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Opinion

Personalized Cancer Medicine: An Organoid Approach

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Personalized cancer therapy applies specific treatments to each patient. Using personalized tumor models with similar characteristics to the original tumors may result in more accurate predictions of drug responses in patients. Tumor organoid models have several advantages over pre-existing models, including conserving the molecular and cellular composition of the original tumor. These advantages highlight the tremendous potential of tumor organoids in personalized cancer therapy, particularly preclinical drug screening and predicting patient responses to selected treatment regimens. Here, we highlight the advantages, challenges, and translational potential of tumor organoids in personalized cancer therapy and focus on gene–drug associations, drug response prediction, and treatment selection. Finally, we discuss how microfluidic technology can contribute to immunotherapy drug screening in tumor organoids.

Highlights

Personalized cancer medicine is an approach to tailoring effective therapeutic strategies for each patient according to a tumor's genomic characterization. There is an urgent demand for research in personalized tumor modeling to confirm the functional aspects of genomic drug response predictions in the preclinical setting.

While different tumor models, such as tumor cell lines and patient-derived tumor xenografts, have been proposed, the drawbacks of each model have limited their applications as personalized tumor models.

A tumor organoid, in which cellular and molecular heterogeneity of tumor cells is preserved, has emerged as a promising platform.

Recently, numerous studies highlighted the application of tumor organoids in personalized cancer medicine in terms of gene–drug association treatment, the identification of new therapies, and prediction of patient outcome.

Importance of Personalized Tumor Models

In conventional approaches to cancer therapy, most patients with a particular type of cancer receive similar 'one-size-fits-all' treatments. However, it has recently become clear that certain treatments work well for some patients but do not show promising results in others. Currently, individualized cancer treatments are progressively improving due to better characterization of the molecular and pharmacogenomic features of tumors. This recent approach, called precision or personalized cancer medicine, can be described as a 'one dose, one patient' treatment.

Each tumor is associated by a heterogeneous tumor microenvironment, which can significantly affect the response to therapy and clinical outcomes. However, the use of **gene–drug association** (see Glossary) treatment strategies may be limited due to a lack of biological understanding of tumor response to drugs [1]. In other words, detecting mutations (e.g., *EGFR* or *PIK3CA* mutations) matched with approved, targeted drugs (e.g., EGFR and PIK3CA inhibitors) does not necessarily mean that the molecular alterations in these pathways are sensitive to the selected therapy [2]. In the field of personalized cancer medicine, the link between functional genomics and pathological data to patient outcome is a major challenge. To address this challenge, different personalized tumor models have been proposed, including cancer cell lines, **patient-derived xenografts (PDXs)**, and 3D culture tumor models, such as organoid culture methods.

Here, we discuss the patient-derived tumor organoid as a novel and promising tumor model in personalized cancer therapy. We highlight the potential and challenges of this model system for preclinical drug screening and predicting patient outcomes in comparison with pre-existing

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tumor models. In addition, we point out how other technologies, such as microfluidic culture systems, can rise to the challenge of testing **immunotherapy** drugs on tumor organoids.

Conventional Tumor Models

Monolayer and 3D Culture Models

The initial results of *in vitro* tumor modeling were obtained from tumor-derived cell lines due to their high-throughput capacity for pharmaceutical drug screening [3]. However, there is evidence [4] showing the limitations of cancer cell lines, such as the lack of tumor and stromal cells, and interactions between the cells and the extracellular matrix (ECM) [4]. In addition, cancer cell lines lack immune–tumor cell interactions *in vitro*, whereas immune cells can dramatically alter the efficacy of cancer therapies in patients. Thus, available cancer cell lines have largely failed to model a tumor microenvironment to evaluate new target and **chemotherapy** medicines [5]. After the emergence of 3D cultures, partial mimicking of the tumor microenvironment allowed researchers to test the functionality of drugs or evaluation of **chemoresistance** in the presence of cell–cell and cell–ECM interactions in a 3D architecture [6]. Nonetheless, these methods also face several limitations (Box 1) and have so far failed in applications to personalized tumor modeling.

PDX Models

Patient-derived tumor xenografts are generated by transplanting a patient's tumor cells to an immune-deficient mouse in a subcutaneous and/or orthotopic manner. These models provide promising platforms for cancer research and drug development [9]. They are widely utilized for drug discovery, biomarker detection, and preclinical drug evaluation [10]. Similar to the original tumor, PDX models can fully recapitulate the interactions of tumor cells with their surrounding stromal cells and the ECM, with the exception of interactions with the immune system. These models can mimic physiological and biochemical effects of drugs against an individual patient's tumors.

PDX models have previously shown potential to investigate resistance mechanisms and determine novel treatment approaches [11–13]. Although the development of PDX models has improved the quality of cancer research and translation of *in vitro* findings to *in vivo*, their application in precision cancer therapy has been restricted. The choice of effective and successful engraftment methods for different types of tumors is the main limitation of PDX models. For instance, in hormone-sensitive breast cancer, the rate of engraftment is very low compared with triple-negative breast cancer [14]. Time is a critical factor in real-time personalized medicine. Generation of a successful PDX model requires 4–8 months, which results in a time delay between engraftment in mice and scheduled treatment regimens for the patient [10].

Generation of specific subtypes of tumors is another limitation of PDX models. The majority of PDX models have been generated from invasive and metastatic tumors, whereas

Box 1. Limitations of Conventional 3D Culture Models

Conventional 3D tumor models, known as tumor spheroids, can be generated from single cell types of cancer cell lines (homotypic spheroids) and/or cocultured with other cell types (heterotypic spheroids). These models have a series of challenges that limit their applications as preclinical tumor models. These challenges include variations in 3D culture methods, lack of immune cell interactions in the culture, and inability to fully mimic the tumor microenvironment in terms of cell types and their spatiotemporal architecture [7]. In terms of gene expression profiles, the comparison between original tumors and corresponding cell line-based tumor spheroids has shown significant differences, where common mutations can be observed in cancer cell lines, whereas rare mutations are not preserved. Thus, cell line-based models are unable to imitate the complete genomic background of tumors and, consequently, the drug response of targeted therapy agents [8].

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nonmetastatic tumors showed engraftment failure in mice (e.g., in colorectal cancer [15] and hormone-sensitive breast cancer studies) [16,17]. Considering the role of the tumor microenvironment in evaluating the drug response [4,18], replacing human stromal cells with murine cells in PDX models could affect drug screening results and consequently, predictions of the drug response [18].

In addition, in PDX models, the absence of immune system components results in an enhanced engraftment rate. This characteristic of immune-deficient mouse models makes them inappropriate for screening and functional analysis of new immune-therapeutic drugs [10]. Despite marked genetic similarities between patient tumors and related PDX models, a series of changes exist in specific genes and drug targets. In the context of modeling targeted therapy in PDX models, induced genomic alterations and rearrangements in tumor cells that result from passaging tumor cells into a new mouse limit their application in targeted therapy and gene–drug association studies [19]. Researchers have sought to overcome these challenges in recent years with the advent of new 3D cell culture methods, known as organoid culture. This has opened up new horizons to use reliable tumor models in cancer research and therapeutics, in particular personalized cancer treatment.

Tumor Organoid as a Preclinical Model for Personalized Cancer Therapy

Currently, there is no consensus definition for 'organoids' (Box 2). To date, different organoid models have been developed for a variety of normal tissues, including the colon and small intestine [30,31], stomach [32–34], liver [35,36], mammary glands [37], retina, and brain [38]. In cancer research, optimizing various culture conditions (Table 1) has resulted in the development of numerous patient-derived tumor organoids, including colon [30], prostate [21,39], gastric [40], breast [37,41], and pancreatic cancers [42,43], in addition to endometrial/ovary carcinomas, uterine carcinosarcoma, urothelial carcinoma, and renal carcinoma [44].

Organoid models have applications in many research areas, such as biomedical research [45], genomic analysis of various diseases, and therapeutic studies [46–48]. Recently, organoids

Box 2. Patient-Derived Tumor Organoids

Organoids are 3D cell cultures that preserve numerous key features of the represented organ. The organoids contain multiple and organ-specific cell types with a spatial architecture similar to that of the corresponding organ. These models can recapitulate some key functions of that organ.

Organoids may be generated from one or a few cells derived from primary tissue samples, adult stem cells, or the directed differentiation of pluripotent stem cells [20–22]. Tumor organoids are 3D cultures of cancerous cells that can be derived from tumor tissues for better mimicking the composition of a tumor in the body [20,21,23–27]. The first organoid culture was reported in murine intestinal cells, further developed for other organs, and eventually translated into human cells [28]. These features of organoids made them useful tools for cancer research and therapy for *in vitro* and clinical studies [29].

Patient-derived tumor organoid culture methods vary depending on the tumor type. A standard and robust technique for primary tumor organoid culture still needs to be developed. Recently, different tissue-specific culture conditions and methods for generation of different tumor organoids have been developed (Table I). The tumor organoid culture is initiated by mechanical and enzymatic digestion of tumor tissue into small pieces, followed by embedding this tissue into a 3D matrix (mostly Matrigel) as a biomimetic scaffold. The cellular architecture of the organoids and its behavior significantly depend on the matrix composition. Matrigel contains laminin, entactin, proteoglycans, and collagen IV. Although it can be enriched with numerous growth factors, reduced growth factor media are mostly used for tumor organoid culture. Paracrine signaling is simulated by a cocktail of different tumor tissue-specific growth factors. The most commonly used growth factors include Wnt3A, R-spondin-1, epidermal growth factor, and bone morphogenetic protein antagonist Noggin. Several additional factors can enhance organoid culture and passage, including Rho-kinase inhibitor Y-27632 and GSK3β-kinase inhibitors. Recently, numerous tissue-specific culture media have been developed

Glossary

Afatinib: a tyrosine kinase inhibitor for EGFR and ErbB 2 (HER2).
APC: adenomatous polyposis coli, a negative regulator of beta catenin and a modulator of its interaction with E-cadherin. Mutations in the *APC* gene can result in colon cancer.
Biopsy: extraction of small pieces of tissue from a specific site (e.g., tumor tissue).
Buparlisib: a targeted therapy drug that inhibits the pan-class I phosphatidylinositol 3-kinase (PI3K) family of lipid kinases.
Chemoresistance: resistance of tumor cells to the effects of chemotherapeutics.
Chemotherapy: a category of cancer treatment that uses one or more anticancer drugs.
Combination therapy: treatment in which a patient is given two or more drugs.
EGFR inhibitors: a type of targeted drug therapy that inhibits EGFR on cancer cells.
FOLFOX regimen: a chemotherapy regimen composed of folinic acid, 5-fluorouracil, and oxaliplatin.
Gene–drug association: selection of a drug based on the genomic abnormalities of a tumor.
HDAC inhibitors: small molecules that inhibit histone deacetylase.
Immunotherapy: a cancer treatment that attempts to stimulate the immune system to destroy tumors.
Interferon regulatory factor 8: a protein that plays a key role in regulation of lineage commitment and maturation of myeloid cells.
Multicellular tumor spheroids: common methods for a 3D culture of cancer cells.
Neoadjuvant chemotherapy: the use of chemotherapy regimens prior to surgery.
Off-label drugs: drugs used as treatment that lack FDA approval.
Olaparib: a poly (ADP-ribose) polymerase (PARP) inhibitor.
Pan-HER inhibitors: small-molecule agents that inhibit HER1, HER2, and HER4 receptors on cancer cells.
Patient-derived xenograft (PDX): the transplantation of patient's tumor cells into immune-deficient mice.
Somatic copy number alterations: also called 'copy number variation' when a section of the genome is

for different tumor organoid cultures. In an attempt to study the hallmarks of cancer on tumor organoids, numerous *in vitro* assays have been introduced (Table II).

Table I. Characterization of Different Cancer Models[a]

Features	Cell lines	PDXs	Organoids
Success rate of initiation	•	••	•••
Expansion	•••	•	••
Cancer subtype modeling	•	–	•••
Biological stability	•	••	••
Genetic manipulation	•••	–	•••
High-throughput drug screening	•••	–	••
Low-throughput drug screening	•••	•	•••
Ease of downstream assays	•••	•	•••
Cost benefits	•••	–	••
Time consumption for modeling	•	•••	•
Ease of maintenance	•••	–	••

Adapted, with permission, from [29].
[a]•••, Best; ••, suitable; •, possible; and –, unsuitable.

Table II. Possible *In Vitro* Assays in Tumor Organoids to Study the Hallmarks of Cancer[a]

Hallmark of cancer	Possible tumor organoid assays
Evading growth suppressors	Proliferation assays and size measurement
Avoiding immune destruction	Coculture assays with immune cells using microfluidic technology
Replicative immortality	Repeat organoid passaging
Tumor-induced inflammation	Treatment with inflammatory cytokines
Invasive and metastasis	96-well trans-well migration and invasion assays
Angiogenesis	Coculture assays with endothelial cells using microfluidic technology
Genome instability and mutation	Whole-genome and/or targeted sequencing.
Resistance to cell death	High-throughput viability assays: MTT, PI
Deregulating cellular energy	Oxygen consumption rate measurement and extracellular acidification rate assays
Sustaining proliferative signaling	Proliferation and viability assays: calcein-AM/PI, CellTiter-Glo

[a]Abbreviations: MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; PI, propidium iodide.

repeated. The numbers of these repeats play key roles in cancer diagnosis and prognosis.
Splenocytes: white blood cells located in the spleen.
Targeted therapy: a cancer treatment that employs small-molecule inhibitors to specifically target cancer cells.

have shown great potential in drug discovery [23], cytotoxicity investigations of new therapeutic compounds [49], *in vivo* modeling of specific cancer metastasis-associated genes [50], and personalized cancer treatments [25,26]. A large body of evidence has provided a proof of concept for applying tumor organoids in personalized cancer therapy, confirming the genomic and functional resemblances between patient-derived tumor organoids and their original specimens [22,26,27,51]. In contrast to conventional cancer models that require large sample sizes (e.g., PDX models), organoids can be cultured from a small sample size, derived from needle **biopsy**, with a high success rate for personalized tumor modeling [43]. In patients with resected tumors, multiple organoids can be generated from different areas of the tumor to better mimic tumor heterogeneity [52,53]. Tumor organoids show tremendous potential for modeling specific cancer subtypes that have unique genomic mutations [54]. Taken together,

Table 1. Developed Culture Conditions for the Generation of Different Tumor Organoids from Tumor Specimens[a]

Cancer type	Aim of study	Source of organoids	Digestion condition	Culture condition	Refs
Prostate	Tumor modeling	Patient tumor specimens (human)	5 ml of 5 mg/ml collagenase Type II + advanced DMEM/F12 (ADMEM/F12)	Growth factor-reduced Matrigel + ADMEM/F12	[21]
Prostate	Lineage and cell transition monitoring	Murine and human prostates: Single cells and bulk	Collagenase Type II + ADMEM/F12	Cells were seeded in growth factor-reduced Matrigel (Corning) and overlaid with medium containing the growth factors (EGF, R-spondin-1, Noggin, TGF-b/ALK inhibitor A83-01, dihydrotestosterone, FGF10, FGF2, prostaglandin E_2, SB202190, nicotinamide, and DHT)	[55]
Breast	Metastasis at tumor's leading edge	Human tumor specimens	Collagenase with or without trypsin + DMEM + DNase	Matrigel or 3D collagen-I, human MEM + insulin, EGF, hydrocortisone, and cholera toxin	[37,56]
Breast	Detect drug response of organoids	Human primary tumor	Macrosuspensions of tissue (50–300 μm) by mechanical cutting of the tissue with a scalpel and surgical scissors	Matrigel + DMEM: F12 + EGF + hydrocortisone + insulin (5 μg/ml) + penicillin–streptomycin. Making gel on coverslips	[57]
Renal	Method for renal carcinoma 3D culture	Renal carcinoma specimens	EGM2 + collagenase Type IV (5 mg/ml), 40–50 min with vortexing at 10-min intervals	Renal ECM scaffold + EGM2	[44]
Colon	Genetic diversity of patient-derived tumor organoids and the original tumor biopsy	Human tumor specimen	N/A	Basement membrane matrix, growth factor-reduced Matrigel + ADMEM/F-12 Hams, penicillin–streptomycin, HEPES, GlutaMAX, R-spondin-conditioned medium, Noggin-conditioned medium, B27, *N*-acetyl-cysteine, nicotinamide, EGF, gastrin, TGFb Type I receptor inhibitor A83-01, p38 MAPK inhibitor (p38i) SB202190, prostaglandin E_2, and Primocin	[22]
Colon	Organoid biobank, personalized medicine	Human colon tumor	N/A	Human intestinal stem Cell medium (HISC): Basal culture medium with Wnt-conditioned medium, R-spondin-conditioned medium, Noggin-conditioned medium, B27, *N*-acetylcysteine, nicotinamide, human EGF, gastrin, A83-01, SB202190, prostaglandin E_2, and Primocin	[26]
Colon	Modeling specific subtype of colon cancer	Human colon and intestinal tumors	Mechanically minced with EDTA cold chelation buffer (distilled water with Na_2HPO_4, KH_2PO_4, NaCl, KCl, sucrose, 5 d-sorbitol, dl-dithiothreitol) for 30 min	Advanced Dulbecco's modified Eagle medium/F12 with penicillin–streptomycin, HEPES, GlutaMAX, B27, gastrin I, and *N*-acetylcysteine	[58]

Table 1. (continued)

Cancer type	Aim of study	Source of organoids	Digestion condition	Culture condition	Refs
Pancreatic	PDAC modeling and drug screening	PDAC tumor specimens	Collagenase + PTOM	Matrigel + pancreatic progenitor and tumor organoid medium (PTOM), DMEM, B27, ascorbic acid, insulin, hydrocortisone, FGF2, all-*trans* retinoic acid, and Y267632 small molecule	[42]
Pancreatic	Organoid model	PDAC tumor specimens	Collagenase II + human complete medium. TrypLE with human CM	Growth factor-reduced Matrigel + human complete medium: ADMEM/F12 medium supplemented with HEPES, GlutaMAX, penicillin/streptomycin, B27, Primocin, *N*-acetyl-l-cysteine, Wnt3a-conditioned medium, R-spondin-1-conditioned medium, Noggin-conditioned medium or recombinant protein, EGF, gastrin, FGF10, nicotinamide, and A83-01	[43]
Stomach	Genomic-based classification of gastric cancer, new driver mutation detection, personalized medicine	Mouse gastric tumor	PBS + EDTA	Growth factor-reduced Matrigel, ADMEM/F12 supplemented with EGF, R-spondin-1, Noggin, Y-27632	[30,40]
Stomach Esophageal	Long-term 3D cultures of human gastric stem cells and bacterial infection study	Human stomach and esophageal tumors	EDTA + cold chelation buffer	Matrigel, ADMEM/F12 supplemented with penicillin/streptomycin, HEPES, GlutaMAX, B27, *N*-acetylcysteine. Gastric medium: Basal medium supplemented with EGF, Noggin-conditioned medium, R-spondin-1-conditioned medium, Wnt-conditioned medium, FGF10, gastrin, A-83-01 (TGF-beta inhibitor). Facultative component: Nicotinamide. Additional components: RHOKi (Y-27632), IGF, p38 inhibitor (SB202190), GSK3β inhibitor PGE_2	[33]
Colon Endometrial Uterine Urothelial Ovary	Personalized tumor modeling	Human patient tumor specimen and PDX	Collagenase IV and trypsin–EDTA	Growth factor-reduced Matrigel, ADMEM with GlutaMAX, B27, penicillin/streptomycin, Primocin, and tumor type-specific growth factors	[44]

[a]Abbreviations: ALK, anaplastic lymphoma kinase; DHT, dihydrotestosterone; DMEM/F-12, Dulbecco's modified Eagle medium/nutrient mixture F-12; ECM, extracellular matrix; EGF, epidermal growth factor; EGM, endothelial cell growth medium; FGF10, fibroblast growth factor-10; FGF2, fibroblast growth factor-2; GSK3β, glycogen synthase kinase 3 beta; HEPES, 4-(2-hydroxyethyl)-1-piperazine-ethane-sulfonic acid; HISC, human intestinal stem cell medium; IGF, insulin growth factor; MAPKs, mitogen-activated protein kinases; MEM, minimum essential medium; PBS, phosphate-buffered saline; PDAC, pancreatic ductal adenocarcinoma; PGE2, prostaglandin 2; PTOM, pancreatic progenitor and tumor organoid medium; RHOKi, rho-associated protein kinase inhibitor; TGFb, transforming growth factor beta.

these key findings suggest that this new model has great potential in personalized cancer therapy, specifically for gene–drug correlation studies, preclinical drug screening of anticancer drugs, and prediction of drug response and patient outcome (Figure 1, Key Figure).

For example, Weeber and colleagues [22] developed a tumor organoid culture condition and sequenced 1977 cancer-related genes across 14 colon cancer organoids and corresponding original tumors. They reported 90% preservation of somatic mutations and DNA copy number profile between the developed tumor organoids and biopsies, which resulted in the successful application of organoids in genomic-based personalized medicine [22]. Until now, the most comprehensive and impressive example of the application of tumor organoids in personalized cancer therapy was reported by van de Wetering and colleagues [26], who established an organoid biobank of 20 colon tumors. Whole-exome sequencing analysis revealed that colon tumor organoids preserved the cancer subtypes detected in the tumor samples. In addition, a comparison of **somatic copy number alterations** in tumor biopsies and related organoids with The Cancer Genome Atlas (TCGA) database showed that genomic alterations found in hypermutated and non-hypermutated clinical samples were represented in tumor organoids [26]. Interestingly, an investigation of drug sensitivities of tumor-derived organoids against the library of 85 therapeutic compounds including chemotherapy and **targeted therapy** agents resulted in the identification of an effective treatment for each individual patient [45]. This study demonstrated that colon tumor cells that contained *TP53* loss of function exhibited resistance to MDM2 inhibitors, and *RAS* mutants were insensitive to the **EGFR inhibitor**. In addition to these findings, they suggested a novel treatment approach for the *RNF43* mutant colon cancer in which colon tumor organoids with the *RNF43* mutation were significantly sensitive to Wnt secretion inhibitors.

Gene–drug association plays a key role in personalized cancer therapy and targeted therapy. The study by van de Wetering and colleagues [26] described a strong correlation between gene mutation status and therapeutic response, known as mutation-based drug sensitivity. Because genomics analysis is insufficient to identify effective therapeutic options for the majority of patients with advanced cancer, drug screening on tumor organoids could illuminate the unknowns of the drug response [27]. To determine the clinical application of tumor organoids in personalized cancer therapy, numerous clinical trials on colon and pancreatic tumor organoids have been conducted (NCT03140592). Modeling specific and rare subtypes of cancer by means of genetically engineered organoids can help to identify an effective therapy for a small proportion of patients [24,47,58–60].

In a comparison study between tumor organoids and its counterpart PDX models, 56 tumor-derived organoid and 19 PDX models were generated from 769 patients with various cancer types. The matched tumor organoids and generated PDX models showed similar histopathological features to their original tumors, which was further validated via whole-exome sequencing in both models [27]. Genomic analysis of organoids in different passages revealed that the tumor organoids preserved genomic alterations of the original tumor during long-term culture. Interestingly, genome sequencing of large numbers of tumor samples revealed that in 85.8% of cases, somatic alterations in cancer genes were not targetable, whereas 9.6% of cases could be targeted by **off-label drugs**, and only 0.4% of detected somatic alterations could be targeted by FDA-approved drugs. This observation highlighted the potential for reliable tumor models to identify novel treatment options [27]. The results from screening of a library of 160 drugs, including FDA-approved chemotherapeutics and targeted agents, showed similar drug responses between tumor organoids and PDX models.

Key Figure

Schema of Organoid-Based Personalized Cancer Therapy

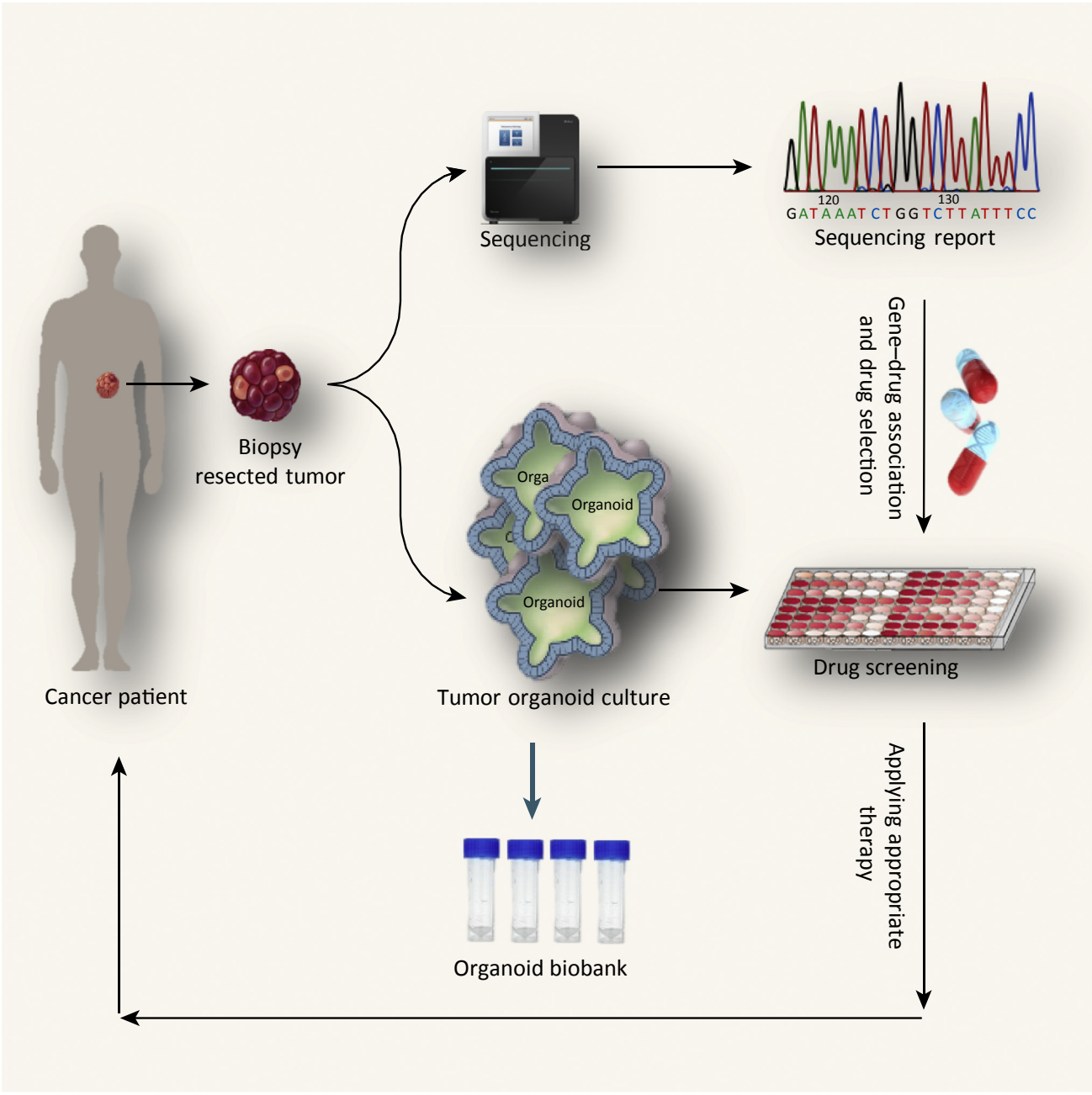

Figure 1. In this approach, the procedure begins with sequencing tumor biopsies or dissected samples by using the next-generation sequencing method and continues with culturing patient-derived tumor organoids, which will be histologically and pathologically compared with the primary tumors before they are subjected to

(Figure legend continued at the bottom of the next page.)

For instance, in endometrial adenocarcinoma, optimal treatment for both organoid and PDX models included the combination of **buparlisib** and **olaparib**. In addition, **afatinib** and other EGFR inhibitors were recorded to be the most effective drugs for advanced stage colon cancer with a mutation in the ***APC*** gene. This result was validated in both tumor models [27]. Interestingly, combination therapy screening on tumor organoids suggested that compared with the **FOLFOX regimen**, a combination of afatinib and **histone deacetylase (HDAC) inhibitors** led to greater inhibition of growth in colon tumors with the *APC* mutation [27]. Targeting mutant *RAS* in patient-derived colorectal cancer organoids identified a relationship between $KRAS^{G12D}$ and insensitivity to the combination of MEK and **pan-HER inhibitors** [25]. The use of a BCl-2 inhibitor in colon organoids overcame resistance to combined therapy of MEK and pan-HER. These results were further confirmed in a PDX model. Therefore, these results suggest that integrating genome data and preclinical drug screening results could improve precision cancer treatment.

Presently, the majority of patients are candidates for second-line therapy with different categories of drugs compared with those used as the initial course of therapy. Organoids derived from individual patients screened with first-line therapy could be tested for second-line therapy to ascertain the best possible treatment option. In support of this idea, Skardal and colleagues [61] used liver tumor hybrid organoids as a screening panel for testing different compounds similar to second line of therapy in liver cancer. Their promising results indicated that organoids were suitable for therapeutic testing *in vitro* [61]. Gao and colleagues [21], in a study of prostate cancer organoids, observed a strong correlation between different therapeutic responses and the genomic profile of individual cancer.

Comparing *in vitro* drug screening results and the patient drug response is another potential application of tumor organoid models. In this context, a large multicenter cohort study on metastatic breast, colon, and nonsmall cell lung cancers (NSCLCs) was conducted by the Foundation Hubrecht Organoid Technology (HUB; Utrecht, The Netherlands, TUMOROID trial: NL49002.031.14). In this study, drug responses in tumor organoids derived from biopsy of the metastatic lesion showed a positive correlation with clinical responses of patients [23]. In addition, the correlation of matched clinical outcome with tumor organoid drug responses has established an *in vitro* threshold for drug response. However, differences between drug dosages in chemotherapy regimens and those used in *in vitro* drug screening assays are challenging issues that remain to be solved.

The selection of patients for appropriate and effective treatment regimens is another main focus of personalized cancer therapy. Patient-derived tumor organoids show great potential to select patients for specific targeted therapy. In this regard, an ongoing prospective clinical study conducted by The Netherlands Cancer Institute (SENSOR study, NL50400.031.14 EudraCT 2014-003811-13) selects colon and NSCLC patients for targeted treatment by means of screening of a panel of targeted agents on patient-derived tumor organoids. In this study, patients with metastatic colorectal cancer and NSCLC with only one standard line of treatment remaining were considered. Tumor organoids of these patient's tumors were screened against eight different targeted therapy agents. After identification of the most active agent with the

drug screening. In parallel, part of the derived organoids will be preserved as a biobank. To determine effective therapeutic strategies, based on the sequencing results and gene–drug association links, high-throughput drug screening of candidate drugs that include standard chemotherapy and targeted therapy agents can be performed in a replicative process.

highest inhibitory effect, the study offered patients the choice to continue therapy with the identified agent.

Tumor Organoid Challenges and Possible Solutions to Overcome Them

Despite the advantages of tumor organoids in personalized cancer therapy, numerous challenges may hamper the implementation of this approach in a clinical setting. In some cancers, the majority of patients are candidates for **neoadjuvant chemotherapy** regimens to shrink tumors prior to surgery. These treatment regimens may result in decreased numbers of tumor cells from the biopsy area and possibly preclude organoid generation in preclinical settings. Therefore, optimizing current culture methods to generate tumor organoids from these types of biopsies will promote organoid culture for a wide variety of patients. Challenges include the lack of immune system elements, other key stromal cells, and vasculature factors in tumor organoid cultures; these can restrict functional testing of immunotherapy drugs and/or stromal targeted agents on tumor organoids. However, to overcome this challenge, other technologies such as microfluidics technology (Box 3) may facilitate coculture of tumor organoids with other cell types to model a complex tumor microenvironment [62].

To date, a wide variety of microfluidic devices have been constructed to assess and model the liver, kidneys, lungs, intestines, heart, smooth muscles, bones, blood vessels, and blood–brain barrier in a microarchitecture setting [63]. These microfluidic analogs have the ability to connect with each other to mimic *in vivo* physiological coupling (called a human-on-a-chip model; Box 3) and to replace animal models in pharmaceutical studies [63]. This has ignited great enthusiasm for the potential of this technology to model immune–tumor cell interactions.

Historically, studies on interactions between immune cells and cancer cells in monolayers were critical for discovering tumor-associated antigens [64,65]. Later, coculturing immune cells and **multicellular tumor spheroids** illustrated new dimensions of tumor immunology [69]. In contrast to 2D cancer–immune interaction, *in vitro* 3D studies not only demonstrated decreased production of tumor-associated antigens in 3D models, but also highlighted the importance of using 3D tumor models in tumor immunology studies, which can be easily captured in microfluidic systems [64]. However, few studies have been conducted using microfluidic systems to investigate immune–cancer cell interactions [64,70–73]. Agliari and colleagues [73] reported a coculture system with microfluidic devices that investigated the migration of immune cells toward tumor cells by exploiting **splenocytes** deficient in the transcription factor **interferon regulatory factor 8**.

Recently, Liu and colleagues [74] conducted a study that employed precision medicine for bladder cancer by coculturing different Matrigel-embedded cell types including human bladder cancer cells (T24), fibroblasts (BJ-5Ta), macrophages (Raw 264.7), and human umbilical vein endothelial cells in a microfluidic device. They observed migration of macrophages toward

Box 3. Introduction of Microfluidic and Organ-On-Chip Technologies

Microfluidics is the science of manipulating fluids in submillimeter channels. This method can control several parameters, such as relative cell and tissue location, fluid flow levels and patterns, mechanical cues, and gradients within a system [63]. The advantages of this technology include (i) smaller sample and material requirements, (ii) enhanced quality of microscopic imaging and quantification of cells, (iii) control over experiments, (iv) low cost of production, and (v) the possibility of a targeted design. These benefits make microfluidic technology a complementary tool for other platforms. A complex tumor microenvironment can be created with the support of this technology and by determining different cell regions [62]. Microfluidic technology can be used to model the metastatic microenvironment, immune–cancer cell interactions, and specific behavior of cancer cells (Figure IA) [64–66]. Organs-on-chips are microfluidic devices that allow the culture of living cells to mimic physiological and functional properties of an organ at micrometer diameters [63]. The human-on-a-chip is an improved method that simulates intracellular relevance and organ interactions, thus providing the possibility of *in vitro* testing of pharmacodynamics and toxicodynamics of drugs (Figure IB) [67].

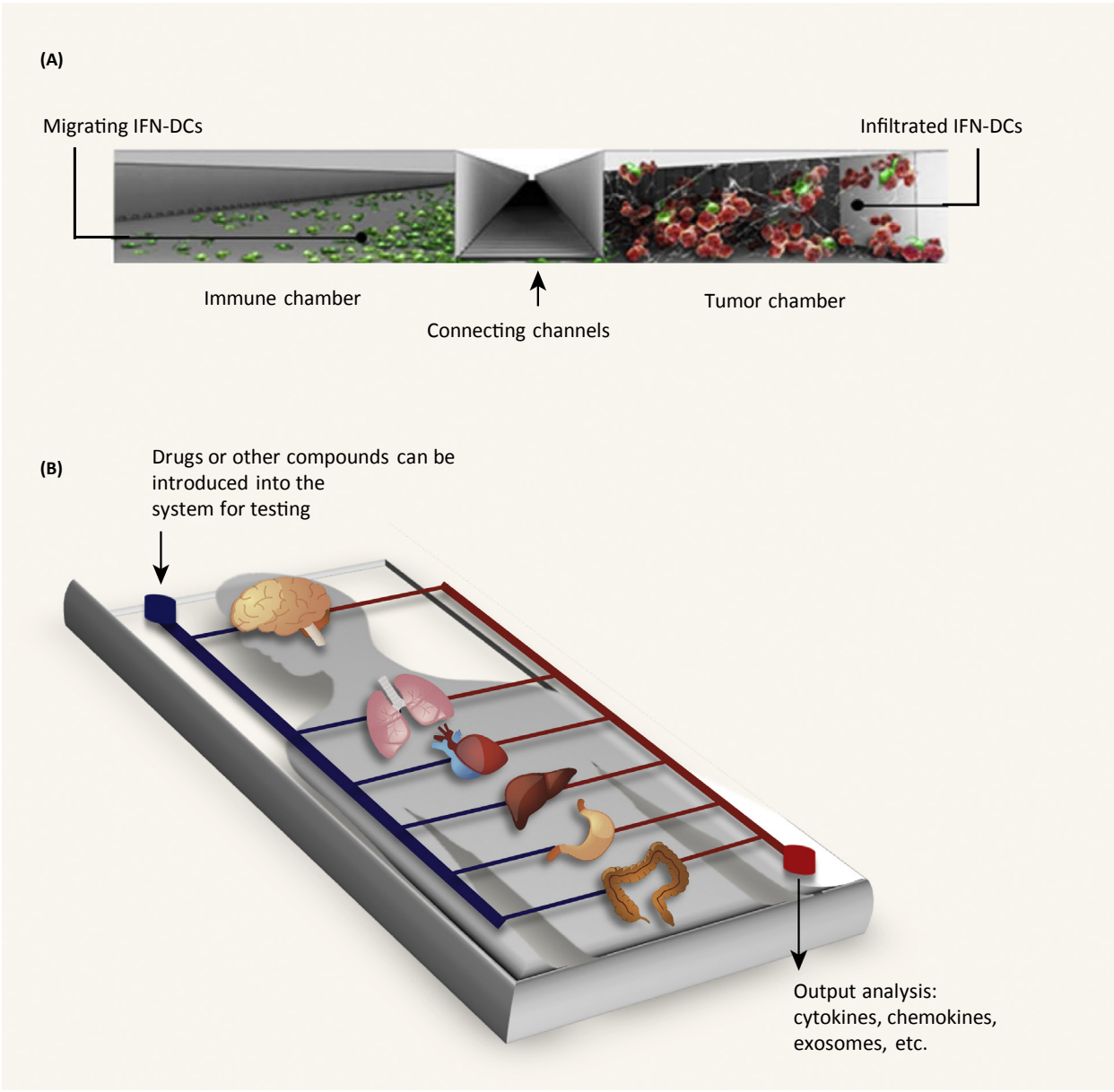

Figure I. Application of Microfluidic Technology in Cancer–Immune Interaction and Organ-On-Chip Concepts. (A) Schematic presentation of microfluidic chip section that shows the migration of interferon-α-conditioned dendritic cells (IFN-DCs) toward the tumor environment by crossing the connecting channels. Reproduced, with permission, from [68]. (B) Graphical presentation of the human-on-chip concept. Microfluidic technology enables the culture of different cells from different organs to approximate a close humanized *in vitro* model.

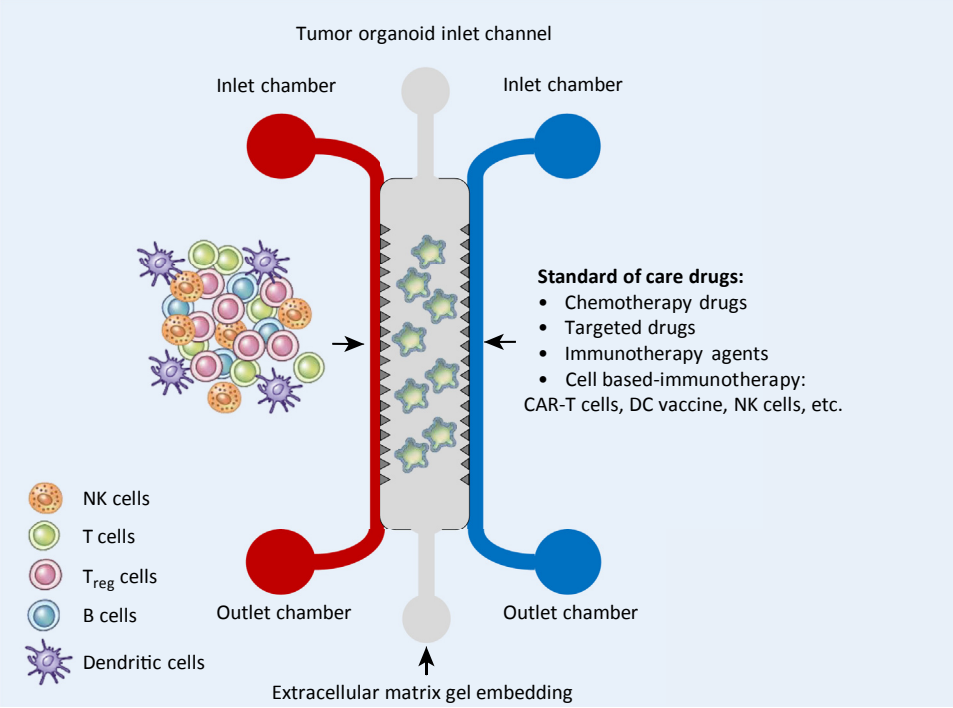

Figure 2. Schematic of Cocultured Tumor-Derived Organoids, with Immune System Elements Derived from the Peripheral Blood of Patients, in a Microfluidic Device. DC, dendritic cell; NK cells, natural killer cells; T_{reg}, T regulatory cells.

cancer cells and screened different chemotherapeutics similar to a neoadjuvant schema in a clinical setting. Their results showed that macrophages in 3D cocultures expressed and released more Arg-1 in the tumor microenvironment, which resulted in alterations in the chemotherapy response [74]. Taken together, in contrast to PDX models, the interaction between tumor organoids and immune cells in microfluidic devices may not only overcome the challenge of screening current immunotherapy drugs, but also can predict the effects of new generation of cancer immunotherapy agents including CAR-T cells, engineered natural killer cells, and dendritic cells on tumor organoids in the preclinical setting (Figure 2).

Concluding Remarks and Future Perspectives

Personalized cancer medicine is devoted to tailoring the most appropriate drug for an individual patient. To achieve this goal, genomic-based drug response prediction has opened new ways to enable better decision making in oncology. Evaluating the functionality of these predictions is difficult because of the lack of representative patient tumor models that can recapitulate all of the key features of original tumors. Patient-derived tumor organoids show great potential for predicting clinical responses to drugs and for selecting treatments. Although numerous questions need to be addressed (see Outstanding Questions), current clinical studies will improve our understanding of the translational application of tumor organoids to the patient.

Here, we have highlighted current studies that assessed the potential application of tumor organoids in precision oncology, in particular, drug response prediction, patient selection, and determination of a novel therapeutic agent. Given that tumor organoids can be generated from

individual cancer patients with close cellular and molecular resemblance to the parental tumor, we believe that tumor organoids will be better, more promising, and more clinically relevant tumor models for personalized cancer therapy compared with pre-existing models. We expect that the use of other technologies, including microfluidics, may overcome the challenge of modeling the immunotherapy response on tumor organoids. In this context, further studies should be designed to coculture tumor organoid and immune system elements derived from individual patients in the microfluidic system and screen immunotherapy drugs.

Outstanding Questions

Can patient-derived tumor organoid models be a useful approach for clinical management of cancer patients?

Can tumor organoids predict the response of chemotherapy regimens and patient outcome?

Can this model be useful for patients who do not have recommended standard treatments?

Can the combination of a microfluidic culture system and tumor organoid model become clinical tools for testing the new generation of immunotherapy drugs and personalized immunotherapy of patients?

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Disclaimer Statement

The authors declare that they have no conflicts of interest in this article.

References

1. Friedman, A.A. *et al.* (2015) Precision medicine for cancer with next-generation functional diagnostics. *Nat. Rev. Cancer* 15, 747–756
2. Voest, E.E. and Bernards, R. (2016) DNA-guided precision medicine for cancer: a case of irrational exuberance? *Cancer Discov.* 6, 130–132
3. Goodspeed, A. *et al.* (2015) Tumor-derived cell lines as molecular models of cancer pharmacogenomics. *Mol. Cancer Res.* 14, 3–13
4. McMillin, D.W. *et al.* (2013) The role of tumour-stromal interactions in modifying drug response: challenges and opportunities. *Nat. Rev. Drug Discov.* 12, 217–228
5. Wilding, J.L. and Bodmer, W.F. (2014) Cancer cell lines for drug discovery and development. *Cancer Res.* 74, 2377–2384
6. Weiswald, L.B. *et al.* (2015) Spherical cancer models in tumor biology. *Neoplasia* 17, 1–15
7. Bartlett, R. *et al.* (2014) Personalized *in vitro* cancer modeling — fantasy or reality? *Transl. Oncol.* 7, 657–664
8. Stein, W.D. *et al.* (2004) A Serial Analysis of Gene Expression (SAGE) Database analysis of chemosensitivity: comparing solid tumors with cell lines and comparing solid tumors from different tissue origins. *Cancer Res.* 64, 2805–2816
9. Tentler, J.J. *et al.* (2012) Patient-derived tumour xenografts as models for oncology drug development. *Nat. Rev. Clin. Oncol.* 9, 338–350
10. Hidalgo, M. *et al.* (2014) Patient-derived xenograft models: an emerging platform for translational cancer research. *Cancer Discov.* 4, 998–1013
11. Leto, S.M. *et al.* (2015) Sustained inhibition of HER3 and EGFR is necessary to induce regression of HER2-amplified gastrointestinal carcinomas. *Clin. Cancer Res.* 21, 5519–5531
12. Bertotti, A. *et al.* (2011) A molecularly annotated platform of patient-derived xenografts ("xenopatients") identifies HER2 as an effective therapeutic target in cetuximab-resistant colorectal cancer. *Cancer Discov.* 1, 508–523
13. Hidalgo, M. *et al.* (2011) A pilot clinical study of treatment guided by personalized tumorgrafts in patients with advanced cancer. *Mol. Cancer Ther.* 10, 1311–1316
14. Zhang, X. *et al.* (2013) A renewable tissue resource of phenotypically stable, biologically and ethnically diverse, patient-derived human breast cancer xenograft models. *Cancer Res.* 73, 4885–4897
15. Tetteh, P.W. *et al.* (2016) Generation of an inducible colon-specific Cre enzyme mouse line for colon cancer research. *Proc. Natl. Acad. Sci. U. S. A.* 113, 11859–11864
16. DeRose, Y.S. *et al.* (2011) Tumor grafts derived from women with breast cancer authentically reflect tumor pathology, growth, metastasis and disease outcomes. *Nat. Med.* 17, 1514–1520
17. Marangoni, E. *et al.* (2007) A new model of patient tumor-derived breast cancer xenografts for preclinical assays. *Clin. Cancer Res.* 13, 3989–3998
18. Klemm, F. and Joyce, J.A. (2015) Microenvironmental regulation of therapeutic response in cancer. *Trends Cell Biol.* 25, 198–213
19. Reyal, F. *et al.* (2012) Molecular profiling of patient-derived breast cancer xenografts. *Breast Cancer Res.* 14, R11
20. Lancaster, M.A. and Knoblich, J.A. (2014) Organogenesis in a dish: modeling development and disease using organoid technologies. *Science* 345, 1247125
21. Gao, D. *et al.* (2014) Organoid cultures derived from patients with advanced prostate cancer. *Cell* 159, 176–187
22. Weeber, F. *et al.* (2015) Preserved genetic diversity in organoids cultured from biopsies of human colorectal cancer metastases. *Proc. Natl. Acad. Sci. U. S. A.* 112, 13308–13311
23. Weeber, F. *et al.* (2017) Tumor organoids as a pre-clinical cancer model for drug discovery. *Cell Chem. Biol.* 24, 1092–1100
24. Clevers, H. and Bender, E. (2015) Q&A: Hans Clevers. Banking on organoids. *Nature* 521, S15
25. Verissimo, C.S. *et al.* (2016) Targeting mutant RAS in patient-derived colorectal cancer organoids by combinatorial drug screening. *Elife* 5, e18489
26. van de Wetering, M. *et al.* (2015) Prospective derivation of a living organoid biobank of colorectal cancer patients. *Cell* 161, 933–945
27. Pauli, C. *et al.* (2017) Personalized *in vitro* and *in vivo* cancer models to guide precision medicine. *Cancer Discov.* 7, 462–477
28. Huch, M. and Koo, B. (2015) Modeling mouse and human development using organoid cultures. *Development* 142, 3113–3125
29. Sachs, N. and Clevers, H. (2014) Organoid cultures for the analysis of cancer phenotypes. *Curr. Opin. Genet. Dev.* 24, 68–73
30. Sato, T. *et al.* (2011) Long-term expansion of epithelial organoids from human colon, adenoma, adenocarcinoma, and Barrett's epithelium. *Gastroenterology* 141, 1762–1772
31. Sato, T. *et al.* (2009) Single Lgr5 stem cells build crypt-villus structures *in vitro* without a mesenchymal niche. *Nature* 459, 262–265
32. Barker, N. *et al.* (2010) Lgr5+ve stem cells drive self-renewal in the stomach and build long-lived gastric units *in vitro*. *Cell Stem Cell* 6, 25–36
33. Bartfeld, S. *et al.* (2015) *In vitro* expansion of human gastric epithelial stem cells and their responses to bacterial infection. *Gastroenterology* 148, 126–136.e6
34. Katano, T. *et al.* (2013) Establishment of a long-term three-dimensional primary culture of mouse glandular stomach

epithelial cells within the stem cell niche. *Biochem. Biophys. Res. Commun.* 432, 558–563

35. Huch, M. *et al.* (2013) *In vitro* expansion of single Lgr5+ liver stem cells induced by Wnt-driven regeneration. *Nature* 494, 247–250
36. Huch, M. *et al.* (2015) Long-term culture of genome-stable bipotent stem cells from adult human liver. *Cell* 160, 299–312
37. Petersen, O.W. *et al.* (1992) Interaction with basement membrane serves to rapidly distinguish growth and differentiation pattern of normal and malignant human breast epithelial cells. *Proc. Natl. Acad. Sci. U. S. A.* 89, 9064–9068
38. Schwarz, J.S. *et al.* (2015) Value of organoids from comparative epithelia models. *Yale J. Biol. Med.* 88, 367–374
39. Drost, J. *et al.* (2016) Organoid culture systems for prostate epithelial and cancer tissue. *Nat. Protoc.* 11, 347–358
40. Wang, K. *et al.* (2014) Whole-genome sequencing and comprehensive molecular profiling identify new driver mutations in gastric cancer. *Nat. Genet.* 46, 573–582
41. Sachs, N. *et al.* (2017) A living biobank of breast cancer organoids captures disease heterogeneity. *Cell* Published online December 5, 2017. http://dx.doi.org/10.1016/j.cell.2017.11.010
42. Huang, L. *et al.* (2015) Ductal pancreatic cancer modeling and drug screening using human pluripotent stem cell- and patient-derived tumor organoids. *Nat. Med.* 21, 1364–1371
43. Boj, S.F. *et al.* (2015) Organoid models of human and mouse ductal pancreatic cancer. *Cell* 160, 324–338
44. Batchelder, C.A. *et al.* (2015) Three dimensional culture of human renal cell carcinoma organoids. *PLoS One* 10, e0136758
45. Xinaris, C. *et al.* (2015) Organoid models and applications in biomedical research. *Nephron* 130, 191–199
46. Dekkers, J.F. *et al.* (2016) Characterizing responses to CFTR-modulating drugs using rectal organoids derived from subjects with cystic fibrosis. *Sci. Transl. Med.* 8, 13
47. Dekkers, F. *et al.* (2012) A functional CFTR assay using primary cystic fibrosis organoids. *Pediatr. Pulmonol.* 47, 289
48. Freedman, B.S. *et al.* (2015) Modelling kidney disease with CRISPR-mutant kidney organoids derived from human pluripotent epiblast spheroids. *Nat. Commun.* 6, 8715
49. Astashkina, A. and Grainger, D.W. (2014) Critical analysis of 3-D organoid *in vitro* cell culture models for high-throughput drug candidate toxicity assessments. *Adv. Drug Deliv. Rev.* 69–70 1–18
50. Roper, J. *et al.* (2017) *In vivo* genome editing and organoid transplantation models of colorectal cancer and metastasis. *Nat. Biotechnol.* 35, 569–576
51. Gao, D. and Chen, Y. (2015) Organoid development in cancer genome discovery. *Curr. Opin. Genet. Dev.* 30, 42–48
52. Baker, L.A. *et al.* (2016) Modeling pancreatic cancer with organoids. *Trends Cancer* 2, 176–190
53. Yachida, S. *et al.* (2010) Distant metastasis occurs late during the genetic evolution of pancreatic cancer. *Nature* 467, 1114–1117
54. Drost, J. *et al.* (2017) Use of CRISPR-modified human stem cell organoids to study the origin of mutational signatures in cancer. *Science* 358, 234–238
55. Karthaus, W.R. *et al.* (2014) Identification of multipotent luminal progenitor cells in human prostate organoid cultures. *Cell* 159, 163–175
56. Cheung, K.J. *et al.* (2013) Collective invasion in breast cancer requires a conserved basal epithelial program. *Cell* 155, 1639–1651
57. Walsh, A.J. *et al.* (2016) Drug response in organoids generated from frozen primary tumor tissues. *Sci. Rep.* 6, 18889
58. Matano, M. *et al.* (2015) Modeling colorectal cancer using CRISPR-Cas9–mediated engineering of human intestinal organoids. *Nat. Med.* 21, 256–262
59. Schwank, G. *et al.* (2013) Functional repair of CFTR by CRISPR/Cas9 in intestinal stem cell organoids of cystic fibrosis patients. *Cell Stem Cell* 13, 653–658
60. Schwank, G. *et al.* (2013) Brief report functional repair of CFTR by CRISPR/Cas9 in intestinal stem cell organoids of cystic fibrosis patients. *Stem Cell* 13, 653–658
61. Skardal, A. *et al.* (2015) Liver-tumor hybrid organoids for modeling tumor growth and drug response *in vitro*. *Ann. Biomed. Eng.* 43, 2361–2373
62. Yin, X. *et al.* (2016) Engineering stem cell organoids. *Cell Stem Cell* 18, 25–38
63. Bhatia, S.N. and Ingber, D.E. (2014) Microfluidic organs-on-chips. *Nat. Biotechnol.* 32, 760–772
64. Boussommier-Calleja, A. *et al.* (2016) Microfluidics: a new tool for modeling cancer–immune interactions. *Trends Cancer* 2, 6–19
65. Hirt, C. *et al.* (2014) "*In vitro*" 3D models of tumor-immune system interaction. *Adv. Drug Deliv. Rev.* 79, 145–154
66. Au, S.H. *et al.* (2016) Clusters of circulating tumor cells traverse capillary-sized vessels. *Proc. Natl. Acad. Sci. U. S. A.* 113, 4947–4952
67. Cirit, M. *et al.* (2016) Human physiome on a chip: merging tissue engineering and systems pharmacology. *FASEB J.* 30, 938.1
68. Parlato, S. *et al.* (2017) 3D microfluidic model for evaluating immunotherapy efficacy by tracking dendritic cell behaviour toward tumor cells. *Sci. Rep.* 7, 1093
69. Feder-Mengus, C. *et al.* (2008) New dimensions in tumor immunology: what does 3D culture reveal? *Trends Mol. Med.* 14, 333–340
70. Hsu, T.-H. *et al.* (2012) The migration speed of cancer cells influenced by macrophages and myofibroblasts co-cultured in a microfluidic chip. *Integr. Biol. (Camb.)* 4, 177–182
71. Businaro, L. *et al.* (2013) Cross talk between cancer and immune cells: exploring complex dynamics in a microfluidic environment. *Lab Chip* 13, 229–239
72. Mattei, F. *et al.* (2014) A multidisciplinary study using *in vivo* tumor models and microfluidic cell-on-chip approach to explore the cross-talk between cancer and immune cells. *J. Immunotoxicol.* 11, 337–346
73. Agliari, E. *et al.* (2014) Cancer-driven dynamics of immune cells in a microfluidic environment. *Sci. Rep.* 4, 6639
74. Liu, P. *et al.* (2015) A bladder cancer microenvironment simulation system based on a microfluidic co-culture model. *Oncotarget* 6, 37695–37705