Patient-derived organoid models help define personalized management of gastrointestinal cancer

Review

Patient-derived organoid models help define personalized management of gastrointestinal cancer

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Background: **The prognosis of patients with different gastrointestinal cancers varies widely. Despite advances in treatment strategies, such as extensive resections and the addition of new drugs to chemotherapy regimens, conventional treatment strategies have failed to improve survival for many tumours. Although promising, the clinical application of molecularly guided personalized treatment has proven to be challenging. This narrative review focuses on the personalization of cancer therapy using patient-derived three-dimensional 'organoid' models.**

Methods: **A PubMed search was conducted to identify relevant articles. An overview of the literature and published protocols is presented, and the implications of these models for patients with cancer, surgeons and oncologists are explained.**

Results: **Organoid culture methods have been established for healthy and diseased tissues from oesophagus, stomach, intestine, pancreas, bile duct and liver. Because organoids can be generated with high efficiency and speed from fine-needle aspirations, biopsies or resection specimens, they can serve as a personal cancer model. Personalized treatment could become a more standard practice by using these cell cultures for extensive molecular diagnosis and drug screening. Drug sensitivity assays can give a clinically actionable sensitivity profile of a patient's tumour. However, the predictive capability of organoid drug screening has not been evaluated in prospective clinical trials.**

Conclusion: **High-throughput drug screening on organoids, combined with next-generation sequencing, proteomic analysis and other state-of-the-art molecular diagnostic methods, can shape cancer treatment to become more effective with fewer side-effects.**

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Introduction

Over the past three decades, there has been a steady decline in overall cancer mortality. This can be attributed to lifestyle modifications, programmes focusing on the early detection of cancer, and more effective and aggressive multidisciplinary treatment strategies[1–3]. For many tumour types, biomarker-directed selection of therapy has led to improved survival. For instance, the use of targeted therapy in gastrointestinal stromal cell tumours and hormone receptor-guided therapy in breast cancer has dramatically improved outcomes for these two diseases[4,5]. Unfortunately, the outcomes for many cancer types remain grim, despite advances in treatment strategies. For example, pancreatic and primary liver cancer are still almost uniformly fatal[6,7].

In traditional models of cancer therapy, histopathologically similar tumours are treated identically regardless of cancer subtype or mutational subtype. This has failed to improve survival for many of the most difficult to treat tumours, and clinical investigators are now looking for ways to select treatment based on the mutational profile of the tumour. As an example, a recent clinical trial[8] has shown that 12 solid cancer types could be treated effectively with immune checkpoint blockade therapy if they possessed the same biological trait: mismatch repair deficiency. Likewise,

Table 1 Published articles on human and murine organoids of resected healthy and diseased gastrointestinal tissue, and protocols for establishing organoids by tissue origin

Tissue	Origin	Disease modelled	Articles	Protocols
Oesophagus	Human	Barrett's oesophagus	14	
	Mouse	Healthy tissue	20	
		Barrett's oesophagus	21	
Stomach	Human	Healthy tissue		22
		Helicobacter pylori infection	23–25	
	Mouse	Healthy tissue	15,26	
Small intestine	Human	Healthy tissue	26–28	29
		Intestinal carcinoma	29,30	29
	Mouse	Healthy tissue	16,26	52,31
		Intestinal carcinoma	32	
Colon/rectum	Human	Healthy tissue	14	
		Colorectal cancer	14,30,33–39	40
	Mouse	Healthy tissue	14	22,31,41
		Colon adenoma/carcinoma	14	42
Pancreas	Human	Healthy tissue		43
		Pancreatic ductal adenocarcinoma	13,44	
	Mouse	Healthy tissue	18	43
		Pancreatic ductal adenocarcinoma	13,44	
Liver	Human	Healthy tissue*	27,45	43
		Hepatocellular carcinoma	29	
	Mouse	Healthy tissue*	19,46	
Biliary	Human	Healthy tissue*	45	43
	Mouse	Healthy*	19	
		Cholangiocarcinoma	47,48	

*Liver bile canaliculi progenitor cells can differentiate into primary hepatocytes and bile duct cells, therefore, the same protocol is used for the generation of normal liver or bile duct organoids. Differentiation is induced using a differentiation medium.

patients with activating non-exon 2 *KRAS* mutations and *NRAS* mutations have a worse prognosis because they do not benefit from antiepidermal growth factor receptor therapy such as cetuximab[9].

Clinical application of molecularly guided personalized treatment in cancer therapy has proven difficult. The extensive use of gene sequencing has resulted in very few actionable mutations[10]; although many new therapeutics are tested every year, very few can be validated clinically[11]. Early results have shown that patient-derived cell lines or xenografts may advance the discovery of new therapeutics as they allow molecular testing for susceptibility to guide chemotherapeutic selection. However, the time required to establish such personalized tumour models and test them for actionable molecular data is a limiting factor[12].

In 2010, a new cell culturing method was developed at the Hubrecht Institute (Utrecht, The Netherlands), which allows the highly efficient and rapid expansion of normal and cancerous cells into three-dimensional 'mini-organs', called organoids[13]. Biopsies, fine-needle aspirations or resection material from healthy or tumour tissue is grown in a basement membrane matrix. Using this method, a patient-specific organoid model can be generated within weeks. This organoid can subsequently be used for extensive diagnostic or therapeutic analysis. In 2015, the method was adapted by researchers at the Cold Spring Harbor Laboratory (Cold Spring Harbor, New York, USA) to model normal and diseased pancreatic ductal tissue. Since then, researchers have established organoid models from many more gastrointestinal cancer specimens derived from the oesophagus[14], stomach[15], small intestine[16], colon[17], pancreas[13,18], liver and biliary epithelium[19] (*Table 1*)[20–48].

This review provides a concise description of the different organoid models that are available for various gastrointestinal cancers, and elaborates on their potential for implementation in clinical practice as a guide for precision medicine. The remaining barriers preventing implementation of an organoid programme to inform therapeutic decisions in a clinical setting are also addressed.

Methods

A PubMed search was conducted to identify relevant articles using the search terms 'gastro-intestinal cancer', 'gastric carcinoma', 'oesophageal carcinoma', 'pancreatic ductal adenocarcinoma', 'cholangiocarcinoma', 'hepatocellular carcinoma', 'organoid' and '3D cell culture'. Definitions of terms used in the review are provided in *Appendix S1* (supporting information).

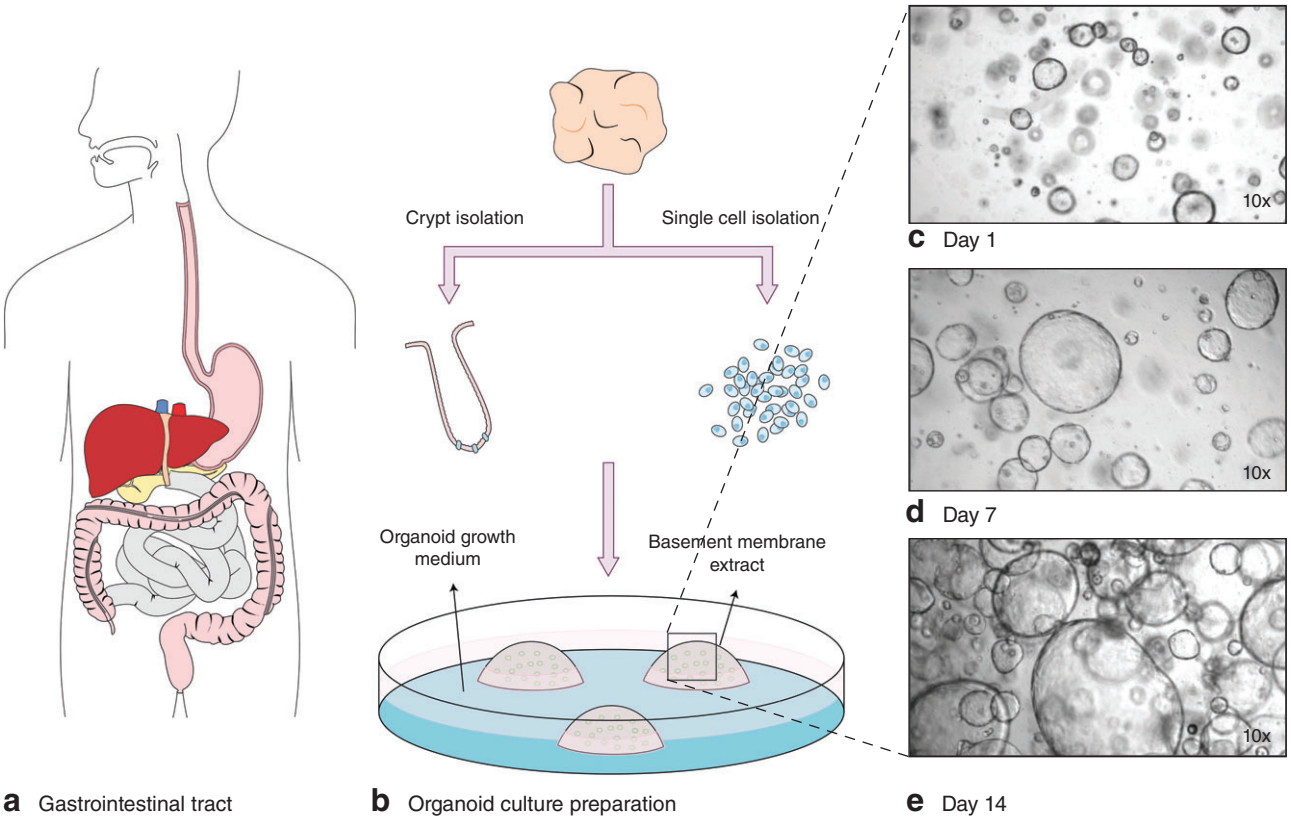

Fig. 1 Organoids can be made from resected healthy or tumour material, or from biopsies. **a** Procedures have been established for the generation of organoids from healthy and diseased oesophagus, stomach, small intestine and colon, pancreatic ducts, bile ducts and liver cells. **b** Procedures to establish organoid cultures depend on tissue type. The general method is as follows: after resection or biopsy, the tissue is either digested to release the crypts (for oesophagus, stomach and intestinal tissue) or until single cells or cell clusters are left. These are subsequently plated in a basement membrane extract and overlaid with organoid growth medium. **c–e** Metastatic pancreatic cancer organoids on day 1 (**c**), day 7 (**d**) and day 14 (**e**). When fully grown, these organoids form hollow spheres with an outer layer of cells and an inner lumen. Budding may occur, but is not shown here (image courtesy of R. Vaes, Maastricht University, Maastricht, The Netherlands)

Introduction to organoid culture

An organoid is a three-dimensional (3D) cell culture that has organ-like properties. Organoids resemble the tissue that they originate from, and can therefore be used as models of healthy and diseased tissue. The fact that they represent the heterogeneity of the tissue of origin is most apparent in normal intestinal organoid cultures, where LGR5+ stem cells give rise to a full complement of differentiated cells such as Paneth cells, enterocytes, goblet cells and enterochromaffin cells. Generally, the term 'organoid' describes primary 3D cultures established from tissue fragments. This should be contrasted with spheroid cultures, which refer to 3D cultures derived from established monolayer cell lines. Other methods to make organoid-like 3D cell cultures have been described and reviewed in the literature. However, these culture systems either lack the ability to generate both normal and neoplastic tissue, or are not as robust and efficacious as the methods described in this review[49].

Organoids can be used as patient-specific avatars of disease (*Fig. 1*). For oesophageal, stomach and intestinal organoids, the tissue is digested until the crypts are separated. These crypts contain LGR5+ stem cells, which, when plated in basement membrane matrix and cultured with organoid medium, expand and differentiate into all the possible cell types for that tissue. For pancreatic, biliary and liver organoids, stem cell precursors are still under investigation. Nonetheless, for these tissues the organoid methods work in a similar way to those for intestinal cultures. Within hours to days, the organoids form cystic structures, with an outer layer of cells and an inner lumen. The time needed to establish an organoid not

only depends on the tissue type, but also differs between patients.

Need for personalized systemic treatment options

Although mortality has declined for colonic cancer, death rates have increased for gastric[50], pancreatic and liver cancer[51]. With incidence rates that are rising steadily, and therapies that remain largely ineffective, it is estimated that in 2020 pancreatic and liver cancer will become the second and third most lethal cancers worldwide[51].

For several gastrointestinal cancers, only locally confined tumours can be treated curatively by resection. However, the vast majority of these tumours are detected after spread of the disease. For gastric cancer, treatment is often restricted to cytotoxic chemotherapy owing to the advanced stage at which the disease is diagnosed[50]. The same is the case for pancreatic cancer, where only 15–30 per cent of patients are eligible for operative exploration[6,51].

Even after surgery, disease recurrence is common and long-term survival for many gastrointestinal cancers remains poor[52–54]. Although standard-of-care systemic therapies have improved outcome for some cancers, such as colorectal cancer[2], the overall survival for other cancer types has been affected only minimally. For pancreatic cancer, for instance, the implementation of adjuvant chemotherapy has increased overall the 5-year survival rate for newly diagnosed patients to 8 per cent[1,6,55]. Although the outcome for colorectal cancer has become much more favourable, in as many as 50 per cent of patients the tumour progresses or recurs under current treatment regimens.

How genetic diversity makes treating cancer difficult

Gene sequencing has shown that for many gastrointestinal cancers there is substantial intertumour and intratumour heterogeneity when it comes to gene mutations[56–63]. For instance, for pancreatic cancer, 16 important gene mutations in distinct pathways have been identified that drive carcinogenesis[57]. Even for colorectal cancer, genetically one of the most homogeneous cancer types, where *APC* mutations occur in 85 per cent of patients[64], it has been shown that metastases show very different mutational profiles from the primary tumour, with absence of driver mutations or resistance predictors[59,60]. This intraindividual and interpersonal heterogeneity explains the limited benefits of current (chemotherapeutic) treatments, as they are based on the outcomes of large and heterogeneous patient cohorts.

In addition, the effect of cancer therapies is diminished by the development of therapeutic resistance. This occurs because drug treatment is a selection mechanism that drives clonal evolution, and the genome of tumours dynamically adapts to develop resistance[59,60,62,65]. This hampers the design and selection of effective drug combinations because these resistance mechanisms often involve multiple mutational events[62,65]. Owing to the genetic diversity that is inherent in cancers, a shift from population-based medicine to personalized or precision medicine is needed[6,57,61].

Previous clinical and preclinical efforts at precision medicine

In general, a framework for precision medicine that is functional in a clinical setting can rely on one of two methods: predictive biomarkers of therapeutic response using immunohistochemistry, DNA and RNA sequencing or molecular profiling, or direct study of *ex vivo* patient-derived tumour models (*Fig. 2*). Although promising, the use of gene sequencing and expression analysis has yet to make its way into routine clinical use. Recently, Le and colleagues[8] have shown that 12 different cancers respond well to PD-1 (programmed cell death protein 1) antibody treatment if the mismatch repair system is deficient. This has led to the first Food and Drug Administration (FDA)-approved treatment based on a common biomarker rather than the location of origin of the tumour[66]. Approximately 5 per cent of adenocarcinomas have microsatellite instability owing to a deficient mismatch repair system, and this can be evaluated using gene sequencing. Treatment of these patients with anti-PD-1 resulted in a radiographic response in half of the patients, with an estimated progression-free 2-year survival rate of 53 per cent[8]. Notably, all included patients had advanced disease or had shown progression under conventional treatment.

Unfortunately, for a variety of reasons, most putative biomarkers for pancreatic cancer have failed to influence clinical practice[67]. Perhaps the most notable attempt at biomarker-based precision therapy, the Australian Pancreatic Cancer Genome Initiative's Individualized Molecular Pancreatic Therapy (IMPaCT) trial, failed to accrue a single patient. This was because of delays in acquiring suitable tumour specimens for molecular analysis and returning high-quality actionable genomic data within a clinically acceptable time frame[68]. The same is the case for the EXaCT-1 trial[33], which was designed to guide therapeutic decision-making for patients with various advanced cancers, including colorectal, pancreatic, stomach and oesophageal cancer, using whole-exome sequencing. This

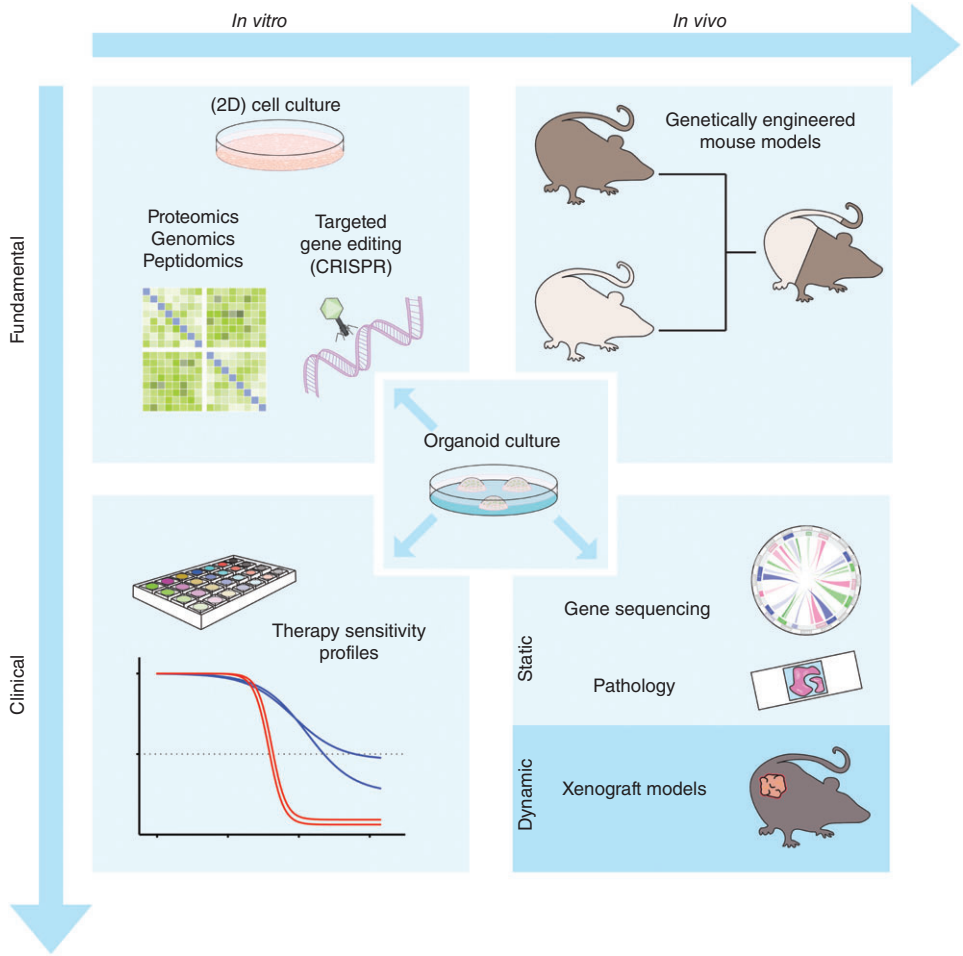

Fig. 2 Fundamental and clinical research applications for patient-specific organoid cultures. Cancer research can be divided into fundamental and clinical research. Although no single method is sufficiently accurate, fast and robust, at present organoids are the most reliable and quickest way to establish patient-specific tissue models. Using personal organoid cultures, multiple techniques can be combined to form a complete picture of the disease. 2D, two-dimensional; CRISPR, clustered regularly interspersed short palindromic repeats (a family of bacterial DNA sequences that, together with the Cas9 enzyme, can be used to induce targeted genetic modification of genes in organisms or, in this case, organoids)

trial analysed 769 tumour–normal pairs from different primary and metastatic tumours, but an FDA-approved drug could be identified for only 0·4 per cent of patients. Using an expanded list of targeted therapies, still only 9·6 per cent of patients had potentially targetable mutations[33,69].

An alternative path to precision medicine in cancer care involves the development of patient-derived models of disease. These avatar models enable molecular testing that can help inform clinicians and aid them in making treatment decisions. The use of two-dimensional cell lines to inform clinical care is already an active area of research in several centres. Early results have demonstrated the promise of this approach; patient-derived cell lines maintain the genetic profile of the 'parent' tumour[70]. However, two-dimensional cell lines lack structural organization and functional differentiation because they are grown in monolayers, which can change therapeutic sensitivity[13,71]. Moreover, the time required to derive actionable molecular data from two-dimensional cell lines and patient-derived xenografts puts constraints on their clinical applicability[12].

Innovation of organoids

Rationale for organoid-based precision medicine in the clinic

Recent advances in cell culture technology, particularly with regard to organoid development, have made

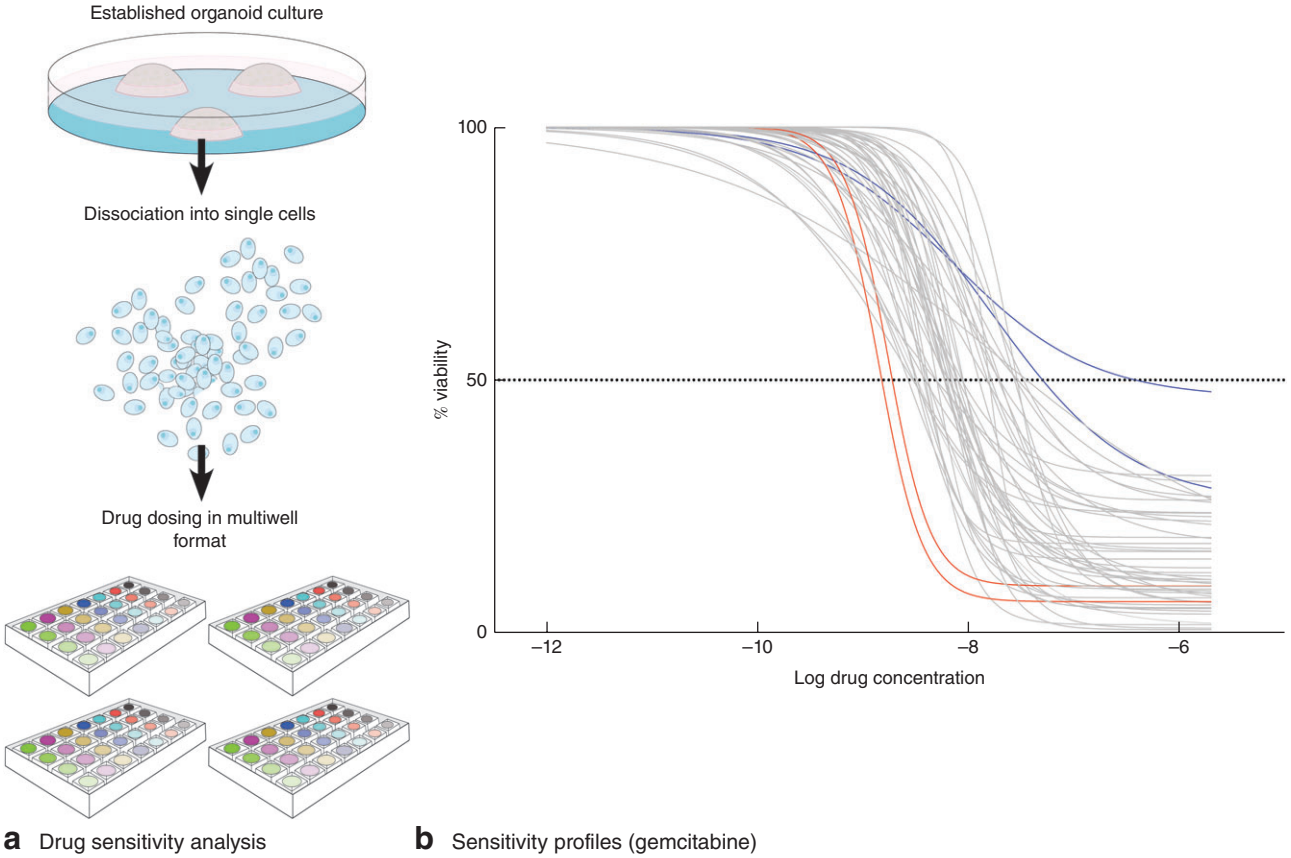

Fig. 3 a A drug sensitivity analysis on organoids can be performed by dissociating the organoids into single cells and plating them at a known density on a multiwell plate (96 or 384 wells). Multiple drugs in varying doses can be added to the organoids to evaluate sensitivity. Each organoid line has a specific sensitivity profile. **b** Multiple sensitivity profiles. Each line represents the viability of an organoid line plotted against concentration of the drug. The two most resistant organoid lines are depicted in blue, and the two most sensitive in red

preclinical modelling of individual patient tumours a viable strategy in personalized medicine initiatives[13]. Organoids can be generated with high efficiency from resected tumours or endoscopic biopsies, and preliminary data suggest that they accurately recapitulate key histological, molecular and genetic characteristics of the tumour of origin[13,42,45]. For instance, pancreatic organoids exhibit ductal- and disease stage-specific characteristics, and organoids transplanted into mice recapitulate the full spectrum of tumour development[13]. For traditional molecular studies, such as sequencing and other 'omics' approaches, an organoid-based approach may be superior to relying on direct studies of patient tumour specimens. This is probably because of the low cancer cellularity exhibited by primary tumour specimens, particularly in cancers that arise from the pancreas. Changes in RNA and DNA profiles of cancer cells may be difficult to detect in surgical resection material when the majority of tissue is made up of stromal cells. Finally, organoid models are stable over many passages, can be cryopreserved and are portable across different laboratory settings. This makes collaboration between different institutions feasible, even across continents, and opens up access to analysis in other laboratories. Moreover, by establishing biobanks, organoid cultures could be made available to other research groups, for future research or for validation of research results.

In fundamental research, experiments with patient-derived organoids have already resulted in discoveries with considerable impact in the field of oncology. Organoid culturing methods provide an ideal way to evaluate co-culture systems of cancer cells with cancer-associated fibroblasts[72] or pathogens[23,47]. They also represent an ideal platform for individualized therapeutic screening (pharmacotyping)[33,73–75]. Preliminary work by the present authors has demonstrated promising results for this approach. For example, organoids can be generated

from primary tumour specimens over an interval of several weeks, and are subsequently amenable for use in standard *in vitro* drug sensitivity assays (*Fig. 3*).

Organoid technology can be used to reduce the time required to develop personalized models of disease. With an immortalized avatar for each patient's tumour, putative biomarkers can be evaluated in real time and compared against other methods of personalized therapeutic selection, such as gene expression and drug sensitivity analyses. Drug sensitivity testing may include cytotoxic agents traditionally used for a particular cancer subtype, as well as agents approved for other cancer subtypes, targeted agents, or experimental therapeutics in a clinical trial setting. Clinically relevant data can rapidly be translated to clinical decisions, and increase chemotherapy response not only by giving justification for the best treatment combinations, but also by reducing ineffective treatment.

Considerations when working with organoids

The organoid culture system described in this review incorporates two basic components: a basement membrane extract that mimics the extracellular environment, and a liquid growth medium that promotes growth and 'stemness' of the organoids (*Fig. 1*, *Table 1*). This method for culturing organoids relies on a basement membrane extract that originates from mouse sarcoma cells, and contains various collagens, laminins and other extracellular matrix components[16]. This biological matrix is essential for the growth of organoid cultures and, although efforts are under way to define tissue-specific synthetic matrix solutions, much more validation has to be done before these can substitute for the biological matrix compounds[76].

A liquid culture medium is added to complement this biological matrix. Different tissues have varying growth requirements, but the basis of the culture media contains WNT and/or RSPONDIN ligands as well as bone morphogenetic protein inhibitor Noggin to promote proliferation of progenitor cells[22]. Addition of epithelial growth factor and tissue-specific mitogens further stimulates the growth of epithelial cultures.

Many organoid culture protocols have been published in the past few years. These should be evaluated carefully when newly attempting organoid culture (*Table 1*) because even slight deviations from these protocols may result in failed organoid isolation or expansion. For instance, the activity of WNT ligands should be monitored, because purified recombinant ligands often do not possess high enough activity to promote organoid growth[27]. In addition to strict matrix and media requirements, there are critical steps in tissue processing that may ruin organoid generation. Tissues obtained from the gastrointestinal tract must be washed to prevent fungal and bacterial contamination of the primary organoid cultures. For pancreas, the risk of microbial contamination is not as high, but the digestion steps should be done carefully because the lysis of acinar cell results in the release of their digestive enzymes, which in turn will lyse any remaining cells. Another critical issue has proven to be the time between resection and organoid isolation. Because not every institute has the facilities to make organoid cultures on site, collaborations with other centres may be desirable. However, the longer the time between resection and organoid isolation, the lower the chance of successful establishment of an organoid culture. Tissue samples can be shipped on ice, in appropriate culture media, but should be processed as soon as possible upon receipt.

For most tissues, organoid culture methods are similar for both normal and tumour cells (*Table 1*). Because of this, tumour organoid isolates may be contaminated by rapidly dividing adjacent normal epithelial cells. In some cases this problem is so substantial that successful isolation of tumour organoids is unlikely[77]. For some tissues, such as colonic carcinoma, this can be overcome by utilizing the ability of cancer cells to divide without the need to be externally stimulated by media components. Removing the media components that are necessary for cell growth of normal tissues (reduced medium) may help prevent normal epithelial cells from overgrowing and outcompeting tumour cells in a culture dish. Ultimately, however, histological assessment of the tissue followed by careful microdissection of tumour cell-rich nodules still provides the best chance for successful isolation of tumour organoids.

Finally, although the quantity of tissue often affects successful isolation of primary cultures, needle biopsies are a sufficient source of cells to generate an organoid culture[13]. For these small biopsies, additional care must be taken when processing tissue as any significant cellular loss will result in failure of organoid isolation. For pancreatic cancer, where only 15–30 per cent of the patients present with resectable disease, this has enabled researchers to generate organoids from early-stage operable cancer as well as late-stage inoperable cancer.

Results of previous efforts to generate organoids

Initial efforts to generate organoids resulted in success rates of between 75 and 83 per cent[13]. Researchers at the Cold Spring Harbor Laboratory have shown that this success rate is higher, and that organoids can be generated from resected pancreatic cancers, endoscopic biopsies or surgical resection material of pancreatic cancer with success rates from 85 to 95 per cent (H. Tiriac and D. Tuveson,

unpublished observations). For fine-needle aspirations, the success rate is about 75 per cent, still greatly exceeding that of conventional culture methods. Organoid isolation is most efficient for cells of ductal origin. After tissue acquisition, the time required to establish and expand the culture sufficiently for molecular study is variable, ranging from as little as 2 weeks to as long as 5 months. Organoids generated from surgically resected specimens generally take less time to expand, as there is usually ample starting material.

Using organoids to inform clinical decisions

For the purposes of this work, clinically actionable data is defined as information gleaned from genetic sequencing, biomarker analysis or drug sensitivity testing that can be used to identify an optimal treatment regimen for individual patients. There are two inherent advantages to the use of organoids as the backbone for molecular characterization in precision medicine. Organoids are stable, immortalized examples of each tumour and can provide clinical scientists with a large quantity of biomass to study. Furthermore, as a cell line, they can be characterized molecularly in contemporary research laboratories using next-generation sequencing methods, gene expression profiling, standard immunohistochemical arrays and a wide variety of 'omics' methodologies.

Although organoids are established more rapidly than two-dimensional cell lines, at present it is probably unreasonable to await organoid-directed therapeutic information for rapidly progressing malignancies such as pancreatic cancer. Therefore, in a clinically relevant paradigm, patients most often begin adjuvant therapies after recovery from surgical resection. In this framework, the 8 weeks needed for surgical recovery can be used to generate organoids, study their molecular subtype, and undertake a clinically meaningful drug screen that individualizes adjuvant therapy for each patient.

Rationale for the selection of therapeutics to study in clinical trials

As discussed previously, data from molecular analysis can be supplemented by organoid pharmacotyping[33–36]. After generation, organoids can be plated in a high-throughput multiwell format and tested against chemotherapeutics. These drug sensitivity assays are used extensively in cancer biology research, and are beginning to gain favour in precision medicine-oriented translational research[12,33]. In recent years, multiple laboratories have explored the possibility of performing drug screening on 3D organoid cell cultures, mostly of colorectal origin, and correlating these with genomic data[33–36,78]. Pauli and colleagues[33] showed that high-throughput drug screening, combined with complete genomic analysis, could identify effective targeted agents, and the optimal combination of these agents for four different cancers. However, none of these studies have correlated the results of such drug-screening assays with clinical data in the form of prospective clinical trials.

The use of organoids to direct therapy for these specific cancer types can first be studied with a focus on the appropriate selection of standard chemotherapeutics. In future work, drug screening should be expanded beyond cytotoxic chemotherapeutics and include novel compounds. Small-molecule inhibitors should be integrated, and optimal combination strategies should be designed. For pancreatic cancer, for example, combinations including gemcitabine should be compared *in vitro* against combinations containing 5-fluorouracil. Similarly, in biliary tract cancers, a variety of agents should be tested with a backbone of gemcitabine.

In this way, early clinical trials will rely solely on standard agents to facilitate the application of the results in the clinic and to satisfy ethical considerations. As the reproducibility and reliability of organoid-directed care are confirmed, further studies should investigate a role for testing non-standard agents, targeted agents and small-molecule inhibitors for each tumour type. In this context, it has to be acknowledged that the current standard systemic combination therapies have historically limited response rates, contributing to the urgency for novel approaches in this set of diseases. Therefore, in highly specialized centres, a more aggressive strategy could be pursued, as informed by organoid data and under the watchful eye of clinician scientists.

Discussion

To improve cancer care, profiling of patient tumour specimens should go beyond classical histopathology and use next-generation sequencing, RNA analysis and other advanced molecular diagnostic approaches such as proteomics to help unravel the molecular pathways that drive a patient's specific cancer. For this purpose, new models are needed that help predict treatment response accurately. 3D tumour organoids can be generated with high efficiency from fine-needle aspirations, biopsies or resection specimens, and have the potential to serve as a personal cancer model. Tumour organoids retain the characteristics of the primary tumour, can be grown indefinitely, are cryopreservable and are transportable for collaborative fundamental scientific as well as clinical projects. Because tumour organoids consist of cancer cells alone, they offer

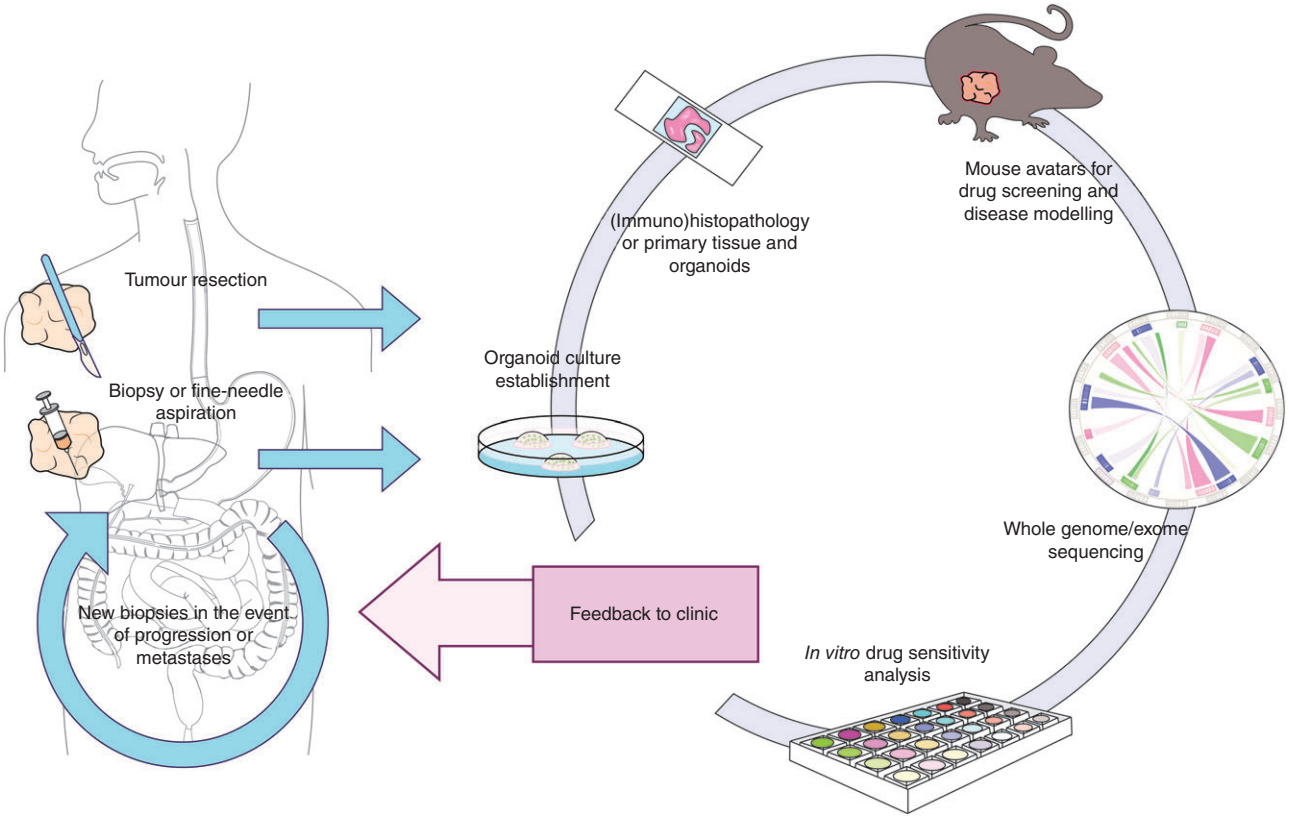

Fig. 4 Model for personalized cancer therapeutics using organoids. Information from gene sequencing, *in vitro* drug analysis and mouse studies can be combined with traditional molecular analyses, such as immunohistochemistry, to establish a complete tumour profile. As the establishment and complete analysis of an organoid line takes only a few weeks, the information could be returned swiftly to the clinic. When patients develop metastases, or the disease relapses, new tumour biopsies can be taken and new organoid lines established

a much higher chance of detecting actionable mutations compared with direct sequencing of the primary tumours. In addition, direct pharmacotyping by organoid-based drug sensitivity testing can be done within weeks.

In many initiatives worldwide, cohorts of patients receiving standard systemic treatment are currently assessed clinically for response, and survival statistics are correlated retrospectively with the molecular tumour profile. Although this strategy helps clarify the relationship between molecular profile and response, it is time-consuming, needs a large group of patients, is logistically difficult and will not immediately benefit patients in need of effective treatment today. Using tumour organoid avatars, together with molecular assessment and drug sensitivity testing, could accelerate the use of personalized anticancer therapy and improve treatment outcomes for current patients. Sampling of tumour tissue can be done at multiple sites, including metastases, but also over time, when patients show resistance to an ongoing therapy or recurrence (*Fig. 4*).

The efficiency of the personal organoid model allows immediate clinical implementation, but the effectiveness in improving patient outcome remains to be proven. Experiments to establish validity are ongoing in multiple institutions, including the Cold Spring Harbor Institute, and show promising results. Therefore, clinical pilot trials validating organoids as a useful clinical tool should not be postponed. The design of adaptive clinical trials, with a treatment arm allocation according to tumour phenotype and organoid pharmacotype, may be more promising and attractive for both patients and clinicians than traditional RCTs with current treatment standards. Additionally, patients are willing to share in the decision-making around their treatment. With the promise of improved quality of life, they accept the uncertainty of using off-label therapies, and are capable of balancing the risks and potential benefits if they understand the prediction model used to guide their personalized treatment. From a societal and medical perspective, it is attractive to use the individualized organoid model to guide treatments even before patients

are exhausted by multiple lines of ineffective, costly and harmful standard treatment regimens.

Institutes with the infrastructure to grow tumour organoids, and with access to state-of-the-art molecular tumour profiling and drug sensitivity testing facilities, can start collaborative clinical pilots today. Increased technical expertise in many newly established organoid facilities, combined with easily sharable frozen vials of patient-derived organoids will result in (inter)national organoid collaborations that could rapidly transform the field of cancer therapeutics.

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References

1 Siegel RL, Miller KD, Jemal A. Cancer statistics, 2017. *CA Cancer J Clin* 2017; **67**: 7–30.
2 Siegel RL, Miller KD, Fedewa SA, Ahnen DJ, Meester RGS, Barzi A *et al.* Colorectal cancer statistics, 2017. *CA Cancer J Clin* 2017; **67**: 177–193.
3 Fisher B, Jeong JH, Bryant J, Anderson S, Dignam J, Fisher ER *et al.*; National Surgical Adjuvant Breast and Bowel Project randomised clinical trials. Treatment of lymph-node-negative, oestrogen-receptor-positive breast cancer: long-term findings from National Surgical Adjuvant Breast and Bowel Project randomised clinical trials. *Lancet* 2004; **364**: 858–868.
4 DeMatteo RP, Ballman KV, Antonescu CR, Corless C, Kolesnikova V, von Mehren M *et al.*; American College of Surgeons Oncology Group (ACOSOG) Intergroup Adjuvant GIST Study team for the Alliance for Clinical Trials in Oncology. Long-term results of adjuvant imatinib mesylate in localized, high-risk, primary gastrointestinal stromal tumor: ACOSOG Z9000 (Alliance) intergroup phase 2 trial. *Ann Surg* 2013; **258**: 422–429.
5 Diaby V, Tawk R, Sanogo V, Xiao H, Montero AJ. A review of systematic reviews of the cost-effectiveness of hormone therapy, chemotherapy, and targeted therapy for breast cancer. *Breast Cancer Res Treat* 2015; **151**: 27–40.
6 Li D, Xie K, Wolff R, Abbruzzese JL. Pancreatic cancer. *Lancet* 2004; **363**: 1049–1057.
7 American Cancer Society. *Cancer Facts & Figures 2017*. American Cancer Society: Atlanta, 2017.
8 Le DT, Durham JN, Smith KN, Wang H, Bartlett BR, Aulakh LK *et al.* Mismatch repair deficiency predicts response of solid tumors to PD-1 blockade. *Science* 2017; **357**: 409–413.
9 Fakih MG. Metastatic colorectal cancer: current state and future directions. *J Clin Oncol* 2015; **33**: 1809–1824.
10 Holch JW, Metzeler KH, Jung A, Riedmann K, Jost PJ, Weichert W *et al.* Universal genomic testing: the next step in oncological decision-making or a dead end street? *Eur J Cancer* 2017; **82**: 72–79.
11 Hay M, Thomas DW, Craighead JL, Economides C, Rosenthal J. Clinical development success rates for investigational drugs. *Nat Biotechnol* 2014; **32**: 40–51.
12 Witkiewicz AK, Balaji U, Eslinger C, McMillan E, Conway W, Posner B *et al.* Integrated patient-derived models delineate individualized therapeutic vulnerabilities of pancreatic cancer. *Cell Rep* 2016; **16**: 2017–2031.
13 Boj SF, Hwang CI, Baker LA, Chio II, Engle DD, Corbo V *et al.* Organoid models of human and mouse ductal pancreatic cancer. *Cell* 2015; **160**: 324–338.
14 Sato T, Stange DE, Ferrante M, Vries RG, van Es JH, Van den Brink S *et al.* Long-term expansion of epithelial organoids from human colon, adenoma, adenocarcinoma, and Barrett's epithelium. *Gastroenterology* 2011; **141**: 1762–1772.
15 Barker N, Huch M, Kujala P, van de Wetering M, Snippert HJ, van Es JH *et al.* Lgr5(+ve) stem cells drive self-renewal in the stomach and build long-lived gastric units *in vitro*. *Cell Stem Cell* 2010; **6**: 25–36.
16 Sato T, Vries RG, Snippert HJ, van de Wetering M, Barker N, Stange DE *et al.* Single Lgr5 stem cells build crypt-villus structures *in vitro* without a mesenchymal niche. *Nature* 2009; **459**: 262–265.
17 Barker N, Clevers H. Leucine-rich repeat-containing G-protein-coupled receptors as markers of adult stem cells. *Gastroenterology* 2010; **138**: 1681–1696.
18 Huch M, Bonfanti P, Boj SF, Sato T, Loomans CJ, van de Wetering M *et al.* Unlimited *in vitro* expansion of adult bi-potent pancreas progenitors through the Lgr5/R-spondin axis. *EMBO J* 2013; **32**: 2708–2721.
19 Huch M, Dorrell C, Boj SF, van Es JH, Li VS, van de Wetering M *et al. In vitro* expansion of single Lgr5+ liver

stem cells induced by Wnt-driven regeneration. *Nature* 2013; **494**: 247–250.
20 DeWard AD, Cramer J, Lagasse E. Cellular heterogeneity in the mouse esophagus implicates the presence of a nonquiescent epithelial stem cell population. *Cell Rep* 2014; **9**: 701–711.
21 Lee Y, Urbanska AM, Hayakawa Y, Wang H, Au AS, Luna AM *et al.* Gastrin stimulates a cholecystokinin-2-receptor-expressing cardia progenitor cell and promotes progression of Barrett's-like esophagus. *Oncotarget* 2017; **8**: 203–214.
22 Miyoshi H, Stappenbeck TS. *In vitro* expansion and genetic modification of gastrointestinal stem cells in spheroid culture. *Nat Protoc* 2013; **8**: 2471–2482.
23 Schlaermann P, Toelle B, Berger H, Schmidt SC, Glanemann M, Ordemann J *et al.* A novel human gastric primary cell culture system for modelling *Helicobacter pylori* infection *in vitro*. *Gut* 2016; **65**: 202–213.
24 Bartfeld S, Bayram T, van de Wetering M, Huch M, Begthel H, Kujala P *et al. In vitro* expansion of human gastric epithelial stem cells and their responses to bacterial infection. *Gastroenterology* 2015; **148**: 126–136.e126.
25 Huang JY, Sweeney EG, Sigal M, Zhang HC, Remington SJ, Cantrell MA *et al.* Chemodetection and destruction of host urea allows *Helicobacter pylori* to locate the epithelium. *Cell Host Microbe* 2015; **18**: 147–156.
26 Yin X, Farin HF, van Es JH, Clevers H, Langer R, Karp JM. Niche-independent high-purity cultures of Lgr5+ intestinal stem cells and their progeny. *Nat Methods* 2014; **11**: 106–112.
27 Tüysüz N, van Bloois L, van den Brink S, Begthel H, Verstegen MM, Cruz LJ *et al.* Lipid-mediated Wnt protein stabilization enables serum-free culture of human organ stem cells. *Nat Commun* 2017; **8**: 14578.
28 Chen Y, Li C, Tsai YH, Tseng SH. Intestinal crypt organoid: isolation of intestinal stem cells, *in vitro* culture, and optical observation. *Methods Mol Biol* 2017; [Epub ahead of print].
29 Blokzijl F, de Ligt J, Jager M, Sasselli V, Roerink S, Sasaki N *et al.* Tissue-specific mutation accumulation in human adult stem cells during life. *Nature* 2016; **538**: 260–264.
30 Drost J, van Jaarsveld RH, Ponsioen B, Zimberlin C, van Boxtel R, Buijs A *et al.* Sequential cancer mutations in cultured human intestinal stem cells. *Nature* 2015; **521**: 43–47.
31 O'Rourke KP, Ackerman S, Dow LE, Lowe SW. Isolation, culture, and maintenance of mouse intestinal stem cells. *Bio Protoc* 2016; **6**: e1733.
32 Onuma K, Ochiai M, Orihashi K, Takahashi M, Imai T, Nakagama H *et al.* Genetic reconstitution of tumorigenesis in primary intestinal cells. *Proc Natl Acad Sci U S A* 2013; **110**: 11 127–11 132.
33 Pauli C, Hopkins BD, Prandi D, Shaw R, Fedrizzi T, Sboner A *et al.* Personalized *in vitro* and *in vivo* cancer models to guide precision medicine. *Cancer Discov* 2017; **7**: 462–477.
34 Verissimo CS, Overmeer RM, Ponsioen B, Drost J, Mertens S, Verlaan-Klink I *et al.* Targeting mutant RAS in patient-derived colorectal cancer organoids by combinatorial drug screening. *ELife* 2016; **5**: pii: e18489.
35 van de Wetering M, Francies HE, Francis JM, Bounova G, Iorio F, Pronk A *et al.* Prospective derivation of a living organoid biobank of colorectal cancer patients. *Cell* 2015; **161**: 933–945.
36 Boehnke K, Iversen PW, Schumacher D, Lallena MJ, Haro R, Amat J *et al.* Assay establishment and validation of a high-throughput screening platform for three-dimensional patient-derived colon cancer organoid cultures. *J Biomol Screen* 2016; **21**: 931–941.
37 Matano M, Date S, Shimokawa M, Takano A, Fujii M, Ohta Y *et al.* Modeling colorectal cancer using CRISPR-Cas9-mediated engineering of human intestinal organoids. *Nat Med* 2015; **21**: 256–262.
38 Weeber F, van de Wetering M, Hoogstraat M, Dijkstra KK, Krijgsman O, Kuilman T *et al.* Preserved genetic diversity in organoids cultured from biopsies of human colorectal cancer metastases. *Proc Natl Acad Sci U S A* 2015; **112**: 13 308–13 311.
39 Fujii M, Shimokawa M, Date S, Takano A, Matano M, Nanki K *et al.* A colorectal tumor organoid library demonstrates progressive loss of niche factor requirements during tumorigenesis. *Cell Stem Cell* 2016; **18**: 827–838.
40 Francies HE, Barthorpe A, McLaren-Douglas A, Barendt WJ, Garnett MJ. Drug sensitivity assays of human cancer organoid cultures. *Methods Mol Biol* 2016; [Epub ahead of print].
41 Fan YY, Davidson LA, Chapkin RS. Murine colonic organoid culture system and downstream assay applications. *Methods Mol Biol* 2016; [Epub ahead of print].
42 Xue X, Shah YM. *In vitro* organoid culture of primary mouse colon tumors. *J Vis Exp* 2013; (**75**): e50210.
43 Broutier L, Andersson-Rolf A, Hindley CJ, Boj SF, Clevers H, Koo BK *et al.* Culture and establishment of self-renewing human and mouse adult liver and pancreas 3D organoids and their genetic manipulation. *Nat Protoc* 2016; **11**: 1724–1743.
44 Walsh AJ, Castellanos JA, Nagathihalli NS, Merchant NB, Skala MC. Optical imaging of drug-induced metabolism changes in murine and human pancreatic cancer organoids reveals heterogeneous drug response. *Pancreas* 2016; **45**: 863–869.
45 Huch M, Gehart H, van Boxtel R, Hamer K, Blokzijl F, Verstegen MM *et al.* Long-term culture of genome-stable bipotent stem cells from adult human liver. *Cell* 2015; **160**: 299–312.
46 Yimlamai D, Christodoulou C, Galli GG, Yanger K, Pepe-Mooney B, Gurung B *et al.* Hippo pathway activity influences liver cell fate. *Cell* 2014; **157**: 1324–1338.
47 Scanu T, Spaapen RM, Bakker JM, Pratap CB, Wu LE, Hofland I *et al.* Salmonella manipulation of host signaling pathways provokes cellular transformation associated with gallbladder carcinoma. *Cell Host Microbe* 2015; **17**: 763–774.

48 Nakagawa H, Suzuki N, Hirata Y, Hikiba Y, Hayakawa Y, Kinoshita H *et al.* Biliary epithelial injury-induced regenerative response by IL-33 promotes cholangiocarcinogenesis from peribiliary glands. *Proc Natl Acad Sci U S A* 2017; **114**: E3806–E3815.

49 Boj SF, Hwang CI, Baker LA, Engle DD, Tuveson DA, Clevers H. Model organoids provide new research opportunities for ductal pancreatic cancer. *Mol Cell Oncol* 2016; **3**: e1014757.

50 Liu X, Meltzer SJ. Gastric cancer in the era of precision medicine. *Cell Mol Gastroenterol Hepatol* 2017; **3**: 348–358.

51 Rahib L, Smith BD, Aizenberg R, Rosenzweig AB, Fleshman JM, Matrisian LM. Projecting cancer incidence and deaths to 2030: the unexpected burden of thyroid, liver, and pancreas cancers in the United States. *Cancer Res* 2014; **74**: 2913–2921.

52 Asiyanbola B, Gleisner A, Herman JM, Choti MA, Wolfgang CL, Swartz M *et al.* Determining pattern of recurrence following pancreaticoduodenectomy and adjuvant 5-flurouracil-based chemoradiation therapy: effect of number of metastatic lymph nodes and lymph node ratio. *J Gastrointest Surg* 2009; **13**: 752–759.

53 Cameron JL, He J. Two thousand consecutive pancreaticoduodenectomies. *J Am Coll Surg* 2015; **220**: 530–536.

54 Winter JM, Cameron JL, Campbell KA, Arnold MA, Chang DC, Coleman J *et al.* 1423 pancreaticoduodenectomies for pancreatic cancer: a single-institution experience. *J Gastrointest Surg* 2006; **10**: 1199–1210.

55 Neoptolemos JP, Palmer DH, Ghaneh P, Psarelli EE, Valle JW, Halloran CM *et al.*; European Study Group for Pancreatic Cancer. Comparison of adjuvant gemcitabine and capecitabine with gemcitabine monotherapy in patients with resected pancreatic cancer (ESPAC-4): a multicentre, open-label, randomised, phase 3 trial. *Lancet* 2017; **389**: 1011–1024.

56 Waddell N, Pajic M, Patch AM, Chang DK, Kassahn KS, Bailey P *et al.* Whole genomes redefine the mutational landscape of pancreatic cancer. *Nature* 2015; **518**: 495–501.

57 Biankin AV, Waddell N, Kassahn KS, Gingras MC, Muthuswamy LB, Johns AL *et al.* Pancreatic cancer genomes reveal aberrations in axon guidance pathway genes. *Nature* 2012; **491**: 399–405.

58 Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. *Cell* 2011; **144**: 646–674.

59 Pietrantonio F, Vernieri C, Siravegna G, Mennitto A, Berenato R, Perrone F *et al.* Heterogeneity of acquired resistance to anti-EGFR monoclonal antibodies in patients with metastatic colorectal cancer. *Clin Cancer Res* 2017; **23**: 2414–2422.

60 Siravegna G, Mussolin B, Buscarino M, Corti G, Cassingena A, Crisafulli G *et al.* Clonal evolution and resistance to EGFR blockade in the blood of colorectal cancer patients. *Nat Med* 2015; **21**: 795–801.

61 Jones S, Zhang X, Parsons DW, Lin JC, Leary RJ, Angenendt P *et al.* Core signaling pathways in human pancreatic cancers revealed by global genomic analyses. *Science* 2008; **321**: 1801–1806.

62 Jamal-Hanjani M, Quezada SA, Larkin J, Swanton C. Translational implications of tumor heterogeneity. *Clin Cancer Res* 2015; **21**: 1258–1266.

63 Chiriac MT, Buchen B, Wandersee A, Hundorfean G, Günther C, Bourjau Y *et al.* Activation of epithelial signal transducer and activator of transcription 1 by interleukin 28 controls mucosal healing in mice with colitis and is increased in mucosa of patients with inflammatory bowel disease. *Gastroenterology* 2017; **153**: 123–138.e128.

64 Markowitz SD, Bertagnolli MM. Molecular origins of cancer: molecular basis of colorectal cancer. *N Engl J Med* 2009; **361**: 2449–2460.

65 Gillies RJ, Verduzco D, Gatenby RA. Evolutionary dynamics of carcinogenesis and why targeted therapy does not work. *Nat Rev Cancer* 2012; **12**: 487–493.

66 Stark A. *FDA Approves First Cancer Treatment for Any Solid Tumor with a Specific Genetic Feature*. US Food and Drug Administration: Silverspring, 2017.

67 Winter JM, Yeo CJ, Brody JR. Diagnostic, prognostic, and predictive biomarkers in pancreatic cancer. *J Surg Oncol* 2013; **107**: 15–22.

68 Chantrill LA, Nagrial AM, Watson C, Johns AL, Martyn-Smith M, Simpson S *et al.*; Australian Pancreatic Cancer Genome I, Individualized Molecular Pancreatic Cancer Therapy Trial Management Committee of the Australasian Gastrointestinal Trials Group (AGITG). Precision medicine for advanced pancreas cancer: the Individualized Molecular Pancreatic Cancer Therapy (IMPaCT) trial. *Clin Cancer Res* 2015; **21**: 2029–2037.

69 Rennert H, Eng K, Zhang T, Tan A, Xiang J, Romanel A *et al.* Development and validation of a whole-exome sequencing test for simultaneous detection of point mutations, indels and copy-number alterations for precision cancer care. *NPJ Genom Med* 2016; **1**: 16019.

70 Knudsen ES, Balaji U, Mannakee B, Vail P, Eslinger C, Moxom C *et al.* Pancreatic cancer cell lines as patient-derived avatars: genetic characterisation and functional utility. *Gut* 2017; [Epub ahead of print].

71 Baker LA, Tiriac H, Clevers H, Tuveson DA. Modeling pancreatic cancer with organoids. *Trends Cancer* 2016; **2**: 176–190.

72 Öhlund D, Handly-Santana A, Biffi G, Elyada E, Almeida AS, Ponz-Sarvise M *et al.* Distinct populations of inflammatory fibroblasts and myofibroblasts in pancreatic cancer. *J Exp Med* 2017; **214**: 579–596.

73 Sachs N, Clevers H. Organoid cultures for the analysis of cancer phenotypes. *Curr Opin Genet Dev* 2014; **24**: 68–73.

74 Yu M, Bardia A, Aceto N, Bersani F, Madden MW, Donaldson MC *et al.* Cancer therapy. *Ex vivo* culture of circulating breast tumor cells for individualized testing of drug susceptibility. *Science* 2014; **345**: 216–220.

75 Coleman SJ, Watt J, Arumugam P, Solaini L, Carapuca E, Ghallab M *et al.* Pancreatic cancer organotypics: high

throughput, preclinical models for pharmacological agent evaluation. *World J Gastroenterol* 2014; **20**: 8471–8481.
76 Gjorevski N, Sachs N, Manfrin A, Giger S, Bragina ME, Ordóñez-Morán P *et al.* Designer matrices for intestinal stem cell and organoid culture. *Nature* 2016; **539**: 560–564.
77 Karthaus WR, Iaquinta PJ, Drost J, Gracanin A, van Boxtel R, Wongvipat J *et al.* Identification of multipotent luminal progenitor cells in human prostate organoid cultures. *Cell* 2014; **159**: 163–175.
78 Schütte M, Risch T, Abdavi-Azar N, Boehnke K, Schumacher D, Keil M *et al.* Molecular dissection of colorectal cancer in pre-clinical models identifies biomarkers predicting sensitivity to EGFR inhibitors. *Nat Commun* 2017; **8**: 14262.

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