Toward a Better Effective Preclinical Model in Immuno-Oncology

Commentary by Alessia Armezzani, PhD 07/01/2020

Animal research has been extensively used in oncology and onco-pharmacology to understand the mechanisms that underpin cancer development, and to design effective tumor treatments.(1) However, the challenges along the path of converting results obtained at the bench into tangible clinical endpoints are numerous and formidable, including the choice of the right model to answer precise immunological questions.(2) Organisms such as the budding yeast *Saccharomyces cerevisiae*, the roundworm *Caenorhabditis elegans*, the fruit fly *Drosophila melanogaster*, the frog *Xenopus laevis*, and the zebrafish *Danio rerio* have made important contributions to our understanding of cancer; however, the laboratory mouse *Mus musculus* remains still the model of election to design specific therapies and evaluate their efficacy prior to clinical trials.(1)

Scientists have now access to a growing range of preclinical mice, each with their specific strengths and limitations.(3) Here, we briefly review the most relevant models used in preclinical cancer research.

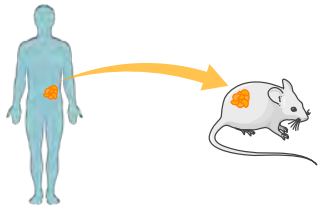
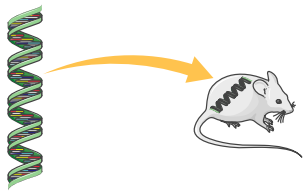
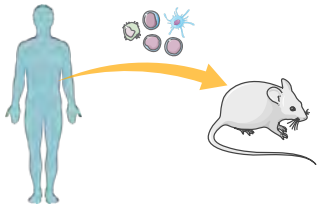
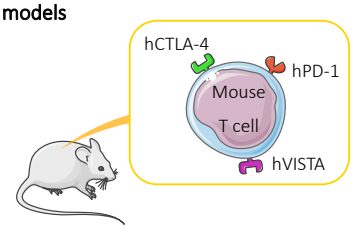
Cell line transplantation models

Cell line transplantation models represent the most commonly used mouse models in onco-immunology. They consist of murine or human cancer cell lines, injected either subcutaneously, orthotopically (to mimic their evolution in a physiological environment), or systemically (to monitor their metastatic spread) in immunocompetent mice.(4) These models are useful to study the pathophysiological relevance of *in vivo* tumor initiation, and for preclinical drug testing.(5) For example, transplantation models have provided important insights into drug resistance mechanisms and novel combination therapies in colorectal cancer.(6) However, such models do not mirror the intra- and inter-tumor heterogeneity of human cancers[1] due to the genetic homogeneity acquired by cell lines through repeated *in vitro* passages, and are therefore poor predictors of therapy responses.(7)

Patient-derived xenografts

Patient-derived xenografts (PDXs) are established by transplanting fresh human tumor biopsies in immunodeficient mice.(9) Unlike cell line transplantation models, PDXs preserve intra- and inter-tumor heterogeneity as observed in cancer patients, and provide clinically valuable data in various tumors, including colorectal cancer, breast cancer, non-small-cell lung cancer, and prostate cancer.(10) As PDXs are engrafted in immunodeficient mice, they lack a normal adaptive immune system: for this reason, the use of these models is typically restricted to chimeric antigen receptor (CAR)-T and engineered T cell therapy studies.(10)

[1]Cancer heterogeneity refers to the existence of subpopulations of cells that display cellular, genetic, and epigenetic variations within a primary tumor and its metastases (intra-tumor heterogeneity), and between tumors of the same histopathological subtype (inter-tumor heterogeneity).(8)

Cancer preclinical model	Advantages	Disadvantages
Cell line transplantation models 	• Simple and low cost • Rapid tumor growth • Highly reproducible phenotypes	• Mouse immune system • Insufficient number of simultaneous spontaneous tumors • Lack of intra- and inter-tumor heterogeneity
Patient-derived xenografts 	• Progressive tumor growth and amplification • Predictive therapeutic value • Maintenance of intra- and inter-tumor heterogeneity	• Immunodeficient model (i. e., no functional mouse immune system) • Physiological tumor microenvironment
Genetically engineered mouse models 	• Faithful recapitulation of human cancer development • Fully functional mouse immune system	• Mouse immune system • Time consuming and expensive • Unexpected and highly variable phenotypes
Human immune system mouse models 	• Studies on human immune cells' function in human tumor tissues	• Potential incompleteness and lack of physiological maturity of reconstituted human immune cells
Humanized immune checkpoint mouse models 	• Fully functional mouse immune system • Proper interaction between stroma, microenvironment, and immune cells	• Mouse immune system

Genetically engineered mouse models

Genetically engineered mouse models (GEMMs) are sophisticated immunocompetent mice harboring constitutive or inducible mutation(s) that lead to tumor development.[5] Such models have provided the scientific community with important insights supporting the immunosurveillance theory, "a natural physiologic function" that allows "recognition and destruction of transformed cells before they grow into tumors, and kill tumors after they are formed."[11, 12] GEMMs closely recapitulate human cancer in terms of genetic composition and crosstalk between tumor cells, stroma, and tumor microenvironment; as such, they are useful to identify tumor-initiating and tumor-promoting events, and are therefore of great importance to unveil the complex mechanisms underlying cancer biology.[13] However, GEMMs do have some limitations: first, the generation and validation of these models is laborious and expensive;

second, these models may possess unpredicted mutations (namely “off-target effects”) caused by the intrinsic properties of the methods used to develop them (e.g., CRISPR/Cas9); third, these models bear biallelic mutations in the target site, and may therefore give rise to embryo lethal phenotypes.[14, 15]

Human immune system mouse models

Immunocompetent mice have been widely used in biomedical research, where they represent effective tools to analyze immune responses directed against engrafted allogeneic tissues. However, critical differences in the genetics and immune systems of mice and humans have precluded certain studies, notably those aiming at assessing drug efficacy. This “gap” has been filled by immunodeficient mice reconstituted with human immune system (HIS).[10] These models have dramatically improved our understanding of the function of the human immune system, and contributed to the study of the complex interactions between myeloid cells, antigen-presenting cells and T cells in reconstituted tumor microenvironments.[16] This has led to the development of novel therapeutics, and the efficacy assessment of immunotherapies prior to translation into the clinic.[3] However, HIS models also have some limitations, including a limited lifespan and incomplete human immune function (e.g., lack of B cell immunoglobulin G responses, underdeveloped lymphoid organs): it is extremely important that these issues are carefully taken into account in the interpretation of the experimental results.[16]

Humanized immune-checkpoint mouse models

Humanized immune-checkpoint (ICP) models are generated by inserting chimeric (i.e., murine and human) ICPs within murine ICP loci.[17] *In vivo* studies conducted in these animals have been instrumental in assessing the efficacy of immuno-oncology compounds directed against ICP, and in developing new immunotherapies for solid cancers such as metastatic melanomas, non-small-cell lung carcinomas and liver cancer.[13, 16] Moreover, ICP models represent powerful tools for studying how compounds modulate immune cell response and/or stroma cells in a physiological microenvironment.[4] However, these mice possess murine immune systems and therefore fail to recapitulate the potential of individual ICP pathways in regulating T cells and, more generally, immune responses.[16]

Recent technological advances have led to the generation of a wide range of new experimental preclinical models; however, many novel oncology drugs have failed to pass phase II programs.[18] This can be attributed, at least in part, to the misleading interpretations of results obtained in models that do not necessarily best answer specific immunological questions.[2] Accordingly, there is an urgent need to develop a line of sight to the clinic at the very early steps of drug discovery projects, as this will help to select appropriate preclinical models, thereby more efficiently translating preclinical research into successful clinical trials.

In our next commentary, we will discuss humanized mouse tumor models in more detail.

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