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Experimental animal modeling for immuno-oncology

Qi-Xiang Li [a,b,*], Gerold Feuer [c], Xuesong Ouyang [a], Xiaoyu An [a,b]

[a] Crown Bioscience Inc., 3375 Scott Blvd, Suite 108, Santa Clara, CA 95054, USA
[b] State Key Laboratory of Natural and Biomimetic Drugs, Peking University, Beijing 100191, China
[c] HuMurine Technologies, Inc., 2700 Stockton Blvd, Rm. 1403, Sacramento, CA 95817, USA

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ABSTRACT

Immuno-oncology (I/O) research has intensified significantly in recent years due to the breakthrough development and the regulatory approval of several immune checkpoint inhibitors, leading to the rapid expansion of the new discovery of novel I/O therapies, new checkpoint inhibitors and beyond. However, many I/O questions remain unanswered, including why only certain subsets of patients respond to these treatments, who the responders would be, and how to expand patient response (the conversion of non-responders or maximizing response in partial responders). All of these require relevant I/O experimental systems, particularly relevant preclinical animal models. Compared to other oncology drug discovery, *e.g.* cytotoxic and targeted drugs, a lack of relevant animal models is a major obstacle in I/O drug discovery, and an urgent and unmet need. Despite the obvious importance, and the fact that much I/O research has been performed using many different animal models, there are few comprehensive and introductory reviews on this topic. This article attempts to review the efforts in development of a variety of such models, as well as their applications and limitations for readers new to the field, particularly those in the pharmaceutical industry.

Contents

Abbreviations: CAR-T, chimeric antigen receptor-T cell; CTL, cytotoxic T lymphocyte; CTLA-4, cytotoxic T lymphocyte antigen 4; FACS, fluorescence-activated cell sorting; GEMM, genetically engineered mouse model; GvHD, graft-versus-host disease; HLA, human leukocyte antigen; HPC, hematopoietic progenitor cell; HSC, hematopoietic stem cell; ICD, immunogenic cell death; IHC, immunohistochemistry; IL, interleukin; I/O, immuno-oncology; KI, knock-in; MDSC, myeloid derived suppressor cell; MOA, mechanism of action; NK, natural killer; PBMC, peripheral blood mononuclear cell; PD, pharmacodynamics; PD-1, programmed cell death protein 1; PD-L1, programmed cell death 1 ligand 1; PDX, patient-derived xenograft; POC, proof of concept; SCID, severe combined immunodeficiency; SOC, standard of care; TIL, tumor infiltrating lymphocyte; TME, tumor microenvironment.

* Corresponding author at: Crown Bioscience Inc., 3375 Scott Blvd, Suite 108, Santa Clara, CA 95054, USA.
E-mail address: henryli@crownbio.com (Q.-X. Li).

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1. Introduction

Despite currently available oncology therapies, including surgery, radiation, chemotherapy and targeted therapies, the majority of certain malignancies are still incurable and unmanageable, perhaps until now, with new immuno-oncology (I/O) therapies appearing on the horizon (Ascierto, Melero, & Ascierto, 2015; Postow, Callahan, & Wolchok, 2012). The discovery of T-cell immuno-inhibitory pathways has uncovered powerful mechanisms by which tumors evade the immune system. These mechanisms are frequently referred to as immune checkpoints or co-inhibitory pathways (Sanmamed, Pastor, et al., 2015; Schreiber, Old, & Smyth, 2011). Targeting immune checkpoints, *e.g.* programmed cell death protein 1 (PD-1), programmed cell death 1 ligand 1 (PD-L1) and cytotoxic T lymphocyte antigen 4 (CTLA-4), has achieved benefits in multiple cancers by blocking immuno-inhibitory signals and enabling patients to produce an effective anti-tumor response. In 2011, the FDA approved a therapeutic antibody that blocks CTLA-4 for the treatment of melanoma, ipilimumab (YERVOY®/BMS), followed by the approval of pembrolizumab (KEYTRUDA®/Merck), the first approved therapeutic antibody targeting PD-1 in 2014 (Hamid et al., 2013; Hodi et al., 2010; "TCGA Research Network: http://cancergenome.nih.gov/,"). Several other therapeutic agents targeting CTLA-4, PD-1 and other immune checkpoints are currently in development; combination treatments with PD-1 and CTLA-4 blocking antibodies have also significantly increased objective response rates in melanoma and are currently in Phase III trials in multiple tumor types (Postow et al., 2015). With rapid clinical development, checkpoint inhibitors have now been approved in several more cancers, including NSCLC, HNSCC, RCC, and classical Hodgkin lymphoma (Ansell et al., 2015; Brahmer et al., 2012; Herbst et al., 2014; Hodi et al., 2010; Powles et al., 2014; Topalian et al., 2012), with the list increasing over time. Thanks to these breakthroughs, certain patients (those without other prospects of long term survival) may now greatly benefit from these new treatments, resulting in long term survival with manageable conditions. These treatments may also represent the coming of age of immunotherapy for cancers, and are now attracting unprecedented intense research which is rapidly changing the landscape of cancer treatments (Pardoll & Drake, 2012; Pardoll, 2012). The significant advantages of cancer immunotherapy include improved safety margin, *e.g.* as seen for checkpoint inhibitors in general (Topalian et al., 2012), and prolonged effect due to immune memory, therefore preventing relapse and metastasis in many cases. It is worth noting certain immunotherapies, *e.g.* chimeric antigen receptor-T cell (CAR-T) therapy can sometimes cause severe toxicity in patients.

2. The need for I/O animal models

Notwithstanding the considerable success of the current checkpoint inhibitors and the great promise of new immunotherapies, many important questions remain to be addressed (Pitt, Vetizou, Daillere, et al., 2016), including: 1) why only subsets of patients respond; 2) what determines response, the host factors (*e.g.* immuno-state of patients including the microenvironment (TME) of the tumors), and/or tumor specific factors (*e.g.* neo-antigens); 3) how to expand patient populations so more patients can benefit (*e.g.* effective combination therapy); and 4) how to discover and validate new I/O targets and agents, including new checkpoint targets/inhibitors. Major hurdles in addressing these key medical questions are the lack of adequate preclinical animal models capable of mimicking patient conditions and predicting responders (and non-responders) to I/O therapies. The ideal preclinical platform needs to be predictive of preliminary safety assessment and efficacy, be reproducible, and have clinical applicability.

Currently traditional therapies, including more recent targeted therapy, have been focused on the nature of tumors, and the most commonly-used experimental cancer models are human xenograft tumors grown in immunocompromised mice (*e.g.* athymic nude mice, and SCID mice), derived from either *in vitro* immortalized cancer cells (cell line derived xenografts) or patient tumors (patient-derived xenografts, or PDXs) (Tentler et al., 2012; Yang et al., 2013). However, both tumors and hosts are critical in today's I/O treatments, and thus the nature of the immunodeficiency of these models renders them generally inadequate for many I/O investigations. At present, preclinical efficacy/safety assessments of immunotherapies, on the other hand, are largely based on the evaluation of surrogate anti-mouse target antibodies using mouse syngeneic or genetically engineered tumor models, with the assumption that the mouse tumor and immunity mimics that of humans (Payne & Crooks, 2007; Takao & Miyakawa, 2015). However, this strategy is limited by that it can only test surrogate molecules that target the mouse immune system/tumors, where inherent differences between the two species occur (Mestas & Hughes, 2004; von Herrath & Nepom, 2005). Recent failures of MAGE-A3 (GSK) and tecemotide (Merck) in late stage clinical trials outline the urgent need for a model system that includes both adequate human tumors and immune cells to achieve a comprehensive understanding of human tumor immunobiology, which is necessary for the development of new immunotherapies. This article attempts to review the advancements of I/O animal modeling (see Table 1 for available I/O models of human and mouse origin).

3. Commonly used mouse strains in cancer models

Most commonly used experimental cancer models are tumors grown in mice, either human tumor xenografts or mouse tumor homografts. Although human xenografts in immuno-compromised mice have been much more widely used in traditional preclinical cancer pharmacology investigations, mouse tumor homografts in immuno-competent syngeneic mice are commonly used in today's I/O research. There are many mouse strains that have been broadly used to support tumor grafts: immunodeficient mice can support human tumor xenografts, and immuno-competent mice can support mouse tumor homografts. For the principle of reductionist experiments, laboratory inbred strains of mice were commonly used for their consistent biological properties and biocompatibility (non-rejection of homografts) (see below). The most commonly used inbred imunocompetent mice are C57BL/6 and BALB/c strains, or those derived from them.

Table 1
I/O animal models of both human and mouse origin.

Platform	Tumor	Immunity	Target	Therapy[b]	Property	Unique utility
Xenograft tumor	Hu	Defective, Mu	Human/Mu[a]	Hu/Mu[a]	Cell line derived, PDX (primary tumor, relevant path.)	Cell therapy (*e.g.* CAR-T), non-T I/O, *etc.*
Syngeneic tumor	Mu	Mu	Mu	Mu	Cell line derived	MOA, surrogate POC
GEMM tumor	Mu	Mu	Mu	Mu	Spontaneous, and primary, relevant path.	Target therapy combo, preventative vaccine
Homograft primary tumor	Mu	Mu	Mu	Mu	"Mouse PDX", primary, relevant path.	Target therapy combo
HuGEMM™	Mu	Mu	Hu	Hu	Partially humanized (target)	Human therapy
Xenograft/humanized	Hu	Hu	Hu	Hu	huPBMC, huHSC, relevant immunity	Human therapy

Note: huHSC: $CD34^+$ cells are usually derived from fetal liver, cord blood or PBMC; POC: proof of concept.
[a] Target immune system.
[b] Assuming species-specific.