ANNUAL REVIEWS

Annual Review of Cancer Biology

Engineering the Immune Microenvironment into Organoid Models

Kathleen A. Luckett[1,2,3] and Karuna Ganesh[1,4]

[1]Molecular Pharmacology Program, Sloan Kettering Institute (SKI), Memorial Sloan Kettering Cancer Center, New York, NY, USA; email: ganeshk@mskcc.org

[2]Weill Cornell/Rockefeller/Sloan Kettering Tri-Institutional MD/PhD Program, New York, NY, USA

[3]Gerstner Sloan Kettering Graduate School of Biomedical Sciences, Memorial Sloan Kettering Cancer Center, New York, NY, USA

[4]Department of Medicine, Memorial Sloan Kettering Cancer Center, New York, NY, USA

Annu. Rev. Cancer Biol. 2023. 7:171–87

First published as a Review in Advance on January 17, 2023

The *Annual Review of Cancer Biology* is online at cancerbio.annualreviews.org

https://doi.org/10.1146/annurev-cancerbio-061421-040659

Keywords

tumor organoids, patient-derived organoids, coculture system, coculture organoids, immune cells, tumor immune microenvironment

Abstract

Organoid models have revolutionized cancer research through their ability to capture the cellular heterogeneity and spatial organization of a tumor in 3D culture. Patient-derived organoids can also mirror responses to therapy in vitro, opening the doors to personalized medicine that can direct clinical decision-making. As cancer immunotherapy has flourished and efforts to develop novel immunotherapies have increased, models that incorporate immune cells into organoid coculture to recapitulate the complexity of the tumor microenvironment faithfully are in high demand. To this end, a wide variety of organoid immune coculture methods have been developed, each differing in the source of immune cells used, types of immune cells maintained in culture, and their specific utility. This review aims to organize these methods into a framework that will aid researchers in choosing the appropriate system for their experimental needs. We also highlight several nonimmune cell types that have been successfully incorporated into organoid culture and the biology these coculture models are poised to interrogate.

INTRODUCTION

Organoid systems that enable the culture of normal and cancer epithelial stem cells and their differentiated progeny in defined, 3D culture conditions have proven to be an important advance in cancer research, more closely recapitulating tumor heterogeneity and plasticity than conventional 2D models, while being more scalable and cost effective than mouse models (Sato et al. 2009, Ootani et al. 2009, Tuveson & Clevers 2019). Organoid modeling has yielded novel insights into the design principles that underpin epithelial homeostasis, regeneration, and cancer, while patient-derived cancer organoids (PDOs) can recapitulate patient-specific responses to some therapies and are currently being evaluated in clinical trials to prospectively guide clinical decision-making in real time (Vlachogiannis et al. 2018, Ganesh et al. 2019, Chalabi et al. 2020, Yao et al. 2020, Ooft et al. 2021). Rooted in stem cell biology, organoid technology initially focused on maintaining and differentiating adult epithelial or induced pluripotent stem cells (iPSCs) and their neoplastic counterparts into a variety of differentiated cell types. The immune microenvironment of a tumor or tissue can be construed as incorporating not only the immediate tissue microenvironment but also constituents of regional lymph nodes, circulating immune cells, and the systemic neurohormonal milieu. However, conventional organoid models have largely lacked stromal cells, including immune cells, that originate from diverse nonepithelial lineages. Yet epithelial development, homeostasis, regeneration, and disease all depend on complex multilineage interactions among distinct cell types to generate and maintain tissue architecture and function (Karin & Clevers 2016, Roberts et al. 2017, Schreurs et al. 2019, Garner & de Visser 2020). The transformative impact of checkpoint immunotherapy in controlling tumor growth in major cancer types has underscored the need to incorporate the immune microenvironment into organoid models to better recapitulate tumor biology (Postow et al. 2015, Wolchok et al. 2017, Scognamiglio et al. 2019, Bar-Ephraim et al. 2020, Yuki et al. 2020, Dao et al. 2022). Such models are being employed not only to define mechanisms of epithelial-immune cross talk in health and disease but also as biomarkers of patient response or resistance to emerging immunotherapies.

ORGANOID MODELING TECHNIQUES

Numerous methods to establish and culture organoids have been developed, each differing in starting material used, tissue processing, culture apparatus, and, consequently, the cell populations that can be maintained in culture. These methods and key advantages and disadvantages of each are summarized in **Table 1**. The most widely adopted organoid culture is the submerged culture method. Primary tissue is enzymatically or mechanically dissociated and cell suspensions are embedded in a matrix. This mixture is plated on plastic dishes in dome-like drops and covered in culture media once the matrix solidifies. Submerged culture allows for rapid generation and expansion of organoids in a matter of days to weeks, and organoids can be cultured for months. Compared to other organoid techniques, submerged culture is relatively user friendly, allows for successive passaging and cryopreservation of organoids, and is easily scaled up for larger experiments. However, endogenous nonepithelial cells from the tissue microenvironment cannot be maintained in submerged culture approaches. Immune and stromal cells must be separately isolated from tissue or from other sources such as peripheral blood and added into the matrix in order to be cocultured.

Other organoid culture methods attempt to address this issue, including spheroid microfluidic cultures and tumor fragment cultures such as air–liquid interfaces (ALIs). Spheroid microfluidic cultures involve gently dissociating tissue into large organotypic tumor spheroids. These patient-derived organotypic tumor spheroids or mouse-derived organotypic tumor spheroids are then embedded in collagen. This collagen mixture is injected into the center of a microfluidic device,

Table 1 Comparison of organoid microenvironment models and coculture techniques

	Top-down reconstitution		Bottom-up reconstitution		
	Spheroid microfluidic culture	**Tumor fragment culture**	**Submerged culture**	**Organoid-on-a-chip**	**iPSC-derived organoids**
Starting material	Murine or human primary tissue				iPSCs generated from various adult somatic cell types through established methods
Processing protocol	Tissue is minced, briefly digested with collagenase, and filtered to a fraction containing immune cells and 40–100 μM of organotypic tumor spheroids.	Tissue is minced into small fragments.	Tissue undergoes thorough mechanical and enzymatic dissociation into a cell suspension.		iPSCs are differentiated toward endodermal, mesodermal, or ectodermal cell fates through treatment with various growth factors and signaling molecules.
Culture apparatus and plating	Mixture is combined with collagen and injected into the center of the culture device, containing media channels on either side.	Fragments are combined with collagen and plated in a 30-mm transwell dish, which is placed in a larger dish containing culture media; the top surface is exposed to air.	Cell suspensions are combined with hydrogel, plated on dishes in droplets, and covered in media.	Organoids can be derived within the chip embedded in a hydrogel compartment, derived elsewhere through submerged culture and later embedded, or derived elsewhere and then later broken up and grown on a hydrogel-coated membrane in a microfluidic channel.	iPSC-derived epithelial organoids are commonly maintained in submerged culture and can be combined with iPSC-derived mesoderm and enteric neural crest cells to generate organoids containing cell types from all three germ layers.
Matrix material	Collagen	Collagen	Most commonly Matrigel	Various natural and synthetic hydrogels	Most commonly Matrigel
Microenvironment modeling	Tissue-endogenous immune cells are preserved in culture.		Cultures lack tissue-endogenous immune cell component; stromal cells sourced elsewhere can be added into culture.		
Microenvironmental cells that have been cocultured	Endogenous T cells, B cells, dendritic cells, MDSCs, and macrophages	Endogenous myofibroblasts, T cells, macrophages, NK cells, and NKT cells	PBMCs, MDSCs, NK cells, fibroblasts, CAR T cells, and microbiota	PBMCs, fibroblasts, endothelial cells, and lymphatics	Smooth muscle, neurons, $Iba1^+$ glial-like cells, endothelial cells, and pericyte-like cells

(*Continued*)

Table 1 (*Continued*)

	Top-down reconstitution		Bottom-up reconstitution		
	Spheroid microfluidic culture	**Tumor fragment culture**	**Submerged culture**	**Organoid-on-a-chip**	**iPSC-derived organoids**
Culture period	5–9 days	Organoids are passaged and maintained >100 days; immune cells decline over a period of 1–2 months.	Epithelial cells are cultured for months; there is a shorter culture of added immune cells.	The length of culture varies depending on chip design (generally short-term culture within the chip).	Multi–germ layer organoids have been cultured for 3–5 weeks.
Applications	■ Short-term drug treatment to screen for responders to therapy ■ Cytokine profiling to identify effectors/ response to therapy	■ Short-term drug treatment to screen for responders to therapy ■ FACS profiling of organoids and T cells after drug treatment	■ Coculturing patient-derived organoids and autologous T cells to screen for organoid killing ■ CAR T cell killing assays against 3D tumor models	■ Modeling immune cell recruitment from the vasculature during inflammation ■ Modeling multiorgan interactions	■ Modeling germ layer assembly during organogenesis ■ Studying the effects of mutations in a germ layer–specific manner ■ Studying cross talk among cell types of each germ layer
Advantages	■ Local in vivo immune microenvironment preserved ■ Antibodies/drugs easily added into microfluidics ■ Patient responses to immunotherapy maintained in culture	■ Local in vivo immune microenvironment preserved ■ TCR repertoire and clonality of tumors maintained ■ Does not require harsh enzymatic digestion ■ Patient responses to immunotherapy maintained in culture	■ Organoids are easily cultured and expanded long term ■ Cell populations of interest can be hand selected for culture ■ Organoids can be cocultured with autologous PBMCs to expand tumor-reactive T cell clones	■ Tight control of parameters such as fluidic shear stress, gradients of nutrients, oxygen, and signaling molecules ■ Microfluidics more closely model vascular perfusion than static culture models ■ Can model recruitment of circulating immune cells into tissue	■ Three–germ layer organoids more closely resemble adult tissues ■ Primary tissue is not needed ■ Potential to add various microenvironmental/ immune cells, as in submerged culture

(*Continued*)

Table 1 (*Continued*)

	Top-down reconstitution		Bottom-up reconstitution		
	Spheroid microfluidic culture	**Tumor fragment culture**	**Submerged culture**	**Organoid-on-a-chip**	**iPSC-derived organoids**
Disadvantages	■ Low reproducibility ■ Short-term culture ■ Requires experienced users ■ Does not model recruitment of circulating immune cells into tissue	■ Does not model recruitment of circulating immune cells into tissue ■ Immune cell populations decline over time	■ Immune cells added in culture do not reflect the tissue-endogenous microenvironment ■ Unable to tightly control the biochemical/biophysical properties of culture ■ Organoids only contain epithelial cells	■ Immune cells added in culture do not reflect the tissue-endogenous microenvironment ■ Cost prohibitive for larger-scale experiments ■ Requires expert users who have been extensively trained	■ Immune cells added in culture do not reflect the tissue-endogenous microenvironment ■ Unable to tightly control the biochemical and biophysical properties of culture
References	Aref et al. 2018, Jenkins et al. 2018, Deng et al. 2018	Li et al. 2014, Neal et al. 2018, Voabil et al. 2021	Dijkstra et al. 2018, Tsai et al. 2018, Courau et al. 2019, Schnalzger et al. 2019, Votanopoulos et al. 2020, Koh et al. 2021, Chan & Ewald 2022, Zhou et al. 2022	Bein et al. 2018, Kasendra et al. 2018, Mazzocchi et al. 2018, Rajasekar et al. 2020, van Riet et al. 2022	Pham et al. 2018, Wörsdörfer, et al. 2019, Park et al. 2020, Eicher et al. 2022

Abbreviations: CAR T cells, chimeric antigen receptor T cells; FACS, fluorescent-activated cell sorting; iPSC, induced pluripotent stem cell; MDSCs, myeloid-derived suppressor cells; NK cells, natural killer cells; NKT cells, natural killer T cells; PBMCs, peripheral blood mononuclear cells; TCR, T cell receptor.

which is fed by media that flow through channels on either side of the collagen layer (Aref et al. 2018, Jenkins et al. 2018). In contrast, tumor fragment cultures call for gently dissociating tissue into small fragments in order to keep some aspects of the in vivo tissue architecture intact (Neal et al. 2018). Both techniques allow for endogenous immune and stromal cells to be maintained in culture for a short period of time. In the study by Jenkins et al. (2018), myeloid and lymphoid cells remained viable in 3D microfluidic culture for 1–2 weeks, while the ALI culture established by Neal et al. (2018) improved upon this time frame and showed the immune cells began to decline over 1–2 months. Both of these methods have allowed for studies with immunomodulatory drugs such as anti-PD1 to interrogate the functionality of endogenous tumor-infiltrating lymphocytes (TILs) in organoid culture.

Emerging from a bioengineering approach, microfluidics-based organoid-on-a-chip devices allow for tight control of many parameters such as pH, oxygenation, nutrient availability, and shear stress, with the ultimate goal of modeling organ-level physiology. The microfluidic systems incorporated into chips also uniquely allow for gradients of nutrients and signaling molecules to be established (Sontheimer-Phelps et al. 2019, Ingber 2022). Chips can be designed to incorporate other cell types beyond epithelial cells, such as endothelial cells, stromal cells, immune cells, and microbiota, potentially making them a useful tool for modeling the microenvironment (Jin et al. 2018, Park et al. 2019). A major drawback of chips that prevents their widespread use is their complexity. They are expensive and require both extensively trained users and additional equipment.

Aside from primary tissues, which contain organ-specific adult stem cell populations within a stem cell niche, organoids can also be generated from iPSCs. These methods incorporate signaling molecules and growth factors to co-opt developmental programs in vitro, making these organoids a useful platform for studying organogenesis in addition to disease pathologies (Azar et al. 2021). While iPSCs can be used to derived epithelial organoids that can be cultured through any of the aforementioned methods, they also have unique applications afforded by their pluripotency. Recently, iPSCs were used to assemble complex gastrointestinal organoids that contained cell types from all three germ layers. These organoids contained smooth muscle cells that were functionally innervated by enteric neuroglia, cell types that primary tissue–derived organoid cultures are devoid of (Eicher et al. 2022). This proof-of-concept work represents an exciting step forward in developing more physiologically relevant organoid models.

RECONSTITUTING THE ORGANOID MICROENVIRONMENT

The incorporation of immune cells into epithelial organoid cultures has allowed researchers to study epithelial-immune cross talk in homeostasis, as well as in the pathogenesis of inflammatory disorders and cancer. Organoid immune coculture approaches can broadly be grouped into two categories: bottom-up reconstitution, where immune cells of interest are added into epithelial cell culture with the goal of reconstituting the immune microenvironment, and top-down reconstitution, which seeks to preserve and culture the existing tissue-infiltrating cells in culture (**Figure 1**). An important factor to consider in cancer organoid immune cocultures is specifically which source of immune cells should be used to best model the tumor immune microenvironment in an in vitro system. The answer may ultimately depend on the goals of the coculture and the questions being asked. For example, while T cells of the same tumor-recognizing clone can be present both intratumorally and in peripheral circulation, their effector statuses have been shown to differ greatly. Intratumoral T cells are often driven to an exhausted phenotype that is largely absent in their matched peripheral counterparts (Lucca et al. 2021). Top-down reconstitution may therefore provide a better model for assessing whether intratumoral T cell exhaustion can

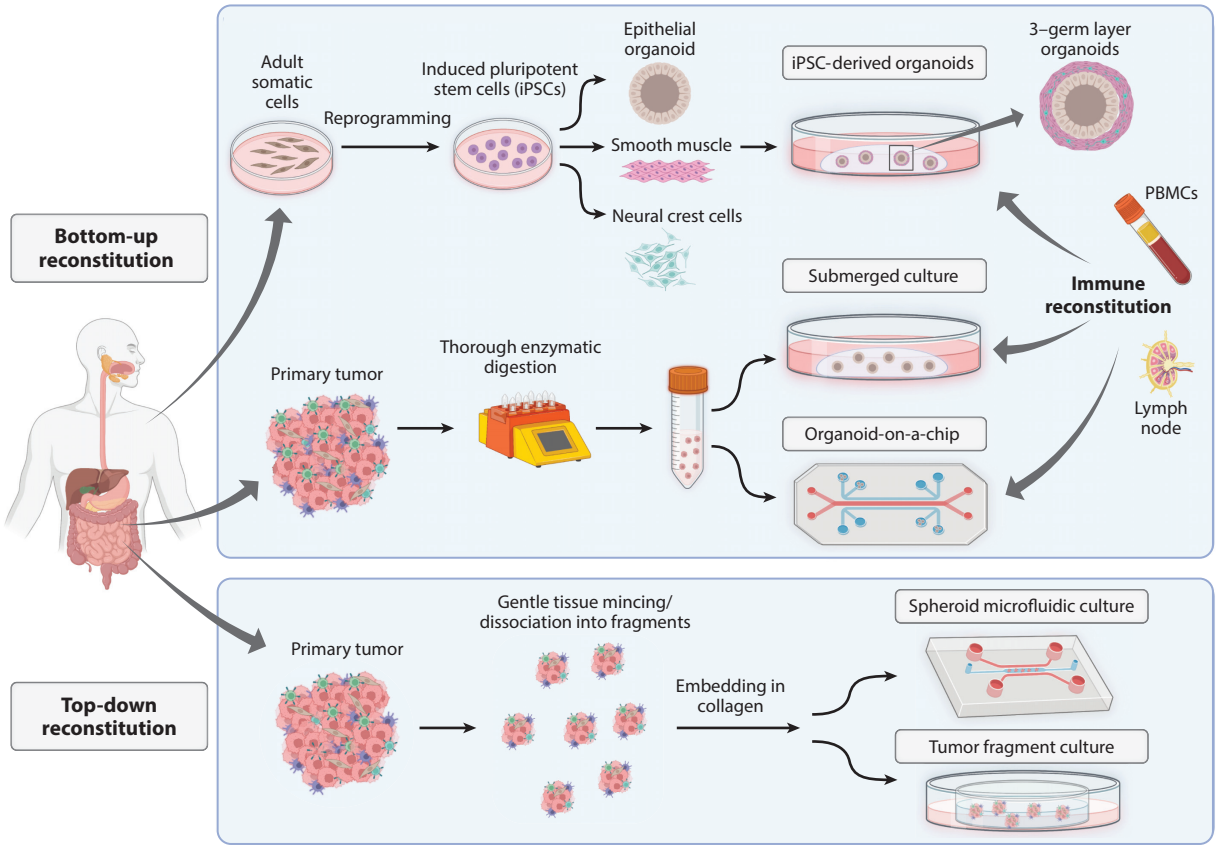

Figure 1

Summary of organoid coculture methods. Organoid microenvironment models can be grouped into bottom-up reconstitution and top-down reconstitution methods. (*Top*) Bottom-up reconstitution methods, including submerged cultures, organoids-on-a-chip, and iPSC-derived organoids, allow users to select specific cell populations of interest to include in coculture to tightly control the identity of the microenvironment. Cells can be sourced from blood or secondary lymphoid tissues such as lymph nodes or differentiated in culture from iPSCs. (*Bottom*) Top-down reconstitution methods, including spheroid microfluidic cultures and tumor fragment cultures, preserve tissue-infiltrating immune and stromal cells in organoid culture for a short period. This provides users a window to interrogate interactions between organoids and endogenous microenvironmental cells, such as assessing the cytotoxicity of tumor antigen–specific T cells or screening for patient-derived organoid response to checkpoint immunotherapy. Abbreviations: iPSC, induced pluripotent stem cell; PBMCs, peripheral blood mononuclear cells. Figure adapted with permission from images created with Biorender.com.

be reversed with immunotherapy, while a bottom-up reconstitution system may provide a better window into the mechanism through which cancer cells induce T cell exhaustion.

Bottom-Up Reconstitution

Reconstituting the immune microenvironment in organoid culture allows researchers to select specific immune cell populations of interest to study. Peripheral blood, spleen, bone marrow, ex vivo expanded TILs, and lymph nodes have all successfully been used as a source of immune cells, primarily in submerged organoid coculture (Chakrabarti et al. 2018, Kong et al. 2018, Dijkstra et al. 2018, Tsai et al. 2018, Votanopoulos et al. 2020). Patient-derived colorectal cancer, non-small-cell lung cancer, and cholangiocarcinoma tumor organoids have been used to expand tumor-reactive T cell clones from matched autologous blood that can effectively kill organoids in

cytotoxicity assays, demonstrating how coculture systems can be used as an individualized model to study interactions between tumor cells and T cells (Dijkstra et al. 2018, Zhou et al. 2022). Coculture of patient-derived melanoma organoids with autologous lymph nodes demonstrated that cocultures had responses to anti-PD1 checkpoint blockade that largely mirrored patients' clinical responses, highlighting the utility of immune organoid cocultures as a platform for drug screening that could inform clinical decision-making (Votanopoulos et al. 2020). In both pancreatic ductal adenocarcinoma and gastric cancer organoid models, depletion of myeloid-derived suppressor cells from coculture improved T cell responsiveness to anti-PD1 therapy (Holokai et al. 2020, Koh et al. 2021). Immune cells have also been successfully cocultured in chip-based devices, specifically monocyte-derived macrophages with human intestinal organoids in a chip-based model of inflammatory bowel disease. Beaurivage et al. (2020) demonstrated that both the epithelial cells and macrophages took on a functional proinflammatory state with a lipopolysaccharide trigger, pointing toward the physiological relevance of this model. Organoid immune cocultures have also proven to be a useful preclinical model in evaluating the efficacy and toxicity of genetically engineered cellular immunotherapies for solid tumors, where chimeric antigen receptor (CAR) T cell development has been hampered by off-target toxicities related to antigen expression in normal tissues. Schnalzger et al. (2019) developed assays to evaluate the killing capacity of CAR-engineered natural killer cells against both normal and tumor PDOs. Their work demonstrates how organoid models are well suited to validate tumor-specific neoantigen targets as well as identify off-target toxicities in the preclinical setting. More recently, Dekkers et al. (2023) developed a coculture system to live-image the activity and behavior of engineered T cells against PDOs. They also utilized single-cell RNA sequencing to identify previously undescribed gene programs associated with potent T cell cytotoxicity or so-called super-engager activity. These studies together highlight how organoid immune coculture models can be harnessed to advance the development of cellular immunotherapies.

Top-Down Reconstitution

In contrast to submerged cultures and organoid-on-a-chip devices, top-down reconstitution approaches such as tumor explant cultures and tumor spheroid microfluidic devices have been shown to maintain a diverse repertoire of intertumoral immune cells in culture for short periods of time. This is a key advantage over bottom-up reconstitution, where cell populations are chosen by the user to add into coculture, potentially biasing the reconstituted microenvironment toward cells of interest. Preserving the tumor microenvironment (TME) in microfluidic culture has allowed researchers to investigate biomarkers of response and mechanisms of resistance to checkpoint blockade in PDOs that track with patient response to therapy (Aref et al. 2018, Deng et al. 2018, Jenkins et al. 2018). Neal et al. (2018) demonstrated that ALI culture of tumor fragments could be used to generate PDOs that preserve a diverse milieu of microenvironmental cells, including cancer fibroblasts, myeloid cells, and lymphoid cells. Notably, the T cells in coculture maintained the T cell receptor repertoire of the original tumor and could respond to checkpoint blockade, highlighting the power of ALI cultures in modeling antitumor T cell responses. A recent study utilizing a droplet-based approach to generate colorectal cancer tumor micro-organospheres demonstrated that both myeloid and lymphoid cell populations were preserved in submerged culture (Ding et al. 2022). These cultures could be used to assess patient responsiveness to immunotherapy. A major disadvantage of these techniques is the inability to maintain microenvironmental cells in culture long term, as most studies have demonstrated a decline in immune cell viability after a few weeks. Furthermore, the ability to genetically modulate individual cell types to ask mechanistic questions may be somewhat limited in top-down cultures relative to bottom-up cultures.

SCAFFOLDS FOR TUMOR MICROENVIRONMENT ENGINEERING

A crucial component of any organoid culture system is the matrix in which the cells are suspended. The matrix supplies both mechanical and chemical cues that are crucial for the signaling, activation, and differentiation of immune and epithelial cells. As the variety of organoid culture techniques provides an enormous amount of flexibility in their culture systems, so too do the available substrates (Fernando et al. 2021). Organoids are generally cultured in hydrogels, which are materials containing a complex 3D network of hydrophilic polymers that form a scaffold. Many organoid systems employ Matrigel, a natural extracellular matrix (ECM)-based hydrogel derived from the culture supernatant of the Engelbreth-Holm-Swarm mouse sarcoma model (Sato et al. 2009). Matrigel contains a variety of basement membrane polymers along with over 1,800 other proteins and is subject to batch-to-batch variability; concerningly, proteins in mouse-derived hydrogels can be immunogenic and have been shown to activate T cells in culture in the absence of organoids (Hughes et al. 2010, Dijkstra et al. 2018, Kaur et al. 2021). Thus, there is a gradual shift toward using alternative hydrogels that could better support the coculture of immune and stromal cell populations. Natural hydrogel alternatives include protein hydrogels like collagen and fibrin, as well as polysaccharide hydrogels like hyaluronic acid and gelatin (Kozlowski et al. 2021). These purified bioreactive materials can create physiologically relevant ECM-like environments that readily support the growth of organoids and microenvironmental cells while removing the heterogeneity and variability seen in Matrigel culture.

Synthetic hydrogels allow users to fine-tune their biochemical and mechanical properties such as the stiffness, elasticity, and pore size—key properties of the ECM all known to effect cellular behavior (Zaman et al. 2006, Miron-Mendoza et al. 2010, Aisenbrey & Murphy 2020). This allows users to better model tissues with specific ECM characteristics unmatched by natural hydrogels, such as the stiff fibrotic stroma of pancreatic ductal adenocarcinoma (PDAC), which was recently modeled more accurately in a PDAC organoid fibroblast coculture model using a custom designed polyethylene glycol (PEG)-based hydrogel (Below et al. 2022). A recent study demonstrated that a PEG hydrogel improved the migratory and chemotactic ability of dendritic cells over Matrigel, increasing dendritic cell organoid physical interactions (Cherne et al. 2021). In 2020, researchers seeking to develop a lymph node–like hydrogel culture system made a PEG- and heparin-based hydrogel loaded with the cytokine CCL21, which allowed for rapid T cell expansion and activation (del Rio et al. 2020). As more complex coculture systems incorporating organoids and multiple microenvironmental cell types are developed, more customizable synthetic or hybrid hydrogel models may be needed to support the unique requirements of these cultures.

RECONSTITUTING THE COMPLEX IMMUNE MICROENVIRONMENT BEYOND IMMUNE-EPITHELIAL INTERACTIONS

Faithful reconstruction of the immune microenvironment of a tissue or tumor must include not only the immune and epithelial cells but also other local and distant stromal cells that modulate complex multicellular interactions. Numerous other cell types, such as endothelial cells, neurons, fibroblasts, and microbiota, interact and cross talk with immune and epithelial cells to shape the tissue's physiology, both in homoeostasis and in disease. Efforts to faithfully recapitulate the TME in organoid cocultures therefore should not neglect to incorporate these components.

Fibroblasts

Cancer-associated fibroblasts (CAFs) are stromal cells that have long been studied for their ability to deposit and remodel the ECM, leading to increased tumor stiffness. Their role in modulating

immunity within the TME is more recently being appreciated. CAFs secrete numerous immunomodulatory cytokines and signaling molecules, and the ECM remodeling they induce has been shown to effect lymphocyte migration within tumors (Sahai et al. 2020). In vitro models that incorporate both immune cells and CAFs are key for studying the biology of cancers with dense CAF infiltration such as PDAC, where they can constitute 80% of the total tumor volume (Feig et al. 2012).

Numerous studies have demonstrated that primary tissue–derived organoids and CAFs can be successfully cocultured (Mäkinen et al. 2022), although few to date have also added immune cells. Tsai et al. (2018) generated PDAC PDOs in a submerged Matrigel culture that included CAFs within the Matrigel and peripheral blood–derived T cells within the surrounding culture media, showing that T cells can invade the Matrigel and travel toward the organoids. More recently Below et al. (2022) developed a custom PEG hydrogel that allowed for successful coculture of murine PDAC organoids, fibroblasts, and bone marrow–derived macrophages. While these proof-of-concept studies demonstrated successful coculture for only a few days, they represent exciting platforms that can potentially be built upon for more robust studies of the complex TME.

Endothelial Cells

The formation of leaky and tortuous vasculature, a hallmark of solid tumorigenesis, is supported by proangiogenic signals produced by both cancer cells and myeloid cells in the TME (Zetter, 1998, Harney et al. 2015, Liang & Ferrara 2016, Potente & Carmeliet 2017). Angiogenesis also impacts tumor immunity—for example, VEGF has known immunosuppressive properties (Yang et al. 2018). Incorporating endothelial cells into epithelial immune cell cocultures could allow for this network of angiogenic signaling and immunomodulation to be interrogated in the hopes of developing novel antiangiogenic therapies.

The most common methods of incorporating endothelial cells into organoid culture—so-called induced angiogenesis or spontaneous vascularization models—involve coculturing organoids with vascular endothelial cells and growth factors that stimulate angiogenesis (Nashimoto et al. 2017, Pham et al. 2018, Holloway et al. 2020). In a more recent induced-angiogenesis organoid system, mesodermal progenitor cells derived from human iPSCs were used to form a functional vasculature. These vessels were complete with endothelial cells, smooth muscle cells, pericyte-like cells, and Iba1$^+$ perivascular macrophage-like cells and were far more complex than previous models that contained endothelial cells exclusively (Wörsdörfer et al. 2019). Such models could provide a platform for studying both the angiogenic cascade and its relationship to the tumor immune microenvironment.

Microbiota

The microbiome is increasingly being recognized for its broad influence on human health in the normal homeostatic balance of immunity and metabolism, as well as in pathologies such as chronic inflammation and cancer (Rooks & Garrett 2016). In the context of disease such as cancer, chronic inflammation induced by microbial species is associated with numerous types of cancer, such as gastric cancer induced by *Helicobacter pylori*, bladder cancer induced by *Schistosoma haemotobium*, and *Fusobacterium nucleatum*–related colorectal cancer (Jain et al. 2021). In contrast to these microbes that have protumor effects, others have been associated with increased T cell infiltration into tumors and improved survival (Cremonesi et al. 2018). The addition of microbial species into organoid immune cocultures could allow for further interrogation of how their pro- and anti-inflammatory influences shape disease.

Strategies for bacterial 3D organoid coculture include microinjecting bacteria into the organoid lumen and, alternatively, reversing the epithelial apical polarity of organoids and

culturing them "apical out" (Williamson et al. 2018, Park & Koh 2022). Microinjection has been utilized to demonstrate that colorectal cancer organoids exposed to an *Escherichia coli*–produced genotoxin harbor a mutational signature also found in a subset of colorectal cancer patients (Pleguezuelos-Manzano et al. 2020). A significant hurdle in intestinal bacteria organoid coculture is that the aerobic conditions needed to support epithelial organoid growth prevent the culture of obligate anaerobes commonly found in the gut. Air trapped within the lumen of the organoid during microinjection prevents long-term coculture of anaerobic bacteria (Williamson et al. 2018). Culture techniques that maintain separate normoxic and hypoxic areas such as organoid-derived 2D monolayer transwell systems can be used, allowing for intestinal epithelial cells and microbes to be cultured in their respective preferred environments (Fofanova et al. 2019, Sasaki et al. 2020). While these monolayer systems destroy the 3D structure of organoids, they do maintain diverse epithelial cell types. Developing methods that allow for sustained cocultures of microbes with organoids that are high throughput, have relative ease of use, and are reproducible will be key for interrogating the interactions among organoids, immune cells, and the microbiome. To date, only a handful of studies have employed a triple coculture of epithelial cells, immune cells, and microbes (Holokai et al. 2019, Jalili-Firoozinezhad et al. 2019, Sachs et al. 2019), and future studies will likely incorporate further multikingdom, multilineage complexity.

Neurons

Neurons secrete a vast array of signaling molecules that can bind to their receptors expressed by tumors and directly support tumor proliferation, migration, and invasion (Wang et al. 2021). What is also increasingly being appreciated is the signaling axis that exists between the nervous system and immune system in the context of cancer—the so-called neuroimmune axis (Kuol et al. 2018, Shurin et al. 2020). Most immune cells express receptors for these same signaling molecules, and several neuropeptides and neurotransmitters have been shown to exert immunosuppressive effects on immune cells or drive protumor immune phenotypes (Sloan et al. 2010, Nissen et al. 2018). Thus, the nervous system can also drive tumor progression indirectly by supporting an immune-evasive TME.

The addition of neurons into organoid coculture could provide an important platform for further research into the emerging area of cancer neuroscience. Organoid cocultures with neurons and glia of the enteric nervous system (ENS) have been accomplished with intestine, stomach, and esophagus organoids using stem cell–derived cell populations (Schlieve et al. 2017, Workman et al. 2017, Park et al. 2020, Eicher et al. 2022). As the epithelial organoids were also stem cell derived, further studies will be needed to determine if this in vitro ENS can innervate mouse and human primary tissue–derived organoids. Given that neurons can be derived from virtually any adult somatic cell types through induced pluripotency, the prospect of coculturing PDOs with autologous iPSC-derived neurons is an exciting future direction (Chambers et al. 2009).

Lymph Nodes

In solid tumors, tumor-draining lymph nodes have historically been viewed rather simply as the location where cancer begins the process of metastasizing to distant organs. Clinical staging is based on the presence or absence of lymph node–positive disease, and indeed, the presence of cancer in lymph nodes is one of the most long-standing and robust prognostic biomarkers of metastatic recurrence and poor survival. More recently in the age of immuno-oncology, lymph nodes are being appreciated for their influence on shaping the TME and clinical responses to immunotherapy (du Bois et al. 2021, van Pul et al. 2021, Reticker-Flynn et al. 2022). Within the immunology and bioengineering fields, efforts to generate in vitro human lymph node models have focused on their

applications in pharmacology and vaccine development. A variety of techniques have been developed utilizing lymph node in-a-dish- and on-a-chip-style cultures, with the most faithful models utilizing surgically resected human lymph nodes that are cultured in slices or disaggregated to form lymphoid organoid–like structures (Shanti et al. 2021, Shim et al. 2019, Wagar et al. 2021).

Combining these research efforts with tumor organoid technology could further improve modeling of the tumor immune environment beyond circulating immune cells and tumor-infiltrating immune cells. Early work in this direction has highlighted the power and applications of such a model, demonstrating that lymph node–derived antigen-presenting cells can interact with both tumor organoids and peripheral blood–derived T cells to drive antigen-specific antiorganoid T cell responses (Votanopoulos et al. 2020). This coculture system was used to screen patient responses to immunotherapy, and further studies are needed to assess long-term stability and applications for mechanistic research.

TOWARD MORE COMPREHENSIVE MODELING OF THE TUMOR IMMUNE MICROENVIRONMENT

For decades, extensively inbred genetically engineered mouse models (GEMMs) have been the backbone of in vivo experimental validation in biomedical research, especially within the fields of cancer biology and immunology. Discoveries made in mice have exponentially deepened our understanding of human biology, and some phenotypic characteristics of specific TMEs can be particularly difficult to recapitulate in human ex vivo models (e.g., the highly desmoplastic microenvironment of PDAC, specific polarized macrophage states) and may benefit from being studied in GEMMs. Mice do offer the advantage of longitudinal tumor development in an intact host, although the extent to which murine models capture the biology of human samples is unclear. Differences between mouse and human biology are likely a contributing factor to the overall ~90% failure rate of drugs in clinical trials (Mestas & Hughes 2004, Mak et al. 2014, Mullard, 2016, Medetgul-Ernar & Davis 2022). While solutions such as humanized mice and patient-derived xenografts offer intermediate solutions to traditional mouse models, there is a clear need for fully human models to bridge the gap between successful translational research and successful clinical trials. Organoid model systems have proven to be a significant advancement in this regard, allowing users to maintain the heterogeneity of cell types within a human tumor as well as incorporate microenvironmental cells to model the complex TME. While it is clear that organoids are in the process of revolutionizing cancer biology research, rigorous validation of these models is key for their potential to be realized.

To date, studies incorporating immune cells into organoid culture systems have largely focused on proof of concept—demonstrating the feasibility of their establishment and the viability of key cell types and, in some cases, validating the competence of organoid immune cocultures to respond to immune-modulatory drugs. As such technologies become more established, the field needs standardized, scalable approaches to assess bidirectional immune and epithelial cell functions and interactions in coculture. Beyond standard viability and cytokine release assays, advanced imaging, spatial transcriptomic, and metabolomic technologies are beginning to yield novel insights into the dynamic interactions between immune and epithelial cells in real time and at high resolution (Genshaft et al. 2021, Dekkers et al. 2023).

As model complexity increases, validating the extent to which ex vivo coculture systems capture the biology observed in vivo also becomes more challenging. One rigorous gold standard for such validation assesses how well a model recapitulates a known clinical phenotype (e.g., comparing patient and PDO immune coculture responses to anti-PD1 checkpoint inhibitor therapy) (Vlachogiannis et al. 2018, Chalabi et al. 2020). However, even when concordant

treatment responses are observed, it cannot be assumed that any other novel aspect of biology uncovered using the same ex vivo model is physiologically or clinically relevant, which requires further validation. In summary, organoid systems are rapidly incorporating a plethora of immune and other stromal cell types, both via reconstitution of individual cell types and from direct tissue explants that retain elements of in situ tissue architecture. A crucial feature of epithelial organoids is their ability to retain a continuum of physiologically relevant cell states within ex vivo culture. Incorporating similar cell state heterogeneity and plasticity among immune cells cocultured with epithelial cells will yield novel insights into epithelial-immune cross talk in health and disease.

DISCLOSURE STATEMENT

K.G. is an inventor on patents related to organoid immune cancer models and methods for targeting metastasis and is a consultant for Seres Therapeutics.

ACKNOWLEDGMENTS

This work was supported by National Institutes of Health (NIH) grants P30CA008748 (Memorial Sloan Kettering Cancer Center), T32GM007739 (to K.A.L.; the Weill Cornell/Rockefeller/Sloan Kettering Tri-Institutional MD-PhD Program), K08CA230213, R37CA266185, U2CCA233284, and U54CA274492 (K.G.), as well as the Burroughs Welcome Career Award for Medical Scientists (K.G.), American Association for Cancer Research NextGen Grant for Transformative Cancer Research (K.G.), Stand Up To Cancer Convergence Award (K.G.), the Josie Robertson Investigator Award (K.G), and a Pershing Square Sohn Prize (K.G.).

LITERATURE CITED

Aisenbrey EA, Murphy WL. 2020. Synthetic alternatives to Matrigel. *Nat. Rev. Mater.* 5:539–51

Aref AR, Campisi M, Ivanova E, Portell A, Larios D, et al. 2018. 3D microfluidic ex vivo culture of organotypic tumor spheroids to model immune checkpoint blockade. *Lab Chip* 18(20):3129–43

Azar J, Bahmad HF, Daher D, Moubarak MM, Hadadeh O, et al. 2021. The use of stem cell-derived organoids in disease modeling: an update. *Int. J. Mol. Sci.* 14:7667

Bar-Ephraim YE, Kretzschmar K, Clevers H. 2020. Organoids in immunological research. *Nat. Rev. Immunol.* (5):279–93

Beaurivage C, Kanapeckaite A, Loomans C, Erdmann KS, Stallen J, Janssen RA. 2020. Development of a human primary gut-on-a-chip to model inflammatory processes. *Sci. Rep.* 10:21475

Bein A, Shin W, Jalili-Firoozinezhad S, Park MH, Sontheimer-Phelps A, et al. 2018. Microfluidic organ-on-a-chip models of human intestine. *Cell. Mol. Gastroenterol. Hepatol.* 5(4):659–68

Below CR, Kelly J, Brown A, Humphries JD, Hutton C, Xu J, et al. 2022. A microenvironment-inspired synthetic three-dimensional model for pancreatic ductal adenocarcinoma organoids. *Nat. Mater.* 21:110–19

Chakrabarti J, Holokai L, Syu L, Steele N, Chang J, et al. 2018. Mouse-derived gastric organoid and immune cell co-culture for the study of the tumor microenvironment. In *Epithelial Cell Culture*, ed. M Baratta, pp. 157–68. New York: Humana

Chalabi M, Fanchi LF, Dijkstra KK, Van den Berg JG, Aalbers AG, et al. 2020. Neoadjuvant immunotherapy leads to pathological responses in MMR-proficient and MMR-deficient early-stage colon cancers. *Nat. Med.* 26(4):566–76

Chambers SM, Fasano CA, Papapetrou EP, Tomishima M, Sadelain M, Studer L. 2009. Highly efficient neural conversion of human ES and iPS cells by dual inhibition of SMAD signaling. *Nat. Biotechnol.* 27(3):275–80

Chan IS, Ewald AJ. 2022. Organoid co-culture methods to capture cancer cell–natural killer cell interactions. In *Natural Killer (NK) Cells*, ed. N Shimasaki, pp. 235–50. New York: Humana

Cherne MD, Sidar B, Sebrell TA, Sanchez HS, Heaton K, et al. 2021. A synthetic hydrogel, VitroGel® ORGANOID-3, improves immune cell-epithelial interactions in a tissue chip co-culture model of human gastric organoids and dendritic cells. *Front. Pharmacol.* 12:707891

Courau T, Bonnereau J, Chicoteau J, Bottois H, Remark R, et al. 2019. Cocultures of human colorectal tumor spheroids with immune cells reveal the therapeutic potential of MICA/B and NKG2A targeting for cancer treatment. *J. Immunother. Cancer* 7:74

Cremonesi E, Governa V, Garzon JF, Mele V, Amicarella F, et al. 2018. Gut microbiota modulate T cell trafficking into human colorectal cancer. *Gut* 67(11):1984–94

Dao V, Yuki K, Lo YH, Nakano M, Kuo CJ, et al. 2022. Immune organoids: from tumor modeling to precision oncology. *Trends Cancer* 8(10):870–80

Dekkers JF, Alieva M, Cleven A, Keramati F, Wezenaar AK, et al. 2023. Uncovering the mode of action of engineered T cells in patient cancer organoids. *Nat. Biotechnol.* 41:60–69

del Río EP, Santos F, Rodriguez XR, Martínez-Miguel M, Roca-Pinilla R, et al. 2020. CCL21-loaded 3D hydrogels for T cell expansion and differentiation. *Biomaterials* 259:120313

Deng J, Wang ES, Jenkins RW, Li S, Dries R, et al. 2018. CDK4/6 inhibition augments antitumor immunity by enhancing T-cell activation. *Cancer Discov.* 8(2):216–33

Dijkstra KK, Cattaneo CM, Weeber F, Chalabi M, van de Haar J, et al. 2018. Generation of tumor-reactive T cells by co-culture of peripheral blood lymphocytes and tumor organoids. *Cell* 174(6):1586–98

Ding S, Hsu C, Wang Z, Natesh NR, Millen R, et al. 2022. Patient-derived micro-organospheres enable clinical precision oncology. *Cell Stem Cell* 29(6):905–17

du Bois H, Heim TA, Lund AW. 2021. Tumor-draining lymph nodes: at the crossroads of metastasis and immunity. *Sci. Immunol.* 6(63):eabg3551

Eicher AK, Kechele DO, Sundaram N, Berns HM, Poling HM, et al. 2022. Functional human gastrointestinal organoids can be engineered from three primary germ layers derived separately from pluripotent stem cells. *Cell Stem Cell* 29:36–51

Feig C, Gopinathan A, Neesse A, Chan DS, Cook N, Tuveson DA. 2012. The pancreas cancer microenvironment. *Clin. Cancer Res.* 18(16):4266–76

Fernando K, Kwang LG, Lim JT, Fong EL. 2021. Hydrogels to engineer tumor microenvironments in vitro. *Biomater. Sci.* 9(7):2362–83

Fofanova TY, Stewart CJ, Auchtung JM, Wilson RL, Britton RA, et al. 2019. A novel human enteroid-anaerobe co-culture system to study microbial-host interaction under physiological hypoxia. bioRxiv 555755. **https://doi.org/10.1101/555755**

Ganesh K, Wu C, O'Rourke KP, Szeglin BC, Zheng Y, et al. 2019. A rectal cancer organoid platform to study individual responses to chemoradiation. *Nat. Med.* 25(10):1607–14

Garner H, de Visser KE. 2020. Immune crosstalk in cancer progression and metastatic spread: a complex conversation. *Nat. Rev. Immunol.* 20(8):483–97

Genshaft AS, Ziegler CG, Tzouanas CN, Mead BE, Jaeger AM, et al. 2021. Live cell tagging tracking and isolation for spatial transcriptomics using photoactivatable cell dyes. *Nat. Commun.* 12:4995

Harney AS, Arwert EN, Entenberg D, Wang Y, Guo P, et al. 2015. Real-time imaging reveals local, transient vascular permeability, and tumor cell intravasation stimulated by $TIE2^{hi}$ macrophage–derived VEGFA. *Cancer Discov.* 5(9):932–43

Holloway EM, Wu JH, Czerwinski M, Sweet CW, Wu A, et al. 2020. Differentiation of human intestinal organoids with endogenous vascular endothelial cells. *Dev. Cell* 54(4):516–28

Holokai L, Chakrabarti J, Broda T, Chang J, Hawkins JA, et al. 2019. Increased programmed death-ligand 1 is an early epithelial cell response to *Helicobacter pylori* infection. *PLOS Pathog.* 15:e1007468

Holokai L, Chakrabarti J, Lundy J, Croagh D, Adhikary P, et al. 2020. Murine- and human-derived autologous organoid/immune cell co-cultures as pre-clinical models of pancreatic ductal adenocarcinoma. *Cancers* 12(12):3816

Hughes CS, Postovit LM, Lajoie GA. 2010. Matrigel: a complex protein mixture required for optimal growth of cell culture. *Proteomics* 10(9):1886–90

Ingber DE. 2022. Human organs-on-chips for disease modelling, drug development and personalized medicine. *Nat. Rev. Genet.* 23:467–91

Jain T, Sharma P, Are AC, Vickers SM, Dudeja V. 2021. New insights into the cancer–microbiome–immune axis: decrypting a decade of discoveries. *Front. Immunol.* 12:622064

Jalili-Firoozinezhad S, Gazzaniga FS, Calamari EL, Camacho DM, Fadel CW, et al. 2019. A complex human gut microbiome cultured in an anaerobic intestine-on-a-chip. *Nat. Biomed. Eng.* 3(7):520–31

Jenkins RW, Aref AR, Lizotte PH, Ivanova E, Stinson S, et al. 2018. *Ex vivo* profiling of PD-1 blockade using organotypic tumor spheroids. *Cancer Discov*. 8(2):196–215

Jin Y, Kim J, Lee JS, Min S, Kim S, et al. 2018. Vascularized liver organoids generated using induced hepatic tissue and dynamic liver-specific microenvironment as a drug testing platform. *Adv. Funct. Mater.* 28(37):1801954

Karin M, Clevers H. 2016. Reparative inflammation takes charge of tissue regeneration. *Nature* 529(7586):307–15

Kasendra M, Tovaglieri A, Sontheimer-Phelps A, Jalili-Firoozinezhad S, Bein A, et al. 2018. Development of a primary human Small Intestine-on-a-Chip using biopsy-derived organoids. *Sci. Rep.* 8:2871

Kaur S, Kaur I, Rawal P, Tripathi DM, Vasudevan A. 2021. Non-matrigel scaffolds for organoid cultures. *Cancer Lett*. 504:58–66

Koh V, Chakrabarti J, Torvund M, Steele N, Hawkins JA, et al. 2021. Hedgehog transcriptional effector GLI mediates mTOR-induced PD-L1 expression in gastric cancer organoids. *Cancer Lett*. 518:59–71

Kong J, Guerra GR, Millen RM, Roth S, Xu H, et al. 2018. Tumor-infiltrating lymphocyte function predicts response to neoadjuvant chemoradiotherapy in locally advanced rectal cancer. *JCP Precis. Oncol.* 2. **https://doi.org/10.1200/PO.18.00075**

Kozlowski MT, Crook CJ, Ku HT. 2021. Towards organoid culture without Matrigel. *Commun. Biol.* 4:1387

Kuol N, Stojanovska L, Apostolopoulos V, Nurgali K. 2018. Crosstalk between cancer and the neuro-immune system. *J. Neuroimmunol.* 315:15–23

Li X, Nadauld L, Ootani A, Corney DC, et al. 2014. Oncogenic transformation of diverse gastrointestinal tissues in primary organoid culture. *Nat. Med.* 20(7):769–77

Liang W, Ferrara N. 2016. The complex role of neutrophils in tumor angiogenesis and metastasis. *Cancer Immunol. Res.* 4(2):83–91

Lucca LE, Axisa PP, Lu B, Harnett B, Jessel S, et al. 2021. Circulating clonally expanded T cells reflect functions of tumor-infiltrating T cells. *J. Exp. Med.* 218(4):e20200921

Mak IW, Evaniew N, Ghert M. 2014. Lost in translation: animal models and clinical trials in cancer treatment. *Am. J. Transl. Res.* 6(2):114–18

Mäkinen L, Vähä-Koskela M, Juusola M, Mustonen H, Wennerberg K, et al. 2022. Pancreatic cancer organoids in the field of precision medicine: a review of literature and experience on drug sensitivity testing with multiple readouts and synergy scoring. *Cancers* 14(3):525

Mazzocchi AR, Rajan SA, Votanopoulos KI, Hall AR, Skardal A. 2018. In vitro patient-derived 3D mesothelioma tumor organoids facilitate patient-centric therapeutic screening. *Sci. Rep.* 8:2886

Medetgul-Ernar K, Davis MM. 2022. Standing on the shoulders of mice. *Immunity* 55(8):1343–53

Mestas J, Hughes CC. 2004. Of mice and not men: differences between mouse and human immunology. *J. Immunol.* 172(5):2731–38

Miron-Mendoza M, Seemann J, Grinnell F. 2010. The differential regulation of cell motile activity through matrix stiffness and porosity in three dimensional collagen matrices. *Biomaterials* 31(25):6425–35

Mullard A. 2016. Parsing clinical success rates. *Nat. Rev. Drug Discov.* 15(7):447–8

Nashimoto Y, Hayashi T, Kunita I, Nakamasu A, Torisawa YS, et al. 2017. Integrating perfusable vascular networks with a three-dimensional tissue in a microfluidic device. *Integr. Biol.* 9(6):506–18

Neal JT, Li X, Zhu J, Giangarra V, Grzeskowiak CL, et al. 2018. Organoid modeling of the tumor immune microenvironment. *Cell* 175(7):1972–88

Nissen MD, Sloan EK, Mattarollo SR. 2018. β-Adrenergic signaling impairs antitumor $CD8^+$ T-cell responses to B-cell lymphoma immunotherapy. *Cancer Immunol. Res.* 6:98–109

Ooft SN, Weeber F, Schipper L, Dijkstra KK, McLean CM, et al. 2021. Prospective experimental treatment of colorectal cancer patients based on organoid drug responses. *ESMO Open* 6(3):100103

Ootani A, Li X, Sangiorgi E, Ho QT, Ueno H, et al. 2009. Sustained in vitro intestinal epithelial culture within a Wnt-dependent stem cell niche. *Nat. Med.* 15(6):701–6

Park CS, Nguyen LP, Yong D. 2020. Development of colonic organoids containing enteric nerves or blood vessels from human embryonic stem cells. *Cells* 9(10):2209

Park NY, Koh A. 2022. From the dish to the real world: modeling interactions between the gut and microorganisms in gut organoids by tailoring the gut milieu. *Int. J. Stem Cells* 15:70–84

Park SE, Georgescu A, Huh D. 2019. Organoids-on-a-chip. *Science* 364(6444):960–65
Pham MT, Pollock KM, Rose MD, Cary WA, Stewart HR, et al. 2018. Generation of human vascularized brain organoids. *Neuroreport* 29(7):588–93
Pleguezuelos-Manzano C, Puschhof J, Rosendahl Huber A, van Hoeck A, Wood HM, et al. 2020. Mutational signature in colorectal cancer caused by genotoxic *pks*$^{+}$ *E. coli*. *Nature* 580(7802):269–73
Postow MA, Callahan MK, Wolchok JD. 2015. Immune checkpoint blockade in cancer therapy. *J. Clin. Oncol.* 33(17):1974–82
Potente M, Carmeliet P. 2017. The link between angiogenesis and endothelial metabolism. *Annu. Rev. Physiol.* 79:43–66
Rajasekar S, Lin DS, Abdul L, Liu A, Sotra A, et al. 2020. IFlowPlate—a customized 384-well plate for the culture of perfusable vascularized colon organoids. *Adv. Mater.* 32(46):2002974
Reticker-Flynn NE, Zhang W, Belk JA, Basto PA, Escalante NK, et al. 2022. Lymph node colonization induces tumor-immune tolerance to promote distant metastasis. *Cell* 185(11):1924–42
Roberts KJ, Kershner AM, Beachy PA. 2017. The stromal niche for epithelial stem cells: a template for regeneration and a brake on malignancy. *Cancer Cell* 32(4):404–10
Rooks MG, Garrett WS. 2016. Gut microbiota, metabolites and host immunity. *Nat. Rev. Immunol.* 16(6):341–52
Sachs N, Papaspyropoulos A, Zomer-van Ommen DD, Heo I, Böttinger L, et al. 2019. Long-term expanding human airway organoids for disease modeling. *EMBO J.* 38(4):e100300
Sahai E, Astsaturov I, Cukierman E, DeNardo DG, Egeblad M, et al. 2020. A framework for advancing our understanding of cancer-associated fibroblasts. *Nat. Rev. Cancer* 20(3):174–86
Sasaki N, Miyamoto K, Maslowski KM, Ohno H, Kanai T, Sato T. 2020. Development of a scalable coculture system for gut anaerobes and human colon epithelium. *Gastroenterology* 159:388–90
Sato T, Vries RG, Snippert HJ, van De Wetering M, Barker N, et al. 2009. Single Lgr5 stem cells build crypt-villus structures *in vitro* without a mesenchymal niche. *Nature* 459(7244):262–65
Schlieve CR, Fowler KL, Thornton M, Huang S, Hajjali I, et al. 2017. Neural crest cell implantation restores enteric nervous system function and alters the gastrointestinal transcriptome in human tissue-engineered small intestine. *Stem Cell Rep.* 9(3):883–96
Schnalzger TE, de Groot MH, Zhang C, Mosa MH, Michels BE, et al. 2019. 3D model for CAR-mediated cytotoxicity using patient-derived colorectal cancer organoids. *EMBO J.* 38(12):e100928
Schreurs RR, Baumdick ME, Sagebiel AF, Kaufmann M, Mokry M, et al. 2019. Human fetal TNF-α-cytokine-producing CD4^{+} effector memory T cells promote intestinal development and mediate inflammation early in life. *Immunity* 50(2):462–76
Scognamiglio G, De Chiara A, Parafioriti A, Armiraglio E, Fazioli F, et al. 2019. Patient-derived organoids as a potential model to predict response to PD-1/PD-L1 checkpoint inhibitors. *Br. J. Cancer* 121(11):979–82
Shanti A, Hallfors N, Petroianu GA, Planelles L, Stefanini C. 2021. Lymph nodes-on-chip: promising immune platforms for pharmacological and toxicological applications. *Front. Pharmacol.* 12:711307
Shim S, Belanger MC, Harris AR, Munson JM, Pompano RR. 2019. Two-way communication between ex vivo tissues on a microfluidic chip: application to tumor–lymph node interaction. *Lab Chip* 19(6):1013–26
Shurin MR, Shurin GV, Zlotnikov SB, Bunimovich YL. 2020. The neuroimmune axis in the tumor microenvironment. *J. Immunol.* 204(2):280–85
Singh N, Gurav A, Sivaprakasam S, Brady E, Padia R, et al. 2014. Activation of Gpr109a, receptor for niacin and the commensal metabolite butyrate, suppresses colonic inflammation and carcinogenesis. *Immunity* 40:128–39
Sloan EK, Priceman SJ, Cox BF, Yu S, Pimentel MA, et al. 2010. The sympathetic nervous system induces a metastatic switch in primary breast cancer. *Cancer Res.* 70(18):7042–52
Sontheimer-Phelps A, Hassell BA, Ingber DE. 2019. Modelling cancer in microfluidic human organs-on-chips. *Nat. Rev. Cancer* 19(2):65–81
Tsai S, McOlash L, Palen K, Johnson B, Duris C, et al. 2018. Development of primary human pancreatic cancer organoids, matched stromal and immune cells and 3D tumor microenvironment models. *BMC Cancer* 18:335
Tuveson D, Clevers H. 2019. Cancer modeling meets human organoid technology. *Science* 364(6444):952–55

van Pul KM, Fransen MF, van de Ven R, de Gruijl TD. 2021. Immunotherapy goes local: the central role of lymph nodes in driving tumor infiltration and efficacy. *Front. Immunol.* 12:643291

van Riet S, van Schadewijk A, Khedoe PP, Limpens RW, Bárcena M, et al. 2022. Organoid-based expansion of patient-derived primary alveolar type 2 cells for establishment of alveolus epithelial Lung-Chip cultures. *Am. J. Physiol. Lung Cell. Mol. Physiol.* 322(4):L526–38

Vlachogiannis G, Hedayat S, Vatsiou A, Jamin Y, Fernández-Mateos J, et al. 2018. Patient-derived organoids model treatment response of metastatic gastrointestinal cancers. *Science* 359(6378):920–26

Voabil P, de Bruijn M, Roelofsen LM, Hendriks SH, Brokamp S, et al. 2021. An ex vivo tumor fragment platform to dissect response to PD-1 blockade in cancer. *Nat. Med.* 27(7):1250–61

Votanopoulos KI, Forsythe S, Sivakumar H, Mazzocchi A, Aleman J, et al. 2020. Model of patient-specific immune-enhanced organoids for immunotherapy screening: feasibility study. *Ann. Surg. Oncol.* 27(6):1956–67

Wagar LE, Salahudeen A, Constantz CM, Wendel BS, Lyons MM, et al. 2021. Modeling human adaptive immune responses with tonsil organoids. *Nat. Med.* 27:125–35

Wang H, Zheng Q, Lu Z, Wang L, Ding L, et al. 2021. Role of the nervous system in cancers: a review. *Cell Death Discov.* 7:76

Williamson IA, Arnold JW, Samsa LA, Gaynor L, DiSalvo M, et al. 2018. A high-throughput organoid microinjection platform to study gastrointestinal microbiota and luminal physiology. *Cell. Mol. Gastroenterol. Hepatol.* 6(3):301–19

Wolchok JD, Chiarion-Sileni V, Gonzalez R, Rutkowski P, Grob JJ, et al. 2017. Overall survival with combined nivolumab and ipilimumab in advanced melanoma. *N. Engl. J. Med.* 377(14):1345–56

Workman MJ, Mahe MM, Trisno S, Poling HM, Watson CL, et al. 2017. Engineered human pluripotent-stem-cell-derived intestinal tissues with a functional enteric nervous system. *Nat. Med.* 23:49–59

Wörsdörfer P, Dalda N, Kern A, Krüger S, Wagner N, et al. 2019. Generation of complex human organoid models including vascular networks by incorporation of mesodermal progenitor cells. *Sci. Rep.* 9:15663

Yang J, Yan J, Liu B. 2018. Targeting VEGF/VEGFR to modulate antitumor immunity. *Front. Immunol.* 9:978

Yao Y, Xu X, Yang L, Zhu J, Wan J, et al. 2020. Patient-derived organoids predict chemoradiation responses of locally advanced rectal cancer. *Cell Stem Cell* 26(1):17–26

Yuki K, Cheng N, Nakano M, Kuo CJ. 2020. Organoid models of tumor immunology. *Trends Immunol.* 41(8):652–64

Zaman MH, Trapani LM, Sieminski AL, MacKellar D, Gong H, et al. 2006. Migration of tumor cells in 3D matrices is governed by matrix stiffness along with cell-matrix adhesion and proteolysis. *PNAS* 103(29):10889–94

Zetter BR. 1998. Angiogenesis and tumor metastasis. *Annu. Rev. Med.* 49:407–24

Zhou G, Lieshout R, van Tienderen GS, de Ruiter V, van Royen ME, et al. 2022. Modelling immune cytotoxicity for cholangiocarcinoma with tumour-derived organoids and effector T cells. *Br. J. Cancer* 127:649–60