Humanized mice for the study of immuno-oncology

Philippe De La Rochere[1], Silvia Guil-Luna[2], Didier Decaudin[3], Georges Azar[4], Sukhvinder Sidhu[4], Eliane Piaggio[1]

[1] INSERM U932, and SiRIC Translational Immunotherapy Team, Translational Research Department, Institut Curie, PSL Research University, Paris F-75005, France.

[2] Maimónides Institute for Biomedical Research of Córdoba (IMIBIC), Córdoba, Spain.

[3] Laboratory of Preclinical Investigation, Translational Research Department, and Department of Medical Oncology, Institut Curie, PSL Research University, Paris F-75005, France.

[4] Sanofi Oncology, 1 Impasse des Ateliers, Vitry Sur Seine, France.

Correspondence: eliane.piaggio@curie.fr (E. Piaggio)

Keyword: Humanized mice, immuno-therapy, pre-clinical research

ABSTRACT

Immunotherapy is revolutionizing cancer treatment, however, complete responses are achieved in only a small fraction of patients and tumor-types. Thus, there is an urgent need for predictive preclinical models to drive rational immunotherapeutic drug development, combinations and minimize failures in clinical trials. Humanized mouse models have been developed to study and modulate the interactions between immune components and tumors of human origin. In this review, we discuss recent advances in the "humanization" of mice to improve the quality of immune reconstitution, the new insights gained into the basic mechanisms and preclinical evaluation of onco-immunotherapies, and also the limitations, which constitute the drivers for the improvement of the models and the increase of their translational power.

Immunotherapy in oncology: the need for preclinical models

Immunotherapies of cancer represent a significant leap forward in the successful treatment of cancer with unprecedented long-term survival rates in a growing number of indications [1]. However, many patients still do not benefit from these immunotherapies, leading to an increased focus on identifying novel immunotherapies or combinations that can prolong responses or convert non-responders. To this effect, there is an increasing demand for more predictive preclinical models to drive rational immunotherapeutic drug development, combinations, and minimize failures in clinical trials.

Rodent models have long been key tools to carry out biomedical research. Given the need of experimental models recapitulating human biology, mice represent one of the most widely used sources of animal models. The four major approaches with mouse models used to assess immunotherapies today include: syngeneic mouse tumor models with fully immune-competent hosts, genetically engineered mouse models (GEMMs), chemically induced models and “humanized” mouse models. While the first three approaches are widely used, one major drawback is that they rely on a mouse immune system, which cannot always recapitulate the human immune response. Preclinical models recapitulating a functional human immune system are therefore highly desirable.

Humanized mouse models, are composed of three elements: **1)** immunodeficient host mice, **2)** human immune cells, and **3)** human tumor cells. This review discusses the advantages and caveats of these humanized mouse models to study cancer immunotherapy.

38

39

40 ## 1. Immunodeficient host mice

41 Since the discovery of ***scid*** (severe combined immunodeficiency) mutated mice
42 in the 1980s [2] and their ability to host human peripheral blood mononuclear cells
43 (PBMC) [3], fetal hematopoietic tissues [4] or hematopoietic stem cells (HSC) [5],
44 immunodeficient mice have steadily become more sophisticated. The study of
45 hematopoiesis has benefited from models using immunodeficient mice, just as the
46 evaluation of infectious diseases, auto-immunity and GvHD (Graft versus Host Disease)
47 [6,7]. Nevertheless, for cancer immunotherapy, a complication arises, as the models
48 must simultaneously tolerate the transplantation of human tumors and human
49 immune cells.

50 The first model that allowed human tumor transplantation was the **nude** mouse (**see**
51 **glossary**), which lacks T cells [8]. But since then, it has become clear that the more
52 immunodeficient the mice, the better the engraftment efficacy, especially in models
53 lacking NK cell activity [9]. The same applies for the reconstitution of the human
54 immune system. Xeno-reactivity towards the human graft, whether tumor or
55 hematopoietic cells, is due to the recognition of the human cells by the mouse innate
56 and adaptive immune systems as foreign. The first approach to avoid xeno-reactivity
57 was the generation of mice lacking T and B lymphocytes due to mutations of immune-
58 related genes: 1) the protein kinase DNA-activated catalytic polypeptide (*Prkdc)* gene
59 mutation (that underlies the *scid* phenotype) which affects DNA repair [2], and 2) the
60 recombination activating genes 1 and 2 (*Rag-1* or *Rag-2*) mutations [10]. The *Rag*
61 mutations disrupt the V(D)J recombination necessary for T and B receptor generation,

62 leading to a block in T and B cell development and survival. The engineering of these
63 mice defective for adaptive murine immunity, allows human hematopoietic
64 reconstitution, although with low and variable levels of engraftment.

65 By comparing human immune reconstitution efficiencies in different mouse
66 backgrounds, the SCID mutation on the **NOD** (Non Obese Diabetic) background
67 showed a clear advantage. The difference observed was driven by accumulated defects
68 in NK cells, macrophage activity and in the complement system, allowing for at least a
69 5 fold better human immune reconstitution compared to the original CB-17 SCID mice
70 [6]. The next step in significantly improving the quality and levels of human immune
71 system reconstitution was achieved by knocking-out the common γ-chain of the IL-2
72 receptor [11,12] (IL-2Rγ_c; shared by the receptors of IL-2, IL-4, IL-7, IL-9, IL-15 and IL-
73 21), allowing for the loss of murine NK cells. The combination of the SCID mutation or
74 RAG KO with the IL-2Rγ_c KO gave rise to a "new generation" of severely
75 immunodeficient mouse models, namely **NSG** (NOD.Cg-*Prkdc*^{scid} *Il2rg*^{tm1Wjl}/SzJ) [6], **NOG**
76 (NOD.Cg-*Prkdc*^{scid} *Il2rg*^{tm1Sug}/JicTac) [6] and **BRG** (BALB/c *Rag2*$^{-/-}$ *IL-2R*$\gamma_c^{-/-}$) mice [13] .

77 Interestingly the C57BL/6 mice carrying the same *Rag and* γ_c KO are still capable
78 of rejecting xeno-grafted human cells [12,14], highlighting the implication of other
79 rejection mechanisms in that particular genetic background. Takenaka *et al.* [14]
80 demonstrated that the NOD's genetic background, but not the C57BL/6's, codes for an
81 allele of *sirp*α that strongly interacts with human CD47 molecule, in contrast to other
82 mice strains [13,14]. Indeed the *sirp*α gene is essentially expressed on myeloid cells
83 and codes for an inhibitory immunoglobulin superfamily transmembrane protein
84 (CD172a) that acts as a "don't eat me signal" when interacting with CD47, its
85 ubiquitously expressed cognate ligand.

86 These findings led to the development of the “next generation“ of humanized
87 immune system (HIS) mice in which, the transfer of the NOD.*sirp*α allele (BALB/c *Rag2*$^{-}$
88 $^{/-}$ *IL-2R*$\gamma_c^{-/-}$ NOD.*sirp*α*:* **BRGS**) [13] or even a human *sirp*α (**SRG**) [15] to other genetic
89 backgrounds, increased their tolerance to human hematopoietic stem cell xeno-graft
90 and justified the noted difference between the C57BL/6 and other mouse genetic
91 backgrounds. These new HIS mice showed more robust reconstitution levels and
92 reproducibility, and allowed the initial studies on immuno-oncology therapies, which
93 nevertheless highlighted an important flaw: immune reconstitution is not optimal and
94 the human myeloid compartment is still largely underrepresented. In the next
95 paragraphs we will describe the main approaches used for immune cell reconstitution
96 in HIS models, and then discuss the novel developments aiming at improving
97 hematopoietic reconstitution in the host mice.

98

99 ## 2. Mice humanization

100 Two major sources of human immune cells are currently used for the
101 establishment of a functional human immune system: i) human peripheral blood
102 mononuclear cells (PBMCs), and ii) human CD34+ HSC; which are used in three types of
103 models with their own advantages and limitations: **Hu-PBL** (peripheral blood
104 lymphocytes), **Hu-CD34+** (also named Hu-SCR for **“**scid-repopulating cell”) and **BLT**
105 **mice** (bone marrow-liver-thymus), described in detail below (**Figure 1A, Key figure**).

106

107 ### *2.1. PBMCs: Hu-PBL model*

108 The simplest and most economic version of humanization consists in engrafting
109 human leukocytes in immunodeficient mice, known as **Hu-PBL**. This approach was first

110 described in 1988 using CB17-*scid* mice [3] and has been widely used for the study of
111 human immune responses in autoimmunity and infectious diseases.
112 Human leukocytes can be obtained from PBMCs, spleen or lymph nodes.
113 Typically, PBMCs are obtained from healthy donors, which are not MHC (major
114 histocompatibility complex)-matched with the tumor graft leading to variations in
115 intrinsic allogenicity. In our hands, including in each experimental group mice
116 reconstituted each with a different PBMC donor is an appropriate strategy to
117 compensate for donor variability. PBMCs can be injected intravenously (i.v.) (most
118 routinely used), intraperitoneally (i.p.), or intrasplenically into adult mice.
119 Among the PBMC inoculum, besides mature human leukocytes, there are a few
120 HSCs, which are unable to colonize the murine host due to the lack of a proper
121 microenvironment. Very low levels of human B cells and myeloid cells are observed,
122 probably due to the lack of the human cytokines required for their survival [16–18].
123 Interestingly, low levels of human IL-1β, GM-CSF, IFN-γ, IL-10, IL-2 and IL-5 have been
124 detected in this model, which may contribute to the survival of the human cells [19].
125 Thus, T cells are the main immune subpopulation that is present and remains
126 functional in the murine host. In our experience, an injection of 20 x 10^6 PBMCs
127 typically results in ~50% of human CD45+ cells in the murine peripheral blood after 4
128 weeks of engraftment. Around 90% of the human CD45+ cells are CD3+ T cells with an
129 activated/memory phenotype and a roughly 1:1 CD4:CD8 ratio, which is maintained 4-
130 6 weeks after PBMC injection **(Figure 2A)**. The main caveat of this model is that it
131 invariably leads to lethal xeno-GvHD [3,11,18], which can be evaluated by body weight
132 loss [20] **(Figure 2B)**. The onset of GvHD is directly correlated with the degree of
133 human T cell engraftment, and previous sub-lethal irradiation accelerates its onset

134 [18]. Thus, the therapeutic observational window is restricted to a few weeks (usually
135 4-6 weeks after PBMCs injection) before evident signs of GvHD [11,18]. Interestingly,
136 CD4+ T cells seem to play a major role in the induction of GvHD in Hu-PBL mice, as
137 depletion of CD4+ cells from PBMCs before inoculation alleviates clinical symptoms
138 and extend mice survival [21].

139

140 ### *2.2. CD34+ stem cells: Hu-CD34+ and BLT models*

141 Another approach to humanization is by the injection of human CD34+ HSCs
142 into newborn or adult immunodeficient recipients: **Hu-CD34+ model** [9], **(Figure 1A)**.
143 The success of engraftment is highly variable, depending on i) HSC source: human
144 umbilical cord blood [11,12], adult bone marrow [22], granulocyte colony-stimulating
145 factor-(G-CSF) mobilized PBMCs [23] or fetal liver [22]; ii) route of injection i.v. or
146 intrafemoral in adult mice; and i.v., intracardiac or intrahepatic in newborn mice; and
147 iii) age, strain and sex of recipient: newborn or young mice (up to 4 weeks of age)
148 allows an accelerated T-cell development in comparison to adult mice [24]. This
149 approach requires sub-lethal γ-irradiation of the host mice to deplete mouse HSCs and
150 facilitate human HSC engraftment. Alternatives to irradiation have been reported,
151 including busulfan [25] and antibody-mediated deletion of mouse progenitor cells [26].
152 Fetal liver has also been used extensively for making the "**BLT model**" (for
153 "bone marrow/liver/thymus") (**Figure 1A**). This model is generated by the
154 transplantation of human fetal liver and thymus tissue into the sub-renal capsule,
155 simultaneously with the i.v. injection of autologous CD34+ cells from the same fetal
156 liver into adult immunodeficient mice [27].

157 In the Hu-CD34+ model, all human hematopoietic lineages are represented, but
158 not all are functionally fully developed [11]. The majority of the human B cells are
159 immature CD5+ B cells, CD4+ T cells show a memory phenotype, and both T and NK cells
160 display some functional impairment [28,29]. The differentiation of the myelo-
161 monocytic lineage is also impaired and monocytes are phenotypically immature [30]
162 (**Figure 2C**). Although the mouse thymus supports human T cell development, the
163 question of MHC restriction is still unclear. Halkias *et al* have shown that the human
164 thymocytes have similar behavior in mouse and human thymic environments and that
165 they serially interact with human hematopoietic cells as well as with mouse tissue in
166 HIS mice thymus [31]. Furthermore, Watanabe et al. [29] have shown that the mouse
167 thymic environment is essential for human T cell development but that the mouse I-A
168 MHC molecule is not, suggesting that human CD4+ TcR repertoire is possibly restricted
169 by HLA class II molecules as well as by murine MHC.

170 In the BLT model, the transplanted human fetal liver and thymus provide a
171 human thymic microenviroment that supports the development of human T cells and
172 their selection on human MHC molecules. However, a positive selection in the thymus
173 occurs exclusively on human cells, and T cells with affinity for mouse MHC are not
174 eliminated, with the consequence of higher incidence of GvHD than seen in other
175 CD34+ HSC engrafted models.

176 Overall, although these models constitute a great advancement, some aspects
177 need to be improved, like the incomplete engraftment of immune cells, the xeno-
178 GvHD and the lack of human cytokines and growth factors. The table below (**Table 1**)
179 compares the different features of Hu-PBMC and Hu-CD34+ models.

180

3. Tumors of human origin: tumor cell lines and PDXs

Both human cell lines and patient-derived-xeno-grafts (PDX) represent relevant preclinical tools for immunotherapy assessment. Importantly, various criteria related to the tumor molecular features and to the experimental design should be taken into account when choosing cell lines or PDXs (reviewed in **Table 2).**

PDXs have been associated with a high predictive value for therapeutic responses to oncology treatments in cancer patients, including chemotherapy and targeted therapy [32]. Moreover, PDXs have been used for *in vivo* therapeutic screening of targeted therapies using a single-mouse schedule [33]. Such an approach, which decreases the number of mice, and costs, is able to (i) identify the best treatment or combination of treatments among all tested in a panel of PDXs, and (ii) validate the efficacy of tested therapies in selected target-specific tumors. Nevertheless, such pre-clinical studies have not yet been developed for immune therapies. Moreover, evaluation of radio, chemo and targeted therapies in HIS mice, in the context of a functional immune system, could be of high interest.

One advantage of PDXs is that they can allow a personalized therapeutic management of cancer patients in the so-called “AVATAR” approach, where a patient’s tumor is grafted into immunodeficient mice and, after in vivo growth and molecular characterization of the tumor, a pharmacological experiment is performed to assess the efficacy of treatments that could be, in a second time, administered to the PDX-originating patient (Figure 1B) [34]. Theoretically, HIS mice could also be used as avatars for the evaluation of immunotherapies. Along this line, Jespersen *et al* have recently shown that adoptively transferred TILs were able to kill autologous PDXs (provided human IL-2 was continuously supplied), and that for the few patients tested,

205 eradication of the tumor was correlated with the objective response to adoptive T cell
206 therapy in the clinic [35]
207
208 ## 4. New developments in HIS mouse models
209 The previously described models are limited in their ability to sustain functional
210 myeloid, NK and B cell populations, which are required for the evaluation of cancer
211 immunotherapies. Thus, we will describe here the different approaches that have been
212 developed to tackle this issue, and are summarized in **Table 3**.
213
214 ### *4.1- Niche preparation for HSC engraftment*
215 HIS models require myeloablative conditioning of the host mice before
216 transplanting human HSCs [23] to create the required space in the host's bone marrow
217 niche for human HSC engraftment. Of note, susceptibility to irradiation is strain
218 dependent: the s*cid* mutation leads to increased sensitivity to radiation-induced DNA
219 damage, than the *Rag1*^{null} or *Rag2*^{null} mice [36]. Recently, the *c-kit* (CD117) mutant
220 mouse has been identified as a suitable host for human HSC engraftment without the
221 need for prior irradiation. As c-kit is involved in HSC maintenance and differentiation,
222 mice harboring the w41 mutation in *c-kit* (**NSGW41** mice) have reduced HSC numbers,
223 which translates into lower competition and better engraftment of human HSCs
224 [37,38]. The NSGW41 mice also sustain more efficient human platelet and erythroid
225 development [37], relevant for the evaluation of platelet activity in the tumor setting.
226 Dendritic cells (DCs) also show impaired reconstitution in HIS mice. Knocking-out
227 *Flt3* (Fms-like tyrosine kinase 3), which essential for DC development, leads to
228 improved human DC development at the expense of the murine counterpart [39]. The

229 resulting humanized **BRGF** (BALB/c *Rag2*$^{-/-}$ *IL-2R*$\gamma_c^{-/-}$ *Flt3*$^{-/-}$) mouse shows better human
230 monocyte and DC development compared to its parental BRG strain, and improved DC
231 homeostasis results in increased numbers of human NK and T cells [39]. Transferring
232 the *Flt3* KO on the BRGS strain further increases NK cell levels and can even allow
233 limited study of human ILC (Innate lymphoid cell) development [40].

234

235 4.2- Improvement of myeloid and Natural Killer cell reconstitution

236 As mentioned previously, human myeloid cells are underrepresented or have
237 maturation and functional defects in the current generation of HIS models [30]. One
238 strategy to increase the number and maturation of myeloid cells is the hydrodynamic
239 injection of plasmids coding for human IL-4, GM-CSF or Flt-3 ligand, or M-CSF [41]. HIS
240 mice of different genetic backgrounds have been knocked-in with human *SCF*, *GM-CSF*,
241 *IL-3*, *TPO* or *SIRP*α. In the NOD background, NSG mice have been knocked-in with
242 human *SCF* (c-kit ligand), *GM-CSF* and *IL-3* (**NSG SGM3**) [42] and NOG mice with human
243 *GM-CSF* and *IL-3* (**NOG-EXL**) [43]. Also, human *IL-3* and *GM-CSF* have been introduced
244 in the BRG background [44]. All these strategies show significant increases in the
245 numbers of myeloid cells and in the function of macrophages [43,44] compared to
246 parental strains.

247 In parallel, the BRG mouse has been knocked-in with the human
248 thrombopoietin gene (*TPO)*, which resulted in higher human HSC engraftment and
249 better myeloid development. Subsequently, the BRG-human TPO mice was knocked-in
250 with the NOD.sirpα, hIL-3 and human M-CSF genes, giving rise to the **MISTRG** mice (M-
251 CSF, IL-3, Sirpα, TPO, Rag2$^{-/-}$ IL-2R$\gamma_c^{-/}$) [45]. MISTRG mice support superior levels of
252 myeloid development, increased differentiation of monocytes, dendritic cells and

253 macrophages, and higher NK development. However these mice: i) develop anemia
254 [45], ii) have shorter lifespans, and iii) exhaust the human graft 3-4 months after
255 transplantation.

256 Supplementation with human IL-2 and/or IL-15 has been attempted to increase
257 NK cell reconstitution. Injection of a DNA vector coding for IL-15 [41] or administration
258 of IL-15/IL-15Rα [46] increased human NK cell numbers in immunodeficient mice.
259 Interestingly, Katano and colleagues developed two mice with favored NK cell
260 differentiation: the **NOG-IL2 Tg**, expressing human IL-2 [47] and the **IL-15-NOG Tg** ,
261 expressing human IL-15 [48]. Also, Flavell's team generated the BALB/c Rag2-/- IL-2rγ_c-
262 /- knock-in for human SIRPα and IL-15 (**SRG-15**) [49], which showed enhanced
263 development and function of NK cells, CD8+ T cells and tissue-resident ILCs.

264

265 *4.3- MHC manipulation*

266 To avoid xeno-GvHD, which can be acute in Hu-PBL mice, or chronic in Hu-SRC
267 mice, different strategies have been developed based on the genetic manipulation of
268 the MHC molecules. Administration of PBMCs into NSG mice lacking mouse class I
269 and/or class II MHC molecules, such as NSG knocked-out for mouse beta-2
270 microglobulin (β2m), a structural component of the MHC class I molecule [18], or NOG
271 knocked-out for mouse MHC class I and class II molecules [50], led to the engraftment
272 of the human immune cells (albeit at poorer rates) and showed limited xeno-GvHD. In
273 the case of Hu-CD34+ mice, the mismatch between human and mouse MHCs, besides
274 inducing GvHD, likely underlies defective T cell function. HSC infusion into NSG mice
275 with homozygous expression of HLA class I heavy and light chains (**NSG-HLA-A2/HHD**)
276 allowed the generation of functional HLA-restricted T cells [51]. Moreover,

277 transplantation of HLA-DR-matched HSC into NOD.Rag1KO.IL-2Rγ_cKO mice transgenic
278 for the HLA class II molecule HLA-DR4 (**DRAG**), highly reconstituted T and B
279 lymphocytes. Furthermore, these mice produced all subclasses of immunoglobulins
280 and of antigen-specific IgGs upon vaccination, demonstrating the critical role of HLA
281 class II molecules in the development of functional T cells capable of ensuring
282 immunoglobulin class switching [52]. A similar observation was found in NOG mice
283 expressing the HLA-DR4 molecules in MHC II-positive cells [53]. More recently, Lone
284 YC's group has generated a mouse combining both murine MHC deficiency and HLA
285 transgene expression named "**HUMAMICE**" (HLA-A2+/+/DR1+/+/H-2-β2m-/-/IAβ-/-
286 /Rag2-/-/IL2Rγ_c-/-/perf-/-) [54]. This mouse has no T and B cells due to the Rag
287 mutation, no NK cells due to IL2Rγ_c mutation and no residual cytolytic activity due to
288 perforin knockout.
289
290 ### *4.4 Humanization of immune checkpoints in immunocompetent mice*
291 An alternative approach to the use of HIS mice for the study of anti-immune
292 checkpoint antibody-based immunotherapies has been the development of humanized
293 target knock-in mice in immunocompetent C57BL/6 or BALB/c mice. The major
294 advantage of these mice is that a clinical candidate can be evaluated in this model,
295 albeit with a fully murine immune system, but there is no need to generate murine
296 surrogates. A growing number of immunocompetent mice genetically modified to
297 express one or more fully human genes or "humanized" knock-ins coding for positive
298 and negative immunomodulatory receptors and ligands such as PD-L1, CD47, BTLA,
299 CD137, TIM3, LAG-3, ICOS, GITR, OX40, OX40L, among others have been generated
300 and are commercially available by different companies. These mice are particularly

attractive for the evaluation of IO checkpoint combinations. Mice expressing "humanized" CTLA-4 or PD-1 molecules [55,56] have been useful to dissociate efficacy and autoimmunity induced by anti-CTLA-4 antibodies [55], and to characterize a clinical candidate anti-PD-1 antibody [56].

5. Pre-clinical evaluation of cancer immunotherapy in humanized models

HIS mice represent one of the most attractive pre-clinical models for screening of immunotherapeutic approaches including cellular and antibody-based immunotherapy, immune checkpoint inhibitors, or even gene therapy. A summary of pre-clinical evaluation of immune-based therapies performed in HIS mice is presented in **Table 4.**

5.1. Cell-based immunotherapy

The recent progress in the use of humanized mice has provided new developments to assess the efficacy of CAR-T cells. Of note, after several preclinical studies, the Food and Drug Administration (FDA) approved the first CAR-T treatment for B-cell acute lymphobastic leukemia in 2017. One of the first studies in this area showed that CAR-T cells designed to recognize mesothelin, an antigen highly expressed on mesothelioma cells, exerted potent antitumor effects on malignant mesothelioma of Hu-PBL-mice [57]. The efficacy of other CAR-T cells evaluated in HIS mice is summarized in **Table 4** [57–65]. However, CART-T therapy has shown serious adverse events such as off-tumor toxicity, cytokine release syndrome or neurotoxicity, which are not reproduced in HIS mice. This is partially due to the lack of the human target expression in normal tissues. The development of more sophisticated HIS models

325 should help to provide safer and more effective CAR-T therapy. For example,
326 transgenic expression of the CAR-T cell targeted human tumor-associated-antigen
327 under the mouse endogenous promoter could help identify off-target effects, as
328 already shown for immunocompetent mice [66]. However, a good understanding of
329 the human target expression is required and validation that the murine equivalent has
330 a similar expression pattern.

331 Adoptive natural killer (NK) cell therapy is also a promising cellular
332 immunotherapy for cancer. Recent progress has been obtained in stimulating NK and
333 NKT cell anti-tumor activity using HIS models in glioblastoma, ovarian, colorectal and
334 pancreatic cancer [67–71].

335

336 *5.2. Immune checkpoint Inhibitors*

337 Different human-specific monoclonal antibodies have been evaluated in HIS
338 models, either as mono or combinatory therapies for different tumor-types, including
339 antibodies directed against CD137, PD1 and/or CTLA-4 [21,72–74] (**Table 4**). Recently,
340 combination of PD-1 checkpoint blockade with CAR T cell infusion was evaluated in an
341 orthotopic mouse model of pleural mesothelioma [75]. However, despite these
342 sporadic successful results for individual models, a wide variety of response is seen in
343 HIS mice treated with immune checkpoint Abs, likely attributed to donor-to-donor
344 variability of immune cells used for these reconstituted HIS models.

345

346 *5.3. ADCC evaluation, bi-specific antibodies and DARPins*

347 HIS models, in which human immune cells mediate the antitumor action of the
348 therapeutic antibodies, allow for the study of human antibody-dependent cellular

349 cytotoxity (ADCC) (**Table 4**). Thus, HIS mice have been used to evaluate anti-CCR4 and
350 anti-CD52 antibodies that acts by NK cell-mediated ADCC in leukemia and lymphoma
351 models [17,47,76,77], as well as antibodies against a surface-expressed protein
352 overexpressed on renal cell carcinoma [78]. Recently, Wege et al., evaluated the
353 potential reinforcing effect of trastuzumab in combination with IL-15 in humanized
354 models of breast cancer [79]. Also, Mahne et collaborators observed in a Hu-CD34+
355 model treated with an anti-GITR mAb a reduced frequency of Tregs and an increase of
356 CD8+ T cell that correlated with the inhibition of tumor growth [80].

357 Bi-specific antibodies targeting T cells to a tumor antigen have been evaluated
358 in humanized preclinical models of colon carcinoma (bi-specific EpCAM/CD3 antibody)
359 [81], lymphoma (bi-specific CD20/CD3 antibody) [82], and ovarian carcinoma (anti-
360 CD3/CLDN6 and anti-CD3/EpCAM) [83,84]. Also, a carcinoembryonic antigen T-cell bi-
361 specific antibody (CEA TCB) has been tested in humanized mice, showing potent
362 antitumor activity in poorly infiltrated solid tumors [85] (**Table 4).**

363 Interestingly, administration of a recombinant adeno-associated virus (AAV)
364 vector displaying designed ankyrin repeat proteins (DARPins) specific for Her2/neu,
365 reduced breast tumor mass and extended survival longer than the antibody Herceptin
366 [86].

367

368 ### *5.4. Cytokine-based therapy*

369 Administration of pro-inflammatory cytokines is a commonly used strategy
370 aimed at boosting the anti-tumor function of effector immune cells. Using HIS mice, IL-
371 15-based immunotherapies stimulated the survival and function of NK cells, leading to
372 significant control of tumor growth, including breast cancer and leukemia [87,88,79].

373 Of note, Wege et al, showed that co-administration of trastuzumab and IL-15 induced
374 breast tumor eradication, but also induced fatal side effects associated to an hyper-
375 activation of the T cells [79].

376

377 ## 6. Concluding remarks and future perspectives

378 In this new exciting era of cancer immunotherapy, the development of HIS
379 models is a promising tool to evaluate novel therapies, to help in the selection/ranking
380 of human-specific immunomodulatory agents, to study combinatory treatments and to
381 guide the design of personalized immunotherapies, 'see Outstanding Questions'.
382 However, although HIS mice recapitulate many aspects of the crosstalk between
383 human cells of the innate and adaptive immune system and tumor, these models still
384 lack some key elements of a complete human immune system. Some major hurdles
385 include MHC incompatibility and lack of species-specific growth factors, cytokines and
386 chemokines to allow the maturation of certain immune subpopulations. Nevertheless
387 the use of HIS models has already yielded considerable data, contributing not only with
388 new insights into basic mechanisms of immunotherapeutics but also allow pre-clinical
389 evaluation of onco-immunotherapies. Understanding the caveats of HIS mice and the
390 increasing genetic optimizations are effectively and actively contributing to the
391 development of improved models with heightened translational power.

Acknowledgments

392 The authors thank Christine Sedlik and Fariba Nemati for their helpful
393 discussion and Inmaculada Guil Luna for the illustrations.

References

394 1 Chen, D.S. and Mellman, I. (2017) Elements of cancer immunity and the cancer-
395 immune set point. *Nature* 541, 321–330

396 2 Bosma, G.C. *et al.* (1983) A severe combined immunodeficiency mutation in the
397 mouse. *Nature* 301, 527–530

398 3 Mosier, D.E. *et al.* (1988) Transfer of a functional human immune system to
399 mice with severe combined immunodeficiency. *Nature* 335, 256–259

400 4 McCune, J.M. *et al.* (1988) The SCID-hu mouse: murine model for the analysis of
401 human hematolymphoid differentiation and function. *Science* 241, 1632–1639

402 5 Lapidot, T. *et al.* (1992) Cytokine stimulation of multilineage hematopoiesis
403 from immature human cells engrafted in SCID mice. *Science* 255, 1137–1141

404 6 Shultz, L.D. *et al.* (2007) Humanized mice in translational biomedical research.
405 *Nat. Rev. Immunol.* 7, 118–130

406 7 Pérol, L. *et al.* (2014) Potential limitations of IL-2 administration for the
407 treatment of experimental acute graft-versus-host disease. *Immunol. Lett.* 162, 173–
408 184

409 8 Fogh, J. *et al.* (1977) One hundred and twenty-seven cultured human tumor cell
410 lines producing tumors in nude mice. *J. Natl. Cancer Inst.* 59, 221–226

411 9 Hudson, W.A. *et al.* (1998) Xenotransplantation of human lymphoid
412 malignancies is optimized in mice with multiple immunologic defects. *Leukemia* 12,
413 2029–2033

414 10 Oettinger, M.A. *et al.* (1990) RAG-1 and RAG-2, adjacent genes that
415 synergistically activate V(D)J recombination. *Science* 248, 1517–1523

416 11 Ito, M. *et al.* (2002) NOD/SCID/gamma(c)(null) mouse: an excellent recipient
417 mouse model for engraftment of human cells. *Blood* 100, 3175–3182

418 12 Traggiai, E. *et al.* (2004) Development of a human adaptive immune system in
419 cord blood cell-transplanted mice. *Science* 304, 104–107

420 13 Legrand, N. *et al.* (2011) Functional CD47/signal regulatory protein alpha
421 (SIRP(alpha)) interaction is required for optimal human T- and natural killer- (NK) cell
422 homeostasis in vivo. *Proc. Natl. Acad. Sci. U. S. A.* 108, 13224–13229

423 14 Takenaka, K. *et al.* (2007) Polymorphism in Sirpa modulates engraftment of

424 human hematopoietic stem cells. *Nat. Immunol.* 8, 1313–1323

425 15 Strowig, T. *et al.* (2011) Transgenic expression of human signal regulatory
426 protein alpha in Rag2-/-gamma(c)-/- mice improves engraftment of human
427 hematopoietic cells in humanized mice. *Proc. Natl. Acad. Sci. U. S. A.* 108, 13218–
428 13223

429 16 Shultz, L.D. *et al.* (2007) Humanized NOD/LtSz-scid IL2 receptor common
430 gamma chain knockout mice in diabetes research. *Ann. N. Y. Acad. Sci.* 1103, 77–89

431 17 Ito, A. *et al.* (2009) Defucosylated anti-CCR4 monoclonal antibody exercises
432 potent ADCC-mediated antitumor effect in the novel tumor-bearing humanized
433 NOD/Shi-scid, IL-2Rgamma(null) mouse model. *Cancer Immunol. Immunother. CII* 58,
434 1195–1206

435 18 King, M.A. *et al.* (2009) Human peripheral blood leucocyte non-obese diabetic-
436 severe combined immunodeficiency interleukin-2 receptor gamma chain gene mouse
437 model of xenogeneic graft-versus-host-like disease and the role of host major
438 histocompatibility complex. *Clin. Exp. Immunol.* 157, 104–118

439 19 Moser, J. *et al.* (2016) Distinct Differences on Neointima Formation in
440 Immunodeficient and Humanized Mice after Carotid or Femoral Arterial Injury. *Sci.*
441 *Rep.* 6, 35387

442 20 Naserian, S. *et al.* (2018) Simple, Reproducible, and Efficient Clinical Grading
443 System for Murine Models of Acute Graft-versus-Host Disease. *Front. Immunol.* 9, 10

444 21 Sanmamed, M.F. *et al.* (2015) Nivolumab and Urelumab Enhance Antitumor
445 Activity of Human T Lymphocytes Engrafted in Rag2-/-IL2Rγnull Immunodeficient Mice.
446 *Cancer Res.* 75, 3466–3478

447 22 Holyoake, T.L. *et al.* (1999) Functional differences between transplantable
448 human hematopoietic stem cells from fetal liver, cord blood, and adult marrow. *Exp.*
449 *Hematol.* 27, 1418–1427

450 23 Shultz, L.D. *et al.* (2005) Human lymphoid and myeloid cell development in
451 NOD/LtSz-scid IL2R gamma null mice engrafted with mobilized human hemopoietic
452 stem cells. *J. Immunol. Baltim. Md 1950* 174, 6477–6489

453 24 Brehm, M.A. *et al.* (2010) Human immune system development and rejection of
454 human islet allografts in spontaneously diabetic NOD-Rag1null IL2rgammanull
455 Ins2Akita mice. *Diabetes* 59, 2265–2270

456 25 Hayakawa, J. *et al.* (2009) Busulfan produces efficient human cell engraftment
457 in NOD/LtSz-Scid IL2Rgamma(null) mice. *Stem Cells Dayt. Ohio* 27, 175–182

458 26 Czechowicz, A. *et al.* (2007) Efficient transplantation via antibody-based
459 clearance of hematopoietic stem cell niches. *Science* 318, 1296–1299

460 27 Lan, P. *et al.* (2006) Reconstitution of a functional human immune system in

461 immunodeficient mice through combined human fetal thymus/liver and CD34+ cell
462 transplantation. *Blood* 108, 487–492

463 28 André, M.C. *et al.* (2010) Long-term human CD34+ stem cell-engrafted
464 nonobese diabetic/SCID/IL-2R gamma(null) mice show impaired CD8+ T cell
465 maintenance and a functional arrest of immature NK cells. *J. Immunol. Baltim. Md*
466 *1950* 185, 2710–2720

467 29 Watanabe, Y. *et al.* (2009) The analysis of the functions of human B and T cells
468 in humanized NOD/shi-scid/gammac(null) (NOG) mice (hu-HSC NOG mice). *Int.*
469 *Immunol.* 21, 843–858

470 30 Gille, C. *et al.* (2012) Monocytes derived from humanized neonatal
471 NOD/SCID/IL2Rγ(null) mice are phenotypically immature and exhibit functional
472 impairments. *Hum. Immunol.* 73, 346–354

473 31 Halkias, J. *et al.* (2015) Conserved and divergent aspects of human T-cell
474 development and migration in humanized mice. *Immunol. Cell Biol.* 93, 716–726

475 32 Izumchenko, E. *et al.* (2017) Patient-derived xenografts effectively capture
476 responses to oncology therapy in a heterogeneous cohort of patients with solid
477 tumors. *Ann. Oncol. Off. J. Eur. Soc. Med. Oncol.* 28, 2595–2605

478 33 Gao, H. *et al.* (2015) High-throughput screening using patient-derived tumor
479 xenografts to predict clinical trial drug response. *Nat. Med.* 21, 1318–1325

480 34 Calvo, E. *et al.* (2017) A Phase I Clinical Trial and Independent Patient-Derived
481 Xenograft Study of Combined Targeted Treatment with Dacomitinib and Figitumumab
482 in Advanced Solid Tumors. *Clin. Cancer Res. Off. J. Am. Assoc. Cancer Res.* 23, 1177–
483 1185

484 35 Jespersen, H. *et al.* (2017) Clinical responses to adoptive T-cell transfer can be
485 modeled in an autologous immune-humanized mouse model. *Nat. Commun.* 8, 707

486 36 Pearson, T. *et al.* (2008) Non-obese diabetic-recombination activating gene-1
487 (NOD-Rag1 null) interleukin (IL)-2 receptor common gamma chain (IL2r gamma null)
488 null mice: a radioresistant model for human lymphohaematopoietic engraftment. *Clin.*
489 *Exp. Immunol.* 154, 270–284

490 37 Rahmig, S. *et al.* (2016) Improved Human Erythropoiesis and Platelet Formation
491 in Humanized NSGW41 Mice. *Stem Cell Rep.* 7, 591–601

492 38 McIntosh, B.E. *et al.* (2015) Nonirradiated NOD,B6.SCID Il2rγ-/- Kit(W41/W41)
493 (NBSGW) mice support multilineage engraftment of human hematopoietic cells. *Stem*
494 *Cell Rep.* 4, 171–180

495 39 Li, Y. *et al.* (2016) A novel Flt3-deficient HIS mouse model with selective
496 enhancement of human DC development. *Eur. J. Immunol.* 46, 1291–1299

497 40 Lopez-Lastra, S. *et al.* (2017) A functional DC cross talk promotes human ILC

498 homeostasis in humanized mice. *Blood Adv.* 1, 601–614

499 41 Chen, Q. *et al.* (2009) Expression of human cytokines dramatically improves
500 reconstitution of specific human-blood lineage cells in humanized mice. *Proc. Natl.*
501 *Acad. Sci. U. S. A.* 106, 21783–21788

502 42 Billerbeck, E. *et al.* (2011) Development of human CD4+FoxP3+ regulatory T
503 cells in human stem cell factor-, granulocyte-macrophage colony-stimulating factor-,
504 and interleukin-3-expressing NOD-SCID IL2Rγ(null) humanized mice. *Blood* 117, 3076–
505 3086

506 43 Ito, R. *et al.* (2013) Establishment of a human allergy model using human IL-
507 3/GM-CSF-transgenic NOG mice. *J. Immunol. Baltim. Md 1950* 191, 2890–2899

508 44 Willinger, T. *et al.* (2011) Human IL-3/GM-CSF knock-in mice support human
509 alveolar macrophage development and human immune responses in the lung. *Proc.*
510 *Natl. Acad. Sci. U. S. A.* 108, 2390–2395

511 45 Rongvaux, A. *et al.* (2014) Development and function of human innate immune
512 cells in a humanized mouse model. *Nat. Biotechnol.* 32, 364–372

513 46 Huntington, N.D. *et al.* (2009) IL-15 trans-presentation promotes human NK cell
514 development and differentiation in vivo. *J. Exp. Med.* 206, 25–34

515 47 Katano, I. *et al.* (2015) Predominant development of mature and functional
516 human NK cells in a novel human IL-2-producing transgenic NOG mouse. *J. Immunol.*
517 *Baltim. Md 1950* 194, 3513–3525

518 48 Katano, I. *et al.* (2017) Long-term maintenance of peripheral blood derived
519 human NK cells in a novel human IL-15- transgenic NOG mouse. *Sci. Rep.* 7, 17230

520 49 Herndler-Brandstetter, D. *et al.* (2017) Humanized mouse model supports
521 development, function, and tissue residency of human natural killer cells. *Proc. Natl.*
522 *Acad. Sci.* 114, E9626–E9634

523 50 Yaguchi, T. *et al.* (2017) Human PBMC-transferred murine MHC class I/II-
524 deficient NOG mice enable long-term evaluation of human immune responses. *Cell.*
525 *Mol. Immunol.* DOI: 10.1038/cmi.2017.106

526 51 Shultz, L.D. *et al.* (2010) Generation of functional human T-cell subsets with
527 HLA-restricted immune responses in HLA class I expressing NOD/SCID/IL2r
528 gamma(null) humanized mice. *Proc. Natl. Acad. Sci. U. S. A.* 107, 13022–13027

529 52 Danner, R. *et al.* (2011) Expression of HLA class II molecules in humanized
530 NOD.Rag1KO.IL2RgcKO mice is critical for development and function of human T and B
531 cells. *PloS One* 6, e19826

532 53 Suzuki, M. *et al.* (2012) Induction of human humoral immune responses in a
533 novel HLA-DR-expressing transgenic NOD/Shi-scid/γcnull mouse. *Int. Immunol.* 24,
534 243–252

535 54 Zeng, Y. *et al.* (2017) Creation of an immunodeficient HLA-transgenic mouse
536 (HUMAMICE) and functional validation of human immunity after transfer of HLA-
537 matched human cells. *PloS One* 12, e0173754

538 55 Lute, K.D. *et al.* (2005) Human CTLA4 knock-in mice unravel the quantitative
539 link between tumor immunity and autoimmunity induced by anti-CTLA-4 antibodies.
540 *Blood* 106, 3127–3133

541 56 Burova, E. *et al.* (2017) Characterization of the Anti–PD-1 Antibody REGN2810
542 and Its Antitumor Activity in Human PD-1 Knock-In Mice. *Mol. Cancer Ther.* 16, 861–
543 870

544 57 Zhao, Y. *et al.* (2010) Multiple injections of electroporated autologous T cells
545 expressing a chimeric antigen receptor mediate regression of human disseminated
546 tumor. *Cancer Res.* 70, 9053–9061

547 58 Abate-Daga, D. *et al.* (2014) A novel chimeric antigen receptor against prostate
548 stem cell antigen mediates tumor destruction in a humanized mouse model of
549 pancreatic cancer. *Hum. Gene Ther.* 25, 1003–1012

550 59 Guedan, S. *et al.* (2014) ICOS-based chimeric antigen receptors program bipolar
551 TH17/TH1 cells. *Blood* 124, 1070–1080

552 60 Song, D.-G. *et al.* (2012) CD27 costimulation augments the survival and
553 antitumor activity of redirected human T cells in vivo. *Blood* 119, 696–706

554 61 Diaconu, I. *et al.* (2017) Inducible Caspase-9 Selectively Modulates the Toxicities
555 of CD19-Specific Chimeric Antigen Receptor-Modified T Cells. *Mol. Ther. J. Am. Soc.*
556 *Gene Ther.* 25, 580–592

557 62 Gill, S. *et al.* (2014) Preclinical targeting of human acute myeloid leukemia and
558 myeloablation using chimeric antigen receptor-modified T cells. *Blood* 123, 2343–2354

559 63 Hombach, A.A. *et al.* (2016) Superior Therapeutic Index in Lymphoma Therapy:
560 CD30(+) CD34(+) Hematopoietic Stem Cells Resist a Chimeric Antigen Receptor T-cell
561 Attack. *Mol. Ther. J. Am. Soc. Gene Ther.* 24, 1423–1434

562 64 Pizzitola, I. *et al.* (2014) Chimeric antigen receptors against CD33/CD123
563 antigens efficiently target primary acute myeloid leukemia cells in vivo. *Leukemia* 28,
564 1596–1605

565 65 Brown, C.E. *et al.* (2012) Stem-like tumor-initiating cells isolated from IL13Rα2
566 expressing gliomas are targeted and killed by IL13-zetakine-redirected T Cells. *Clin.*
567 *Cancer Res. Off. J. Am. Assoc. Cancer Res.* 18, 2199–2209

568 66 Globerson-Levin, A. *et al.* (2014) Elimination of Progressive Mammary Cancer
569 by Repeated Administrations of Chimeric Antigen Receptor-Modified T Cells. *Mol. Ther.*
570 22, 1029–1038

571 67 Hoogstad-van Evert, J.S. *et al.* (2017) Umbilical cord blood CD34+ progenitor-

572 derived NK cells efficiently kill ovarian cancer spheroids and intraperitoneal tumors in
573 NOD/SCID/IL2Rgnull mice. *Oncoimmunology* 6, e1320630

574 68 Geller, M.A. *et al.* (2013) Intraperitoneal delivery of human natural killer cells
575 for treatment of ovarian cancer in a mouse xenograft model. *Cytotherapy* 15, 1297–
576 1306

577 69 Lee, S.J. *et al.* (2015) Natural killer (NK) cells inhibit systemic metastasis of
578 glioblastoma cells and have therapeutic effects against glioblastomas in the brain. *BMC*
579 *Cancer* 15, 1011

580 70 Veluchamy, J.P. *et al.* (2017) In Vivo Efficacy of Umbilical Cord Blood Stem Cell-
581 Derived NK Cells in the Treatment of Metastatic Colorectal Cancer. *Front. Immunol.* 8,
582 87

583 71 Ames, E. *et al.* (2015) NK Cells Preferentially Target Tumor Cells with a Cancer
584 Stem Cell Phenotype. *J. Immunol. Baltim. Md 1950* 195, 4010–4019

585 72 Fisher, T.S. *et al.* (2012) Targeting of 4-1BB by monoclonal antibody PF-
586 05082566 enhances T-cell function and promotes anti-tumor activity. *Cancer Immunol.*
587 *Immunother. CII* 61, 1721–1733

588 73 Ashizawa, T. *et al.* (2017) Antitumor Effect of Programmed Death-1 (PD-1)
589 Blockade in Humanized the NOG-MHC Double Knockout Mouse. *Clin. Cancer Res. Off.*
590 *J. Am. Assoc. Cancer Res.* 23, 149–158

591 74 Ma, S.-D. *et al.* (2016) PD-1/CTLA-4 Blockade Inhibits Epstein-Barr Virus-Induced
592 Lymphoma Growth in a Cord Blood Humanized-Mouse Model. *PLoS Pathog.* 12,
593 e1005642

594 75 Cherkassky, L. *et al.* (2016) Human CAR T cells with cell-intrinsic PD-1
595 checkpoint blockade resist tumor-mediated inhibition. *J. Clin. Invest.* 126, 3130–3144

596 76 Pallasch, C.P. *et al.* (2014) Sensitizing protective tumor microenvironments to
597 antibody-mediated therapy. *Cell* 156, 590–602

598 77 Leskov, I. *et al.* (2013) Rapid generation of human B-cell lymphomas via
599 combined expression of Myc and Bcl2 and their use as a preclinical model for biological
600 therapies. *Oncogene* 32, 1066–1072

601 78 Chang, D.-K. *et al.* (2015) Human anti-CAIX antibodies mediate immune cell
602 inhibition of renal cell carcinoma in vitro and in a humanized mouse model in vivo.
603 *Mol. Cancer* 14, 119

604 79 Wege, A.K. *et al.* (2017) IL-15 enhances the anti-tumor activity of trastuzumab
605 against breast cancer cells but causes fatal side effects in humanized tumor mice
606 (HTM). *Oncotarget* 8, 2731–2744

607 80 Mahne, A.E. *et al.* (2017) Dual Roles for Regulatory T-cell Depletion and
608 Costimulatory Signaling in Agonistic GITR Targeting for Tumor Immunotherapy. *Cancer*

609 *Res.* 77, 1108–1118

610 81 Wulf-Goldenberg, A. *et al.* (2011) Intrahepatically transplanted human cord
611 blood cells reduce SW480 tumor growth in the presence of bispecific EpCAM/CD3
612 antibody. *Cytotherapy* 13, 108–113

613 82 Smith, E.J. *et al.* (2015) A novel, native-format bispecific antibody triggering T-
614 cell killing of B-cells is robustly active in mouse tumor models and cynomolgus
615 monkeys. *Sci. Rep.* 5, 17943

616 83 Stadler, C.R. *et al.* (2016) Characterization of the first-in-class T-cell-engaging
617 bispecific single-chain antibody for targeted immunotherapy of solid tumors
618 expressing the oncofetal protein claudin 6. *Oncoimmunology* 5, e1091555

619 84 McCormack, E. *et al.* (2013) Bi-specific TCR-anti CD3 redirected T-cell targeting
620 of NY-ESO-1- and LAGE-1-positive tumors. *Cancer Immunol. Immunother. CII* 62, 773–
621 785

622 85 Bacac, M. *et al.* (2016) CEA TCB: A novel head-to-tail 2:1 T cell bispecific
623 antibody for treatment of CEA-positive solid tumors. *Oncoimmunology* 5, e1203498

624 86 Münch, R.C. *et al.* (2015) Off-target-free gene delivery by affinity-purified
625 receptor-targeted viral vectors. *Nat. Commun.* 6, 6246

626 87 Cany, J. *et al.* (2013) Natural killer cells generated from cord blood
627 hematopoietic progenitor cells efficiently target bone marrow-residing human
628 leukemia cells in NOD/SCID/IL2Rg(null) mice. *PloS One* 8, e64384

629 88 Vallera, D.A. *et al.* (2016) IL15 Trispecific Killer Engagers (TriKE) Make Natural
630 Killer Cells Specific to CD33+ Targets While Also Inducing Persistence, In Vivo
631 Expansion, and Enhanced Function. *Clin. Cancer Res. Off. J. Am. Assoc. Cancer Res.* 22,
632 3440–3450