Development and Applications of Patient-Derived Xenograft Models in Humanized Mice for Oncology and Immune-Oncology Drug Discovery

Bhavna Verma,1 Michael Ritchie,1 and Maria Mancini1

1Champions Oncology, Inc., Baltimore, Maryland

With the recent approval of four novel immune oncology agents for the treatment of various cancers, the emerging power of this drug class has been substantiated. However, the full potential of such agents is yet to be realized, with only a fraction of the patient population responding to these drugs. A more advanced pre-clinical and translational research platform may increase our understanding of the mechanisms associated with immune-mediated cancer cell death, thereby facilitating the design and development of more generally efficacious agents and drug regimens. Described in this report are the nuances, advantages, and limitations of such a research approach. © 2017 by John Wiley & Sons, Inc.

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INTRODUCTION
Immune oncology (IO), a therapeutic approach that exploits a patient’s immune system to reduce or eliminate tumor burden, is not a new concept. Attempts have been made for decades to harness the power of the human immune system to eradicate tumor growth, but to no avail. As the understanding of the immune system advanced, approaches were developed to induce the system to recognize tumor cells as a foreign pathogen. While there were early indications that this might be an effective strategy, there remained a fundamental lack of understanding of how tumors evade recognition by the immune system. Success was finally achieved with the identification of the immune checkpoints, the gatekeepers responsible for modulating the immune system (Pardoll, 2012). These proteins were found to play an essential role in sustaining self-tolerance to prevent damage to peripheral tissue during a normal immune-mediated response. Recently approved therapeutics, including Ipilimumab, Atezolizumab, Pembrolizumab, and Nivolumab take advantage of these findings, inducing the immune system to attack tumor cells by interfering with immune checkpoint proteins (Chuk et al., 2017; Hazarika et al., 2017; Ledford, 2011; Ning et al., 2017). The development of this novel class of therapeutics represents a major breakthrough in oncology, providing therapeutic options for a subset of previously untreatable tumors. Often, these checkpoint inhibitors display long-lasting effects and, in some cases, completely eradicate the tumor (Chuk et al., 2017; Hazarika et al., 2017; Ledford, 2011; Ning et al., 2017). Although significant progress has been made in this therapeutic area, efforts must continue to exploit this approach by designing newer agents that are more efficacious over a broader patient population, as the current IO compounds are effective only for a subset of patients with advanced metastatic non–small cell lung cancer (NSCLC), melanoma, colorectal carcinoma, head and neck carcinoma,
renal cell carcinoma, and bladder cancer. To this end, it is necessary to develop more predictive pharmacology models that can be employed for the preclinical identification of such drugs. Provided here is a review of the use of the humanized immune system (HIS) patient-derived xenograft (PDX) platform as an assay for screening IO drug candidates. Emphasis is placed on considering the advantages and limitations of this approach, with discussion of recent advances made to improve the utility of this preclinical drug discovery platform.

DEVELOPMENT OF HUMAN IMMUNE SYSTEM MODELS

Humanization of the Mouse Model

Humanization of the mouse is required for performing a HIS PDX experiment (Fig. 14.41.1). To achieve this, it is important to begin with a genetically modified mouse background that has impaired B, T, and NK cell populations to prevent rejection of the reconstituted human immune system. The NOG (NOD.Cg-Prkdcscid Il2rgtm1Sug/JicTac) or NSG (NOD.Cg-PrkdcscidIl2rgtm1Wj/l/SzJ) mouse has been found useful for immune humanization (Pearson, Greiner, & Shultz, 2008). These mice are of NOD/SCID background with the IL-2R \( \gamma \) truncated or deleted, respectively. To reconstitute the human immune system, CD34\(^+\) hematopoietic stem cells (HSCs) are injected via the tail vein into sub-lethally irradiated 3- to 4-week-old mice (see Aryee, Shultz, Greiner, Brehm, & Jurczyk, 2015, 2016, for a detailed protocol on the humanization of these mice). The different sources of HSCs are detailed in the commentary below. Peripheral blood mononuclear cells (PBMCs) may also be used to humanize mice. While the technique for reconstitution is identical to that employed for stem cells, the PBMCs often produce graft-versus-host disease more quickly than fetal liver- or umbilical cord-derived CD34\(^+\) cells (Ali et al., 2012). This restricts the utility of using PBMCs with PDX because of the more limited time for performing a study. For this reason, this platform is most commonly used with cell line xenografts. Therefore, for the remainder of this discussion, emphasis is placed on humanized mice reconstituted with fetal-liver or umbilical-cord CD34\(^+\) cells in the context of PDX. Following injection of CD34\(^+\) cells, mice are monitored for 10 weeks, with the level of humanization evaluated by flow cytometry to assess the peripheral blood levels of human CD45 (huCD45), human CD3 (huCD3), and human CD19 (huCD19) gated within the huCD45 compartment, identifying human T and B cell populations, respectively. A minimum threshold of 25% huCD45-expressing
lymphocytes in peripheral blood is the industry standard for demonstrating the level of humanization needed to launch an immune response. Only mice exhibiting this percentage or more of humanization are used for an HIS PDX study. Successful humanization depends largely on the source and quality of CD34+ HSCs. The likelihood of successful engraftment is enhanced by using only cells that exhibit 90% or greater viability and CD34+ enrichment (unpub. observ.).

There are some important issues to consider with respect to the individual donor and tissue source used for collecting the CD34+ HSCs. For example, because it is possible to obtain more CD34+ cells from fetal liver from a single donor than from umbilical cord blood, more humanized mice can be generated from the former than the latter. On average, 50 HIS mice can be produced from a single liver donor. However, donor-to-donor variability is an important factor in the humanized mouse platform, and therefore it is ideal to utilize multiple donors within an experimental cohort. For this reason, CD34+ cells derived from umbilical cord blood are more commonly used. Other technical considerations for maximizing the system are described below.

**Patient-Derived Xenograft (PDX) Development and Considerations**

The PDX models are generated from a sample of human tumor removed without in vitro expansion and implanted into immunocompromised mice. The tumor tissue is typically implanted subcutaneously in the rear flank of the mouse. Tumor length and width are measured using a digital caliper, and tumor volume is calculated using the equation \( V = L \times W^2 \times 0.52 \). The PDX is monitored until it reaches a volume of 800 to 1200 mm³. It is then excised, cut into multiple fragments, and re-implanted into additional animals (referred to as “passaging”) for expansion and banking (Gao et al., 2015). Tumor fragments are implanted in this manner through multiple rounds to generate a sufficient number of tumor-bearing animals for drug testing. The methods used to develop a PDX model are described by Kim, Evans, Wang, Abbruzzese, Fleming, and Gallick (2009), and DeRose et al. (2013).

Models generated in this way have proven to be highly representative of the human tumor. That is, genetic profiles remain intact, tumor heterogeneity is retained, and the tumor/stroma architecture is maintained (Stebbing et al., 2014). For this reason, in vivo pharmacology studies of potential oncology agents using PDX platforms generate data that reliably predict patient responses. There have been several reports on PDX models being used successfully to prospectively guide the selection of therapeutic regimens that have proven effective in patient populations (Davies et al., 2016; Garralda et al., 2014; Stebbing et al., 2014). Furthermore, retrospective analyses of PDX models derived from patients with known clinical treatments and responses have accurately represented the respective patient responses, and/or modeled the overall clinical population response rate (Corcoran et al., 2015; Gao et al., 2015).

The PDX model that will be used in the humanized platform should be prepared in parallel with the human immune system reconstitution of the cohort of mice. To accomplish this, the selected PDX model should be grown in immunocompromised pre-study mice so that the actively growing or “warm” tumors can be directly transferred to mature humanized mice. It is therefore important to have a good understanding of the growth rate of the selected PDX model, because its preparation must be timed precisely. The number of humanized mice that should be implanted with the PDX fragment depends on the PDX ‘take rate’ (the percentage of tumors which grow in mice after implantation into pre-study mice) and the confidence interval of the unchallenged growth curve. The take rate of a PDX is significantly lower than that of immortalized cell lines, averaging approximately 40% to 60% for a tumor implanted from cryopreservation. The take rate is significantly higher (~80%) for a warm tumor that is passaged. Furthermore, take rates can vary across indications. Rapidly growing PDX tumors might be ready for study in 2 to 3 months, whereas tumors with slower growth kinetics may require up to 6 to 8 months to generate a sufficient number of tumor-bearing animals for a study. If a PDX model has a low take rate and/or a wide confidence interval, suggesting a wide range of growth rates for an unchallenged tumor, a 2× overage of implanted mice is suggested. That is, if 30 mice are needed for a study, 60 humanized mice should be implanted with PDX fragments. Because some individual PDX fragments grow slowly or not at all after implantation, preparation of twice the number of implanted mice needed for the study should ensure that a sufficient quantity of tumor-bearing humanized mice are available for study. A 1.2 to 1.5× overage is suggested if the PDX model has a high take rate and a narrow growth confidence.
Figure 14.41.2  Current challenges with HIS PDX studies. (A) Under normal conditions, immune cells recognize a tumor as self through multiple antigen interactions between the immune cell and tumor, such as immune checkpoints and HLA proteins. Current approved immune oncology agents block the interaction between immune checkpoint proteins, thereby allowing the immune cell to destroy the tumor. (B) Human immune system PDX studies employ a system in which the immune system and tumor are derived from different individuals. Thus, HLA proteins are mismatched, and the immune system does not recognize the tumor as self.

interval. Once the implanted tumor fragments reach a mean size of $\sim 100 \text{ mm}^3$, with an acceptable range being 80 to 200 mm$^3$, the mice should be randomized for the IO screening assay.

An important consideration in selecting a PDX model is the ‘self’ versus ‘non-self’ signals expressed between the model and the reconstituted human immune system. One ‘self’ identifying mechanism is mediated by the major histocompatibility complex (MHC) or human leukocyte antigen in human (HLA) cell surface proteins (Fig. 14.41.2A). These proteins are encoded by HLA genes on chromosome 6 (Kanda et al., 2013). There are three different HLA gene groups (HLA-A, HLA-B, and HLA-C) and six HLA genes groups in HLA complex II (HLA-DPA1, HLA-DPB1, HLA-DQA1, HLA-DQB1, HLA-DRA, and HLA-DRB1). There are many different specific HLA alleles for each HLA gene that help create a specific HLA gene signature that differs from one person to the next. When immune cells encounter a cell that expresses a different pattern of HLA genes, it is recognized as non-self, and a T cell–mediated response for destruction is initiated. This is an important consideration when dealing with tissue rejection requiring the use of immunosuppressants that occurs as the result of an organ transplant. The same is true for a HIS study. While it is very difficult to find a PDX model and a humanized mouse model that are 100% HLA matched, unless developed from the same donor (known as an autologous model), for most HIS PDX studies the investigators have sought to haplotype match the common HLA serotypes, e.g., the common HLA-A2. As a consequence, these studies employ a platform where the HLA serotypes between the PDX and immune component are not 100% matched, with a consequent allorejection of the tumor (Fig. 14.41.2B). While the degree of rejection is not yet clearly defined, arguments are made that the reconstituted human immune system is responding to the presence of a ‘foreign’ cell type when encountering and rejecting the human tumor in the mouse. Thus, there is ongoing debate within the field about whether haplotype matching between the PDX and the CD34$^{+}$ donor cells contributes to a successful HIS study (Shultz et al., 2010; Yao et al., 2016). Additional studies comparing the outcomes of haplotype-matched and autologous HIS platforms where the PDX model and immune system are derived from the same patient will be revealing in this regard.

In Vivo Pharmacology Studies Employing the HIS Model

Two conditions must be met before initiating a study. The first is that the individual tumors implanted into HIS mice must fall within the 80- to 200-mm$^3$ randomization
Figure 14.41.3  Tumor measurements from a representative HIS PDX study. A NSCLC PDX model is grown in CD34\(^+\)-generated humanized mice, the mice are treated with vehicle or Ipilimumab (anti-CTLA4) at a dose of 10 mg/kg via i.p. administration every 4 days for 24 days, and tumor measurements are recorded. Data generated internally by Champions Oncology.

Figure 14.41.4  T-cell proliferation in peripheral blood post-study analysis. Peripheral blood is drawn from mice treated with vehicle or Ipilimumab at study termination and the level of lymphocyte activation in response to Ipilimumab treatment is assessed. Flow cytometry is employed to analyze the levels of human CD8\(^+\) cytotoxic and CD4\(^+\) Th1 cells present in the peripheral blood. Data generated internally by Champions Oncology.

Once this occurs in a sufficient number of animals for the treatment groups, the engrafted PBMCs are assessed again in all animals using flow cytometry to determine baseline huCD45\(^+\) levels. This done so that treatment groups can be populated with animals based on both tumor volume and level of huCD45\(^+\) engraftment. It is critical to observe the health of the mice on a daily basis while administering the IO agent, as the presence of the human immune system may unmask toxicities that are not typically observed in studies using immune-deficient mice. An example of a HIS PDX study assessing the activity of Ipilimumab against a NSCLC PDX model is shown in Figures 14.41.3 to 14.41.6. In this study, Ipilimumab (anti-CTLA4) was administered i.p. six times at 10 mg/kg every 4 days. Note that the Ipilimumab-treated tumors rapidly increase in size when treatment is initiated before showing regression (Fig. 14.41.3). It is possible this transient increase in size is indicative of immune cell infiltration into the tumor, which has been reported clinically and has prompted a revision of the Response Evaluation Criteria in Solid Tumors (RECIST) criteria for IO-related clinical trials (Chiou & Buroto, 2015). Tumor growth should be assessed on at least a bi-weekly basis until the end of
**Figure 14.41.5**  
T cell activation markers at study endpoint. Peripheral blood, splenocytes, and dissociated tumor cells are removed from CD34⁺-derived humanized mice treated with vehicle or anti-CTLA4 for analysis of lymphocyte activation or tumor infiltration; samples are drawn at the end of a 28-day study. Flow cytometry is employed to analyze various T cell populations present within these samples. Data generated internally by Champions Oncology.

<table>
<thead>
<tr>
<th>T cell subtype</th>
<th>Untreated (n=6)</th>
<th>α-CTLA4 (n=7)</th>
<th>p-value*</th>
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<tr>
<td>huCD3⁺</td>
<td>47.0 ± 42.9</td>
<td>89.9 ± 8.40</td>
<td>p &lt; 0.05</td>
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<td>huCD4⁺+huCD69⁺ (helper)</td>
<td>29.0 ± 15.5</td>
<td>46.2 ± 14.3</td>
<td>p &lt; 0.05</td>
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<td>huCD8⁺ (cytotoxic)</td>
<td>5.70 ± 4.10</td>
<td>16.6 ± 16.5</td>
<td>ns</td>
</tr>
<tr>
<td>huCD4⁺+huCD25⁺ (regulatory)</td>
<td>19.7 ± 17.8</td>
<td>8.60 ± 4.70</td>
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</tr>
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</table>

**Whole blood**

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<th>p-value*</th>
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<td>huCD3⁺</td>
<td>49.1 ± 9.30</td>
<td>76.7 ± 9.20</td>
<td>p &lt; 0.001</td>
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<tr>
<td>huCD4⁺+huCD69⁺ (helper)</td>
<td>13.5 ± 18.6</td>
<td>13.3 ± 10.3</td>
<td>ns</td>
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<td>huCD8⁺ (cytotoxic)</td>
<td>6.80 ± 2.50</td>
<td>16.2 ± 17.5</td>
<td>ns</td>
</tr>
<tr>
<td>huCD4⁺+huCD25⁺ (regulatory)</td>
<td>10.8 ± 3.70</td>
<td>19.7 ± 6.00</td>
<td>p &lt; 0.01</td>
</tr>
</tbody>
</table>

**Spleen**

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<th>T cell subtype</th>
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<th>α-CTLA4 (n=7)</th>
<th>p-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>huCD3⁺</td>
<td>18.9 ± 8.60</td>
<td>49.0 ± 15.6</td>
<td>p &lt; 0.001</td>
</tr>
<tr>
<td>huCD4⁺+huCD69⁺ (helper)</td>
<td>11.8 ± 2.60</td>
<td>20.3 ± 4.50</td>
<td>p &lt; 0.01</td>
</tr>
<tr>
<td>huCD8⁺ (cytotoxic)</td>
<td>2.60 ± 1.20</td>
<td>5.90 ± 5.60</td>
<td>ns</td>
</tr>
<tr>
<td>huCD4⁺+huCD25⁺ (regulatory)</td>
<td>8.90 ± 3.40</td>
<td>11.0 ± 2.20</td>
<td>ns</td>
</tr>
</tbody>
</table>

* Student’s t-test; ns = not significant

**Figure 14.41.6**  
T cell infiltration in HIS PDX studies. Tumors were removed and analyzed at study termination by immunohistochemistry to assess T cell infiltration to demonstrate a mechanistic response to treatment with Ipilimumab. Immunohistochemistry is employed to analyze tumor infiltrating lymphocytes present after completing an HIS PDX study. Utilizing an antibody targeted at FOXP3 in FFPE tumor sections has demonstrated that T regulatory cells do have a basal level of infiltration, nor do they infiltrate a tumor upon the dosing of anti-CTLA4. Alternatively, the utilization of antibodies targeting CD3, CD8, and CD69 in FFPE tumor sections demonstrates that there is a large increase in the infiltration of CD3 T cells, CD8 cytotoxic T cells, and CD69 myeloid cells after the administration of anti-CTLA4. Data generated internally by Champions Oncology.
The HIS PDX study endpoints commonly utilized are summarized in Table 14.41.1. Three factors are usually assessed with a HIS PDX study: (1) tumor response to the IO agent, (2) immune response to the IO agent in the periphery, and (3) immune response to the IO agent within the tumor. The HIS PDX study usually concludes 28 days after the first injection of drug or test agent, with all remaining mice euthanized and analyzed at the same time. Because humanization is persistent, the studies may be performed for longer than 28 days (Pearson et al., 2008). At the conclusion of treatment, peripheral blood, bone marrow, and spleen can be extracted from euthanized mice for post-study flow cytometry analysis, with any remaining tumors removed for post-study flow cytometry and IHC analyses, as detailed below. If sufficient tumor material remains, it can be processed for both immunohistological and flow cytometry analyses to search for changes in key cellular subtypes or other markers of interest. These analyses provide additional data that may provide further mechanistic and clinical insights.

**Post-Study Analyses**

The analysis of HIS PDX data is aimed at determining how the human immune system reacted to the IO agent and, in turn, how the immune system interacted with the tumor. Accordingly, the HIS PDX post-study analysis can be broken into two segments: (a) immune cell activation, and (b) immune cell infiltration into the tumor. It is important to assess data from a multidimensional perspective and to correlate any tumor regression, or lack thereof, with the post-study analysis. Post-study peripheral blood and bone marrow samples are used to evaluate the level of immune cell activation after administration of the IO test agents. Cell surface markers (Figs. 14.41.4 and 14.41.5) aid in understanding the level of immune cell activation and proliferation.

The cell surface markers can also be employed for the analysis of dissociated tumor cells to identify the extent of tumor-infiltrating
lymphocytes (TILs). When available, post-study tumor fragments can be processed as FFPE for IHC analysis of TILs. Shown in Figure 14.41.6 is a series of cell surface markers which can be used to evaluate TIL subpopulations via IHC.

Syngeneic Models

The preclinical development and validation of IO agents has heavily utilized syngeneic animal models which involve the murine immune system and murine tumors. In essence, spontaneous or induced murine tumors are developed in vivo and then extracted from the mice and propagated in vitro. They are then re-implanted into immunocompetent mice of a genetic background from which the tumors were originally derived. Thus, the tumor can re-grow in the presence of an intact immune system that recognizes it as ‘self’. Syngeneic models are popular because they are inexpensive and easy to use. However, they are limited in their ability to predict the human immune response (Mestas & Hughes, 2004; Rangarajan & Weinberg, 2003). Further, investigators often must rely on the use of murine homologs of therapeutic agents that react with murine antigens rather than the experimental IO agent, which may not cross-react to murine antigens. For example, the murine RMP-14 clone of PD-1 antibody, which recognizes and binds murine PD-1, is used widely in syngeneic tumor models to assess its therapeutic efficacy. As an alternative to using surrogate agents, investigators developed systems in which ectopic expression of the human antigen is expressed on the tumor cells. Burova et al. (2017) studied the in vivo activity of REGN2810, a fully human, hinge-stabilized IgG4 which does not cross-react with murine PD-1 in a human PD-1 knock in mice. However, these systems are limited by the possibility of abnormal antigen expression levels on the tumor, thereby biasing results towards a strong, non-physiological response. Therefore, while this is a relevant preclinical platform for studying potential IO agents, it should be employed only with a keen understanding of its limitations and the potential for false positive or negative results.

Adoptive Transfer and Cell Line Xenografts

Pharmacological models in which human PBMCs are injected directly into growing tumors of immortalized human cell line xenografts (CLX) are often utilized as alternatives to syngeneic animal models (Rosenberg, Spiess, & Lafreniere, 1986). This adoptive transfer of established and mature immune systems into the microtumor environment allows investigators to explore whether a test agent induces immune-mediated killing within the tumor microenvironment. Although this approach can provide some mechanistic insight with respect to the mechanism of tumor killing (i.e., whether blocking a particular immune checkpoint induces an effective immune response), it may also bias the results and provides little in the way of new information on how a systemic and replenishing immune system can localize, infiltrate, and eradicate a tumor. The use of humanized mice is growing in popularity because it avoids the limitations of the syngeneic and adoptive transfer platforms (Hiramatsu et al., 2003; Pearson et al., 2008).

Early iterations of HIS platforms involved the use of immortalized tumor cell lines grown as solid tumors within the humanized mice (Brunda et al., 1996). While this delivered a novel alternative to the syngeneic and adoptive transfer platform, tumor CLXs do not fully recapitulate the heterogeneity and global gene expression represented in tumor cell biology. Hence, CLX models were replaced with patient-derived xenografts (PDX) to establish the next-generation preclinical human immune system (HIS) platform.

HIS PDX STUDY CONSIDERATIONS

The HIS PDX pharmacology approach has a number of elements and limitations that must be considered before undertaking a study. Ultimately, the experimental design will dictate the source of CD34⁺ cells and the mouse background utilized in a HIS PDX.

CD34⁺ Source

The results of a HIS PDX study rely not only on the efficacy of the test agent and the biology of the tumor being evaluated, but also on the activity of the immune system within the humanized mouse. As described above, there are limitations to consider when selecting the source of CD34⁺ HSCs. Investigators differ about whether a large cohort of mice derived from a single donor, or many small cohorts of mice derived from different donors, is the most suitable study design. While having a single donor can reduce donor-to-donor variability, a response obtained from animals derived from a single donor may not be
Table 14.41.2  Sources of Humanized Mice

<table>
<thead>
<tr>
<th>Model</th>
<th>Mouse strain</th>
<th>Human cells</th>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD34-FL</td>
<td>NSG/NOG</td>
<td>CD34⁺ from fetal liver (FL)</td>
<td>• Long life before onset of GVHD&lt;br&gt; • Good reconstitution (T/B cells)&lt;br&gt; • Large cohorts of mice due to the high volume of CD34⁺ cells.</td>
<td>• Might not be optimal for myeloid cells&lt;br&gt; • Cannot perfectly match to tumor&lt;br&gt; • Use of fetal tissue</td>
</tr>
<tr>
<td>CD34-UC</td>
<td>NSG/NOG</td>
<td>CD34⁺ from umbilical blood (UB)</td>
<td>• Long life before onset of GVHD&lt;br&gt; • Good reconstitution (T/B cells)&lt;br&gt; • No fetal tissue</td>
<td>• Might not be optimal for myeloid cells&lt;br&gt; • Cannot perfectly match to tumor&lt;br&gt; • Small cohorts</td>
</tr>
<tr>
<td>PBMCs</td>
<td>NSG/NOG</td>
<td>PBMCs from adult humans</td>
<td>• Potential for autologous HIS pharmacology study</td>
<td>• Short life before onset of GVHD</td>
</tr>
</tbody>
</table>

representative of the larger population. Therefore, utilizing several small cohorts of humanized mice derived from different donors in a single study makes it possible to randomize against different immune donors, and to avoid drawing conclusions from a single active or weak immune system.

There are two different sources of human CD34⁺ cells available for humanizing mice (Table 14.41.2): fetal liver and umbilical cord blood. While each source has its own advantages and disadvantages for humanizing mice, the engraftment of the human immune system is similar for both, and both provide ample time for analysis before the onset of graft versus host disease (Lepus et al., 2009). However, whereas a single source of fetal liver cells can reconstitute the immune system of up to 50 mice, a single source of umbilical cord blood–derived CD34⁺ cells can usually only reconstitute the human immune system of 5 to 10 mice. In some instances, enough umbilical cord blood–derived CD34⁺ cells are generated from a single donor to humanize 30 mice. Testing experimental agents in multiple PDX models across multiple immune donors provides a wealth of information for characterizing the activity of an IO test agent.

Selection of a Mouse Background for Humanization

The methods and data detailed in this unit largely describe the generation and utility of HIS mice on a NOG or NSG background. These animals are appropriate for studying approved IO agents, as such drugs harness the anti-tumor power of the lymphoid compartment of the immune system. The human lymphoid compartment reconstitutes within the NOG or NSG mouse very well and allows for productive experimentation on known IO therapeutics. However, a problem with this standard HIS mouse model is that the myeloid compartment of the human immune system does not reconstitute well in the NOG or NSG mouse (Jangalwe, Shultz, Mathew, & Brehm, 2016). Therefore, when testing experimental agents designed to act on or modify the myeloid compartment, a transgenic mouse strain is needed to improve the engraftment of the human myeloid component (Table 14.41.3; Aryee et al., 2015). The NOG-EXL and NSG-SGM3 mice provide this enhanced background by the use of transgenes that drive expression of human granulocyte/macrophage-colony stimulating factor and human interleukin-3. The NSG-SGM3 also expresses the human stem cell factor transgene. Humanized mice generated from these transgenic backgrounds have a greater ability to recapitulate the myeloid lineage of the human immune system. Agents blocking granulocyte-derived arginase activity to maintain extracellular arginine levels to restore T cell proliferation can be used to study the outcomes in these mouse strains (Wesolowski, Markowitz, & Carson, 2013).
Table 14.41.3  Humanized Mouse Backgrounds

<table>
<thead>
<tr>
<th>Model</th>
<th>Mouse strain</th>
<th>Human cells</th>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>NOG-EXL</td>
<td>Transgenic: Human IL3 and GM-CSF</td>
<td>CD34+ from FL or UCB</td>
<td>• Better reconstitution of the myeloid lineage</td>
<td>• Longer generation time</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>• Costs more than NOG/NSG</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>• Not available off the shelf</td>
</tr>
<tr>
<td>NSG-SGM3</td>
<td>Transgenic: Human IL3, GM-CSF, SF</td>
<td>CD34+ from FL or UCB</td>
<td>• Better reconstitution of the myeloid lineage</td>
<td>• Longer generation time</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>• Cost higher than Taconic EXL mouse</td>
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<tr>
<td></td>
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Future Directions

An autologous HIS PDX model is one in which the PDX model and immune system within the humanized mouse are taken from the same patient. Creation of this model is logistically challenging. The use of fetal liver or umbilical cord blood CD34+ HSCs prohibits the procurement from the same patient of tumor tissue for the development of a PDX model. It is, however, possible to isolate CD34+ HSCs from peripheral blood or bone marrow of adult cancer patients thereby allowing for the congruent isolation of clinically relevant tumor tissue and CD34+ HSC from the same donor. However, the quantities of CD34+ HSCs that can be isolated from an adult patient are extremely low. It is not possible to isolate sufficient CD34+ HSCs from a patient’s peripheral blood to humanize even a single mouse. While the bone marrow is another potential source of CD34+ HSCs, this is a difficult and invasive procedure. Furthermore, in vitro methods that effectively expand the isolated CD34+ population while maintaining pluripotency must be perfected to have a CD34+ cell count high enough for humanization. These considerations make the isolation of CD34+ HSCs for humanization of a mouse immune system a challenging, although not impossible, task.

An alternative method for generating an autologous system for PDX studies is the use of an induced-pluripotent stem cell (iPS) intermediate to derive CD34+ HSCs from a cancer patient. This approach harnesses the iPS technology developed by Yamanaka (Takahashi et al., 2007) for reprogramming of terminally differentiated cell types to a pluripotent state that can then be directed down an alternative cell lineage. During this process, the genetic profile of the patient is maintained with the reprogrammed cells being recognized as ‘self’. The iPS technology is now routine, with iPS cells having been re-differentiated to virtually all tissues types, including the immune lineage, that are currently in more than 25 regenerative medicine clinical trials (https://clinicaltrials.gov). Developing an autologous HIS model through an iPS intermediate involves the extraction of both a tumor and skin biopsy from the same patient. The tumor biopsy is used to develop a PDX model, while the skin biopsy is employed for developing an iPS line. The latter is then differentiated in vitro to a CD34+ HSC cell line used to humanize the mouse immune system, thereby creating a platform where the immune system and tumor components are derived from the same patient. As the iPS lines are immortal, they can be used multiple times, thereby reducing the costs of frequently procuring expensive fetal liver or umbilical cord-derived CD34+ cells. Furthermore, this technology avoids the ethical issues associated with using CD34+ cells derived from fetal liver.

SUMMARY

While the preclinical models for studying immune-oncology agents are still in the early stages of development and have yet to be fully characterized, their utility in advancing agents in this rapidly evolving field is established. Incorporation of these platforms into ongoing translational study designs will further elucidate the mechanisms of immune response and accelerate the discovery and development of more generally efficacious therapeutic agents.
CONFLICT OF INTEREST
All authors are employees of Champions Oncology, Inc.

LITERATURE CITED


INTERNET RESOURCES

CD34+ Humanized mice.


Insights from the hunog production lab—product integrity by M. Seiler.