Cancer Cell

CellPress

Voices

Models for Immuno-oncology Research

The interactions between cancer cells and immune cells are complex and context dependent. Choosing the right model to study these interactions is a crucial step in the development of immunotherapies. From cell co-cultures to organoids, organs-on-chip, and a variety of mouse models, experts share their model of choice for immuno-oncology research and discuss their strengths and caveats.

Tumor Immunity in Organoids

Calvin J. Kuo
Stanford University School of Medicine, California

The transformative impact of immunotherapy has been accompanied by an equal recognition of its limitations. Developing new immunotherapies and understanding resistance to current approaches could be greatly aided by human *in vitro* model systems that embody the diversity and interactions between tumor stromal immune populations. Organoid methods are now widely used to culture cancer biopsies but typically only contain tumor cells and not immune components.

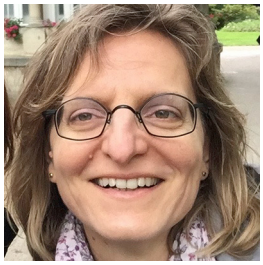
I've been very interested in the creation of organoids that not only contain epithelial cells but also maintain a rich stromal diversity. Over the years, we have generated organoids using an air-liquid interface (ALI) method that allows tumors to be grown as a cohesive unit preserving cancer cells en bloc with their native stroma without requiring artificial reconstitution. These ALI organoids intrinsically possess the fibroblasts and diverse immune populations of the parental tumors, including T, B, and NK cells and macrophages, even preserving T cell receptor diversity. We've started to use such organoids as more holistic models of the tumor microenvironment and to model immune checkpoint blockade. It has been interesting to observe that anti-PD-1 checkpoint inhibitors can initiate anti-tumor immune responses within such organoids from subsets of patients, in a manner that seems independent of tumor PD-L1 expression status. There's a long way to go, but potentially these organoids could serve as *in vitro* avatars to examine immunotherapeutic sensitivity and resistance, test new treatments, and predict individualized responses.

Organoids and Immunotherapy

Emile Voest
The Netherlands Cancer Institute, Amsterdam, the Netherlands

Immunotherapy has great therapeutic potential, but a better understanding of who will benefit from this treatment is highly needed. There is an abundance of models to study immunotherapy, but not one model entirely recapitulates the complexity of the human immune system. Tumor organoids closely resemble the patient's tumor and are an improvement over generating patient-derived cell lines. A clear advantage of organoids is that you can use a fully autologous *ex vivo* model with tumor cells, immune cells, and stromal cells, all from the same patient. Also, findings can be correlated with the patient's clinical outcome. These models provide insights into how T cells interact with tumors, allow for neo-antigen discovery on an individual basis, and have the potential to advance T cell therapy. Organoids also shed light on the contributions of other immune cells, such as macrophages, natural killer cells, and gamma-delta T cells. However, as with every model, there are limitations to the organoids. Not all tumors can be used to generate tumor organoids, tumor biopsies reduce the success rate of generating organoids over resections, culture conditions are very specific, and many growth factors are required to maintain the cultures but may also impact other cells with which the organoids are cultured. Growth rates may be slow (e.g., breast cancer) or tumor purity uncertain due to overgrowth of normal epithelial cells (e.g., lung cancer). Before you embark on using organoids as an immunotherapy model, it is therefore advised to understand their potential and pitfalls. Once you have established that, organoids can be a great immune-oncology tool.

Tumor-on-Chip Breakthrough



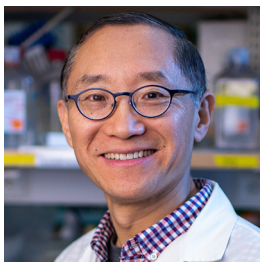
Maria Carla Parrini
Institut Curie, Inserm U830, France

A critical problem in the development of effective anti-cancer treatments is the lack of clinically relevant model systems. Conventional cell cultures or animal models fail to accurately predict drug responses in humans, as they do not properly mimic the complexity of the tumor microenvironment. This is why clinical trial success rate in oncology is dramatically low (<4%).

The emerging technology of organ-on-chip (OoC), and specifically of tumor-on-chip (ToC), was born from the combination of cell biology, microfabrication, and microfluidics. ToC platforms are generated by co-culturing tumor and stroma cells (immune cells, endothelial cells, fibroblasts) within 3D biomimetic matrices in microfluidics devices, also called "chip." They are immunocompetent, in that they recapitulate the interplay between immune and cancer cells. They can be personalized by introducing patient-derived autologous primary cells, and they can be treated with drugs and visualized in real time by video microscopy. ToC is a disruptive approach to investigate the drug-dependent plasticity of tumor ecosystems and the mechanisms underlying immunotherapy resistance.

Moreover, ToC technology contributes to the reduction of the need for animal testing, supporting the 3R principles of replacement, reduction, and refinement. A ToC experiment is less expensive than an animal one, is faster, is more ethically acceptable, and is potentially more predictive. In the future, ToC-based companion diagnostic tests might be valuable for several purposes, such as to differentiate patients that are responders from non-responders to immunotherapies, to define efficient doses and combination therapies.

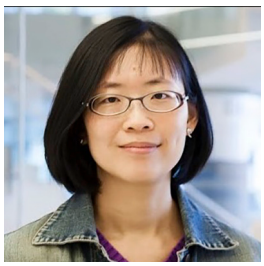
Model and Clinical Integration



Weiping Zou
University of Michigan School of Medicine, Michigan

Immunotherapy has seen unprecedented success in cancer therapy. Current challenges in tumor immunology include identifying novel "checkpoints" as potential therapy targets and overcoming immunotherapy resistance. To this end, wide adoption of multiple culture systems, complementary animal models, and studies in patients with cancer are essential to understanding the molecular and cellular mechanisms shaping spontaneous and therapy-induced tumor immunity. Several mouse and human tumor cell lines (including primary human cancer cells), tumor tissues, and immune cell subsets from different compartments (including the tumor microenvironment) are often included in the *ex vivo* and *in vitro* culture systems. Tumor progression and therapy in syngeneic immunocompetent murine models and human chimeric NOD-scid IL2rg^{tmWjl}/J (NOD-scid IL-2Rγ^{null}) (NSG) mice with adoptive transplantation of human immune cells (particularly T cells) and human tumor tissues are frequently and practicably applied in cancer immunology research. Obviously, no single model or approach accurately mimics and recapitulates the complexity of patients with cancer. Indeed, each model entails specific drawbacks and is subject to the influence of other parameters. Thus, it is critical to extend and validate our data in primary cells and tissues from cancer patients. In addition, it is strongly encouraged to link our observations to genetic, therapeutic, pathological, and clinical information. Inclusion of multiple *ex vivo*, *in vitro*, and *in vivo* models with clinical information generates complementary and confirmatory data, thereby making a compelling case in our understanding of cancer immunity and immunotherapy.

Mice Advance Cancer Research



Michele W.L. Teng
QIMR Berghofer Medical Research Institute, Australia

Experimental mouse models allow for a controlled validation of the efficacy and safety profiles of novel immunotherapy drugs in a complex biological organism and can easily be genetically manipulated. As Rolf Zinkernagel said, "It's either legs up or down (for the mouse)!" Mechanism of action studies in mouse models provide valuable information to guide biomarker development and to understand in which tumor microenvironments any particular drug might be most efficacious. Scheduling of immunotherapies with other standards of care or their potential to induce toxicities can be evaluated using appropriate tumor models or mouse strains. Using the 4T1.2 and E0771 spontaneously metastatic breast cancer models, my group found that neoadjuvant immunotherapy was superior to adjuvant immunotherapy in the context of cancer surgery. Our findings provided the rationale for new comparative trials of neoadjuvant and adjuvant immunotherapies in different cancer types, which subsequently have corroborated our pre-clinical findings.

Nevertheless, we must be mindful of the caveats associated with mouse tumor models. The real skill lies in validating hypotheses and mechanisms across a breadth of models that best answer the scientific questions you pose. This requires both an in-depth understanding of tumor models and the clinical setting being modeled. Sadly, expertise and skills in mouse experimentation have declined with increased time demands and the cost of such work caused by continual expansion of monitoring, reporting, and other regulatory requirements. Ironically, this comes at a time when big data lacks functional answers and patients' ability to benefit from advances (bench to bedside) has never been greater.

The Truth about Mouse Models

Tim F. Greten
Center for Cancer Research, National Cancer Institute, Maryland

Medical oncology has dramatically changed with the approval of various immunotherapies. The development of any of these treatments would have been impossible without understanding the immunological mechanisms and testing potential therapies in murine animal models. While initial mouse models relied on immunocompetent syngeneic tumor transplantation, newer studies have focused on the actual clinical scenario of cancer patients. Human tumors are implanted into immunodeficient mice together with a "pseudo" human immune system consisting of PBMC or CD34^{+} stem cells. However, most of these models rely on subcutaneous implantation methods and lack the specific organ-dependent tumor microenvironment. Furthermore, intra- and inter-tumor diversity are very hard to model in mice. Understanding the role of the microbiome in anti-tumor immunity, tumor growth, and responses to immunotherapy is more challenging. Mice kept under laboratory conditions differ in their immune responses from conventional mice found in the wilderness. So why do we still rely so much on mouse models? Well, they still remain the closest we can come to the patient and have proven to be highly effective in helping to understand immunological mechanisms. It is important to keep in mind that even the most sophisticated mouse models using orthotopic, human-derived tumor cells in immune-compromised mice with a humanized immune system and a specific microbiome remain only a model to answer a very specific question. Only a clinical trial will expose the ultimate truth of whether a new treatment approach is successful in patients with cancer, and this is what makes experimental medicine one of the most exciting research areas.

CellPress

Humice and Immunotherapy

Karolina Palucka
JAX Cancer Center, The Jackson Laboratory for Genomic Medicine, Connecticut

Immunocompetent mice have provided translational utility in dissecting cancer-intrinsic pathways and conserved immunological mechanisms, including T cell checkpoints. However, critical differences in certain components of the immune systems of mice and humans, particularly myeloid cells, lead to species-specific tumor-host interactions. These differences can be overcome by conducting preclinical studies in immunodeficient humanized mice (Humice) engrafted with human cells or tissues. Humice enable *in vivo* mechanistic investigations of immunotherapy toxicity and of therapeutic effector function in genetically diverse contexts. For example, treatment of leukemia-bearing Humice with chimeric antigen receptor T cells revealed a key role for monocytes in cytokine release syndrome. Pembrolizumab efficacy studies using patient-derived xenografts in Humice showed strikingly similar responses to those in cancer patients. The development of a robust, functional human immune system in Humice following engraftment of hematopoietic progenitor cells is a major goal for the field. Progress remains constrained by suboptimal development of lymphoid architecture; impaired class switching and affinity maturation of immunoglobulins; and species specificity in major histocompatibility antigens, hematopoietic growth factors, and cytokines. Nevertheless, CRISPR-based engineering has recently facilitated more-precise humanization at the genetic level, including expression of putative or validated human therapeutic targets. Improved Humice will manifest an increasingly human immune context and will bring about the identification of new immunological targets with enhanced translational potential.

Furry Test Tubes and Avatars

Saar Gill
University of Pennsylvania, Pennsylvania

Every biologist knows that a molecule is not a cell, a cell is not an organ, an organ is not an organism, and a mouse is not a human. All of these models must be called upon at some stage in order to understand the complexity of the interaction between cancer and the immune system. To reduce this complexity, I try to go back to the fundamentals of pharmacology: pharmacokinetics describes trafficking, proliferation, and contraction or exhaustion of the adoptively transferred immune cells. Pharmacodynamics is reflected in adverse effects and in determinations of tumor response or resistance. Living drugs such as engineered cell therapies may be subject to a third law: impact of the disease on the drug. Thus, cell therapies may behave differently in different patients, since the cancer under treatment or its attendant microenvironment can exert a dominant effect over the administered therapeutic product. For this reason, my preferred vehicle for studying these interactions is the immunodeficient "patient-derived xenograft" (PDX) mouse model, whereby immunodeficient mice are engrafted with primary patient-derived hematologic malignancy cells and treated with engineered human cells such as chimeric antigen receptor (CAR) T cells or macrophages, as a sort of avatar of the patient. In order to circumvent some of the limitations of this "furry test tube" model, I first "humanize" the mice by engrafting them with primary human hematopoietic and immune cells. This approach provides the minimal information required for testing immunotherapeutics in the only model that matters, a human being with cancer.

Studying Cancer Immunobiology

Nikhil S. Joshi
Department of Immunobiology, Yale University School of Medicine, Connecticut

Tumors develop for months or years before cancer is diagnosed, and throughout that time, tumor cells interact with the cells in their tissue microenvironment, including immune cells. Tumor development (and potentially their responses to future therapy) is critically shaped by these interactions, but most of the immunobiology of these early stages remains underexplored. In part, this is due to the lack of suitable animal models for studying these processes. Most transplantable tumor models reflect late-stage cancers, and spontaneous or genetically engineered mouse (GEM) cancer models often lack sufficient neoantigen burdens to drive meaningful anti-tumor T cell responses. As a consequence, we have significant data about the phenotypes and functions of immune cells in advanced tumors, but we have a poor understanding of how these immune responses developed and about the roles that immune cells play in early disease.

We and others have focused on the development of novel tumor models to address these gaps. Programming developing tumors in GEM models to express known model antigens or to have higher somatic mutation rates (i.e., through deletion of MMR genes) can profoundly change immune infiltrates. We developed the NINJA model, which creates known neoantigens *de novo* in GEM models. This model has been particularly useful for our studies on how endogenous tumor-specific T cells in tumors change between early and late tumors and has revealed mechanisms for how their responses are sustained. Investigating these early immune processes is informing our understanding of how immunosurveillance suppresses early tumors and on how breakdowns in immune control lead to cancer.