REVIEW ARTICLE

Humanized Mouse Models for Immuno-Oncology Research: A Review and Implications in Lung Cancer Research

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ABSTRACT

Cancer immunotherapy has brought significant clinical benefits to patients with cancer, including those with lung cancer. Patient-derived tumor xenograft mouse models have become the preferred animal model for translational cancer research and preclinical studies. Given the unmet need for improved predictive models in immuno-oncology, humanized mouse models which are co-engrafted with both human tumors and immune system components have been used to investigate novel immunotherapeutics. These models have similarly been used to predict immune-related adverse events and to develop predictive biomarkers. This review summarizes key concepts related to humanized mouse models. We highlight the various approaches to generate them, factors that are critical to successfully establishing such models, their respective limitations, and considerations in model selection for preclinical lung cancer immuno-oncology research and therapeutic studies.

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Keywords: Lung cancer; Immunotherapy; Humanized mouse models; Patient-derived xenograft

Introduction

Immune checkpoint inhibitor (ICI) therapy has been found to have considerable clinical benefits in NSCLC and has become the new standard of care in patients with metastatic disease. It has more recently emerged as a standard treatment in the neoadjuvant,[1] adjuvant,[2,3] and post-chemoradiation consolidation therapy setting in nonmetastatic patients.[4] Cancer immunotherapy has expanded beyond ICIs to novel approaches, including cancer (mRNA) vaccines,[5] immune cell engagers,[6] and adoptive cell therapies (ACTs).[7] Nevertheless, preclinical immuno-oncology (IO) research faces challenges due to the paucity of clinically relevant human cancer models.[8] In early IO drug development, syngeneic mouse tumors, nonhuman primates (cynomolgus monkeys),[8] and cell line-derived xenograft (CDX) mouse models[9] have been used. In addition, however, these animal models do not appropriately recapitulate the complexity of the patient tumor immune microenvironment (TME),[10] highlighting an unmet need for "humanized" animal models with

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human immune system (HIS) elements to model immunotherapeutic interventions and potential adverse events accurately. Patient-derived tumor xenograft (PDX) models in immune-deficient mice have been most frequently used for non-IO preclinical studies[11] to investigate drug efficacy, predictive biomarkers, and drug-resistant mechanisms.[12] This review summarizes the state of science in humanized PDX models with a focus on their utility in lung cancer IO research.

Methods

A PubMed search was conducted on manuscripts published between January 2018 and July 2023 using the keywords "humanized mouse model for immuno-oncology/immunotherapy." We excluded articles involving mouse models with hematologic malignancies or nonhumanized models and review articles. Ultimately, 89 of 197 reports were selected in this review, including 61 reports on humanized PDX models (Supplementary Fig. 1).

Factors in the Establishment of Humanized PDX Models

To establish successful humanized PDX models, it is necessary to consider graft (patient tumor), host (mouse), and immunologic factors to align the humanization strategy with the study's purpose. These are summarized in Figure 1 and Supplementary Tables, with some additional details described in the Supplementary Materials.

Graft (Tumor) Factors. Lung cancer is one of the most investigated cancer types using humanized PDX models (Fig. 1). Currently, several preclinical studies for IO drugs have used humanized CDX models with lung cancer cell lines, such as A549, H292, and HCC827. Although CDX models are less time consuming, culturing primary cells in vitro before engraftment can cause vital loss of heterogeneity within the tumor population. In comparison, PDX models better recapitulate the original tumor's characteristics. Nevertheless, some PDX models can take significantly longer to initiate growth and slower growth rates[37] (Fig. 2).

Host (Mouse) Factors. Highly immunodeficient first-generation mouse strains, such as NOD/*Prkdc*^{scid}, *IL2rg*$^{-/-}$ (NSG), NOD/*Prkdc*^{scid}, *IL2rg*^{null} (NOG), NOD/*Rag1*$^{-/-}$, *IL2rg*$^{-/-}$ (NRG), and BALB/c/*Rag2*$^{-/-}$, *IL2rg*$^{-/-}$, *SIRPA*^{NOD} (BRGS), are crucial for growing human tumors in mice without xenogeneic rejection. NSG is the most frequently used strain for humanization studies. This model uses a NOD/SCID background and includes a deletion of an interleukin 2 receptor gamma chain (IL2Rγ). This inhibition disrupts cytokine signaling and the development of immune repertoire, leading to a higher engraftment success rate of human cells and tissues in the NSG mice as compared with the SCID or NOD/SCID mice.[43] New-generation mouse strains have been developed to improve PDX and HIS engraftment in these mice. These next-generation mice incorporate numerous genetic modifications.[7] The strains for humanized mouse models used in the reviewed IO studies are summarized in Table 1 and Supplementary Tables 1 and 2.

Humanized PDX Models

The objective of humanized mouse models is to develop an HIS in immunocompromised mice that recapitulates complex human tumor-immune interactions and to avoid significant graft-versus-host disease (GvHD) when conducting studies using PDX tumors. The three most common humanized mouse models include the Hu-PBMC, Hu-HSC, and Hu-BLT models. Each model is distinct regarding immunologic profiling, engraftment protocol, strengths, and limitations (Fig. 2, and Supplementary Materials). The advantages and disadvantages of each humanization strategy are described in Table 2.

Hu-PBMC Model. The most frequently used source of mature human ICs is peripheral blood mononuclear cells (PBMCs) isolated from circulating blood or occasionally from lymph nodes and spleen.[44] Human PBMCs include lymphocytes, monocytes, dendritic cells (DCs), and natural killer (NK) cells. Compared with the others, the infusion of PBMCs is the simplest and fastest humanization method. The Hu-PBMC model can support tumor antigen-specific T-cell–directed immune reactions when the PBMC donor and tumor are human leukocyte antigen (HLA) matched[16] or when PBMCs autologous to the implanted tumor are used.[13]

Before infusion, mice can be irradiated with a low dose (2–2.5 Gy) to eradicate mouse ICs (Fig. 2), although its advantage is uncertain.[45] Two to four weeks after PBMC infusion, human ICs are detectable in the mouse peripheral blood. The Hu-PBMC model is predominantly engrafted with CD3$^+$ T-cells, and their survival is sustained by the cytokines released from PBMCs (e.g., IFNγ, IL-1β, IL-2, IL-5, and IL-10).[38,46] A lack of other cytokines may result in low levels of B cells and other myeloid populations, as B cells are mildly sustained for several weeks in the mouse spleen and bone marrow, whereas NK and myeloid cells can survive for only the first few days.[45,46] Four weeks after injection of approximately 2 $\times$ 10^7 PBMCs into NSG mice, 50% of the CD45$^+$ ICs in mouse peripheral blood are of human origin, with 90% being CD3$^+$ T-cells and a 1:1 ratio of CD4$^+$-CD8$^+$ T-cells; they are maintained during 4 to 6 weeks post-engraftment.[47] Nevertheless, PBMCs from

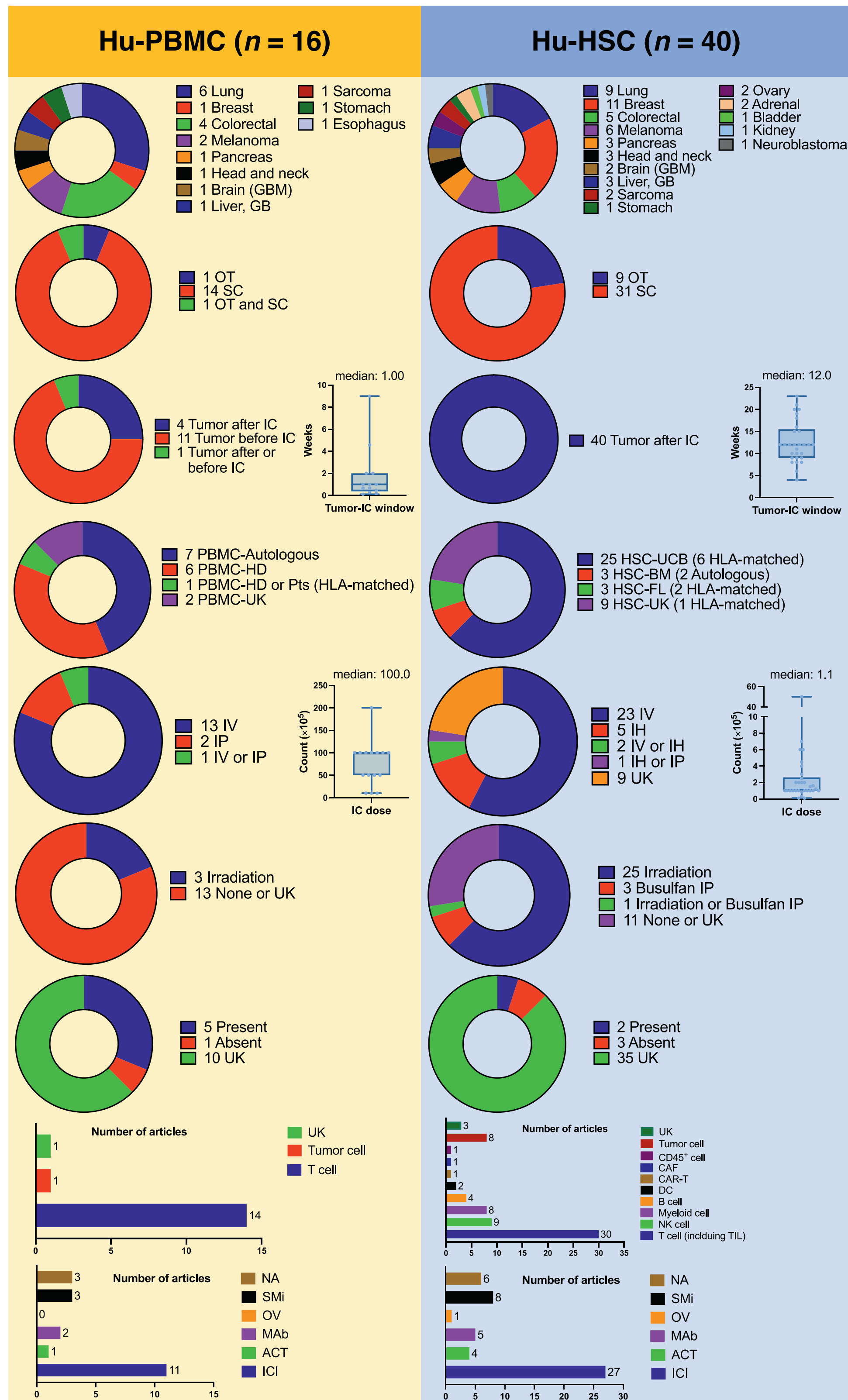

Figure 1. Profiles of graft, host, and immunologic factors that have been reported in humanized PDX models. The characteristics are described according to each category of humanization: Hu-PBMC and Hu-HSC. Articles reporting the use of both Hu-PBMC and Hu-HSC models in the same study ($n = 2$) or the use of other humanization types ($n = 3$) were excluded. ACT, adoptive cell therapy; BM, bone marrow; CAF, cancer-associated fibroblast; CAR-T, chimeric antigen receptor T-cell; DC, dendritic cell; FL, fetal liver; GB, gall bladder; GBM, glioblastoma; HD, healthy donor; HLA, human leukocyte antigen; Hu-HSC, human CD34$^+$ hematopoietic stem cell model; Hu-PBMC, human peripheral blood mononuclear cell model; IC, immune cell; ICI, immune checkpoint inhibitor; IH, intrahepatic; IP, intraperitoneal; IV, intravenous; MAb, monoclonal antibody; NA, not applicable; NK, natural killer; OT, orthotopic; OV, oncolytic virus; Pts, patients; PDX, patient-derived xenograft; SC, subcutaneous; SMi, small molecule inhibitor; TIL, tumor-infiltrating lymphocyte; UCB, umbilical cord blood; UK, unknown; xGVHD, xenogenic graft-versus-host disease.

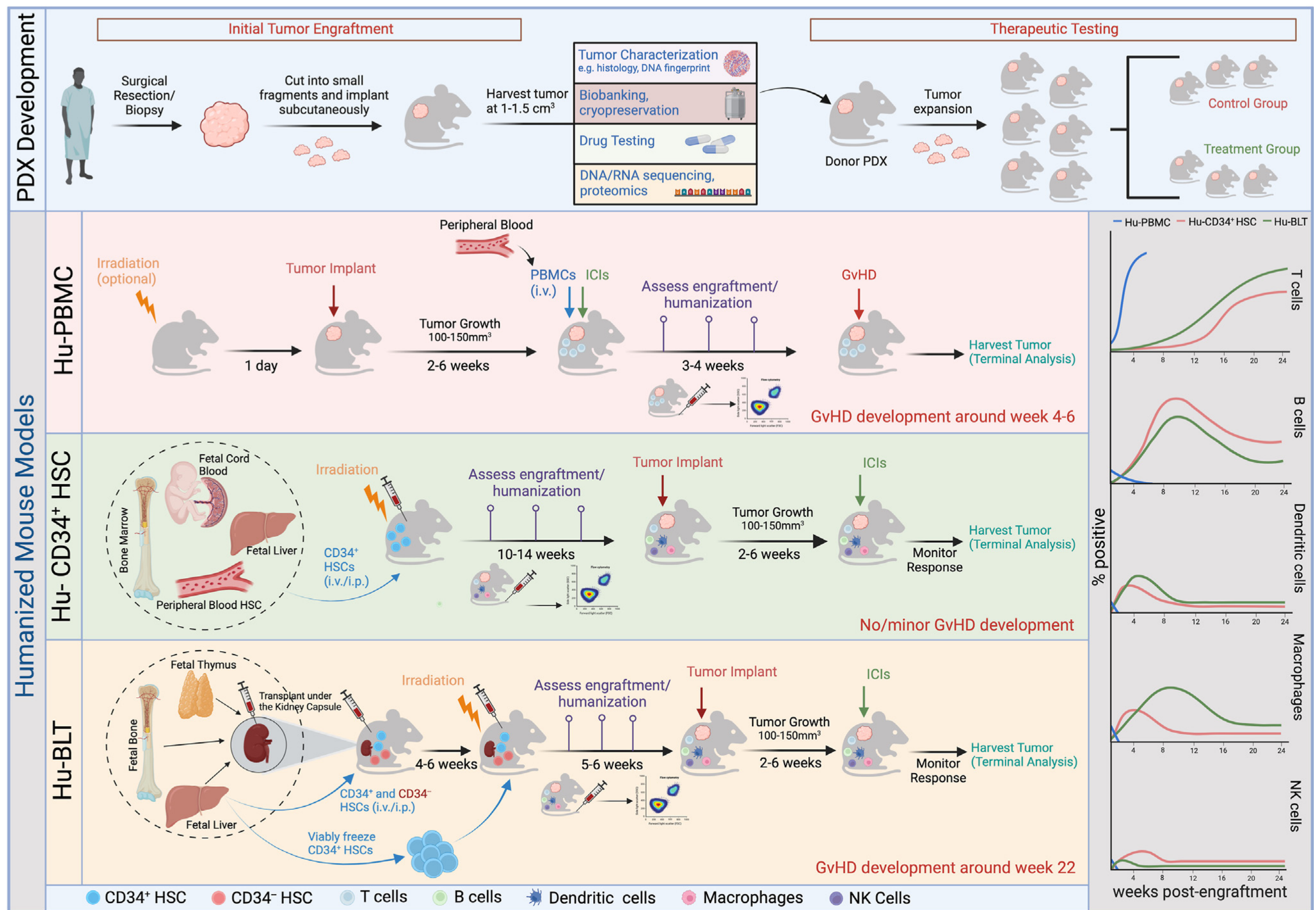

Figure 2. Establishment of humanized mouse models. Schematic representation of the development of PDX models for therapeutic testing and establishment of three humanized immune systems. The source of human immune cells differs in each model and forms the basis of how each system can evaluate ICIs and plays a significant role in their strengths and limitations. The right panel of the figure compares immune cell development in the three humanized immune systems in the NSG mouse strain.[7,15,38-42] GvHD, graft-versus-host disease; Hu-BLT, human bone marrow, liver, and thymus model; Hu-CD34^{+}HSC, human CD34^{+} hematopoietic stem cell model; Hu-PBMC, human peripheral blood mononuclear cell model; ICIs, immune checkpoint inhibitors; i.p., intraperitoneal; i.v., intravenous; NK, natural killer; PDX, patient-derived xenograft.

different donors have variable engraftment rates, and an increased proportion of human lymphocytes in the mouse blood does not necessarily lead to greater antitumor activity,[48] suggesting that fewer PBMCs might also be feasible.

The major limitation of this model is the inevitable and relatively rapid development of lethal xenogeneic GvHD in mice. This limits the therapeutic window to 4 to 6 weeks post-PBMC injection, and systemic inflammation may confound antitumor immune responses.[49]

Hu-CD34^{+} HSC Model. Hematopoietic stem cells (HSCs) are characterized by CD34 expression on the cell surface, and they can be isolated from human bone marrow, peripheral blood, umbilical cord blood (UCB), or fetal liver. UCB is the most common source of CD34^{+} HSCs due to their higher density.[50] The source of CD34^{+} HSCs seems to affect the functionality of T-cells in mice, as fetal CD34^{+} HSCs produce ICs with greater tolerance than those from adult-derived CD34^{+} HSCs.[51] This method also requires sublethal irradiation of the recipient to deplete the mouse HSCs and facilitate human HSC engraftment. Post-radiation, mice are injected with approximately 100,000 CD34^{+} HSCs, which will home to the bone marrow. Approximately weeks 4 to 6, human CD45^{+} cells can be detected in the mouse peripheral blood.[38] Migration of human T-cell precursors to mouse thymus[52] leads to positive and negative selection and prevents the mature T-cell populations from attacking the murine tissue, alleviating GvHD.[53]

CD34^{+} HSC infusion leads to a more complete hematopoietic immune system than the Hu-PBMC model, as it gives rise to both innate and adaptive IC populations.[54] Nevertheless, this model has significant deficits in functional B and T-cell immune

Table 1. Selected Immunodeficient Mouse Models and Humanization Strategies for Immuno-Oncology Preclinical PDX Studies in Solid Tumors

Drugs	Humanization Types/Mouse Strains /Drugs (*n*)					
	Hu-PBMC (*n* = 11)		Hu-HSC (*n* = 27)		Others[a] (*n* = 3)	
Immune checkpoint inhibitors (*n* = 41)	NCG (2)	Anti-PD-1 (2)	BRGS (5)	Anti-PD-1 (2), Anti-PD-1 (+Cabozantinib) (1), Anti-PD-1 (+HDAC inhibitor) (1), Anti-PD-1 and anti-CTLA-4 (1)	NOD-SCID (1)	Anti-PD-L1 (1)
	NOD-SCID (1)	Anti-PD-L1 (+Afatinib and FGFR inhibitor) (1)	DRAG (1)	Anti-PD-1 (1)	NOG, IL2-NOG (1)	Anti-PD-1 (1)
	NOG (1)	Anti-PD-1 (1)	NCG (1)	Anti-PD-1 (+VEGFR2 inhibitor) (1)	NSG (1)	Anti-PD-1 (+TILs) (1)
	NPI (1)	Anti-PD-1 (1)	NOG (1)	Anti-PD-1 (+Anti-FAP ADC) (1)		
	NSG (5)	Anti-PD-1 (1), Anti-PD-1 (+CDK4/6 inhibitor) (1), Anti-PD-1 (+VEGFR2 inhibitor) (1), Anti-PD-L1 (+BsAb[b]) (1), Anti-CTLA-4 (1)	NOG-EXL (2)	Anti-PD-1 (+PLK1 inhibitor) (1), Anti-CD47 (+CAR-M) (1)		
	NSI (1)	Anti-PD-L1 (1)	NPI (1)	Anti-PD-1, anti-PD-L1, and anti-CTLA-4 (1)		
			NSG (17)	Anti-PD-1 (8), Anti-PD-1 (+anti-IL-34) (1), Anti-PD-1 (+PLK1 inhibitor) (1), Anti-PD-1 (+PI3K inhibitor) (1), Anti-PD-1 and anti-CTLA-4 (5), Anti-PD-1 and anti-CTLA-4 (+CAR-NK) (1)		
			NSG-SGM3 (2)	Anti-PD-1 (1), Anti-PD-1 (+PLK1 inhibitor) (1)		
	Hu-PBMC (*n* = 1)		Hu-HSC (*n* = 4)		Others[c] (*n* = 2)	
Adoptive cell therapy (*n* = 7)	NSG (1)	Fas-encoding plasmid (1)	MISTRG (1)	NK cell (+anti-GD2) (1)	NSG (2)	TILs (+anti-PD-1) (1), CAR-T (+BiTE[d]) (1)
			MITRG (2)	NK cell (+anti-GD2) (1)		
			NOG-EXL (2)	CAR-T (+MWA) (1), CAR-M (+anti-CD47) (1)		
			NSG (2)	NK cell (+anti-GD2) (1), CAR-NK (+anti-PD-1 and anti-CTLA-4) (1)		
	Hu-PBMC (*n* = 2)		Hu-HSC (*n* = 4)		Others[e] (*n* = 2)	
Monoclonal antibodies (*n* = 8)	NOG (1)	BiTE[f] (1)	MISTRG (1)	Anti-GD2 (+NK cell) (1)	NSG (2)	BiTE[g] (1), BiTE[d] (+CAR-T) (1)
	NSG (1)	BsAb[b] (+anti-PD-L1) (1)	MISTRG6 (1)	Anti-VEGF-A (1)		
			MITRG (1)	Anti-GD2 (+NK cell) (1)		
			NOG (1)	Anti-FAP ADC (+anti-PD-1) (1)		
			NSG (2)	Anti-GD2 (+NK cell) (1), Anti-IL-34 (+anti-PD-1) (1)		

(continued)

Table 1. Continued

Drugs	Humanization Types/Mouse Strains /Drugs (*n*)					
	Hu-PBMC ($n = 11$)		Hu-HSC ($n = 27$)		Others[a] ($n = 3$)	
	Hu-PBMC ($n = 3$)		Hu-HSC ($n = 8$)		Others ($n = 0$)	
Small molecule inhibitors ($n = 11$)	NOD-SCID (1)	Afatinib and FGFR inhibitor (+anti-PD-L1) (1)	BRGS (2)	Cabozantinib (+anti-PD-1) (1), HDAC inhibitor (+anti-PD-1) (1)		
	NSG (2)	CDK4/6 inhibitor (+anti-PD-1) (1), VEGFR2 inhibitor (+anti-PD-1) (1)	NCG (1)	VEGFR2 inhibitor (+anti-PD-1) (1)		
			NOG-EXL (1)	PLK1 inhibitor (+anti-PD-1) (1)		
			NSG (4)	cPLA2 inhibitor (1), PLK1 inhibitor (+anti-PD-1) (1), PI3K inhibitor (+anti-PD-1) (1), FGFR1-3 and FGFR4 inhibitors (1)		
			NSG-SGM3 (1)	PLK1 inhibitor (+anti-PD-1) (1)		
	Hu-PBMC ($n = 0$)		Hu-HSC ($n = 1$)		Others ($n = 0$)	
Oncolytic virus ($n = 1$)			NPI (1)	HSV-1 OV (1)		

Note: References for individual studies are described in Supplementary Table 2.
[a]TILs ($n = 2$), lymphocytes ($n = 1$).
[b]Bispecific antibody: EGFR-specific 4-1BB-agonistic trimerbody ($n = 1$).
[c]TILs ($n = 1$), Hu-PBMC and Hu-HSC ($n = 1$).
[d]Bispecific T-cell engager: HER2-specific ($n = 1$).
[e]Hu-PBMC and Hu-HSC ($n = 2$).
[f]Bispecific T-cell engager: DLL3-specific ($n = 1$).
[g]Bispecific T-cell engager: CEA/HER2-specific ($n = 1$).
ADC, antibody-drug conjugate; CAR-M, chimeric antigen receptor-macrophage; CAR-NK, chimeric antigen receptor-natural killer cell; CAR-T, chimeric antigen receptor T-cell; CDK4/6, cyclin-dependent kinase 4/6; CEA, carcinoembryonic antigen; cPLA2, cytosolic phospholipase A2; CTLA-4, cytotoxic T-lymphocyte-associated protein 4; DLL3, delta-like ligand 3; FAP, fibroblast activation protein; FGFR, fibroblast growth factor receptor; GD2, disialoganglioside; HDAC, histone deacetylase; Hu-HSC, human CD34+ hematopoietic stem cell; Hu-PBMC, human peripheral blood mononuclear cell; HSV-1, herpes simplex virus type-1; IL, interleukin; MWA, microwave ablation; NK, natural killer; OV, oncolytic virus; PDX, patient-derived xenograft; PD-1, programmed cell death protein 1; PD-L1, programmed death-ligand 1; PI3K, phosphoinositide 3-kinase; PLK1, polo-like kinase 1; TILs, tumor-infiltrating lymphocytes; VEGFR2, vascular endothelial growth factor receptor 2; VEGF-A, vascular endothelial growth factor-A.

Table 2. Advantages and Disadvantages of Humanization Strategies

Factors	Hu-PBMC	Hu-HSC	Hu-BLT
Preconditioning	−/+	+ (sublethal irradiation or busulfan)	+ (sublethal irradiation or busulfan)
B-cell	−/+ (low level)	+ (++ with IL-6)	+
T-cell	+++	++ (not HLA restricted)	+++
NK cell	−/+	−/+ (+++ with IL-15 or FLT3L)	−/+
Myeloid cell	−/+	+ (++ with SCF, GM-CSF, IL-3)	++
Dendritic cell	−/+	+ (++ with FLT3L)	+
Advantages	Easy to prepare, fast to establish	Easy to prepare	Presence of human thymus (HLA-restricted T-cells)
	HLA-restricted T-cells	Stable and long-term multilineage hematopoiesis	Multilineage hematopoiesis
	Drug screening and trials (e.g., ICIs, CAR, BsAbs)	Primary immune response (IgM/IgG)	Primary immune response
		Mucosal engraftment	Mucosal engraftment
Disadvantages	Prone to have GvHD	No HLA restriction	Surgery with human fetal tissue
	Short duration (<3 mo)	Limited Ig class switching	Possibility of GvHD
	No primary immune response	Low NK cells and myeloid cells	Limited Ig class switching
	Lack of B and myeloid cells	Long duration of immune differentiation (>10 wk)	Low NK cells
	No multilineage hematopoiesis		Long duration of immune differentiation (>10 wk)

BsAbs, bispecific monoclonal antibodies; CAR, chimeric antigen receptor; FLT3L, FLT3 ligand; GM-CSF, granulocyte-macrophage colony-stimulating factor; GvHD, graft-versus-host disease; HLA, human leukocyte antigen; Hu-BLT, human bone marrow, liver, and thymus model; Hu-HSC, human $CD34^+$ hematopoietic stem cell model; Hu-PBMC, human peripheral blood mononuclear cell model; ICIs, immune checkpoint inhibitors; Ig, immunoglobulin; IL, interleukin; NK, natural killer; SCF, stem cell factor.

populations.[55] Most B cells are immature due to B cell maturation and differentiation being blocked at the transition phase, resulting in the accumulation of B cell precursors in the spleen.[56] There is also incomplete development of innate immune lineage and the absence of thymic HLA expression, which is essential for developing HLA-restricted T-cells.[7] Although the Hu-$CD34^+$ HSC model requires a more extended establishment period (Fig. 2), it presents a superior advantage with a more fully constituted, albeit immature, immune system, with the rare occurrence of GvHD development.

Hu-BLT Model. The Human Bone marrow, Liver, and Thymus (Hu-BLT) model involves injection of the $CD34^+$ HSCs in combination with surgical implantation of the human fetal liver and thymus under the mouse kidney capsule[57,58] (Fig. 2). This approach results in the development of multilineage hematopoietic reconstitution which includes T-cells, B-cells, DCs, macrophages, and myeloid cells. It also supports the development of T-cell subtypes, including $CD4^+$, $CD8^+$, and regulatory T (T_{reg}) cells. As opposed to the Hu-HSCs, within the Hu-BLT model, T-cells mature within the transplanted human thymus, leading to the education of human T-cells and resulting in HLA-restricted T-cell development.[59] Although there is a negative selection of T-cells in the fetal thymus in these models, it is exclusive to the human peptide-MHC complexes, and T-cells harboring affinity for mouse MHC are not eliminated, leading to a greater risk of GvHD development in the Hu-BLT versus Hu-$CD34^+$ HSC model. GvHD may shorten the lifespan of BLT mice as it develops up to 35% by 22 weeks post-implantation.[60]

Although this model is likely the most complete humanized mouse model, it is very complex to develop and poses many challenges, including technical complexity and limitations in accessing thymic tissue. Notably, Smith et al.[39] revealed that the Hu-BLT model can be propagated through the secondary transfer of bone marrow cells and human thymus implants from the donor Hu-BLT mouse to 4 to 5 propagated Hu-BLT (proBLT) mice, thus presenting a possibility of expansion of these models without requiring new fetal tissues and $CD34^+$ HSCs.

Humanized PDX Models in Lung Cancer Research

Preclinical Testing for Novel Immunotherapy. Humanized mouse models have been used to assess the efficacy and safety of various cancer immunotherapies in solid tumors, including NSCLC (Table 1 and Supplementary Table 2). The U.S. FDA's Division of Applied Regulatory Science has also advanced humanized mouse models,

Table 3. Current Status of Humanized Mouse Models Used in Preclinical Immuno-Oncology Studies of Lung Cancer Based on Factors for Establishing Xenograft Models

	Graft		Host		Immunologic						
Author [Year][ref.]	Subtype (Stage)	Tumor Source	Mouse Strain	Tumor Implant[a]	Type of IC	Inject Route	IC Source	IC Dose	Preconditioning	Successful IC Graft	Drugs or Intervention
PDX ($n = 15$)											
Lin et al. [2018][13]	NSCLC	Surgery	NSI	SC, before IC	PBMCs	IV	Autologous (patients)	5×10^6	Irradiation	hCD45+ CD3+ >50% in PB	Anti-PD-L1
Wang et al. [2018][14]	NSCLC	Surgery	NSG	SC, after IC	$CD34^+$ HSCs	IV	Allogeneic, partially HLA-matched (FL)	1×10^5	Irradiation	hCD45+ cells >25% in PB	Anti-PD-1
Meraz et al. [2019][15]	NSCLC	UK	NSG	SC, after IC	$CD34^+$ HSCs	IV	Allogeneic, HLA-matched (UCB)	1×10^5	Irradiation	hCD45+ cells >25% in PB	Anti-PD-1
Pyo et al. [2019][16]	NSCLC, EML4-ALK	UK	NSG	SC, after IC	PBMCs	IV	Allogeneic, HLA-matched (HD or patients)	1×10^7	UK	hCD45+ hCD3+, hCD45+ hCD14+ cells (%) in PB	Anti-PD-1
Fabre et al. [2020][17]	NSCLC (IV)	Biopsy (CTG0860)	NOG	SC, after IC	$CD34^+$ HSCs	IV	Allogeneic (commercial)	UK	UK	UK	Anti-PD-1, anti-FAP ADC
Hama et al. [2020][18]	NSCLC	Surgery	NSG	SC, after IC	$CD34^+$ HSCs	IV	Allogeneic (FL)	1×10^5	Irradiation	hCD45+ cells >25% in PB	Anti-PD-1, anti-IL-34
Chen [2021][19]	NSCLC (IIIA/IB: EGFRm,FGFRm)	Surgery	NOD-SCID	SC, before IC	PBMCs	IV	Allogeneic (HD)	1×10^7	UK	UK	Anti-PD-L1, afatinib, FGFR inhibitor
Compte et al. [2021][20]	NSCLC, EGFRm, TP53m (early)	Surgery (TP103)	NSG	SC, before IC	PBMCs	IP	Allogeneic (HD)	1×10^7	UK	UK	BsAb (EGFR/4-1BB-bispecific), anti-PD-L1
Giffin [2021][21]	SCLC	UK	NOG (PDX), NSG (CDX)	SC (PDX) or IV (CDX, orthotopic - lung and liver), before IC	Ex vivo expanded T-cells from PBMCs	IV	Allogeneic (HD)	2×10^7	UK	UK	BiTE (DLL3/CD3-bispecific)
Marín-Jiménez et al. [2021][22]	SCLC	UK	BRGS	SC, after IC	$CD34^+$ HSCs	IV	Allogeneic (UCB)	$0.2\text{-}0.6 \times 10^6$	Irradiation	hCD45+ cells >25% in PB	Anti-PD-1, anti-CTLA-4
Cao [2022][23]	NSCLC	UK	NOG-EXL	SC, after IC	$CD34^+$ HSCs	IV	Allogeneic (UCB)	UK	UK	UK	CAR-T (AXL-specific) and MWA
DeAngelis et al. [2022][24]	NSCLC	Surgery (LG1306)	NSG	SC, after IC	$CD34^+$ HSCs	UK	Allogeneic (UCB)	UK	UK	UK	Anti-PD-1
Oswald et al. [2022][25]	NSCLC (I-IV)	Surgery, Biopsy	huNSG (commercial)	SC, after IC	$CD34^+$ HSCs	IV	Allogeneic (UCB)	UK	Irradiation	hCD45+ cells (%) in PB	Anti-PD-1, anti-CTLA-4
Wu et al. [2022][26]	UK	UK (purchased)	NPI	SC, before IC	PBMCs	IV	Allogeneic (commercial)	1×10^7	UK	hCD45+ cells (%) in PB	Anti-PD-1
Xu [2022][27]	NSCLC	Surgery	NPI	SC, after IC	$CD34^+$ HSCs	IV	Allogeneic, partially HLA-matched (UCB)	1×10^4	IP Busulfan	hCD45+ cells > 25% in PB	Anti-PD-1, Anti-PD-L1, Anti-CTLA-4

(continued)

Table 3. Continued

Author [Year][ref.]	Graft		Host		Immunologic						Drugs or Intervention
	Subtype (Stage)	Tumor Source	Mouse Strain	Tumor Implant[a]	Type of IC	Inject Route	IC Source	IC Dose	Preconditioning	Successful IC Graft	
CDX ($n = 9$)											
Li [2018][28]	NSCLC	Cell lines (H292, HCC827)	NSG	SC, before (T-cells)/ simultaneously (PBMCs)/after (CD34+ HSCs) IC	Ex vivo expanded T-cells/PBMCs/ $CD34^+$ HSCs	IV (T-cells)/SC (PBMCs, HSCs)	Allogeneic (PBMCs - HD, HSCs - UCB)	2.5×10^6 (T-cell)	UK	UK	Anti-PD-L1
Wang [2019][29]	NSCLC	Cell lines (H292)	NOG	SC, after IC	PBMCs	IV	Allogeneic (commercial)	2×10^6	UK	UK	Anti-PD-1
Yao [2019][30]	NSCLC	Cell lines (H460, A549)	NSG	SC, before IC	Ex vivo expanded DNTs	IV	Allogeneic (HD)	2×10^7	UK	UK	DNTs + IL-2 +/− IL-15
Kotanides [2020][31]	NSCLC	Cell lines (H292, HCC827)	NSG	SC, before IC (T-cells)/ simultaneously (PBMCs)	Ex vivo expanded T-cells / PBMCs	IV (T-cells)/SC (PBMCs)	Allogeneic (commercial)	3×10^6 (T-cells)/ 0.5×10^6 (PBMCs)	UK	UK	BsAb (PD-1/PD-L1-bispecific)
Rios-Doria [2020][32]	NSCLC	Cell lines (H1975, H1299, A549, HCC827)	huNSG (commercial)	SC, after IC	$CD34^+$ HSCs	IV	Allogeneic (UCB, commercial)	UK	UK	UK	Anti-PD-L1
Rowe [2020][33]	NSCLC	Cell line (A549)	HuNCG (commercial)	SC, after IC	$CD34^+$ HSCs	IV	Allogeneic (UCB)	UK	Irradiation	UK	Anti-PD-1, Anti-CTLA-4
Taromi et al. [2022][34]	SCLC (III-IV)	Cell line (H69)	MITRG	OT (intra-thoracic), after IC	$CD34^+$ HSCs	IH	Allogenic (UCB)	2×10^5	Irradiation	hCD45+ cells (%) in PB	CAR-T (AC133-specific), CD73 inhibitor, anti-PD-1
Diwanji [2023][35]	NSCLC	Cell line (H358)	NSG	SC, after IC	FL, thymus, $CD34^+$ HSCs (Hu-BLT)	Surgery (FL, thymus)/IV (HSCs)	Allogeneic (Advanced Biosciences Resources)	UK	UK	UK	Anti-PD-1, anti-IL-1β
Meraz et al. [2023][36]	NSCLC, EGFRm (III-IV)	Cell line (H1975, H1975-OsiR)	NSG	SC, after IC	$CD34^+$ HSCs	IV	Allogeneic, HLA matched (UCB)	1-2 $\times 10^5$	Irradiation	hCD45+ cells > 25% in PB	Osimertinib, PDK1 inhibitor

[a]Tumor implantation site (OT or SC) and sequence related to immune cell injection (before or after).

ADC, antibody-drug conjugate; BiTE, bispecific T-cell engager; BsAb, bispecific antibody; CAR-T, chimeric antigen receptor T-cell; CDX, cell line-derived xenograft; CTLA-4, cytotoxic T-lymphocyte-associated protein 4; DLL3, delta-like ligand 3; DNTs, double-negative T-cells; EGFRm, epidermal growth factor receptor mutation; FAP, fibroblast activation protein; FGFRm, fibroblast growth factor receptor mutation; FL, fetal liver; HD, healthy donor; HLA, human leukocyte antigens; HSCs, hematopoietic stem cells; Hu-BLT, human bone marrow, liver, and thymus model; IC, immune cell; IH, intrahepatic; IL, interleukin; IP, intraperitoneal; IV, intravenous; MWA, microwave ablation; OT, orthotopic; PB, peripheral blood; PBMCs, peripheral blood mononuclear cells; PD-1, programmed cell death protein 1; PDK1, 3-phosphoinositide-dependent kinase 1; PD-L1, programmed death-ligand 1; PDX, patient-derived xenograft; TP53m, tumor protein p53 mutation; SC, subcutaneous; UCB, umbilical cord blood; UK, unknown.

especially using $CD34^+$ human stem cells, for pharmacology and toxicity evaluation of biologics, biosimilars, and small-molecule drugs.[61] Table 3 presents the current preclinical studies using humanized mouse models, especially in lung cancer, based on factors critical for successful model establishment.

Immune Checkpoint Inhibitors. ICIs, particularly those affecting the immunomodulatory function of T-cells (e.g., anti-programmed cell death protein 1 [PD-1] or programmed death-ligand 1 [PD-L1]), are the most studied drugs using humanized mice. NSCLC PDX models with Hu-$CD34^+$ HSC mice using UCB were used to investigate the antitumor effects of PD-1 inhibitors.[15] Pembrolizumab or nivolumab induced significant tumor regression in humanized PDX models, irrespective of the HLA status of $CD34^+$ HSC donors; no response was found in nonhumanized PDX mice. Anti–PD-1 therapy was linked to higher active $CD8^+$ T-cells and lower myeloid-derived suppressor cells (MDSCs) and T_{reg} cells in the splenocytes of treated mice compared with untreated controls,[15] suggesting the functionality of human ICs activated by ICIs in humanized PDX mice for tumor control.

Humanized mouse models are valuable for discovering biomarkers associated with responsiveness to ICIs limited to a narrow population of patients. NSCLC PDX models using Hu-$CD34^+$ HSC mice were used to recapitulate clinical differences between tumors categorized as hot (>5% TILs) or cold (<5% TILs) before ICI treatment in their cytokine profiles, molecular genetic aberrations, stromal content, and PD-L1 expression in tumor cells.[25] Moreover, ICI treatment, including anti–PD-1, anti-cytotoxic T-lymphocyte-associated protein 4 (CTLA-4), or the combination, enhanced the infiltration of $CD45^+$ ICs and TILs, particularly in cold tumors, and reduced stromal content across all tumors, indicating that unique TILs and stromal signatures could serve as reliable immunohistochemistry markers in preclinical studies.[25]

Antibody-Drug Conjugates, Bispecific Antibodies, and Bispecific T-cell Engagers. Antibody-drug conjugates (ADCs) are among the fastest-growing classes of novel therapeutics for patients with lung cancer with previous treatments.[62] The antitumor efficacy of fibroblast activation protein alpha (FAP-α)-targeting ADC (OMTX705) connected to a novel cytolysin (TAM470) was assessed in anti–PD-1-resistant NSCLC PDX models using NOG mice engrafted with $CD34^+$ HSCs.[17] The OMTX705 ADC exhibited complete and lasting antitumor activity as a monotherapy and when combined with an anti–PD-1 antibody. Notably, there was an increase in $CD8^+$ T-cell and $CD8^+CD25^+$ memory T-cell infiltration, alongside a reduction in $CD4^+CD25^+$ T_{reg} cell infiltration within PDX tumors, primarily when ADC was used in combination with anti–PD-1 therapy, suggesting TME modulation through the recruitment of $CD8^+$ cytotoxic T-cells.[17]

Bispecific antibodies (BsAbs) are designed to bind two different antigens or epitopes, simultaneously targeting two receptors or engaging ICs with cancer cells.[63] EGFR-positive NSCLC PDX models growing in the Hu-PBMC model were used to evaluate a humanized Fc-free EGFR-targeted 4-1BB-agonistic trimerbody ($4\text{-}1BB^{N/C}$ EGFR), composed of three anti-human 4-1BB antibody fragments (scFv) and three anti-human EGFR antibodies (V_{HH}).[20] Antitumor activity in vivo was noted, together with less severe GvHD-associated hepatotoxicity, a concern previously associated with the Fc region in IgG-based 4-1BB agonists, and an increase in tumor-infiltrating $CD8^+$ T-cells was noted in NSCLC PDX tumors.[20] LY3434172, a bispecific IgG1 monoclonal antibody with an Fc effector-null backbone targeting both PD-1 and PD-L1, was studied for its antitumor activity and immunomodulatory effects in NSCLC CDX models using Hu-PBMC mice or ex vivo expanded T-cells.[31] This PD-1-PD-L1 BsAb had significant antitumor efficacy in a dose-dependent manner compared with the parent antibody (anti–PD-1 or -PD-L1) monotherapy options or their combinations.[31]

Bispecific T-cell engagers (BiTEs) engage any $CD3^+$ T-cells regardless of MHC-TCR interaction and promote the proliferation of effector T-cells on binding to cytotoxic T-cells and tumor cells, resulting in effective tumor lysis.[64] Tarlatamab (AMG757), a half-life–extended BiTE targeting both delta-like ligand (DLL) 3 on cancer cells and CD3 on T-cells, is among the most promising BiTEs for recurrent SCLC. A preclinical study on AMG757 explored its efficacy and immunomodulatory function in SCLC PDX and orthotopic CDX models engrafted with PBMC-derived ex vivo expanded $CD3^+$ T-cells using NOG and NSG mice, respectively. Tarlatamab significantly boosted human T-cell activation and infiltration into tumors, including lung and liver metastases, and led to complete tumor regression in both SCLC PDX and CDX models.[21] In the following phase I and II studies (DeLLphi-300 and -301), tarlatamab had durable responses and promising survival outcomes with manageable safety profiles.[65]

Small-Molecule Inhibitors and Cytokine Therapies. Treatment with small molecules that interfere with intracellular negative regulators of the antitumor immune response associated with T-cell receptor (TCR) and cytokine signaling, COX2-PGE2 axis, cGAS-STING pathway, or adenosine pathway harboring CD73, has emerged as an innovative therapeutic strategy, either as monotherapy or

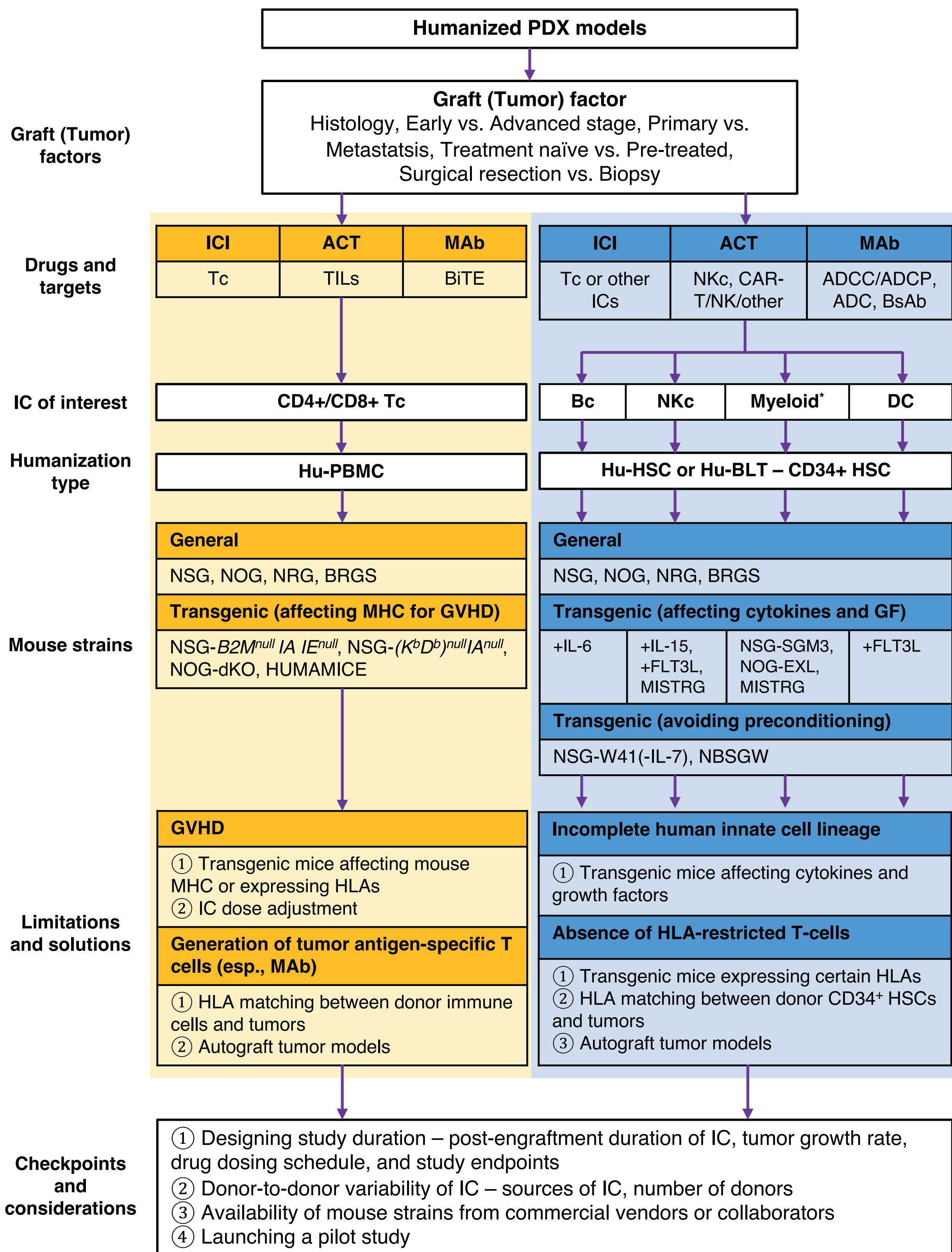

Figure 3. Optimized strategy for establishing preclinical humanized PDX models in solid tumors. *Monocytes, macrophages, and neutrophils. ACT, adoptive cell therapy; ADC, antibody-drug conjugate; ADCC, antibody-dependent cellular cytotoxicity; ADCP, antibody-dependent cellular phagocytosis; Bc, B cell; BiTE, bispecific T-cell engager; BsAb, bispecific antibody; CAR, chimeric antigen receptor; DC, dendritic cell; FLT3L, FLT3 ligand; GF, growth factor; HLAs, human leukocyte antigens; Hu-BLT, human bone marrow, liver, and thymus; Hu-HSC, human CD34+ hematopoietic stem cell; Hu-PBMC, human peripheral blood mononuclear cell; ICI, immune checkpoint inhibitor; ICs, immune cells; IL, interleukin; MAb, monoclonal antibody; MHC, major histocompatibility complex; NKc, NK cell; PDX, patient-derived xenograft; Tc, T0cell; TILs, tumor-infiltrating lymphocytes; xGVHD, xenogenic graft-versus-host disease.

in combination with ICIs.[66] Orthotopic CDX models with chemoresistant SCLC cell lines in CD34^{+} HSC-engrafted MITRG-SKI mice were used to reveal the efficacy of AC133-specific CAR T-cells in combination with anti–PD-1 and CD73 inhibitor (adenosine 5'-(α,β-methylene) diphosphate).[34] These models identified CD73 and PD-1-PD-L1 as immune-escape mechanisms, including CAR T-cell exhaustion, and revealed that triple immunotherapy enhanced the long-term complete response compared with monotherapies. In addition, AC133-positive cancer stem cells and PD-L1^{+}-CD73^{+} myeloid cells were identified as potential biomarkers for combination immunotherapy, as their proportions in xenograft tumors were significantly enriched after chemotherapy.[34] In addition to small molecules targeting negative regulators of immune responses, various kinase inhibitors (cabozantinib, palbociclib, rigosertib, apatinib, regorafenib, infilgratinib) and anti-inflammatory agents (cPLA2 inhibitor) have also been evaluated for their immunomodulatory effects or antitumor immunity, either as monotherapy or in combination with other classes of immunotherapy, using humanized xenograft mouse models in various solid tumors, including lung cancers (Table 1, Supplementary Tables 2 and 3).

Several cytokines are in preclinical or clinical development stages, often combined with other immunotherapeutic options. The antitumor effect of inhibiting IL-1β, a potent proinflammatory cytokine, was studied in NSCLC CDX models implanted with H358 cells using Hu-BLT NGS mice.[35] The anti–IL-1β antibody, canakinumab, improved the effectiveness of pembrolizumab but did not have efficacy as a monotherapy. Anti–IL-1β treatment alone or combined with anti–PD-1 led to substantial TME remodeling, with increased CD8^{+} T-cell tumor infiltration and decreased immunosuppressive cells (e.g., monocytes, MDSCs). Transcriptomic analysis in this study revealed significant differentially expressed genes associated with cancer-associated fibroblast (CAFs) and changes in CAF populations, particularly a reduction in inflammatory CAFs after canakinumab, suggesting that TME remodeling after IL-1β inhibition may stem from mesenchymal cells, including fibroblasts.[35] Although the potential utility of canakinumab in this preclinical study did not uniformly align with outcomes of phase III clinical trials (e.g., CANOPY-A, CANOPY-1),[67,68] humanized xenograft mouse models may offer a useful platform for applying cytokines to develop effective immunotherapy and identify optimal combination agents for various stages, treatment lines, and cancer types.

Cancer Vaccines. Vaccines have long been considered as a possible therapeutic option for cancer. The purpose of cancer vaccines is to educate the immune system to detect and destroy existing cancer cells resistant to other therapies or minimal residual cancer cells that could give rise to tumor recurrence. Depending on the source of the antigen, vaccines can be classified into oncolytic virus, nucleic acid, peptide, or cell-based vaccines. Similar to the antigens present in viruses and bacteria, cancerous cells can also express these tumor-associated antigens (TAA) or neoantigens, leading to high tumor immunogencity.[69] Although vaccines are very effective in preventing infectious diseases, developing cancer vaccines is challenging. Sipuleucel-T (Provenge; Dendreon) is the first FDA-approved therapeutic cancer vaccine, which targets prostatic acid phosphatase (PAP), an antigen that is highly expressed in prostate cancer.[70] Talimogene laherparepvec, or T-VEC (Imlygic; Amgen) is the first FDA-approved oncolytic virus composed of a genetically modified herpesvirus with GM-CSF gene, which is used by intralesional injection against advanced melanoma.[71] In addition, other therapeutic “off-the-shelf” or personalized vaccine candidates using mRNA and neoantigens are currently in clinical trial investigations.[72]

Humanized immune mouse models can provide an effective platform for screening neoantigens to develop targeted vaccines. In a study of CD133-positive glioblastoma (GBM) tumors isolated from patients, a human CD133 mRNA vaccine was transfected into the DCs and resulted in increased IL-12 secretion and a greater efficiency at activating the T-cells.[73] In this study, NOG mice were humanized by injecting CD34^{+} HSCs. After 40 days of HSC injection, these mice bearing intracranial human GBM tumors were randomized into the following three groups: (1) mice that received vaccination of CD133 transfected DCs; (2) mice that received DCs without mRNA transfection; and (3) mice that received phosphate-buffered saline. The population of CD3^{+}, CD4^{+}, and CD8^{+} T cells was significantly higher in group 1, and the median survival time of group 1 was 60 days, whereas those of groups 2 and 3 were 40 and 38 days, respectively. Immunohistochemical staining of the brain tissue revealed that there was no residual tumor in the humanized mice vaccinated with CD133 mRNA transfected DC, whereas both groups 2 and 3 had large tumors with infiltration of CD3^{+} T cells. Xie et al.[74] used the Hu-PBMC model with NCG mice and transplanted luciferase-positive Nalm6, a human acute lymphoblastic leukemia (ALL) cells, 2 weeks after humanization. After a week, these humanized mice were then vaccinated with Eps8- (an antigen highly expressed in ALL patients) and PD-1 antibody-loaded microcapsules, and the therapeutic performance of this co-encapsulated vaccine was studied. A single dose of these microcapsules led to significant inhibition of the luciferase signal, and mice had a prolonged survival time (five of eight mice were still alive after 6 weeks), whereas all the mice in the control groups died within 5 weeks. These studies revealed that humanized mouse models can provide a robust and cost-saving

platform to conduct an early evaluation of novel vaccine candidates and test their efficacy before clinical trials.

Overcoming Limitations to Optimize Humanized PDX Models in Lung Cancer

To enhance the success of a preclinical study with IO drugs using humanized PDX models, it is essential to weigh the advantages and disadvantages of each humanization strategy (Table 2) and the type of immunodeficient mice (Supplementary Table 1). Figure 3 outlines the selection of critical components, including ICs of interest, humanization type, and mouse strains, in establishing humanized PDX models.

Graft-Versus-Host Disease. In the Hu-PBMC model, GvHD usually develops within 4 to 8 weeks of PBMC engraftment and is caused by the rapid expansion of human $CD8^+$ T-cells that start to target the mouse tissues through recognition of MHC-I proteins in the mouse cells.[75] Increased engraftment rate of PBMCs led to reduced survival times, likely due to the abundance of T-cells in these mice leading to GvHD onset and development of cytokine storm.[48] Development and severity of GvHD can be affected by several factors: dose of PBMCs, transgenic mouse strains, or preconditioning (Supplementary Materials). GvHD onset in the Hu-PBMC model can be delayed by injecting a smaller number of PBMCs (1–5 versus 10 million).[48] The lack of MHC-I or -II molecules in mice can prevent GvHD development in the Hu-PBMC models. MHC-deficient strains can be generated by the knockouts of beta-2 microglobulin ($\beta 2m^{null}$) or MHC heavy chain alleles, such as H2-K and H2-D $(K^bD^b)^{null}$ or H2-IA and H2-IE (Supplementary Table 1). To mitigate the occurrence of GvHD and extend the therapeutic window, transgenic mice with inactivated mouse MHC genes (e.g., NSG-MHC-I or -II KO, NOG-dKO) have been used in preclinical IO research (Fig. 3, Supplementary Tables 1 and 2). A NOG-MHC-dKO mouse model revealed no predominant signs of GvHD until 12 weeks post-PBMC injection, highlighting the utility of dKO models for evaluating immunotherapies.[76]

Incomplete Development of Innate Cell Lineage. Both Hu-PBMC and Hu-$CD34^+$ HSC models lack complete reconstitution of all human immune populations, as the mouse cytokine environment cannot support the full development of all human immune lineages.[47] This is due to limited cross-reactivity between human cytokine receptors and mouse cytokines. Exogenous delivery of Flt3-L or GM-CSF into the Hu-$CD34^+$ HSC model can enhance the survival and proliferation of the myeloid cells,[77] whereas exogenous delivery of plasmids encoding human IL-15 with Flt3-L, GM-CSF with IL-4 and Flt3-L, and M-CSF resulted in increased NK cell, DC, and macrophage and monocyte activation and development, respectively.[78] Despite the usefulness of these cytokines in IC expansion and differentiation, exogenous delivery leads to nonphysiological concentrations and can cause aberrant cell development and trafficking. Therefore, genetic modifications through knock-in of genes to increase the innate IC populations have also been investigated. HIS with multilineage hematopoiesis can be achieved in Hu-HSC models using mice with transgenic expression of key cytokines and growth factors for individual IC components, such as MISTRG, MISTRG6, MITRG, NOG-EXL, and NSG-SGM3 for human myeloid cell development and MISTRG and NSG with transgenic expression of IL-15 (NSG-Tg [Hu-IL15]) for NK cell development (Fig. 3, Supplementary Tables 1 and 2).

Absence of HLA Expression. In the Hu-HSC model, T-cell development in the early phase can be restricted by a lack of HLA expression in the murine thymus, resulting in the absence of HLA-restricted T-cells. This leads to T-cells that cannot recognize the antigen presented by HLA^+ cells in the peripheral tissues. HLA expression in the thymus is also essential for human T-cell development and maturation, which consequently is integral for B cell stimulation toward immunoglobulin class switching and antibody selection.[79] To overcome these hurdles without the technical challenges of the Hu-BLT model, transgenic mice expressing HLA class I or II (e.g., DRAG, DRAGA) have been introduced in several studies (Fig. 3, Supplementary Tables 1 and 2). The expression of transgenic HLA class I in mice can improve $CD8^+$ T-cell response in an HLA-A2–restricted manner; however, reconstitution of T- and B-cell response and functionality remained a severe limitation due to lack of HLA-restricted CD4+ T-cell help.[80,81] Alternatively, transgenic expression of HLA class II, with or without expression of class I, increased the proportion of functional B- and T-cells with appropriate immune responses, including immunoglobulin class switching and antigen-specific antibody production.[82,83]

Other Considerations. ICs evaluated in preclinical IO studies are chosen based on the target of investigational drugs, and the selection of humanization strategy depends on the experiment's hypothesis and the ICs under investigation (Fig. 3). For instance, if the mechanism of action of an investigational drug focuses on targeting mature human ICs, particularly T-cells, and the study's end points are centered on interactions between mature human ICs and tumors, adult PBMCs may be the optimal source for engrafting a mature HIS into immunodeficient mice. In contrary, Hu-HSC models can lead to the

development of various immune lineages to support DC-mediated T-cell priming and provide stable and long-term humanization with minimal risk of GvHD. Nevertheless, multiple factors may affect the engraftment of human ICs into mice, including the mouse age, source of human ICs, dose of injected ICs, and a preconditioning regimen. In addition, the time required for engraftment of ICs according to humanization models (Fig. 2), tumor-specific growth kinetics, drug dosing schedule, and study end points should be considered when determining the duration of preclinical studies using humanized mouse models.

Donor-to-donor variability can be observed in ICs used for engraftment and implanted tumors. Although humanized PDX models can closely mimic the tumor biology, immunology, and drug responses of the parent tumor,[37] the efficacy of investigational drugs may vary, even among different PDXs derived from the same patient's tumor. Furthermore, the intrinsic variability of ICs obtained from various donors and sources (e.g., $CD34^+$ HSCs from UCB, fetal liver, bone marrow, or peripheral blood) may influence the overall engraftment rates, the kinetics of human IC engraftment, and the therapeutic response to IO drugs. Therefore, Verma et al.[38] advised the creation of separate parallel batches of cohorts of mice engrafted with ICs from at least three or four different donors. Ultimately, it is recommended that a pilot experiment be conducted to consider all aspects of the current capacity and facilities, ensuring the protocol optimization for the relevant preclinical IO study is confirmed.

Future Perspectives and Conclusion

Humanized mouse models have emerged as a promising tool for evaluating the efficacy of novel and already approved immunotherapeutic agents, especially beneficial in studying combination treatment strategies. For instance, double-negative T-cells (DNTs), characterized by the expression of CD3 in the absence of CD4 and CD8, account for 3% to 5% of mature T-cells in the peripheral blood. Yao et al.[30] reported that using humanized NSG mice, treatment with ex vivo expanded DNTs can lead to tumor regression in NSCLC CDX models. Notably, infusion of allogenic DNTs in NSG mice does not induce GvHD. Owing to their unique characteristics, DNTs are currently in clinical investigation for their potential role as "off-the-shelf" adoptive cellular therapies (NCT03027102).

Although adoptive cell therapy, including CAR cell therapy, has not been successful for most solid tumors, lung cancer emerges as a promising candidate for cell therapy due to its biological characteristics of many somatic mutations and tumor neoantigens. Nevertheless, CAR cell therapy faces challenges within the solid tumor context, including physical barriers impeding immune infiltration, and the hypofunction of engineered ICs due to potential resistance mechanisms, such as antigen escape, tumor heterogeneity, immunosuppressive TME, and T-cell exhaustion.[84] Humanized mouse models have been of great value for the preclinical evaluation of CAR cell therapies in vivo in terms of efficacy, toxicity, persistence, exhaustion, and immune responses around TME and for optimizing CAR designs, including in vivo CAR cell generation.[85] In a study using NOG-EXL mice engrafted with $CD34^+$ HSCs, Cao et al. developed AXL-directed CAR T-cells, revealing significant, albeit modest, antitumor activity in both subcutaneous and pulmonary metastasis mouse models of AXL-positive NSCLC xenografts. Combination with microwave ablation significantly enhanced tumor regression synergistically without toxicities in humanized PDX models, augmenting the activation, infiltration, and persistence of AXL-CAR T-cells through TME remodeling.[23] Based on preclinical studies, many clinical trials in the early phase are evaluating the efficacy of CAR-T therapies in lung cancer targeting tumor antigens, such as MSLN (NCT02414269), MUC-1 (NCT03525782), GPC3/TGFβ (NCT03198546), PD-L1 (NCT03330834), and ROR1 (NCT02706392).

As discussed, significant challenges in establishing humanized mouse models remain an area of improvement. Instead of rodents, a chicken embryo model with its chorioallantoic membrane has re-emerged as a relevant PDX model for studying tumor biology, including angiogenesis, immunology, and preclinical research of conventional and novel treatment modalities, possessing advantages over other models, including cost-effectiveness, time efficiency, and simplicity.[86]

Despite all their limitations, humanized mouse models have provided the most predictive platform to mimic and study various aspects of cancer immunology and immunotherapy. Studies using these systems have already given us a significant understanding of crosstalk with IC lineages and cancer cells and a mechanistic understanding of immunotherapies. With the rising interest in immunotherapies for cancer treatment, the efficient and robust establishment of these models is a significant need, as they can be used as preclinical models for evaluating novel IOs.

CRediT Authorship Contribution Statement

Cheol-Kyu Park: Conceptualization, Methodology, Formal analysis, Data curation, Writing - Original draft, Writing - Review and Editing.

Maryam Khalil: Conceptualization, Methodology, Formal analysis, Data curation, Writing - Original draft, Writing - Review and Editing.

Nhu-An Pham: Conceptualization, Writing - Review and Editing, Project administration

Stephanie Wong: Conceptualization, Writing - Review and Editing.

Dalam Ly: Conceptualization, Methodology, Writing - Review and Editing.

Adrian Sacher: Conceptualization, Writing - Review and Editing.

Ming-Sound Tsao: Conceptualization, Writing - Review and Editing, Project administration, Funding acquisition.

Disclosure

The authors declare no conflict of interest.

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Supplementary Data

Note: To access the supplementary material accompanying this article, visit the online version of the *JTO Clinical and Research Reports* at www.jtocrr.org and at 10.1016/j.jtocrr.2024.100781.

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