The Human Touch:

Improving *In Vivo* Evaluation of Biologics with Humanized Immune System Models

By **Paul Volden, PhD**

THERAPEUTICS BASED ON biological materials present both opportunities and challenges: The need for specific precision to engage the human target that improves efficacy and reduces off-target effects also poses difficulties when studying a biologic in a preclinical *in vivo* setting. Yet, through humanization of a mouse's immune system, it is possible to enhance and improve *in vivo* evaluation of certain biologics. This article reviews the challenges of preclinical research on biologics, the utility of humanized immune system (HIS) models in exploring these promising therapeutics, and the factors to consider when selecting a HIS model for preclinical biological therapeutic development.

Biologics to the Forefront

The emphasis on cell and gene therapies, therapeutic proteins such as antibodies, vaccines, and other biologics in drug discovery is expanding at an incredible pace. Between 2010 and 2015, a time when small molecule drug discovery was relatively flat, the biologics pipeline grew at a compound annual growth rate of 8.3 percent.[1] While early efforts primarily focused on proteins, such as antibody-based drugs, the category of therapeutic biologicals has expanded greatly to include other classes of compounds, such as RNA therapies, and cellular therapies such as CAR-T (chimeric antigen receptor T cells).

The trend is likely to continue, as pharmaceutical and biotech drug discovery pipelines reflect an increased focus on this category of therapeutics.

As noted in the 2019 Pharmaprojects Pharma R&D Annual Review, biologics already represented four out of 10 drugs in the pipeline in 2019.[2] Looking ahead, the Global Biologics Market Report (released in December 2020 by FutureWise Market Research) forecasts this market to reach $424 billion worldwide by 2027.

It is not surprising that the research community is investing significant resources to the study of biologics, since these therapeutics offer advantages from both an efficacy and safety perspective. For one, pharmaceuticals based on biological materials tend to be highly specific to their intended human target. The high degree of specificity improves the accuracy of these therapeutics and can result in therapies that have fewer off-target effects, lower levels of toxicity, and a reduced incidence of drug-drug interactions. Additionally, biologics have tended to transition successfully from bench to clinic. Between 2013 and 2015, biologics had an 18 percent cumulative probability of success from Phase 1 to commercial launch vs. 9 percent for small molecule drugs.[1] Another advantage is that biologics can add value to a company's intellectual property beyond its small molecule drug portfolio.

While these advantages have prompted investigators to devote substantial resources to the study of biologics, their complexity can equate to potentially longer research timelines, higher discovery and development costs, and possible hurdles in the preclinical research phase. While biologics may have fewer adverse events and side effects compared to small molecule therapies, they do have unique safety concerns, often related to their mechanism of action, and these can be difficult to evaluate in traditional preclinical systems.

Preclinical Challenges of Biologics Drug Discovery

The same specificity that makes biological therapeutics such as monoclonal antibodies efficacious in humans can, unfortunately, limit or eliminate the relevant therapeutic biology in test species (such as mice and rats), resulting in preclinical research obstacles. To overcome this challenge, investigators historically have taken one of two approaches in early drug discovery: Produce surrogate drugs that can work within a rodent model, or genetically engineer a model to express the relevant human target. Both approaches can be costly and require long lead times.

More recently, the use of HIS mouse models has become an increasingly common alternative that can reduce the overall study timeline and costs. The term "HIS mouse" refers to a broad category of models in which an immunodeficient mouse is engrafted with human immune cells. These may be mature cells, such as peripheral blood mononuclear cells (PBMCs) from an adult donor, or immature stem cells, such as human CD34+ hematopoietic stem cells (HSCs) from neonatal umbilical cord blood, which differentiate into a variety of immune cell types post-engraftment. Super immunodeficient strains such as the NOG mouse are typically used as hosts for humanization; these strains lack adaptive immunity, have deficiencies in innate immunity, and have strain-specific alleles which promote engraftment of human hematopoietic cells. (**Table 1**)

The human immune cells represented in a HIS model may encompass a single cell type or a range of immune cells from different cell lineages. While the presence of a more diverse variety of immune cells may inherently suggest a better model, this factor alone does not determine a model's suitability for a particular research application. As described later in this article, selection of a HIS model for biologics research – or any research endeavor – should be based on a wider set of criteria, and the optimal model must be fit-for-purpose, balancing presence of the target cell(s) and validated utility for a particular application, cost, and timeline.

Some HIS models, such as those based on PBMCs, are relatively straightforward to develop, requiring only a few weeks' time. However, these mice only live for a short period, limiting the types of research for which they are applicable. Other HIS models offer longer study windows, but the resulting human immune cell types supported differ based on the host strain employed.

Over time more sophisticated HIS models have emerged, designed to facilitate the exploration of the human myeloid cell compartment, natural killer (NK) cells, and other cell types. One example is the huNOG-EXL mouse model (see https://www.taconic.com/mouse-model/nog-exl-hgm-csfhil-3-nog). The host strain used for this HIS model, the NOG-EXL, transgenically expresses two human cytokines – human granulocyte

Table 1: Commonly used humanized immune system (HIS) models

HIS model	Host strain	Donor cells	Advantages	Disadvantages
huPBMC-NOG	NOG	Human peripheral blood mononuclear cells	Good model of T cell function with mature human cells Relatively easy to generate Model ready to use immediately after engraftment	Limited persistence of other human cell types Short study window due to development of graft vs. host disease (GvHD)
huPBMC-B2m-NOG	B2m-NOG	Human peripheral blood mononuclear cells	Delayed GvHD development provides longer study window compared to huPBMC-NOG Good model of T cell function with mature human cells Relatively easy to generate Model ready to use immediately after engraftment	Limited persistence of other human cell types Short study window due to development of graft vs. host disease (GvHD)
huNOG	NOG	Human hematopoietic stem cells	Long-term, stable engraftment of human cells Development of multiple human lineages, including T and B cells as well as low levels of myeloid and NK cells	More technically challenging to generate Full human cell reconstitution takes significant time, which can delay study start
huNOG-EXL	NOG-EXL	Human hematopoietic stem cells	Higher overall engraftment and higher levels of myeloid cells compared to huNOG Long-term, stable engraftment of human cells Development of multiple human lineages, including T, B and NK cells and various myeloid cells	Generally shorter lifespan compared to huNOG due to anemia / macrophage activation syndrome More technically challenging to generate Full human cell reconstitution takes significant time, which can delay study start

macrophage colony stimulation factor (hGM-CSF) and human interleukin-3 (hIL-3) – which are critical for myeloid cell development and differentiation. The NOG-EXL is engrafted with human CD34+ HSCs to generate the huNOG-EXL (the humanized version), and this host strain displays both higher overall engraftment as well as improved myeloid cell differentiation compared to the base NOG model.[3] (**Figure 1**)

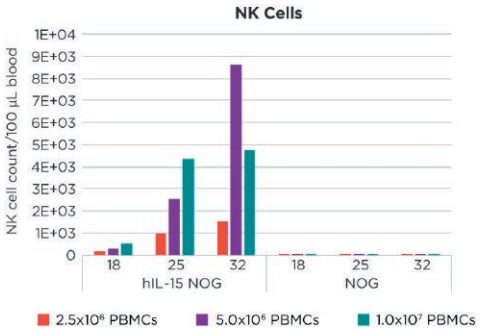

Figure 1: Human immune cell engraftment in hIL-15 NOG vs NOG mice

Data from peripheral blood analysis of NK cells performed at 18, 25, and, 32 days post PBMC engraftment.

Adapted from source material that can be viewed in Volden et al., 2018 @ https://info.taconic.com/hubfs/paulv/Taconic%20Data/PT1101-PV-1804-PD-AACR%20Poster%2087x40%20-%204-3-18.pdf. Or ESMO Open, Volume 3, Supplement 2, 30 June–3 July 2018, Page A312, ESMO Open, Poster Presentation, Animal Models of Cancer; PO-218 Cytokine-transgenic NOG mice engrafted with human peripheral blood cells support natural killer cell expansion, M.Paterka, P.Volden, A.Tanaka, D.Gimnopoulos, I.Fichtner

HIS Model Utility and Applications

Several characteristics of HIS models make them well suited to overcoming the inherent challenges of studying human-specific drugs within a non-human host. The ability to support engraftment of a wide range of human cell types and both normal and diseased tissues, to express human cytokines that are critical to intercellular signaling, and to harbor the multitude of human molecules expressed by the engrafted human components make HIS models effective and powerful tools for evaluating biologics, and feasible alternatives to either genetically engineering a model that expresses the desired drug target or developing surrogate test articles. These characteristics, in turn, spur greater use of these models in a range of biologic research applications.

One area of research in which HIS models have become established as enabling tools for drug discovery and development is immuno-oncology. Given that the traditional xenograft models often employed in cancer studies lack the immune cells targeted by immuno-therapeutics, it is no surprise that HIS models have emerged as useful for immuno-oncology research. At the same time, HIS models are applicable to a much wider variety of therapeutic areas and disease states – from colitis[4] and rheumatoid arthritis,[5] to human-specific infectious diseases, such as HIV.[6] In studies evaluating biologics across this varied spectrum of research applications, HIS models have been demonstrated to faithfully mimic clinical responses.

Prostate cancer is one of many areas of oncology in which HIS models are helping to advance research. Anti-androgen therapy is the standard of care for prostate cancer, but rodent tumor models do not always accurately model drug responses seen in the clinic. This phenomenon poses challenges for investigators studying anti-androgen treatment as monotherapy or in combination with other therapeutics. For example, in a standard xenograft model of prostate cancer, treatment with enzalutamide paradoxically increased metastases relative to controls.[7] However, when researchers at the University of Michigan employed a HIS mouse model of prostate cancer, the humanized mice treated with enzalutamide had significantly reduced metastases, similar to the outcome seen in humans.[8] This more translationally-relevant model permits study of combination therapies such as enzalutamide plus immunotherapies such as pembrolizumab.

HIS models also have reproduced clinical responses to cell-based oncology therapies. In one study, investigators obtained tumors from cancer patients treated using adoptive cell transfer, then engrafted them onto mice along with autologous tumor-infiltrating lymphocytes (TILs). Tumors from both responder and non-responder patients were used. When TILs from non-responder patients were transferred to immunodeficient NOG mice expressing human IL-2, no significant anti-tumor effects were observed. When TILs from responder patients were used, the tumors were completely eradicated in mice with sufficient hIL-2 levels. This effect was not seen in a base NOG host strain but required the next generation hIL-2 NOG host strain to support sufficient TIL expansion *in vivo*.[9] Hence, this result demonstrates the ability for a model engrafted with both tumor tissues and human immune cells to recapitulate the human disease and the clinical response.

Cancer researchers are increasingly investigating therapeutic oncological vaccines intended to elicit an immune response against tumor antigens. One of the many oncological vaccine approaches under investigation is the use of dendritic cells to express tumor-associated antigens, thereby prompting T cells to infiltrate cancer cells. A HIS model served as a critical tool in a preclinical study on a dendritic cell-based cancer vaccine at Cedars-Sinai Medical Center. Researchers developed a model engrafted with both human glioblastoma cells and a humanized immune system. Mice vaccinated with dendritic cells expressing the tumor antigen CD133 had significantly improved survival relative to those treated with non-transfected dendritic cells or control mice, demonstrating the effectiveness of a cell-based vaccination approach targeting the cell-surface antigen CD133.[10]

HIS models also have proven their utility in evaluating biologics targeted at infectious disease. Because vaccines are widely administered to large populations of healthy people to prevent infectious diseases, safety evaluation is imperative to understand potential adverse events. While it is challenging to study human adverse reactions to a vaccine in preclinical research, HIS models are helping to overcome this limitation. Researchers tested two HIS mouse models to assess their feasibility in evaluating the safety of an influenza vaccine candidate. Increased expression of certain genes in the lungs of rodents was previously identified as associated with vaccination with influenza vaccines known to cause more adverse events, but the applicability of these biomarkers to humans was not clear. In a human PBMC-engrafted HIS mouse model, expression of the human versions of these biomarker genes increased after vaccination with a reference vaccine known to have a high rate of adverse events, suggesting that evaluation of biomarkers in HIS models could serve as an effective tool for evaluating vaccine safety.[11]

Safety studies also are vital to the evaluation of emerging biological therapeutics such as monoclonal antibodies and cell and gene therapies. Preclinical test species, however, may not adequately model human safety risks. In a highly publicized example, when the monoclonal antibody therapy candidate TGN1412 was administered in clinical trials, it induced life-threatening cytokine release syndrome, despite inducing no remarkable side effects in preclinical studies with non-human primates, even at extremely high doses. Later, to evaluate the predictability of mouse models in identifying risks associated with immune-modulating drugs, researchers administered clinically relevant doses of TGN1412 to HIS mice engrafted with PBMCs. Treated mice rapidly developed lethal illness characterized by lymphopenia and release of human cytokines,[12] suggesting that HIS models could be a critical tool for identifying potential safety liabilities prior to the clinic.

Assessing a biological therapy's pharmacokinetic (PK) profile is critical to evaluating it from both an efficacy and a safety perspective. Though it can be challenging to model human PK in a mouse, HIS models may have some utility in this area. To assess the toxicity of immune checkpoint inhibiting

antibodies, researchers investigated the *in vivo* pharmacokinetics and whole-body distribution of zirconium-89 (^{89}Zr) labeled pembrolizumab, which targets PD-1. Immune system-humanized and non-humanized NOG mice with xenografts of human A375M melanoma cells were treated with ^{89}Zr-pembrolizumab. Through PET (positron-emission tomography) imaging and *ex vivo* biodistribution studies, investigators observed a high uptake of ^{89}Zr-pembrolizumab in tissues containing human immune cells, including the spleen, lymph nodes, and bone marrow, with uptake higher in these lymphoid tissues than in tumors. This distribution was significantly different from what was seen in control NOG mice. This proof-of-concept work supports further studies in humans directed at determining whether tracer uptake correlates to patient response or observed toxicities.[13]

Selecting HIS Models for Biologics Research

Choosing a HIS model to support a biologics study is not a straightforward task, in part because these models vary greatly in the types of human immune cells they support and their functionality. Factors such as the specific human immune cells required for the particular study and the requisite functional aspects of these cells should be primary considerations.

One should also consider the inherent advantages and drawbacks of each type of HIS model. For instance, CD34+ HSCs can differentiate into a variety of human immune cell types post-engraftment, which can be especially useful for studies looking at interactions between different immune cell types. However, HSCs can be challenging to obtain, with only a limited quantity available from a single donor. Not only does this prove an obstacle when the research objective requires a longitudinal study; it also reduces researchers' ability to limit inter-study variability. Additionally, the relatively scarce supply of these cells results in a higher cost for HIS models that use HSCs. These models also have a relative disadvantage in that significant time is required for the engrafted cells to differentiate, but this is offset by a long period of stable engraftment which offers a good study window. The timelines for each of these can vary by host strain and other factors.

In contrast, HIS models that are not based on HSCs, such as those that use human PBMCs, tend to rely on donor cells that are more readily available, enabling investigators to share those cells across studies and to limit donor-related variability. In addition, for studies that cannot be conducted effectively in an HSC-based HIS model – for instance, evaluating NK-cell based immunotherapies – alternative HIS models are often the better choice. Since limited uptake and survival of NK cells has been demonstrated in standard HIS models, researchers have turned to engrafting human NK cells into a host engineered to support longer term NK cell survival, often accomplished through the expression of human interleukin 15 (hIL-15). These hIL-15 models enable successful *in vivo* evaluation of monoclonal antibodies which depend on NK cells for their efficacy as well as the engineered NK cell therapies. (**Figure 2**)

Study-specific needs such as required human immune cells and desired study window will drive selection of an appropriate HIS host strain. For instance, HIS models in which certain murine genes have been deleted can provide a longer study window for PBMC-engrafted models. Strains that express certain human cytokines enhance engraftment or improve the functionality of certain human cells. The specific objective of the study will determine the importance of such host properties.

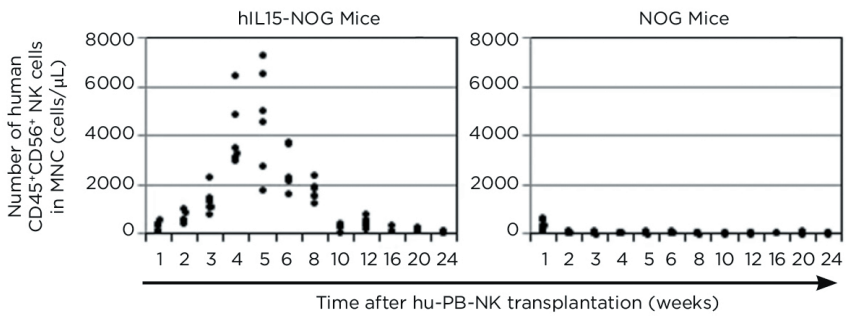

Figure 2: hIL-15 NOG mice engraft and expand human NK cells

Expansion of transferred human NK cells in NOG mice expressing human IL-15. X-irradiated NOG and hIL-15 NOG mice received human NK cells from peripheral blood magnetic cell sorting. Blood was collected and analyzed by FACS every week for 24 weeks after transfer.

Adapted from source material that can be viewed in Katano et al., 2017, Katano, I., Nishime, C., Ito, R. et al. Long-term maintenance of peripheral blood derived human NK cells in a novel human IL-15- transgenic NOG mouse. Sci Rep 7, 17230 (2017). https://doi.org/10.1038/s41598-017-17442-7

Summary

As biologics research continues to accelerate, therapeutics based on biological materials will constitute a growing percentage of drug candidates in the R&D pipeline and approved drug portfolio. Researchers will continue to rely on sophisticated HIS models that demonstrate significant utility in biologics research and an ability to faithfully mimic clinical responses across multiple disease states. Consequently, preclinical models that support research and translational *in vivo* studies will remain a pressing need. JoPM

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