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Humanized mouse models for immuno-oncology research

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Abstract

Immunotherapy has emerged as a promising treatment paradigm for many malignancies and is transforming the drug development landscape. Although immunotherapeutic agents have demonstrated clinical efficacy, they are associated with variable clinical responses, and substantial gaps remain in our understanding of their mechanisms of action and specific biomarkers of response. Currently, the number of preclinical models that faithfully recapitulate interactions between the human immune system and tumours and enable evaluation of human-specific immunotherapies in vivo is limited. Humanized mice, a term that refers to immunodeficient mice co-engrafted with human tumours and immune components, provide several advantages for immuno-oncology research. In this Review, we discuss the benefits and challenges of the currently available humanized mice, including specific interactions between engrafted human tumours and immune components, the development and survival of human innate immune populations in these mice, and approaches to study mice engrafted with matched patient tumours and immune cells. We highlight the latest advances in the generation of humanized mouse models, with the aim

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Author contributions

J.C., H.B., M.O.S., D.L.G., L.D.S. and M.A.B. researched data for the article; J.C., H.B., D.L.G., J.G.K., F.I., L.D.S. and M.A.B. contributed substantially to discussions of content; J.C., H.B., L.D.S. and M.A.B. wrote the article; and all authors reviewed and/or edited the manuscript before submission.

Competing interests

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of providing a guide for their application to immuno-oncology studies with potential for clinical translation.

Introduction

Human immune system homeostasis and the processes occurring within the tumour microenvironment (TME) are complex, posing substantial challenges for studies of interactions between immune cells and tumours and of alterations in immune cell phenotype and function following anticancer treatment[1,2]. Moreover, non-haematopoietic cells in the TME, including fibroblasts and epithelial and endothelial cells, have the capacity to produce immunomodulatory factors, also influencing interactions between the tumour and the immune system[3]. Mouse models of cancer are crucial for our basic understanding of human tumour biology but often do not accurately recapitulate basic tumour biology, the TME, and response and resistance to therapy when compared with outcomes in clinical studies[4,5]. In addition, ex vivo experiments with tumour organoids and patient-derived tumour fragments can be used to study the TME but lack the physiological complexity associated with in vivo systems[6,7]. This situation has led to a growing interest in research using humanized mice, which are immunodeficient mice co-engrafted with human tumours and key components of the immune system[8].

The emergence of humanized mouse models was driven by the development of immunodeficient strains homozygous for the *Prkdc*^{scid} mutation (where scid stands for severe combined immunodeficiency)[9,10], or with *Rag1* (ref. [11]) or *Rag2* (ref. [12]) deficiency (all of which eliminate host adaptive immunity owing to defective recombination of genes that encode antibodies and T cell receptors), and/or with disruption of the *Il2rg* locus (encoding IL-2 receptor subunit-γ) to reduce host innate immunity[9-12]. Severely immunodeficient mice combining these alterations support efficient engraftment of human immune system components as well as the growth of patient-derived xenografts (PDXs) and cell line-derived xenografts (CDXs)[13,14]. The currently available humanized mouse models are promising and innovative platforms for human immuno-oncology research. Numerous researchers have used these models to study interactions between human tumours and the immune system and to evaluate the efficacy of immunotherapies[15]. However, key aspects of humanized mouse models restrict their application to the study of antitumour immunity, including the limited development of mature innate immune cell populations (monocytes, macrophages, conventional and plasmacytoid dendritic cells (DCs), and natural killer (NK) cells), lack of HLA molecules in standard immunodeficient mouse strains, inability to generate robust antigen-specific antibody responses, lack of haemolytic complement in mice with a non-obese diabetic (NOD) background, and a dearth of lymph node structures and germinal centres[16-18].

In this Review, we provide an overview of the latest advances in the generation of functional human immunity in humanized mouse models and present examples of their application in human immuno-oncology research (Table 1). Although substantial progress has been made to improve the translational potential of studies using humanized mice in clinical oncology, no single model exists that reproduces all aspects of human biology. These mouse

models are most useful when applied to directly address a specific question; thus, herein, we discuss the importance of selecting the most appropriate humanized mouse platform for each experiment.

Utility of humanized mouse models

Humanized mouse models are being used to test and validate a broad range of immunotherapeutic approaches[19,20], including treatment with monospecific, bispecific and trispecific antibodies[21-23], adoptive cell therapies[24] (such as cells engineered to express chimeric antigen receptors (CARs))[25], small-molecule inhibitors or agonists[26], and oncolytic viruses[27]. As discussed later, the efficacy of first-generation immune-checkpoint inhibitors (ICIs) targeting PD-1–PD-L1, CTLA4 or LAG3 has been demonstrated in several humanized mouse models[28-31]. These platforms have also been used to screen other novel therapeutic agents for their ability to control tumour growth in vivo[32-36]. The FDA has stated their interest in using humanized mouse platforms to test the efficacy and safety of novel therapeutic agents[37]. Indeed, the current FDA-approved CAR T cell products were originally studied in humanized mouse models[38]. In addition to CAR T cell products, other cell-targeted approaches tested in humanized mice include bispecific T cell engagers and dual affinity retargeting molecules[39,40]. Finally, humanized mouse models might also be effective tools to study combination immunotherapies[41].

The study of malignant haematopoietic disorders, leading to the identification of leukaemia stem cells and leukaemia-initiating cells[42], is another important application of humanized mouse models. Initial studies in this area[43-45] have been instrumental to advance our understanding of the mechanisms underlying the development of and explore therapeutic strategies for human leukaemias[46-49].

Human immune system engraftment

Several strategies have been developed to engraft human immune system components into immunodeficient mice[50]. The optimal design of experimental strategies with humanized mice requires a comprehensive understanding of the engraftment protocols and, importantly, their overall strengths and limitations. Indeed, the specific cell population used for engraftment affects the relative abundance of different human immune cell types in mice (typically assessed by staining for CD45, a surface marker for haematopoietic cells and their precursors; Fig. 1). Given the complexity and time-consuming nature of engraftment protocols[51], a clear definition of the experimental question along with careful selection of the optimal engraftment strategy and immunodeficient mouse model are essential. Here, we provide a general overview of the engraftment protocols used to generate several types of humanized models used in immuno-oncology research and highlight specific innovations for each approach, in particular related to the development of different immune cell types (Table 1). For conciseness, in the following sections we tend to refer to each mouse model only by their name (see Table 2 for detailed information).

Hu-PBL-SCID mice

A straightforward method for engrafting human immune system components into SCID mice is the direct infusion of mature human immune cells, resulting in human peripheral blood leukocyte (Hu-PBL) SCID mice[52]. Although mature human immune cells can be obtained from several tissues (including the spleen), peripheral blood mononuclear cells (PBMCs) are the most commonly used source. PBMCs are readily available, easy to work with and can be obtained in large numbers. Human $CD3^+$ T cells, including both the $CD4^+$ and $CD8^+$ subsets, are the most abundant cell population that survives the engraftment process[53], whereas $FOXP3^+CD25^+CD127^{low}$ regulatory T (T_{reg}) cells are detectable for the first 2–4 weeks post-injection but then become undetectable in the circulation[54]. Human innate cell populations, including myeloid and NK cells, survive for the first few days within mice but quickly become undetectable in blood and tissues (Fig. 1). Human $CD19^+$ B cells are maintained at low levels in specific sites, including the spleen and bone marrow, for several weeks. In addition, human IgGs are detectable in the peripheral blood of Hu-PBL-SCID mice for their whole lifespan[55].

Immuno-oncology studies in Hu-PBL-SCID mice—Hu-PBL-SCID mice have been used extensively for the study of interactions between human immune cells and human tumours[56]. One example includes a report of tumour infiltration of human T cells in a glioblastoma CDX growing in NOG mice (knockout for *Prkdc* and *Il2rg*), which was directly visualized using PET. This model could potentially enable monitoring of the TME during exposure to therapeutic agents[57].

The PBMCs engrafted into Hu-PBL-SCID mice can be either autologous or allogeneic with implanted PDXs and allogeneic with injected CDXs[58] (Table 3). Tumour growth was suppressed in NSG (knockout for *Prkdc* and *Il2rg*) mice expressing SCF, GM-CSF (also known as CSF2) and IL-3 (NSG-SGM3) implanted with an ovarian cancer PDX and autologous PBMCs that were exposed to anti-PD-1 and anti-CTLA4 antibodies but not with anti-PD-1 antibody exposure alone, compared with non-exposed, non-engrafted NSG-SG3M PDX mice[59]. Tumour growth was also impaired in BRG mice (knockout for *Rag2* and *Il2rg*) implanted with a gastric carcinoma PDX and autologous PBMCs that were exposed to an anti-PD-1 antibody and/or an antibody against TNFRSF9 (also known as CD137) compared with non-exposed mice[60]. Although such ‘autograft’ Hu-PBL-SCID models have the advantage of supporting studies of HLA-restricted tumour antigen-specific T cells, this approach is logistically complicated and depends on the availability of patients with cancer who can donate both blood and tumour samples for preclinical studies.

An alternative autologous approach involves using ex vivo-expanded tumour-infiltrating lymphocytes (TILs) derived from the same tumour tissue as the PDX instead of PMBCs[1,59,61]. TILs isolated from samples from patients with melanoma were expanded ex vivo and injected into NOG mice expressing *IL2* (hIL2-NOG) that had been engrafted with an autologous melanoma PDX, leading to eradication of the tumour[62]. In another study, ex vivo-expanded TILs exposed to human IL-2 were injected into NSG-SGM3 mice bearing an autologous ovarian cancer PDX, leading to impaired tumour growth compared

with non-injected NSG-SGM3 PDX mice and potentiating the effect of a combination of anti-PD-1 and anti-CTLA4 antibodies[59].

Allograft Hu-PBL-SCID models (Table 3) have also proved useful to test a number of antitumour agents in vivo. NSG mice implanted with renal cell carcinoma CDXs and engrafted with allogeneic human PBMCs were used to test an antibody against carbonic anhydrase 9, a prototypic tumour-specific antigen expressed by renal cell carcinomas. This antibody inhibited tumour growth, which was correlated with tumour infiltration of human T cells and a more limited infiltration of human NK cells[63]. Exposure to anti-PD-1 antibodies reduced tumour burden in NSG, NOG and NSI (knockout for *Prkdc* and *Il2rg*) mice implanted with lung cancer CDXs or PDXs[64-67], and in NPG mice (knockout for *Prkdc* and *Il2rg*) implanted with osteosarcoma CDXs[68], all engrafted with allogeneic human PBMCs. Exposure to a novel bispecific antibody targeting both human PD-1 and PD-L1 also reduced tumour burden in NSG mice implanted with a lung cancer CDX and engrafted with allogeneic PBMCs[69]. Combination approaches have also been successfully tested in this model[60].

Hu-PBL-SCID models that lack GVHD—A key feature of the Hu-PBL-SCID model is that engrafted human T cell populations mediate acute xenogeneic graft-versus-host disease (GVHD) in these mice[53] (Fig. 1). In mouse models, the kinetics of GVHD development depend on several factors, including the use of preconditioning regimens (such as irradiation), which accelerates engraftment, and the number of PBMCs injected, which directly correlates with the resulting level of human cell chimerism. T cell-mediated GVHD is lethal and limits the time frame for performing experiments in standard immunodeficient mice. The Hu-PBL-SCID model is an effective platform for testing agents that suppress human T cell responses, including antibody-based therapies[70-72], T_{reg} cell-based therapies[73-75] and cytokines[71,76]. The human T cell response in Hu-PBL-SCID mice is predominantly directed against mouse MHC molecules. Inactivation of the genes encoding mouse MHC class I (heavy chains H2-K and H2-D and β_2-microglobulin[77]) and class II (H2-IA and H2-IE) molecules in NSG and NOG mice resulted in the NSG-MHC-DKO and NOG-dKO models. All these models have limited occurrence of GVHD and thus enable experiments with an extended time window[54,78-80]. HUMAMICE, another mouse model with reduced GVHD, was developed on a C57BL/6 background engineered to be deficient for *Rag2, Il2rg, Perf* (encoding perforin), *B2m* and *IA*, and to express HLA-A2 and HLA-DR1 (ref. [81]). Engraftment of HLA-$A2^+$ and HLA-$DR1^+$ PBMCs in HUMAMICE enabled the development of human $CD4^+$ and $CD8^+$ T cells and $CD19^+$ B cells with no GVHD and with robust generation of antigen-specific antibody responses. An important consideration regarding MHC-deficient mice, however, is that *B2m* (which encodes β_2-microglobulin) is required for the expression of mouse IgG receptor FcRn large subunit p51, which increases the half-life of circulating IgGs[82-84]. Accordingly, the half-life of human IgGs was substantially lower in NSG mice lacking *B2m* than in NSG and NSG-lacking K^bD^b mice, which might directly affect the efficacy of antibody-based approaches in this model[54]. In another study, engraftment of PBMCs in NOG mice expressing *IL4* led to a shift in the predominant T cell phenotype from $CD8^+$ T cells to $CD4^+$ T helper 2

cells, suppressed the onset of GVHD, and enabled the generation of antigen-specific IgG responses[85].

Anti-PD-1 antibodies can limit the growth of either human lymphoma or glioblastoma cell lines in NOG-dKO mice engrafted with allogeneic PBMCs[79,86]. In another study, a small-molecule inhibitor of STAT3 promoted tumour infiltration of human $CD8^+$ T cells and inhibited the growth of human glioblastoma CDXs in NOG-dKO mice engrafted with allogeneic human PBMCs[26]. Unexpectedly, the combination of the same STAT3 inhibitor and an anti-PD-1 antibody had a reduced ability to enhance tumour infiltration by human immune cells and inhibit tumour growth compared with either agent alone in NOG-dKO mice implanted with a human pancreatic tumour cell line and engrafted with allogeneic PBMCs[87]. Finally, a bispecific antibody targeting human B7-H4 and CD3 promoted tumour infiltration of activated $CD8^+$ T cells and reduced tumour growth in NSG-MHC-DKO mice implanted with a breast cancer cell line and engrafted with allogeneic PBMCs[88].

CAR therapies in Hu-PBL-SCID mice—In the past few years, a number of studies have shown that CAR T cell-based approaches[25,50,89-91] inhibit tumour cell growth in various Hu-PBL-SCID models. For example, CAR T cells targeting HER2 show efficacy against melanoma cells in hIL2-NOG[91]. Furthermore, CAR NK cell strategies have also documented the antitumour effects of NK cells derived from induced pluripotent stem cells (iPSCs)[92]. This study showed that NK cells derived from iPSCs display potent tumour-specific cytotoxic activity in NSG mice and promote T cell infiltration of tumours in Hu-PBL-SCID models. The use of iPSCs derived from patients with cancer to generate human immune cells and haematopoietic stem and progenitor cells (HSPCs) could provide unlimited quantities of these cell populations for engraftment into humanized mice bearing autologous tumours as well as novel platforms for the testing of new therapies[93,94].

Hu-SRC mice

Engraftment of immunodeficient mice with human $CD34^+$ HSPCs gives rise to human SCID repopulating cell (Hu-SRC) mice, which have a more complete haematopoietic system than Hu-PBL-SCID mice, including innate immune cells, adaptive immune cells, and low numbers of red blood cells and platelets[95]. Before the introduction of immunodeficient mice harbouring mutations within the *Il2rg* locus, the ability to engraft $CD34^+$ HSPCs and achieve a high degree of human immune cell chimerism for the development of functional innate and adaptive immune cells was limited[96]. For example, NOD-SCID mice are amenable to engraftment of $CD34^+$ HSPCs but the resulting levels of $CD34^+$ cells in their bone marrow is very low, leading to limited and variable development of a peripheral human immune system[10,97-99]. $CD34^+$ HSPCs are most easily and reliably obtained from umbilical cord blood (UCB) and can also be obtained from peripheral cells mobilized in response to G-CSF (also known as CSF3), fetal liver tissue and bone marrow[100]. A variety of parameters affect engraftment of $CD34^+$ HSPCs into immunodeficient mice, including the genetic background of the strain, age of the recipient, source of $CD34^+$ HSPCs, injection route, number of $CD34^+$ HSPCs injected and the preconditioning regimen used[51,52,101-103]. Moreover, researchers have proposed that the source of $CD34^+$ HSPCs influences the functionality of human T cells developed in engrafted mice, and fetal $CD34^+$

HSPCs give rise to T cells with greater immune tolerance than those developing from adult-derived $CD34^+$ HSPCs[104]. $CD34^+$ HSPC engraftment into SCID mice gives rise to both innate and adaptive cell lineages (Fig. 1); however, the engraftment of $CD34^+$ HSPCs into standard NSG, NRG (knockout for *Rag1* and *Il2rg*), NOG and BRG models has several limitations such as (1) incomplete development of mature human innate cell lineages (monocytes, macrophages, DCs and NK cells); (2) limited overall functionality of human B cells (reduced ability to produce antigen-specific IgGs and undergo class switch and affinity maturation); and (3) an absence of HLA expression, required for the development of HLA-restricted T cells. A number of genetic alterations have been introduced in immunodeficient mouse strains to enhance human immune system development and function following $CD34^+$ HSPC engraftment (Table 1).

A key aspect for the survival of $CD34^+$ HSPCs following engraftment into SCID mice is the need for host preconditioning to deplete endogenous immune cells. Standard protocols include irradiation or treatment with an alkylating agent (such as busulfan). Moreover, several novel immunodeficient mouse strains have been generated that do not require preconditioning owing to the introduction of genetic alterations such as mutations within the *Kit* locus, which encodes a receptor tyrosine kinase that binds to KIT ligand (also known as SCF) and has a crucial role in haematopoiesis[105,106]. Exposure of mice to anti-KIT antibodies transiently enables the engraftment of $CD34^+$ HSPCs and immune system chimerism without the need for preconditioning with irradiation; this phenotype is sustained in mice harbouring *Kit* mutations[107,108]. For example, NSG mice harbouring the Kit^{W41J} mutation (NSG-W41) are amenable to engraftment of $CD34^+$ HSPCs and develop a human immune system without being irradiated; additionally, they have enhanced human myeloid differentiation, human erythropoiesis and thrombopoiesis compared with irradiated NSG mice[109,110]. Transgenic expression of human IL-7 in NSG-W41 mice enhances expansion of functional human T cells in the periphery after $CD34^+$ HSPC engraftment compared with non-transgenic NSG-W41 mice[111]. *C57BL/6J-*Kit^{W41J} mice have been crossed with standard NSG mice resulting in non-albino NBSGW mice[112,113], which are also amenable to engraftment of $CD34^+$ HSPCs and immune system development in the absence of irradiation. In comparison with NSG mice, NBSGW mice have improved erythropoiesis. C57BL/6J-Kit^{Wv} mice carrying the loss-of-function Kit^{Wv} mutation have been crossed with BRGS mice (which are deficient for *Sirpa,* encoding SIRPα, the receptor for CD47)[114]. The resulting mouse model, $BRGSK^{Wv}$, does not require irradiation to develop a human immune system after $CD34^+$ HSPC engraftment and has enhanced human erythropoiesis and thrombopoiesis compared with BRGS mice[115].

Immuno-oncology studies in Hu-SRC mice—Hu-SRC mice have been used to evaluate both the TME (including phenotype and function of infiltrating immune cells, and spatial relationships with tumour cells) and antitumour activity of immunotherapies[116,117]. Given the logistical challenges of obtaining autologous bone marrow or G-CSF- and/or GM-CSF-mobilized peripheral blood samples from patients with cancer, most Hu-SRC models are allografts, although several groups have generated autograft Hu-SRC models[118,119] (Table 3). In one such study[120], tumour tissue from two patients with metastatic melanoma were used to establish a PDX model and autologous $CD34^+$ HSPCs were isolated from

blood from the same patients (after mobilization of these cells from the bone marrow with G-CSF). Although the number of human $CD45^+$ cells varied between individual recipient mice, tumour growth was delayed in mice engrafted with autologous $CD34^+$ HSPCs relative to non-engrafted mice and to mice engrafted with mismatched, allogeneic $CD34^+$ HSPCs (obtained from UCB). Moreover, this delay correlated with increased tumour infiltration of human immune cells. An alternative approach based on genetically modified $CD34^+$ HSPCs was used to create an autograft humanized mouse model of acute myeloid leukaemia (AML)[121]. $CD34^+$ HSPCs were transduced with a lentiviral vector encoding a mutant form of NPM1 (an alteration detected in ~30% of adults with AML), and NSG mice engrafted with these engineered $CD34^+$ HSPCs developed both human AML and functional immune systems. In this autograft model, a bispecific T cell engager targeting CD3 and CD123 resulted in depletion of $CD123^+$ leukaemia-initiating cells compared with mice receiving phosphate-buffered saline and with mice receiving the bispecific T cell engager and depleted of T cells with an anti-CD3 antibody. Another approach for modelling interactions between autologous immune systems and tumour xenografts involves the generation of humanized mouse models with T cells expressing a T cell receptor (TCR) specific for an HLA-restricted tumour-specific antigen. One example of this model was developed in NSG mice expressing HLA-A24 that were engrafted with HLA-matched $CD34^+$ HSPCs transduced with a lentiviral vector encoding an HLA-A24-restricted TCR specific for a WT1 antigen[122]. In these mice, MHC-tetramer staining revealed the development of WT1-specific $CD8^+$ T cells in the thymus and periphery. These $CD8^+$ T cells were reactive against the WT1 peptide and displayed cytotoxicity against a $WT1^+$ leukaemia cell line. Although autograft Hu-SRC mouse models provide a promising platform to study interactions between immune cells and tumours, they pose substantial technical and logistical challenges and are limited in scope.

Allograft Hu-SRC mouse models have also been used to study human immune system–tumour interactions and test immunotherapies. In one such study, ten different CDXs from a range of tumour types were implanted into NSG mice engrafted with allogeneic UCB $CD34^+$ HSPCs[116]. All ten CDXs grew in the engrafted mice and human $CD45^+$ immune cell infiltrates were detectable in all tumours; however, the numbers of infiltrated cells varied substantially between tumours, leading to some of them being classified as ‘hot’ or ‘cold’ depending on the level of infiltration. The composition of the immune infiltrates seemed to be tumour-type specific and not influenced by the HSPC donor factors. In six out of these ten CDX models, an anti-PD-L1 antibody reduced tumour growth and this reduction was observed both in tumours classified as ‘hot’ or ‘cold’. Allogeneic $CD34^+$ HSPC-engrafted NSG mice also support the growth of PDXs, including specimens from patients with lung, breast, or bladder cancer or sarcoma[28]. In a study, seven PDXs from these tumour types were generated in $CD34^+$ HSPC-engrafted NSG mice and had growth kinetics comparable to those in non-engrafted NSG mice[28]. Human $CD45^+$ infiltrates were detectable in Hu-SRC mice, although the number of $CD45^+$ cells varied for each PDX model and variable responses were also observed for specific HSPC donors[28]. In addition, exposure to an anti-PD-1 antibody delayed the growth of four of those seven PDXs, although the efficacy varied by $CD34^+$ HSPC donor[28]. In the same study, depletion of human $CD8^+$ T cells (using a human-specific anti-CD8 antibody) abrogated the growth-suppressive activity of anti-PD-1 antibodies in a triple-negative breast cancer (TNBC) CDX, indicating that $CD8^+$

T cells are key mediators of the effect of ICIs[28]. Interestingly, the level of HLA match between PDX tumours and CD34$^+$ HSPC donors did not influence the growth kinetics or effects of anti-PD-1 antibodies[28]. Another study also demonstrated prolonged antitumour activity of anti-PD-1 antibodies in an NSG mouse TNBC CDX model engrafted with CD34$^+$ HSPCs[123]. Other studies have demonstrated the utility of allograft Hu-SRC mouse models to study the activity of ICIs in lung cancer PDXs and CDXs[124], hepatocellular carcinoma PDXs and CDXs[66,125], ovarian cancer CDXs[64], dedifferentiated liposarcoma PDXs[126], and TNBC PDXs and CDXs[127,128].

Overcoming limitations of Hu-SRC mice—Despite the substantial progress achieved using Hu-SRC mice in immuno-oncology research, the interpretation of datasets remains challenging. The existence of donor variability among the CD34$^+$ HSPCs used for engraftment affects tumour growth patterns and the efficacy of ICIs[28]. Moreover, in Hu-SRC mice, ICIs do not always have activity against PDXs from some tumour models and therapeutic efficacy is most often observed with malignancies that show responses in the clinical setting[30,65,129]. However, activity in different PDXs from the same tumour type can be variable[127]. Although these challenges might be explained by the inherent variability between tumours and CD34$^+$ HSPC donors, the limitations of humanized mouse models might also contribute.

Immunodeficient mice harbouring *Il2rg* mutations have impaired development of lymphoid tissue inducer (LTi) cells, and thus have limited development of lymph node structures[130,131]. This feature is considered a key contributor to the inability of *Il2rg*^{null} mice to generate potent antibody responses following engraftment with CD34$^+$ HSPCs. In the past few years, two groups have generated humanized mouse models with improved lymph node development. One of these models is based on the expression of *Il2rg* in NOG mice under control of the endogenous *Rorc* promoter, thus enabling expression of the IL-2Rγ subunit (a subunit shared by several interleukin receptors) in a LTi lineage. The resulting NOG-*pRorc-γc* mice have increased numbers of mouse LTi cells and improved generation of lymph node structures after CD34$^+$ HSPC engraftment compared with NOG mice[132]. The other model is based on the expression of mouse thymic stromal lymphopoietin (TSLP) in BRGS mice under control of the *Krt14* promoter, a model referred to as BRGST[133]. TSLP is a cytokine secreted by epithelial cells that is functionally and structurally similar to IL-7 and binds a heterodimeric receptor comprising the IL-7Rα chain and TSLPR[134]. The function of TSLP was originally characterized in *Il7*-deficient immunocompetent mice, in which transgenic expression of TSLP restored the generation of LTi cells and lymph node development[135]. Compared with BRGS mice, BRGST mice had improved development of lymph nodes and thymic structures after CD34$^+$ HSPC engraftment[133], which increased the percentages of follicular helper T cells and generation of antigen-specific antibody and T cell responses.

HLA expression in Hu-SRC mice—In the past few years, several new mouse models have been generated that express HLA class I and class II molecules. In Hu-SRC mice, human T cell development occurs in the mouse thymus and, therefore, thymocyte education is driven primarily by mouse MHCs[136]. In numerous studies, transgenic expression of

HLA class I and/or II in humanized mouse models improved the development and survival of human $CD8^+$ and $CD4^+$ T cells, resulting in enhanced of antigen-specific immune responses[137-146]. NSG mice expressing HLA-A2 and HLA-DR1 (NSG-A2/DR1) engrafted with HLA-matched $CD34^+$ HSPCs were infected with an adenovirus encoding the hepatitis C virus-derived protein NS3, resulting in antigen-specific, HLA-A2-restricted $CD8^+$ T cell responses, higher levels of neutralizing antibodies for both NS3 and the adenovirus, and viral clearance compared with infected NSG mice. NSG-A2/DR1 also had a partial ability to control viral load in the liver[147]. NRG mice expressing HLA-A2 and HLA-DR4 (DRAGA) and engrafted with HLA-matched $CD34^+$ HSPCs had enhanced human T cell and B cell function and generated more robust antigen-specific $CD8^+$ T cell responses after immunization with the influenza A virus-derived peptide GLI than those in NRG mice[148]. $CD34^+$ HSPC-engrafted DRAGA mice have been used in studies of other pathogens, including *Plasmodium falciparum*[149], influenza virus and *Orientia tsutsugamushi*[150-152]. BRGS mice expressing HLA-A2 and HLA-DR2 engrafted with HLA-matched $CD34^+$ HSPCs had accelerated development of human T cells as well as enhanced development of functional T and B cells and were able to generate antigen-specific T cells and IgGs after immunization with a modified vaccinia virus Ankara vector encoding a human immunodeficiency virus (HIV)-derived polyprotein[153].

The expression of HLA molecules and engraftment with HLA-matched tumours and $CD34^+$ HSPCs in Hu-SRC mice could facilitate the use of this model in immuno-oncology research. In many cases, however, achieving complete HLA matching between the engrafted immune system and the implanted tumour is challenging; therefore, difficulties in preventing the activation of alloreactive T cells following tumour implantation remain.

BLT mice

A fundamental disadvantage of the Hu-SRC model is the lack of human thymic epithelium for HLA-based education and selection of human T cell populations[154]. Perhaps the most complete, and accordingly, most complex method of human immune engraftment involves the implantation of human fetal liver $CD34^+$ HSPCs and autologous fetal thymus tissue into SCID mice, generating a model referred to as bone marrow, liver, thymus (BLT) or Thy/Liv. This method enables the growth of a human thymus-like structure that supports HLA-restricted T cell development. The BLT model is derived from the SCID-hu model, which was developed to study HIV infection and involves the implantation of human fetal thymus tissue under the kidney capsule of CB17-SCID mice accompanied by intravenous injection of autologous fetal liver cells[155]. The thymus engraftment protocol was refined by co-implanting an autologous fetal liver fragment with the thymus under the kidney capsule and injecting fetal liver-derived $CD34^+$ HSPCs, resulting in the BLT mouse model[156-158]. This model has been used to study the selection of human T cells within the thymus and has provided functional insights into thymopoiesis[159] and the negative selection of autoreactive T cells[160-163]. Moreover, BLT mice support the robust development of peripheral human T cells, including conventional $CD4^+$ and $CD8^+$ T cells and functional $CD4^+$ T_{reg} cells. Nevertheless, the functional and maturation status of human B cells and innate immune cells remains limited in BLT mice[164,165]. Several studies have shown that BLT mice can generate human T cell responses following a prime–boost immunization protocol and can

generate T cell responses and limited antibody responses after infection with HIV, dengue virus or Epstein–Barr virus[156,166-170]. The development of a GVHD-like wasting syndrome that substantially reduces the lifespan of BLT mice is a concern related to this model[171-173]. The development of this syndrome varies between laboratories and across specific SCID mouse strains[174,175]. Notably, BLT mice generated on a C57BL/6 *Rag2*^{null} *Il2rg*^{null} *Cd47*^{null} background do not develop the GVHD-like syndrome and have been used in experiments to study long-term infection with and the generation of immunity to HIV[176,177]. However, the presence of the human thymic organ environment seems to promote the development of chronic GVHD in most BLT models excepting C57BL/6 *Rag2*^{null} *IL2rg*^{null} *CD47*^{null} mice, leading to tissue inflammation and fibrosis ~15 weeks post engraftment. Additionally, the limitations in accessibility to adequate tissue for engraftment make the BLT model difficult to implement for large-scale studies.

The challenges associated with generating and using BLT mice, including the need for survival surgery, access to tissues for transplant and development of GVHD-like wasting disease, have limited the application of this model in human immuno-oncology research[178]. Two groups have used an NSG-BLT model to validate the transduction of human fetal liver CD34$^+$ HSPCs with lentiviral vectors encoding an HLA-A2-restricted TCR specific for MART-1, a melanoma-related antigen, and the subsequent expression of this MART-1–TCR in human CD8$^+$ T cells in vivo[179,180]. Both studies showed that MART-1–TCR-expressing CD8$^+$ T cells in NSG-BLT mice could control the growth of an implanted HLA-A2$^+$ melanoma. NSG-BLT mice have also been used to demonstrate the antitumour activity of ex vivo activated human NK cells against poorly differentiated human oral squamous carcinoma stem cells[181]. Another study showed that human UCB-derived mesenchymal stem cells do not impair the ability of NSG-BLT mice to control the growth of tumours derived from the injection of transformed human fibroblasts[182]. NSG-BLT mice have also been used to model the growth of human leukaemia cells in the presence of an autologous immune system[183]. This model was created by transduction of the fetal liver CD34$^+$ HSPCs with *MLL–AF9,* a fusion gene detected in some human leukaemias[184], leading to the spontaneous development of B cell acute lymphoblastic leukaemia.

Models to study human innate immunity

Conventional humanized mouse models develop substantial numbers of human T and B cells but those of innate immune cell lineages, including myeloid cells (monocytes, macrophages and myeloid-derived suppressor cells), DCs and NK cells, are more limited[185]. Innate immune cell subsets have central roles in tumorigenesis[186,187], in the maintenance of the TME[188], and in response and resistance to immunotherapies[189-191]. The limited development of the human innate immune system in humanized mouse models, including NSG, NRG, NOG and BRGS, is largely related to the absence of human cytokines key for innate immune cell homeostasis and to minimal cross-species reactivity of mouse cytokines[192]. In the past few years, however, several humanized mouse models have been created to enhance the development of human innate immune cells through transgenic expression or injection of human cytokines[19].

Innate myeloid models

Myeloid cells are often abundant in the TME[193], where they typically promote tumour development, immune evasion and resistance to immunotherapies[194]. Multiple humanized mouse models have been developed specifically to promote the development of myeloid lineage cells. These models are important because, although most immunotherapies are primarily directed at cytotoxic T lymphocytes, several strategies targeting myeloid cell populations in tumours are being actively developed[195].

NSG-SGM3 mice—NSG and NRG SGM3 mice were initially developed to improve the engraftment of AML PDXs and to test systemic therapies for this malignancy[196-198]. $CD34^+$ HSPC-engrafted NSG-SGM3 mice and NSG-SGM3-BLT mice have elevated numbers of human myeloid cells and human $CD4^+$ T_{reg} cells compared with similarly engrafted NSG mice[165,199,200]. However, in some studies, $CD34^+$ HSPC-engrafted NSG-SGM3 mice developed a lethal macrophage-activation syndrome characterized by the release of human IL-6 (ref. [201]) and therefore have defective maintenance of long-term haematopoiesis[202]. Similarly, NSG-SGM3-BLT mice develop phenotypes consistent with haemophagocytic lymphohistiocytosis[203]. Humanized NSG-SGM3 mice have been used to effectively study the adverse effects of therapies based on antibodies (including but not limited to ICIs) and of CAR T cell therapies[204-206]. The results of the CAR T cell studies suggested a role for both IL-1 and IL-6 produced by myeloid populations in the pathogenesis of cytokine-release syndrome[204,206]. $CD34^+$ HSPC-engrafted NSG-SGM3 mice have also been used to test the activity of CAR macrophages against $HER2^+$ ovarian cancer CDXs and in promoting inflammation and T cell activity[207].

MISTRG mice—The MITRG model was developed using a knock-in strategy to express human M-CSF (also known as CSF1) and GM-CSF, IL-3, and thrombopoietin on the *Rag2*$^{-/-}$*Il2rg*$^{-/-}$ mouse background[208]. Subsequently, MITRG mice were modified to express human SIRPα, resulting in MISTRG mice in which phagocytosis of human cells by mouse phagocytes is reduced compared with MITRG mice[209]. $CD34^+$ HSPC-engrafted MISTRG mice developed functional populations of human monocytes, macrophages, and conventional and plasmacytoid DCs[208]. Humanized MISTRG mice support the growth of human melanoma CDXs that are infiltrated with M2-like macrophages, a model in which exposure to bevacizumab inhibits tumour growth. $CD34^+$ HSPC-engrafted MISTRG mice also support metastasis from melanoma CDXs, which does not occur in similar mice that do not express M-CSF (ISTRG mouse), suggesting that human macrophages are required for metastasis[210]. Primary leukaemic cells from patients with favourable-risk AML cells have been shown to engraft well and were serially passaged in MISTRG mice[211]. In another study, MISTRG mice engrafted with patient-derived human myelofibrosis stem and progenitor cells develop a myelofibrosis phenotype, which was abrogated upon exposure to the JAK inhibitor ruxolitinib[212]. Bone marrow-derived $CD34^+$ HSPCs from patients with myelodysplastic syndromes also engraft well in MISTRG mice, leading to the development of a human haematopoietic system with immunophenotypes and dysplastic features similar to those associated with these syndromes[213]. Notwithstanding, the robust development of human myeloid cell populations in humanized MISTRG mice can result in increased

phagocytosis of mouse erythroid cells and anaemia, limiting the lifespan and potential experimental time window of this model[208].

IL-6-based models—IL-6 is a pleiotropic cytokine that can both promote and inhibit inflammation and also promotes maturation of B cells[214]. In $CD34^+$ HSPC-engrafted BRGS mice transgenic for *IL6,* immunization with ovalbumin results in the generation of human IgGs specific for this protein[215]. $CD34^+$ HSPC engraftment of NOG mice transgenic for *IL6* (NOG-IL-6) enables efficient development of a human immune system[216]. $CD34^+$ HSPC-engrafted NOG-IL-6 mice have higher numbers of human monocytes and macrophages compared with engrafted NOG mice but a substantial proportion of these myeloid cells are HLA-DR^-, consistent with an immature phenotype. A head and neck cancer CDX implanted into $CD34^+$ HSPC-engrafted NOG-IL-6 mice was found to have high levels of infiltration with M2-like macrophages with immunosuppressive function. The expression of human *IL6* in MISTRG mice using a knock-in approach results in a model that supports efficient engraftment with human multiple myeloma CDXs and primary multiple myeloma specimens[217].

FLT3L-based models—FLT3 ligand (FLT3L) has a key role in the development of several myeloid cell populations, including DCs[218,219]. Injection of either recombinant human FLT3L cytokine or an adenovirus encoding *FLT3LG* increases the numbers of monocytes, macrophages and conventional, plasmacytoid DCs and $CD56^+$ NK cells in $CD34^+$ HSPC-engrafted SCID mice deficient in *Flt3* (refs. [220,221]). In these studies, the FLT3L-supplemented humanized mouse models had a greater ability to generate antigen-specific immunity. A subsequent study demonstrated the utility of FLT3L-supplemented humanized mice in immuno-oncology research[222]. In this study, injection of human FLT3L into $CD34^+$ HSPC-engrafted NSG-SGM3 mice improved the development of human $CD141^+$ DCs and enhanced the activity of anti-PD-1 antibodies (alone or together with a TLR3 agonist) to suppress the growth of melanoma CDXs[222].

Human NK cell models—NK cells are an attractive target for immuno-oncology research[223]; however, the development and survival of human NK cells are limited in most humanized mouse models[24,224]. IL-15 is a key cytokine for the development, survival and function of NK cell populations; thus, injection of IL-15–IL-15Rα/Fc complexes into $CD34^+$ HSPC-engrafted BRG mice transiently promotes the development of functional human NK cells[225]. Transgenic expression of *IL15* results in the development of functional human NK cells that can mediate antibody-dependent cell-mediated cytotoxicity against Burkitt lymphoma CDXs in BRGS mice[226] and of cytotoxic human NK cells that delay the growth of melanoma PDXs in $CD34^+$ HSPC-engrafted NSG mice[227]. Co-expression of *IL7* and *IL15* also enhances the development of cytotoxic human NK cells in $CD34^+$ HSPC-engrafted NSG mice[228]. In NOG mice, transgenic expression of *IL15* provides proliferative signals that support the survival of human NK cells isolated from blood, which had antibody-dependent cell-mediated cytotoxicity against a $HER2^+$ gastric cancer CDX[229]. $CD34^+$ HSPC-engrafted FLT3L-supplemented mice also have increased numbers of NK cells, albeit the majority of these are $CD56^{bright}$ NK cells with an immature phenotype[220,221]. MISTRG mice engrafted with $CD34^+$ HSPCs also develop functional

human NK cells through a mechanism proposed to be mediated by transpresentation of IL-15 by human macrophages[208].

Current landscape of humanized models

The selection of the most appropriate mouse model is an essential consideration to optimize the translational potential of studies with humanized mice. Unfortunately, no single humanized mouse recapitulates every aspect of the immune landscape within the TME[5,230]. The complexity of generating humanized mouse models and the limitations of each specific model need to be carefully considered and assessed to ensure the highest probability of an effective study[185,231]. Several parameters should be contemplated when designing experiments with humanized mice.

Engraftment strategy and immune cells of interest

The selection of an engraftment strategy for immuno-oncology studies should be guided by the therapy being evaluated. For example, the activity of CAR-based cell therapies has traditionally been assessed using mature immune cells from the peripheral blood. Individual immune cell subsets, including T cells and NK cells, are isolated, engineered to express a CAR and then injected into tumour-bearing immunodeficient mice[25]. In the past few years, technological developments have enabled the isolation of human immune cells (including T, NK and myeloid cells) from $CD34^+$ HSPC-engrafted mice for transduction of CAR constructs[232-234]. Given that both $CD4^+$ and $CD8^+$ T cells are the dominant $CD45^+$ subset in Hu-PBL mice, immunotherapies exploiting human T cells can be effectively tested in these models, although human $CD4^+$ T_{reg} cells are generally not abundant after PBMC engraftment[54]. T cell-directed therapies can also be tested in Hu-SRC or BLT mice but the kinetics of T cell development in these models need to be considered (Fig. 1). Hu-SRC models also support the development of B cells and innate immune cell subsets, which can be targeted therapeutically.

Selection of mouse strain

The development and survival of specific haematopoietic lineages will also be directly influenced by the cytokines and growth factors that are expressed by the immunodeficient mouse strain that is engrafted. This point is crucial for the success of an experiment and should determine which mouse strain is used as well as the engraftment protocol. As described, the expression of human cytokines in Hu-SRC models promotes the development of innate immune cells, which has resulted in remarkable progress in this field (Table 1). Several models, including MISTRG, NSG-SGM3, NOG-EXL (expressing GM-CSF and IL-3)[235] and FLT3L-based models, support human myeloid development and are promising models for studies of tumour-associated macrophages and DCs (Table 1). NK cell development, survival and function are substantially enhanced in mice expressing *Il15* and in MISTRG mice. The development of functional human T cells can be greatly improved in autograft models (Table 1). Finally, humanized models expressing *IL6* have shown promise to improve B cell function and enhance the development of innate immune cells and, therefore, might be useful tools for studies of tumour-specific antibody responses and antibody-dependent cellular phagocytosis.

Additional parameters to consider when selecting a humanized mouse model include: (1) whether the functionality of the immune cells of interest has been validated after engraftment; (2) the availability of the mouse strain from a collaborator or commercial source; and (3) the longevity of the engrafted mice. All of these factors will determine the feasibility of an experimental approach and its translational potential for evaluating immunotherapies. A final recommendation is to perform pilot validation experiments with the selected mouse model to confirm that it is appropriate for the planned studies.

Future directions

The majority of the experimental approaches that we have discussed rely on the use of mismatched tumours and immune systems. These allograft tumour models (Table 3) have been successfully used to characterize the human immune cells infiltrating the TME and to study immune system–tumour interactions. The resources used to create allograft models, including HSPCs and patient-derived cancer specimens, are readily abundant; however, even with partial HLA matching between donor immune cells and tumours, the tumour will be recognized as allogeneic. In addition, the generation of HLA-restricted tumour antigen-specific T cells is challenging owing to the HLA mismatch and lack of a human thymic epithelium that supports T cell development. Autograft tumour models (Table 3) have several advantages that would potentially enable the generation of HLA-restricted, tumour antigen-specific T cell responses and would reproduce the phenotype and therapeutic response in patients more faithfully than allograft models[61,62,120,236-239]. An autograft Hu-SRC model would require the presence of HLA class I and II molecules for optimal T cell development and function. An important obstacle for the autologous model is the limited availability of haematolymphoid cells and HLA-matched tumour specimens[65]. An additional limitation of engrafting mice with $CD34^+$ HSPCs isolated from patients is the requirement for large numbers of HSPCs for the robust development of a human immune system[103,144]. The generation of immunodeficient mice that have reduced barriers for engraftment of $CD34^+$ HSPCs[213], including mice harbouring *Kit* mutations (such as Kit^{W41J})[112] and MISTRG mice[202,208], could enable more efficient engraftment of these cells. The use of patient-derived $CD34^+$ HSPCs will also enable the evaluation of patient-specific responses to therapeutic interventions, including gene therapies. The collection of HLA-matched tumour samples and haematolymphoid cells requires the appropriate infrastructure and oversight (Fig. 2). Currently, most of the available humanized models lack mature human neutrophils, which have important roles in cancer[240]. The replacement of *Csf3* (encoding G-CSF) with *CSF3* in MISTRG mice lacking *Csf3r* promotes the development of human neutrophils[241]. Further development of humanized models supporting neutrophil engraftment will provide unique tools to study the role of granulocytes in tumour biology.

Conclusions

Despite substantial progress in the development of humanized mice for immuno-oncology research, these models still have limitations. A growing number of studies have proven that the currently available humanized models are useful tools to study the ability of immune cell populations to limit tumour growth in vivo and to evaluate a variety of

immunotherapies in patients with cancer. Nevertheless, the effects of these therapies in humanized mice are variable and questions remain regarding the translational potential of these observations. Advances in the development of humanized mouse models to support enhanced human haematolymphoid engraftment using reduced numbers of donor cells and, ultimately, to facilitate the development of $CD34^+$ HSPCs from iPSCs will help to increase the translational potential of humanized mice in immuno-oncology research.

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Key points

Humanized mouse models of cancer are immunodeficient mice co-engrafted with human tumours and immune cells, and are used in immuno-oncology research with potential for clinical translation.

The limitations of humanized mouse models include restricted development of mature innate immune cells, a lack of HLA molecules, limited ability to generate antigen-specific antibody responses, and a dearth of lymph node structures and germinal centres.

Given the complexity of generating humanized mice for experimental studies, the advantages and limitations of each specific model need to be carefully considered and assessed to ensure the highest probability of an effective study.

Next-generation humanized mouse models are being generated to better recapitulate the development of human innate and adaptive immunity. The development and use of novel humanized mouse platforms will accelerate the discovery and testing of new immunotherapies for patients with cancer.

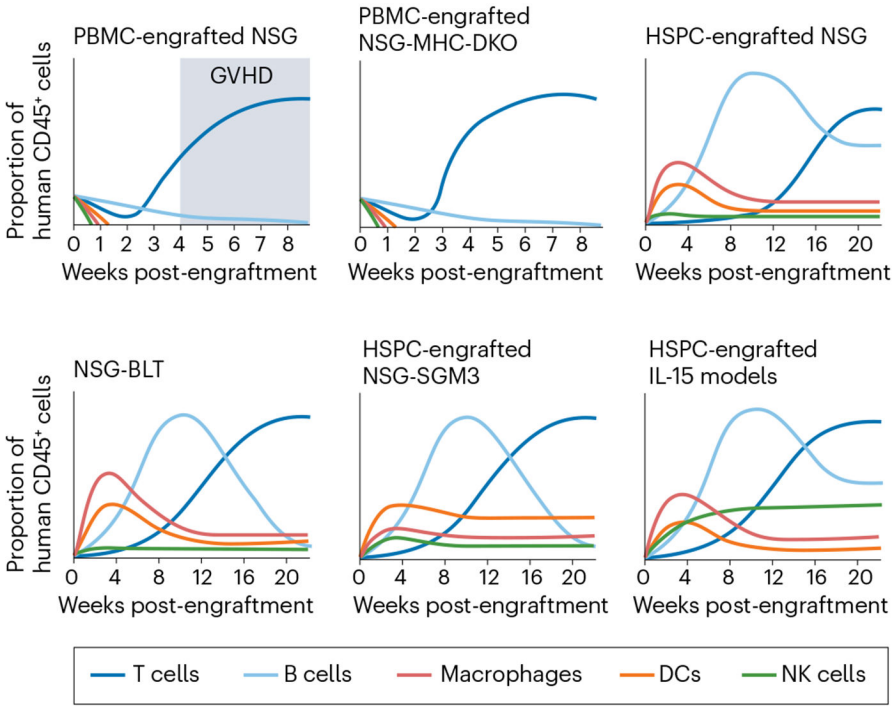

Fig. 1 |. Dynamics of human immune system development in humanized mouse models

The charts show the development of various human immune cell lineages in selected humanized mouse models [53,54,102,171,199,225-227]. The onset of xenogeneic graft-versus-host disease (GVHD) after peripheral blood mononuclear cell (PBMC) engraftment of NSG mice is depicted (grey shading) but the kinetics of GVHD onset and mouse mortality are dependent on the specific PBMC donor, total number of PBMCs injected, injection route and use of any preconditioning regimen such as irradiation [53]. BLT, bone marrow, liver, thymus; DC, dendritic cell; HSPC, haematopoietic stem and progenitor cell; NK, natural killer; SGM3, SCF, GM-CSF and IL-3.

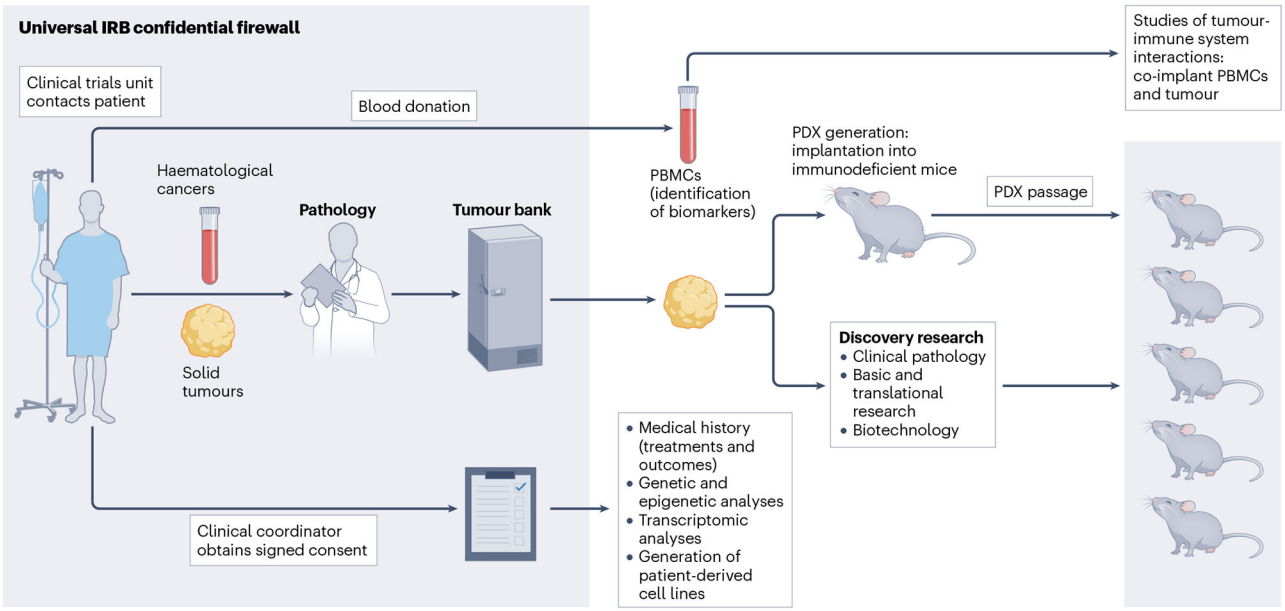

Fig. 2 |. Building resources for human tumour avatar studies

The generation of resources to study patient-derived xenografts (PDXs) and their interactions with human immune system components has several logistical requirements. A crucial aspect is the creation of a universal institutional review board (IRB) and confidential firewall. The firewall process involves obtaining informed consent of participating donors under an IRB-approved protocol, and requires the participation of clinical pathologists to examine collected tumour materials and of staff appropriately trained to manage IRB documents and a tumour bank as well assigned donor consent. Depending on the details of the informed consent, tumour tissues, blood samples and patient history can be made available to clinicians and researchers. PDX tumours could be subsequently propagated and expanded in immunodeficient mice for experiments. PBMC, peripheral blood mononuclear cell.

Table 1 |

Humanized mouse models and their suitability for studies of human immune cell function

Cell type of interest	Additional genetic alterations	Advantages	Limitations	Refs.
Hu-PBL-SCID[53,138]				
T cells	NA	Transfer of mature human donor T cells	Rapid development of GVHD	
	B2m, H2-K, H2-D, IA and/or *IE* deficiency	Delayed onset of GVHD; transfer of mature human donor T cells	Limited development of other cell types	54,78
B cells	NA	Transfer of mature human donor immune repertoire	Rapid development of GVHD; low levels of human B cells; limited de novo antibody response	55
Myeloid cells	NA	NA	Minimal number of myeloid cells	
NK cells	NA	NA	Minimal NK cell survival	
	IL15 expression	Increased NK cell survival	NA	229
DCs	NA	NA	Minimal number of DCs	
Hu-SRC[242]				
T cells	NA	NA	T cell education in absence of human thymic epithelial cells	
	HLA class I and class II	HLA-restricted T cell development	NA	137-145
B cells	NA	NA	Limited class switching; low levels of IgG	17
	IL6 expression	Improved antigen-specific B cell responses (high levels of IgG, class switch)	NA	215,216
Myeloid cells	NA	NA	Low number of human myeloid cells	
	SGM3 expression	Increased number and function of myeloid cells	Anaemia	199
	Human IL-3, GM-CSF, M-CSF, thrombopoietin and/or SIRPα expression	Increased number and function of myeloid cells	Anaemia; decreased lifespan	208
	Human IL-3 and GM-CSF expression	Increased number and function of myeloid cells	NA	243
NK cells	NA	NA	Minimal number and limited function of NK cells	
	Human IL-15, IL-7, IL-2, SIRPα and/or FLT3L expression	Increased number and function of NK cells	NA	225-227
DCs	NA	NA	Low frequency and reduced function of DCs	
	Human SGM3, or IL-3 and/or GM-CSF expression	Increased number and function of DCs	NA	199,235
	Human FLT3L expression	Increased number and function of DCs	NA	220,221

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Cell type of interest	Additional genetic alterations	Advantages	Limitations	Refs.
BLT[156]				
T cells	NA	Human thymic education of functional T cells	Limited availability of donor tissues	
B cells	NA	NA	Low levels of IgG	
	Human SGM3 expression	Improved antigen-specific B cell response (high levels of IgG, class switching)	Haemophagocytic lymphohistiocytosis	165
Myeloid cells	NA	Increased frequency and function of myeloid cells; enhanced longevity	NA	
DCs	NA	NA	Minimal number of DCs	
NK cells	NA	NA	Minimal number and limited function of NK cells	
DCs	NA	NA	Low frequency and reduced function of DCs	
	Human SGM3, or IL-3 and/or GM-CSF expression	Increased frequency and function of DCs	NA	175

BLT, bone marrow, liver, thymus; DC, dendritic cell; FLT3L, FLT3 ligand; GVHD, graft-versus-host disease; Hu-PBL-SCID, human peripheral blood leukocyte severe combined immunodeficiency; Hu-SRC, human severe combined immunodeficient repopulating cell; NA, not applicable; NK, natural killer; SGM3, SCF, GM-CSF and IL-3.

Table 2 |

Selected humanized immunodeficient mouse models used in immuno-oncology

Common name	Strain nomenclature	MGI ID[a]	Commercial source and repository number or refs.
General strains			
B-NDG	NOD.Cg-*Prkdc*^{scid} *Il2rg*^{tm1Bcgen}/Bcgen	7329796	BCGEN:110586
BRG	C;129S4-*Rag2*$^{tm1.1Flv}$ *Il2rg*$^{tm1.1Flv}$/J	4887943	JAX:014593
C57BL/6-RG	B6.Cg-*Rag2*^{tm1Fwa} *Il2rg*^{tm1Wjl}/Tac	6438675	TAC:4111
CIEA BRG	C.Cg-*Rag2*^{tm1Fwa} *Il2rg*^{tm1Sug}/JicTac	6438540	TAC:11503
CIEA NOG	NOD.Cg-*Prkdc*^{scid} *Il2rg*^{tm1Sug}/JicTac	6438369	TAC:NOG
NCG	NOD/ShiLtJGpt-*Prkdc*^{em26Gpt} *Il2rg*^{em26Gpt}/GptCrl	6156913	CRL:572
NOG	NOD.Cg-*Prkdc*^{scid} *Il2rg*^{tm1Sug}/Jic	5431525	[9]
NPG	NOD.Cg-*Prkdc*^{scid} *Il2rg*^{tm1Vst}/Vst	7329740	BVB
NRG; NOD-RG	NOD.Cg-*Rag1*^{tm1Mom} *Il2rg*^{tm1Wjl}/SzJ	3804477	JAX:007799
NSG	NOD.Cg-*Prkdc*^{scid} *Il2rg*^{tm1Wjl}/SzJ	3577020	JAX:005557
NSI	NOD.Cg-*Prkdc*^{scid} *Il2rg*^{em1Pli}/Pli	7329032	[244]
Strains with genetic modifications affecting MHC			
DRAGA	NOD.Cg-*Rag1*^{tm1Mom} *Il2rg*^{tm1Wjl} Tg(HLA-A/H2-D/B2M)Dvs Tg(HLA-DRA,HLA-DRB1*0401)39-2Kito/J	6478985	JAX:035855
HUMAMICE	STOCK *Rag2*^{tm1Fwa} *B2m*^{tm1Unc} *Prf1*^{tm1Sdz} *H2-Ab1*^{tm1Doi} *Il2rg*^{tm1Sug} Tg(HLA-A/H2-D/B2M)1Bpe Tg(HLA-DRA*0101,HLA-DRB1*0101)1Dma/Orl	6198377	EMMA:06327
NOG-dKO or NOG-*B2M*^{null}, *IA*^{null}	NOD.Cg-*B2m*^{tm1Unc} *Prkdc*^{scid} *H2-Ab1*^{tm1Doi} *Il2rg*^{tm1Sug}/Jic	7329748	[78]
NOG I-Abeta <−/−>	NOD.Cg-*Prkdc*^{scid} *H2-Ab1*^{tm1Doi} *Il2rg*^{tm1Sug}/Jic	5431530	[78]
NSG-A2/DR1	NOD.Cg-*Prkdc*^{scid} *Mcph1*$^{Tg(HLA-A2.1)1Enge}$ *Il2rg*^{tm1Wjl} Tg(HLA-DRA*0101,HLA-DRB1*0101)1Dmz/Alpl	7330165	[147]
NSG-*B2M*^{null} *IA IE*^{null}	NOD.Cg-*B2m*^{tm1Unc} *Prkdc*^{scid} *H2*$^{dlAb1-Ea}$ *Il2rg*^{tm1Wjl}/SzJ	6271996	JAX:030547
NSG-(*K*^{b}*D*^{b})^{null}*IA*^{null}	NOD.Cg-*Prkdc*^{scid} *H2-K1*^{tm1Bpe} *H2-Ab1*^{em1Mvw} *H2-D1*^{tm1Bpe} *Il2rg*^{tm1Wjl}/SzJ	6197202	JAX:025216
Strains with genetic modifications for cytokine overexpression			
BRGST	B6.Cg-*Rag2*^{tm1Fwa} *Sirpa*$^{Idd13.2\text{-}NOD/ShiLt}$ *Il2rg*^{tm1Wjl} Tg(*Krt14-Tslp*)#Dfk/Pas	7329944	[133]
hIL2-NOG	NOD.Cg-*Prkdc*^{scid} *Il2rg*^{tm1Sug} Tg(CMV-IL2)4-2Jic/JicTac	6438606	TAC:13440
MISTRG	C;129S4-*Rag2*$^{tm1.1Flv}$ *Csf1*$^{tm1(CSF1)Flv}$ *Csf2/Il3*$^{tm1.1(CSF2,IL3)Flv}$ *Thpo*$^{tm1.1(TPO)Flv}$ *Il2rg*$^{tm1.1Flv}$ Tg(SIRPA)1Flv/J	5555040	JAX:017712

Common name	Strain nomenclature	MGI ID[a]	Commercial source and repository number or refs.
MISTRG6	C;129S4-*Rag2*$^{tm1.1Flv}$ *Csf1*$^{tm1(CSF1)Flv}$ *Csf2/Il3*$^{tm1.1(CSF2,IL3)Flv}$ *Thpo*$^{tm1.1(TPO)Flv}$ *Il2rg*$^{tm1.1Flv}$ Tg(SIRPA)1Flv/J	NA	217
NOG-EXL	NOD.Cg-*Prkdc*^{scid} *Il2rg*^{tm1Sug} Tg(SV40/HTLV-IL3,CSF2)10-7Jic/JicTac	6438862	TAC:13395
NSG-SGM3	NOD.Cg-*Rag1*^{tm1Mom} *Il2rg*^{tm1Wjl} Tg(CMV-IL3,CSF2,KITLG)1Eav/J	5566536	JAX:024099
Strains with other genetic modifications			
BRGS	B6.Cg-*Rag2*^{tm1Fwa} *Sirpa*$^{Idd13.2\text{-}NOD/ShiLt}$ *Il2rg*^{tm1Wjl}/Kyu	7329783	209,245
BRGSK$^{W\text{-}v}$	B6.Cg-*Rag2*^{tm1Fwa} *Sirpa*$^{Idd13.2\text{-}NOD/ShiLt}$ *Kit*^{Wv} *Il2rg*^{tm1Wjl}/Kyu	7329802	115
NBSGW	NOD.Cg-*Kit*^{W41J} *Tyr*$^{+}$ *Prkdc*^{scid} *Il2rg*^{tm1Wjl}/ThomJ	5613001	JAX:026622
NOG-*pRorc γc*	NOD.Cg-*Prkdc*^{scid} *Il2rg*^{tm1Sug} Tg(Rorc-Il2rg)#Takah/Takah	7330081	132
NSG-W41	NOD.Cg-*Kit*$^{W\text{-}41J}$ *Prkdc*^{scid} *Il2rg*^{tm1Wjl}/WaskJ	5779915	JAX:026497
TKO	B6.129(Cg)-*Rag2*^{tm1Fwa} *Cd47*^{tm1Fpl} *Il2rg*^{tm1Cgn}/KhasJ	5528885	176

[a]To retrieve records, enter ‘MGI:’ and the ID number (with no spaces in between) in the Quick Search box at http://www.informatics.jax.org. BCGEN, Byocytogen; BVB, Beijing Vitalstar Biotechnology; CRL, Charles River Laboratories; EMMA, European Mouse Mutant Archive; ID, identification; JAX, Jackson Laboratory; MGI, Mouse Genome Informatics; NA, not available; TAC; Taconic.

Table 3 |

Approaches to study interactions between human immune components and human tumours

Tumour model	Implanted/engrafted human components		Comments	
	Tumour	Immune system	Advantages	Disadvantages
Allograft	HLA mismatched with immune system: PDX and CDX	PBMCs, HSPCs, and human fetal liver $CD34^+$ HSPCs and autologous fetal thymus tissue	Enable studies of immune regulatory properties of the tumour microenvironment Abundance of immune cells and HSPCs Enable modelling graft versus tumour response	Immune system allogeneic to tumour No HLA-restricted tumour antigen-specific T cell responses
Autograft	Autologous to immune system: PDX	PBMCs, HSPCs expanded from PBMCs following mobilization with G(M)-CSF or bone marrow samples, and tumour-infiltrating lymphocytes	Immune system autologous to tumour Potential for HLA-restricted tumour antigen-specific T cells	Require generation of PDX Limited availability of immune cells and HSPCs from patients With HSPC engraftment, development of HLA-restricted T cells will be limited

CDX, cell line-derived xenograft; G(M)-CSF, G-CSF and/or GM-CSF; HSPCs, haematopoietic stem and progenitor cells; PBMCs, peripheral blood mononuclear cells; PDX, patient-derived xenograft.