CD47 on the C57BL/6 $Rag2^{-/-}$ $\gamma c^{-/-}$ background, providing additional 15-20 weeks of healthy longevity [52]. Nonetheless, the existence of the mismatched HLA between immunocytes and tumor tissues is prone to immunologic rejection, suggesting that the engraftments for generation should preferably be derived from the same donor or carry out the HLA-matching detection. Thus, these complex techniques and ethical problems have become the most serious obstacles for its extensive usage due to the sophisticated surgical skills and required abortion material.

An overview of characteristics in these mice strains is outlined in **Table 1** and **Figure 2**.

Next-generation of humanized mouse model

Although the technology of humanization is increasingly maturing, several key limitations have been amplified, powerfully impelling the development of genetic modification.

For example, the decisive ablation before HSC transplantation should be performed for supplying sufficient space for HSC cultivation in the bone marrow. Since the maintenance of HSCs is relevant to the expression of c-Kit (CD117), NBSGW mice, which carried the mutation of

Table 1. Comparison of establishments of the humanized immune mouse model

	Hu-PBL-SCID	Hu-SRC-SCID	Hu-BLT-SCID
Source of immunocytes	PBMCs	Bone marrow; UCB; FL; MPB	FT; FL
Immune reconstitution	Mainly T cells (with activated phenotype)	Multiple of human hematopoietic lineages	Most completely functional immune system
Advantages	Easy and quick establishment; mature functional T cells	Injection to newborns increase reconstitution; long experimental window without GVHD	HLA-restricted T cells; development of mucosal human immune system
Limitations	Lack B or myeloid cells; development of GVHD within 4-6 weeks	H2-restricted T cells; Long period of cell differentiation; indispensable sub-lethal γ-irradiation	Long period of cell differentiation; indispensable sub-lethal γ-irradiation; increased possibility of GVHD; sophisticated technique and required material

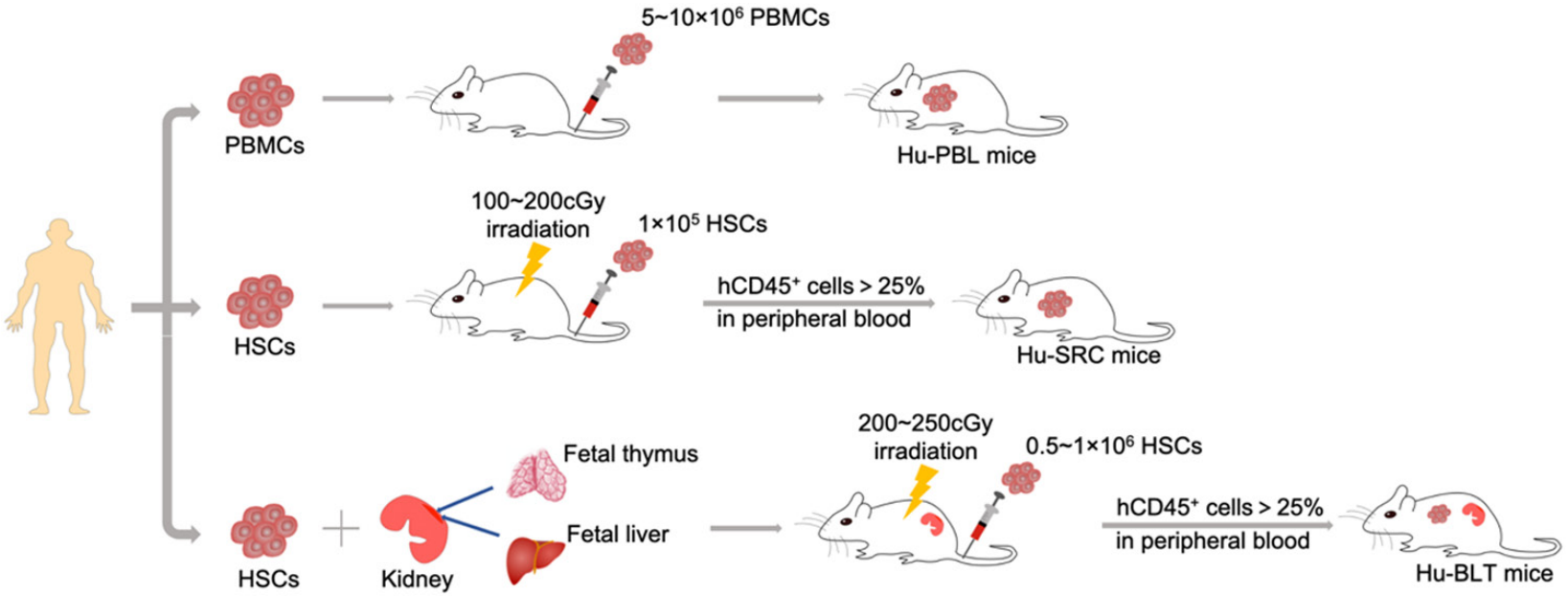

Figure 2. Establishment of the humanized immune mouse model.

w41 in c-Kit, could overcome the inconvenient irradiation, and convert to a better HSC engraftment [53, 54]. Meanwhile, the support of 5- to 12-fold higher rates of erythropoiesis and platelet development in NBSGW mice, compared with irradiated NSG mice, created bright potentials for studying human HSC differentiation and pathophysiology [55, 56].

In addition, the lack of cross-reactivity of cytokines caused the minimal production of functional lymphoid cell differentiation [57]. This highlights that limitations are mainly due to the lack of IL-3, IL-4, IL-7, IL-15, stem cell factor (SCF), and thrombopoietin (TPO) [58]. In order to circumvent such limitations, replacing the coding genes of mice with human genes could induce a targeted expression. For example, it was reported that increased frequency of human NK cells were confirmed in human IL-7 and IL-15 double knock-in NSG mice engrafted with human HSCs [59]. Furthermore, the encoding-gene knock-ins of different cytokines and growth factors, including IL-3, SCF, macrophage colony-stimulating factor (M-CSF), granulocyte macrophage colony-stimulating factor (GM-CSF), TPO, or signal-regulatory protein alpha (SIRPα), into conventional humanized mouse models, produces NSG-SGM3, NOG-EXL and MISTRG mice for increasing the proportion of lymphoid cells (the details are shown in **Table 2**) [60-62]. Moreover, the immature T and B cells in the humanized mice engrafted with HSCs hardly resulted in the IgG antibody response to antigen stimulation. Qingfeng Chen et al. investigated that GM-CSF and IL-4 treatment could correct the defect by stimulating effective Ag-specific CD4+ T cell priming and inducting a significant level of B cells response, generating a novel mouse model for studying antibodies against clinically relevant targets [63]. The differentiation of IL-17-producing T-helper 17 (Th17) cells is generally stimulated by appropriate cytokines, including IL-6, IL-1β, TGF-β, and IL-23. Compared with non-expressive mice, it was observed that there was an aggressive expansion of IL-17- and interferon-gamma (IFN-γ)-producing pathogenic Th17 cells in the skin of the mouse models expressing human IL-1β and IL-23 [64]. In a pathogenesis study of airway inflammation, the administration of human IL-33 could activate Th2 and mast cells in the humanized mouse model, thereby driving the occurrence of asthmatic, as reported by Ryoji Ito et al. [65]. From these interesting observations, it could be inferred that among the various obstacles in the development and differentiation of immunocytes, the conservation of cytokines, which play one of the most important roles, could avoid the bias in the immunocyte maturation process.

A limitation in the present study was the mismatch of MHC among the species. In the case of hu-PBL mice, this might be charged to severe GVHD and the defect of T cell function. Thus, the administration of knock-out for murine class I and class II MHCs ($\beta 2m^{null}$ and $IA\beta^{null}$), which is also known as NOG-MHC double knockout (NOG-dKO) mice, was generated for milder xenograft rejection and GVHD, enabling a long-term window for experiments [66]. A more precise evaluation of anti-tumor T-cell response in immune checkpoint therapies was produced in NOG-dKO mice, when compared to NOG mice, due to the absence of the GVHD-induced nonspecific T-cell activation [67]. The further development of the transgenic engineered humanized mouse model was elucidated through the introduction of HLA class I and/or HLA class II molecules, which greatly promoted human HLA-restricted B- and T-cell functions [68, 69], and antigen-specific IgG responses, making it a suitable model for investigating candidate vaccines and immunotherapies [70, 71].

The comparisons of genetic modification among these mice strains are summarized in **Table 2**.

Preclinical applications for cancer immunotherapies in the humanized mouse model

Reproduction of the TME in hu-PDX mouse model

Tumor growth and therapeutic efficacy has been considered to be extremely associated to the tumor microenvironment (TME), which comprised of blood vessels, lymph vessels, fibroblast, stromal cells, immunocytes and the extracellular matrix (ECM) [76]. The topics and demands about this complex milieu are increasingly dominant in oncobiology, which is of great significance not only for the tumor development and metastasis, but also for the diagnosis, prevention and prognosis. Furthermore, the utility of humanized mice with cancer cell lines has

Table 2. Transgenic platforms for the humanized immune mouse model

Name	Transgenic molecules	Advantages	Limitations	Reference
NBSGW	c-Kit	Without irradiation; development of erythropoiesis and platelet	Not reported	[56]
NSG-SGM3	SCF, GM-CSF, IL-3	Stable HSCs engraftment; increased monocytes, macrophages and DCs	Impaired stem cell function; short-term reconstitution	[72]
NOG-EXL	GM-CSF, IL-3	Stable HSCs engraftment; increased monocytes, macrophages and DCs	Increased anemia	[73]
MISTRG	GM-CSF, IL-3, M-CSF, TPO, SIRPα	Stable HSCs engraftment; increased monocytes, macrophages and DCs; improved NK cells development	Increased anemia; shorter lifespan	[62]
NOG-dKO	$\beta2m^{null}$, $IA\beta^{null}$	Decreased GVHD	Less T-cell differentiation	[37]
NSG-HLA	HLA class I and II	HLA-restricted B- and T-cell functions; antigen-specific IgG responses	Pre-selection of HLA in donor cells	[74, 75]

Table 3. Application of the humanized mouse model for immunotherapy

Disease	Tumor origin	Immune reconstitution	Therapy	Reference
Leukemia	CDX	HSCs	WT-1 TCR-T therapy	[86]
Melanoma	CDX	HSCs, FT, FL	F5 TCR-T therapy	[88]
B-ALL	PDX	HSCs, FT, FL	Anti-CD19 CAR-T therapy	[90]
Pancreatic cancer	CDX	PBMCs	PSCA CAR-T therapy	[92]
Gastric cancer	CDX	PBMCs	chA21-4-1BBz CAR-T therapy	[93]
NSCLC	CDX	PBMCs	EGFR CAR-T therapy	[94]
TNBC	PDX	HSCs	Anti-PD-1 therapy	[101]
NSCLC	CDX&PDX	PBMCs, HSCs	Anti-PD-1/PD-L1 therapy	[102]
Colorectal cancer	PDX	HSCs	Anti-PD-1 therapy	[103]
Osteosarcoma	CDX	PBMCs	Anti-PD-1 therapy	[100]
Lymphoma	CDX	HSCs, FT, FL	Anti-PD-1/CTLA-4 therapy	[104]
Ovarian cancer	PDX	TILs	Anti-PD-1 therapy	[105]
HCC	PDX	HSCs	Anti-PD-1/CTLA-4 therapy	[9]
Mesothelioma	CDX	PBMCs	CAR-T + anti-PD-1 therapy	[106]

been identified for immunotherapeutic evaluation, and the absence of individual tumor heredity directly limits the potential for personalized cancer treatment. Since the PDX model maintains the gene expression profiles and drug responses of patient-derived tumors, this could successfully recapitulate the TME for drug targets with genomic diversity, which is powerfully impelled to be the most reliable human tumor model [77].

Despite the rough persistence of tumor heterogeneity in PDX into immunosuppressed mice, the accurate assessment of immunotherapies is hurdled without the stable existence of human tumor-infiltrating lymphocytes (TILs), proceeding to the forward step of humanized PDX mouse models. Since the humanized PDX mouse model partially reproduces a TME similar to humans, the cytokines and chemokines released by tumor cells, stromal cells and TILs could regulate angiogenesis, metastasis and immune responses [78]. For example, myeloid-derived suppressor cells (MDSCs), which is a heterogeneity group with significant immunosuppressive activity in TME, emerges as a relative T cell inactivation, and this might due to the secretion of MDSC-induced reactive nitrogen species (RNS), and the induction of regulatory T cells (Tregs) in the presence of IFN-γ and IL-2 [79, 80]. With the most lethal effect on cancer cells, CD8+ cytotoxic T lymphocytes (CTLs) also have multiple cross-talks with immunocytes, MDSCs, tumor-associated macrophages (TAMs) and cancer-associated fibroblasts (CAFs) through the promotion of cytokine secretion in TME [81]. A recent evidence from murine and clinical trials suggested that the dysfunctional states of intratumoral T cells might contribute to the multifaceted suppressive signals in TME, such as soluble mediators, metabolic factors and hypoxia [82].

Thus, understanding the role of TME in tumorigenesis and development might provide new strategies for tumor therapies. As the culmination of model technology, the simultaneous implantation of PDX and humanization recapitulates the interplay between the tumor and immunocytes, which especially brings about the increasing capacity for individual hypothesis outcomes [83]. The application examples are detailed in **Table 3**.

Humanized mouse model for genetic-modified T cells

Adoptive cellular therapy (ACT) refers to the expansion of the immunocompetent cells in vitro, and the reinjection back to the patients themselves, which stimulate the immune response for targeted killing tumor cells [84]. ACT infusion is mainly based on engineered T cells that express transgenic T cell receptors (TCRs) or chimeric antigen receptors (CARs), improving the affinity with tumor-associated antigens (TAAs) [85]. Although ACT has achieved ideal benefits for cancer patients, with the constant improvement of experimental recipients in vivo, the mechanism and safety of TCR-T/CAR-T cell

therapy in exerting an explicitly potent antitumor effect was explored in the humanized mouse model, rather than in immunodeficient mice.

TCRs are natural antigen receptors that occur on the surface of T cells, which bind its cognate tumor antigen through the MHC-peptide complex. This is a strategic fulcrum for TCR-T cell therapy, which introduces the selected TCR gene sequence encoding the recognizing specific TAAs into T cells, enabling T cells without tumor recognition ability to effectively kill tumor cells. The related optimization research of transgenic TCRs has been carried out in humanized mice. Yuho Najima et al. recently conformed the development of transgenic Wilm's Tumor-1 (WT-1) specific TCRs in HLA-I transgenic NSG mice transplanted with HSCs. WT-1 specific CTLs retained the capacity for proliferation, and exerts an antigen-specific cytokine response, amplifying the anti-tumor function [86]. The analogic results were supported by Francesca Giannoni et al. and Dimitrios N Vatakis et al. in the hu-BLT mouse model, in which transgenic CTLs specific for MART-1 (melanoma antigen recognized by T cells) were optimized for long-term function and the generation of tumor-targeted mature T cells in melanoma immunotherapy [87, 88].

CARs serve as a "plug" for T cells to identify the targeted proteins on the surface of cancer cells, without the restriction of MHC. At present, CAR-T cell therapy has produced remarkable results in the treatment of B-cell lymphoma, leukemia, and other hematological malignancies, but these were less in solid tumors [89]. Chun-Hui Jin et al. generated a genetically modified CAR targeting CD19 in the hu-BLT mouse model with primary acute B-lymphoblastic leukemia (B-ALL), providing a proof-of-principle for the potential utility to characterize the host immunological changes associated with CAR-T cell therapy [90]. In addition, the introduction of a chimeric costimulatory motif, such as the most commonly used CD28 and CD137 (4-1BB), would trigger optimal signaling to induce CAR-T cell response, when compared to a CAR without co-stimulation. For example, Pratiksha Gulati et al. used the hu-SRC mouse model, and indicated the distinct capability of Δ-CD28/CD3ζ CAR-T cells to eradicate tumor growth and persist a durable antineoplastic activity [91]. Although CAR-T therapy has not achieved ideal clinical efficacy in solid tumors, this has made promising prospects in immunodeficient mice in various tumor CARs specific for prostate stem cell antigen (PSCA), chA21-4-1BBz, epidermal growth factor receptor (EGFR), etc. [92-94]. However, the safety of CAR-T cell therapy is frequently associated with treatment-related adverse reactions, including cytokine-release syndrome (CRS) and neurotoxicity. Marco L Davila et al. elucidated that these are primarily determined by human monocyte/macrophage-derived IL-1 and IL-6 [95]. Even though conventional hu-NSG mice could serve as an ideal model for preclinical efficacy studies, the identification of CRS (including cell function and cytokine production) could be tested in the hu-SGM3 strain, but not in hu-NSG mice, which might be due to the dependence of GM-CSF [96].

Humanized mouse model for immune checkpoint blockade therapy

The recognition and lethal effectiveness of immunocytes on target cells play a key role in immuno-oncology, which could be evaded by tumor cells through multi-approaches. Inhibitory receptors and signaling pathways, which are officially known as immune checkpoints, mainly include programmed cell death protein-1 (PD-1), cytotoxic T-lymphocyte-associated protein-4 (CTLA-4), T cell immunoglobulin-3 (TIM-3) and lymphocyte activation gene-3 (LAG-3), and are regulatory molecules that possess the suppression of excessively activated T cells from attacking self-tissues, thereby preventing the occurrence of autoimmune effects. The monoclonal antibodies of these immune checkpoints could cut the brakes from the self-recognition on T cells for activated function [97]. PD-1 and CTLA-4 inhibitors have won the Nobel Prize in physiology or medicine in 2018, changing the setup of cancer treatment. However, there are typical differences between mouse and human DNA in the expression fragment of immune checkpoints. The remarkably unique superiority of the humanized mouse model in the study of immune checkpoint inhibitors has been confirmed, in order to evaluate the effectiveness of individual clinical consultation [98]. Simultaneously, based on homologous recombination by CRISPR-Cas9, a new generation of human PD-1xLAG-3 knock-in mice, which

replaced the mouse Pdcd1 gene with human PDCD1 to express human PD-1 protein, also accounted as a great place [99]. The potential outcome from these models was to permit the assessment of human immune response, including the analysis of tumor volume, lymphocyte proportion, cytokines, and etc. (the details are shown in **Table 4**). In the administration of antibodies against PD-1 and CTLA-4, the mono- or combination therapy revealed the significant tumor inhibition in humanized mice bearing CDX or PDX triple-negative breast cancer (TNBC), non-small cell lung cancer (NSCLC), colorectal cancer (CRC), osteosarcoma, lymphoma, ovarian cancer, and hepatocellular carcinoma (HCC) [100-105]. Furthermore, Leonid Cherkassky et al. elucidated that the simultaneous induction of the PD-1 blockade with CAR-T cell therapy could enhance the efficacy of monotherapy, promoting the onco-immunotherapy into the advanced era of comprehensive treatment [106].

Humanized mouse model for NK cells therapy

NK cells are identified as one of the most principal segments of tumor immune surveillance. The activation of NK cells mainly through cytokines and other ex-stimulation is associated with more powerful antitumor function. Thus, efforts on the onco-immune research have increasingly focused on the NK cells activity and related cytokines therapy [111].

In HCS-engrafted NSG mice transplanted with human breast cancer cells, Anja K Wege et al. reported the further expansion of NK cell accumulation in all lymphoid and extra lymphoid tissues, which are dependent on the IL-15 treatment [5]. In addition, hIL-7 and hIL-15 double knock-in improved the account of NK cells engraftment in NSG mice injected with HSCs [59]. In contrast to humanized NSG mice, human IL-15 and SIRPα knock-in mouse models injected with HSCs based on the $Rag2^{-/-}$ $IL2rg^{-/-}$ background, which is known as the humanized SRG-15 mice strain, demonstrated the highly identical expression of inhibitory receptors on NK cells to humans [112]. Using the humanized SRG-15 mouse model, it was observed that the dramatic maturation and tissue residence of NK cells aimed to boost the preclinical research of NK-cell targeted therapies.

Future perspectives

In recent years, cancer treatment has made revolutionary progress, along with the striking increase of curative and survival ratio in cancer patients. As an effective tool for immunotherapeutic evaluation, the humanized mouse model could similarly reproduce the human immune microenvironment for simulating the process of tumor occurrence, development and metastasis. Particularly, the introduction of human immunocytes in the PDX mouse model improved the predictability of potential preclinical therapies in individuals. However, its establishment and application still face many challenges, including the MHC incompatibility between immunocytes and tumors, the residual murine innate immunocytes, and the lack of specific-specific cytokines. In order to address these gaps, several modifications with transgenic MHC or tissue implantation resulted in the increase in functionality level and complete subsets of the human immune system, but with more sophisticated technology. In addition, the application evaluation of targeted immunotherapy benefited from humanized mice. However, the effect of immunomodulatory therapy needs to be further explored. Thus, the long-term revolution for optimization of humanized mouse model in onco-immunology would be bound to provide an unprecedented platform for cancer immunotherapy.

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Disclosure of conflict of interest

None.

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Table 4. Pharmacodynamic assessment of immune checkpoint therapies in the humanized mouse model

Drug name	Target	Mouse model	Tumor character	Assessment	Reference
Nivolumab	PD-1	BRG-SIRPα, engrafted with HSCs	TNBC, CRC	Inhibition of tumor growth curve; increased expression of IFN-γ+ and HLADR+ on hCD8+T cells	[103]
Pembrolizumab	PD-1	NSG, engrafted with HSCs	Dedifferentiated liposarcoma	Inhibition of tumor growth curve; increased number of hCD3+hCD8+, hCD8+IFNγ+ T cells and hCD56+Ki-67+ NK cells	[107]
Sintilimab	PD-1	NOG, engrafted with PBMCs	NCI-H292	Inhibition of tumor growth curve and tumor weight; upregulation of IL-2; increased number of hCD3+, hCD8+, hCD8+IFNγ+ T cells; an increase in hCD8+T/hTregs ratio	[108]
Atezolizumab	PD-L1	NSG, engrafted with HSCs	A375, A549, Caki-1, H1299, H1975, etc.	Inhibition of tumor growth curve; upregulation of hCD3+, hCD4+, and hCD8+ T cells; variable expression of PD-1 on T cells and PD-L1 on tumor cells and macrophages	[109]
Nivolumab Ipilimumab	PD-1 CTLA-4	NSG, engrafted with HSCs	Nasopharyngeal carcinoma	Inhibition of tumor growth curve; upregulation of IFN-γ, IL-6; increased expression of HLA-DR+ on hCD8+ T cells; a decrease in hCD4+/hCD8+ ratio	[110]

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