Something old, something new... a marriage of technologies for immuno-oncology *in vivo* studies

Therapeutic successes in immuno-oncology have spurred much excitement and research in the field. Discovering and validating novel therapeutic targets to add to the immuno-oncology arsenal will require refined preclinical models that meet the needs for suitability, scalability and clinical relevancy throughout the various phases of target discovery and drug validation.

By Dr Philip Dubé

The use of animal models in oncology has undergone a revolution with the advent of cancer immunotherapy, and has become a powerful tool in understanding the fundamental biology of tumour-immune cell interactions and identifying therapeutic targets.

Immuno-oncology has led to a major shift in the way that researchers approach cancer. For decades, the emphasis was on cytotoxic agents that directly targeted tumour cells. From this perspective, where the focus was solely on the tumour, drug development relied on *in vitro* screens or cells engrafted into an immunodeficient animal. In the latter, the host animal might be considered merely a vessel for growing tumours, with little regard for how they interacted.

These studies, by design, did not allow researchers to probe the vital role that the immune system has in controlling malignant cells. In contrast, the essence of immuno-oncology requires researchers to probe directly how tumours interact with immune cells and other stromal cells within the host and the tumour microenvironment. From a commercial standpoint, this poses several important business challenges: What models should be used for decision-making during the various stages of immuno-oncology preclinical development? Which are better suited for discovery and optimisation versus validation? How relevant will these models be in the clinic? How would the necessary changes affect development costs and time to market? These challenges have spawned a new generation of animal models that blend old and new technologies to advance development efforts in immuno-oncology.

The right fit

Animal models have the potential to transform the immuno-oncology field, but only if the model type is matched to the research objective and specific phase of discovery or pre-clinical development. Immuno-oncology drug discovery can greatly benefit from two very different animal model types: syngeneic tumour models, a traditional platform proven effective for decades, and humanised models, a newer platform enjoying great success in various therapeutic areas, including oncology.

For discovery of novel immuno-oncology targets, syngeneic models, in which a mouse tumour is implanted in an immunocompetent mouse, are invaluable since the tumour and immune system can be engineered to probe novel pathways. In contrast, validation studies benefit from humanised and patient-derived xenograft (PDX) models since these tumours more closely match human cancer and immune biology. In choosing syngeneic versus humanised models for immuno-oncology research, it is critical to evaluate and ultimately select the

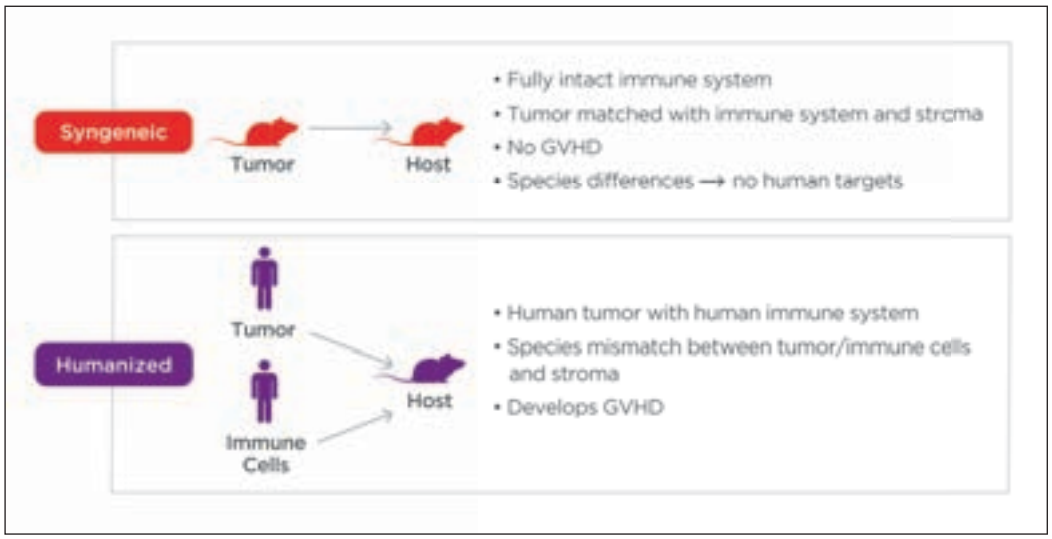

Figure 1
Different strategies for *in vivo* modelling in immuno-oncology research and development

right model for specific research objectives throughout various phases of drug development.

Syngeneic tumour models are an old and established oncology platform. They were first developed in the 1940s and 1950s, when scientists implanted spontaneously-derived tumours from inbred mice into genetically-identical (ie syngeneic) recipient mouse hosts. Given that these tumours originated from the same genetic background, they could grow and not be rejected outright by the host immune system, which made it possible to test cancer therapies *in vivo* for the first time.

Despite their age, syngeneic models are powerful tools for studying how tumours interact with the immune system. The first conclusive proof that the immune system could inhibit tumour growth came from syngeneic models, showing a role for both humoral immunity and T-cells in this effect. These early syngeneic studies essentially gave rise to the modern concept of immuno-oncology; however, they quickly fell out of favour upon the introduction of immunocompromised mice and the advent of xenograft models with human tumour cell lines, with the assumption that these would be more informative of human disease.

A resurgence for syngeneics

Today, however, syngeneic tumour models are making a comeback in modern immuno-oncology research and drug development. There are several important advantages to consider in these models when considering their use in immuno-oncology drug discovery.

Firstly, syngeneic models include a complete and functional immune system, which is critical for studying immune-tumour interactions. Mouse hosts for syngeneic tumours are fully immunocompetent and possess the cells and tissues necessary to initiate tumour immune responses. In contrast, certain immune cells in humanised models may be poorly developed or absent since the mouse host cannot fully support the engraftment or development of these cells.

Secondly, the genetics of the tumour cells and the host are matched in syngeneic models, allowing for improved research into interactions between tumour cells, immune cells, the associated stroma and humoral effects. These interactions influence tumour growth, immune cell infiltration and antibody responses, which are all potential therapeutic avenues. In syngeneic models, there are no genetic differences that might interfere with these interactions and how they contribute to relevant pathways for immuno-oncology discovery. While humanised models allow the tumour and immune system to interact, species differences will hinder how these interact with other tissues of the mouse host.

Finally, syngeneic models are a cost-effective discovery platform. At their most basic level, these models only require a normal inbred mouse and a tumour cell line of matched genetic background – this inexpensive option is at least 50 times less expensive compared to equivalent humanised models. This makes syngeneic models easily scalable to test multiple different tumours and drug candidates.

With at least 130 different murine tumour cell lines available, ranging in tissue of origin, immunogenicity and metastatic ability, syngeneic models provide researchers with a powerful arsenal to study immuno-oncology targets for multiple types of tumours. Using these approaches, syngeneic

models were critical in the identification and validation of CTLA-4 and PD-1 as therapeutic targets, leading to the development of FDA-approved agents such as Yervoy® (Ipilimumab), Opdivo® (Nivolumab) and Keytruda® (Pembrolizumab).

What makes syngeneic models so powerful in ongoing research endeavours is the combination of established murine tumour cell lines with modern and emerging genetically-engineered models (GEMs). Existing immunology GEM models make it possible to study how specific pathways (eg immune cell subsets, cytokines or specific T-cell responses) contribute to immune control of tumour growth. This makes use of a wide range of established immunological models and applies these to oncology studies to identify fundamental aspects of how the immune system regulates tumour biology.

While these immunological approaches provide an important step towards uncovering basic biology in this system, mouse genetic engineering advances make it possible to also discover and validate novel targets. Checkpoint blockade inhibitors, such as Keytruda®, are arguably only the tip of the iceberg of potential immuno-oncology targets, and syngeneic models provide the flexibility and scalability to interrogate biological pathways in developing the next generation of immuno-oncology drugs. By engineering mice with specific alterations in discrete immunological pathways, this provides avenues to discover novel targets for therapeutic intervention.

Syngeneic models play an important role in basic discovery efforts, but how can they contribute to target validation efforts? Once again, the flexibility of mouse models and the marriage of new technologies are an asset. Mice can be easily genetically engineered to express human oncogenes and humanised immuno-oncology targets, either through transgene insertion or targeted knockout and replacement strategies. The advent of clinically-relevant oncogene GEM models makes it possible to study how specific mutations observed in clinical samples can impact tumour immunosurveillance, such as the BRAF V600E mutation in

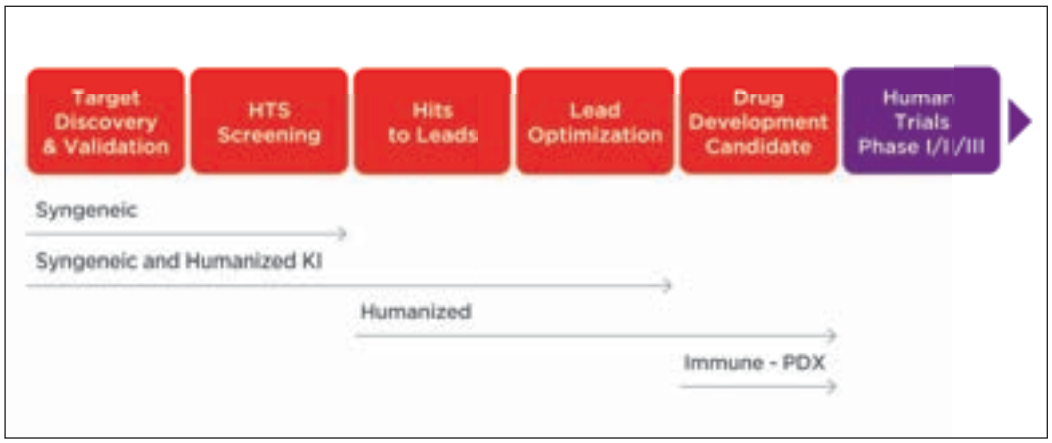

Figure 2
Preclinical objectives drive the decision to use syngeneic or humanised models throughout different phases of drug discovery

melanoma. Furthermore, this flexibility makes it possible to develop mouse GEM models with human gene knock-ins (KI) for therapeutic targets in validation studies. This is particularly useful when evaluating human-specific monoclonal antibodies or in the instances when human and mouse biology differ.

The emerging role of humanised models

Syngeneic models in mice offer a flexible, scalable and cost-efficient platform for drug discovery and early validation in immuno-oncology. Despite these attributes, these models are inherently non-human, and species-specific differences may hinder their ultimate applicability to the clinic. Fortunately, there are now several humanised models that provide additional benefit to validation efforts.

Humanised models are the newest tools for immuno-oncology research. While xenograft models with implanted tumour cell lines are well-established, a major roadblock to studying tumour-immune interactions in these models had been the relative inability to establish long-term engraftment of hematopoietic lineages of the human immune system. The development of super immunodeficient mice (eg NOD.Cg-Prkdcscid Il2rgtm1Sug/JicTac) that allow for the engraftment of both tumours and immune cells, has opened new and exciting avenues for immuno-oncology research. These mice support robust tumour growth and the engraftment of human lymphoid cells, making them ideal to study how human T-cells interact with human tumour grafts. Next generation super immunodeficient mice improve on this base model and allow for the engraftment of a more complete human immune system. These mice express a variety of human cytokines transgenes that support additional immune cell lineages to test how these cells interact with tumours. For example, transgenic mice expressing the cytokines IL-3 and GM-CSF make it possible to test agents that act indirectly (through myeloid cells) to control how T-cells respond to tumour antigens. The future refinement of mouse models with increasingly complete and sophisticated humanised immune systems will be powerful tools to study human immune responses to human tumours.

Humanised models fall into two basic categories: humanised immune system + human tumour cell lines, or humanised immune system + patient-derived xenografts (Immune-PDX). Hundreds of the human tumours cell lines that were established and validated in standard xenograft models can be used to test tumour-immune interactions in these humanised models.

Tumour cell lines have the advantage of well-characterised growth and performance, the opportunity for genetic modification, and the ability to scale up through *in vitro* propagation. However, a major criticism of using any tumour cell line is that these have been selected by growth *in vitro* and lack the cellular heterogeneity seen in primary tumours in patients. In PDX models, excised tumours are implanted and propagated *in vivo*, and maintain many of the characteristics of the primary tumour, including cellular heterogeneity. PDX tumours can also be selected and expanded *in vitro* to create characterised cell lines to address specific cell populations within a tumour. Combining PDX with human immune cell engraftment may prove to be a highly relevant model to validate immuno-oncology applications. This model even makes it possible to study how immune cells and a tumour from the same patient interact.

While humanised models may be better suited for validation studies, they are inherently less flexible to answer specific research questions, and are

more expensive and less scalable for screening due to the fact that immunodeficient mice need to be engrafted with both a human immune system and a tumour. There are several other important limitations to be considered with humanised models.

First is potential rejection by the engrafted human immune system. Human leukocyte antigen (HLA) mismatches between the engrafted immune system and tumour may lead to erroneous anti-tumour activity. Since these studies generally require that the hematopoietic cells and tumour cells come from different individuals, there is a real risk that immune rejection of the tumour may occur. At best, this effect may be minor and affect test and control subjects equally; at worst, this could potentially interfere with test compound efficacy and affect model performance.

Secondly, there is an inherent time limitation for these types of models. Immunodeficient hosts engrafted with human hematopoietic cells are prone to developing graft-versus-host disease (GvHD) since human immune cells attack mouse tissues. In general, this limits the length of time that these models can be reliably employed before the onset of clinical disease. While this may not be an issue for fast growing tumour cell lines, this is problematic for slow growing tumours, including many PDX models, which often take many months to establish and respond to treatment. Understanding the limitations and the power of humanised models for immuno-oncology will help to inform decisions about the appropriate uses of these models in the discovery and validation pipeline.

Objectives drive the decision

Animal models are an important tool in the discovery pipeline for immuno-oncology therapies. Like any tool, these models must be used appropriately and judiciously. The high failure rate of oncology drugs in the clinic has led many to question the value of animal models. One issue here is that preclinical *in vivo* studies often rely on a relatively small number of models given the time and money involved. (Contrast this with high throughput *in vitro* screens that can quickly and cheaply screen hundreds to thousands of different tumour cell lines for responses to candidate drugs.) Increasing the number of *in vivo* models involved in discovery phases will go a long way to increase their predictive power. Studies have clearly shown that preclinical results are more likely to inform clinical success when drug candidates are evaluated in a number of different and complementary *in vivo* models. It is becoming clear that decisions to advance drugs to clinical development should be based on strong results throughout multiple experimental *in vivo* models. While this means more money and increased time to market, this is also critical to fully validate and optimise drug candidates and reduce subsequent clinical failures. This concerted approach in evaluating preclinical results through expanded *in vivo* modelling will likely lead to a greater return on investment with increased chances for subsequent clinical success.

For immuno-oncology, this concerted approach means identifying potential targets in syngeneic models, preferably through multiple different experimental paradigms, involving a range of different tumour lines. Overinterpretation of results from a single study, or of small effect sizes, should be viewed with caution. A valuable next step would include the use of genetically-engineered models to confirm therapeutic mechanisms, or the use of human KI GEM models to validate and optimise targeting strategies. Humanised immune system models round out the pre-clinical phase by adding important validation data for identified lead compounds or targets. Here again, research should focus on multiple sources of immune cells and tumours to minimise biases and increase the predictive power of these studies.

By combining established oncology approaches with genetic engineering and humanisation approaches, animal models give us the ability to discover and validate the next generation of immuno-oncology drugs. These models are becoming more complicated and highly refined, and choosing appropriate applications will require a deep knowledge of the biology involved and its utility in each phase of drug discovery and development. DDW

***Dr Philip Dubé** is a Field Applications Scientist with Taconic Biosciences. Philip has degrees in Pharmacology and Physiology from the University of Toronto and has a depth of expertise in animal models of immunology, oncology and microbiome research.*