Organoids in cancer research

Jarno Drost[1] * *and Hans Clevers*[1,2,3]

Abstract | The recent advances in in vitro 3D culture technologies, such as organoids, have opened new avenues for the development of novel, more physiological human cancer models. Such preclinical models are essential for more efficient translation of basic cancer research into novel treatment regimens for patients with cancer. Wild-type organoids can be grown from embryonic and adult stem cells and display self-organizing capacities, phenocopying essential aspects of the organs they are derived from. Genetic modification of organoids allows disease modelling in a setting that approaches the physiological environment. Additionally, organoids can be grown with high efficiency from patient-derived healthy and tumour tissues, potentially enabling patient-specific drug testing and the development of individualized treatment regimens. In this Review, we evaluate tumour organoid protocols and how they can be utilized as an alternative model for cancer research.

Karyotype
The number and appearance of chromosomes in the nucleus of a cell.

Feeder cells
A layer of cells that is used to support the growth of a cell culture (that is, stem cell cultures) by secretion of important growth factors into the culture medium.

Matrigel
A mouse-derived ex vivo basement membrane substitute that is used to support 3D growth of organoid cultures.

[1]*Princess Máxima Centre for Paediatric Oncology, Utrecht, Netherlands.*

[2]*Hubrecht Institute, Royal Netherlands Academy of Arts and Sciences (KNAW) and UMC Utrecht, Utrecht, Netherlands.*

[3]*Oncode Institute, Utrecht, Netherlands.*

**e-mail: j.drost@prinsesmaximacentrum.nl*

https://doi.org/10.1038/s41568-018-0007-6

Over the past decades, our knowledge of the origin of cancer has increased immensely. Despite substantial progress in the treatment of (certain types of) cancer, it remains a major worldwide health problem[1]. The number of cancer deaths may be reduced by preventive measurements and early detection. In addition, the development of new, more targeted therapies offers opportunities. One of the major hurdles for the development of novel treatment regimens is the challenge of translating scientific knowledge from bench to bedside, which is mainly due to the fact that many cancer models only poorly recapitulate the patient's tumour[2], and as a consequence, many drugs that perform well in cancer models ultimately fail in clinical trials[3]. Although animal cancer models have provided important insights into the basics of cancer, their generation is time consuming, and it is argued that these models often do not faithfully recapitulate pathogenic processes in patients. For example, the histological complexity and genetic heterogeneity of human cancers are typically not reflected in genetically engineered mouse models of cancer[4].

Commonly used human cancer models include cancer cell lines and primary patient-derived tumour xenografts (PDTXs). Cancer cell lines are derived from primary patient material and have contributed tremendously to cancer research. However, they have several drawbacks. For instance, their generation from primary patient material is very inefficient and involves extensive adaptation and selection to in vitro 2D culture conditions. As only rare clones are able to expand and can be maintained over many passages, the derived cell lines may have undergone substantial genetic changes and no longer recapitulate the genetic heterogeneity of the original tumours. Other limitations of cell lines include the absence of normal tissue-derived control cell lines as reference and the lack of stromal compartments (TABLE 1). A recently developed method called 'conditional reprogramming' facilitates the establishment of 2D cell cultures from normal and tumour epithelial cells with high efficiency[5]. These cultures can be maintained long term and retain a stable karyotype. The procedure involves the presence of a RHO kinase inhibitor and fibroblast feeder cells[5] (TABLE 1). PDTXs have the advantage of mimicking the biological characteristics of the human tumour much better than in vitro culture models. PDTXs are generated by transplanting freshly derived patient material subcutaneously or orthotopically into immunodeficient mice. The ability to serially transplant tumour tissues into increasing numbers of animals allows for preclinical testing of novel therapies for cancer treatment (TABLE 1). Limitations of PDTXs include the use of animals and limited engraftment efficiencies for subsets of patient tumours. Moreover, the approach is expensive, time consuming and resource consuming, and PDTXs may undergo mouse-specific tumour evolution[6,7].

Recently developed 3D culture technologies have led to the development of novel and more physiological human healthy tissue and cancer models. Upon embedment into a 3D matrix, tissue-derived adult stem cells can be grown with high efficiencies into self-organizing organotypic structures, termed organoids. In 2009, Sato et al.[8] demonstrated that 3D epithelial organoids can be established from a single leucine-rich repeat-containing G protein-coupled receptor 5 ($LGR5)^+$ intestinal stem cell. Upon embedment into Matrigel, cells are cultured under serum-free conditions mimicking the in vivo stem cell niche (involving R-spondin 1 (a WNT

Table 1 | **Comparison of the described preclinical cancer models**

Feature	Cancer cell lines	Conditional reprogramming	Adult stem cell-derived organoids	PDTX
Success rate of initiation	±	+++	+++	++
Ease of maintenance	+++	++	++	±
Resource consumption	Low	Low	Medium	High
Expansion	+++	+++	++	+
3D growth	±	±	++	+++
Retention of phenotypic features in vitro	–	–	++	+++
Retention of genetic features in vitro	±	++	++	++
Representation of cancer spectrum	+	+[a]	++	++
Amenable to genetic modification	+++	NA	+++	–
Matched normal controls	–	++	+++	–
Tumour–stroma interactions	–	–	–	++
Incorporation of an immune system	–	–	±[b]	–
Genetic cancer modelling (initiation and progression)	–	NA	+++	–
Low-throughput drug screens	+++	+++	+++	+
High-throughput drug screens	+++	+++	++	–
Biobanking	–	++	+++	–

Respective features were judged as best (+++), suitable (++), possible (+), not very suitable (±) or unsuitable (–). NA, not available; PDTX, patient-derived tumour xenograft. [a]Only in epithelial tumours. [b]The immune system could be implemented by co-culturing organoids with haematopoietic cells[66,68]. Table adapted and updated with permission from REF.[116], Elsevier.

agonist and ligand of LGR5 (REFS[9,10]), epidermal growth factor (EGF) and the bone morphogenetic protein (BMP) inhibitor noggin). Consequently, LGR5$^+$ intestinal stem cells grow out as organotypic, highly polarized epithelial structures with proliferative crypt and differentiated villus compartments[8]. This culture protocol formed the starting point for other organoid culture protocols of multiple mouse and human epithelia, including colon[11,12], liver[13], pancreas[14], prostate[15,16], stomach[17], fallopian tube[18], taste buds[19], salivary glands[20], oesophagus[21], lung[22], endometrium[23] and breast[24] (reviewed in REF.[25]). Organoids can be expanded long term, can be cryopreserved and genetically modified and remain genetically and phenotypically stable. This allows for a wide range of applications in cancer research. Instead of culturing organoids in medium containing niche-recapitulating and tissue-specific growth factors, the Kuo laboratory[26] pioneered an organoid culture system using an air–liquid interface with stromal support cells as a source of essential growth factors. Organoids can also be derived from induced pluripotent stem cells (iPSCs)[25]. However, the efficiency of generating iPSC-based cancer models from patients may depend on cancer type and the presence or absence of specific oncogenic mutations, potentially selecting for outgrowth of tumour subclones and loss of the genetic heterogeneity of the tumour it is derived from[27]. Generally, it appears more practical to grow tumour organoids directly from cancers than to involve an intermediate iPSC step.

In this Review, we discuss the use of the adult stem cell-derived organoid technology in both basic and translational cancer research. We highlight the approaches to exploit patient-derived tumour organoid biobanks for drug development and personalized medicine and evaluate the application of organoid technology as an experimental tumour model. Finally, we discuss the limitations and potential of exploiting organoids for cancer research.

Organoids for translational research

Living organoid biobanks. The ability to grow organoids with high efficiency from healthy human adult stem cells has paved the way to grow organoids from patient-derived tumour tissue. So far, we and others have shown that long-term organoid cultures can be established from primary colon[11,28,29], oesophagus[11], pancreas[14,30], stomach[17], liver[31], endometrium[23] and breast[24] cancer tissues, as well as from metastatic colon[32], prostate[33,34] and breast[24] cancer biopsy samples. Importantly, these studies have shown that tumour-derived organoids — both phenotypically and genetically — resemble the tumour epithelium they were derived from. Tumour organoids do not grow faster per se than their matching normal organoid counterparts and, counterintuitively, in many cases even grow at slower rates, possibly owing to higher rates of mitotic failures and subsequent cell death[35,36]. Therefore, the overgrowth of tumour organoids by healthy epithelial organoids derived from remaining normal tissue present in tumour biopsy samples needs to be avoided. Hence, it is essential to initiate tumour organoid cultures using either pure tumour material or to grow the samples under selective culture conditions. For example, in the vast majority of colorectal cancers (CRCs), activating mutations in the WNT signalling pathway are present[37]. In these cases, pure tumour organoid cultures can be obtained by using

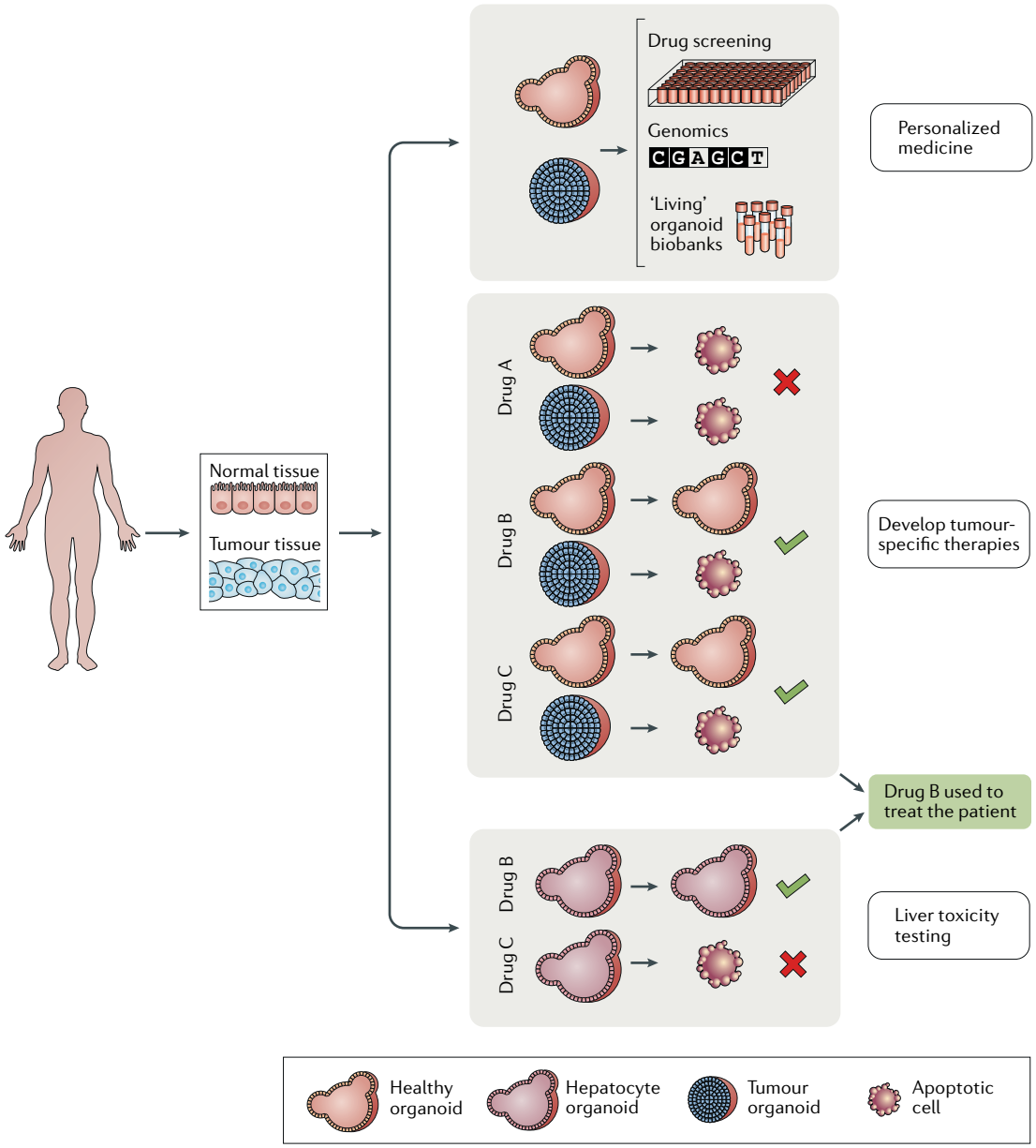

Fig. 1 | **Organoid cultures for personalized cancer treatment and drug development.** Organoids can be established from patient-derived healthy and tumour tissue samples (upper panel). The organoid cultures can be genetically characterized and used for drug screening, which makes it possible to correlate the genetic background of a tumour with drug response. Organoids can be cryopreserved and stored in living organoid biobanks. The establishment of organoids from healthy tissue of the same patient (middle panel) gives the opportunity to develop less toxic drugs by screening for compounds that selectively kill tumour cells while leaving healthy cells unharmed. Self-renewing hepatocyte organoid cultures may be used to test for hepatotoxicity — one of the causes of drug failure in clinical trials — of a potential new drug (lower panel). In this example, drug B seems most suitable for treating the patient as it specifically kills tumour organoids and does not induce hepatotoxicity.

culture medium lacking WNT and R-spondins[11], which are essential growth factors for healthy tissue-derived colon organoids. Similarly, tumours harbouring mutations in the EGF receptor (EGFR) signalling pathway can be selected by EGF withdrawal[29,35,38]. The small molecule nutlin-3, which stabilizes p53 by disrupting the binding of p53 to its negative regulator E3 ubiquitin ligase MDM2, has been used as a strategy to remove *TP53*-wild-type (WT) healthy cells from *TP53*-mutant cancer organoid cultures[24,35]. When such selection methods are not available, using pure tumour cells as starting material is a prerequisite.

Currently, large collections of patient-derived tumour and matching healthy organoids are generated and biobanked. These resources can be used to determine whether organoids have predictive value for drug responses for individual patients (FIG. 1). One of the earliest efforts to store large collections of primary tumour cultures was undertaken by Inoue and colleagues[39]. A collection of cultures derived from patients with CRC

was assembled using a method to expand tumour tissues as tumour spheroids in vitro. This culture method did not sustain the growth of healthy colon epithelium[39]. More recently, a tumour organoid biobank derived from patients with CRC was generated that consists of a set of 20 genetically diverse tumour organoid cultures and their matching normal tissue-derived organoids[28]. Integrating genomic and monotherapy drug-response data demonstrated that only one of the tumour organoid cultures was sensitive to the inhibitor of WNT secretion LGK974 (REF.[40]) owing to a mutation in the WNT antagonist E3 ubiquitin ligase *RNF43* (REFS[28,41,42]). Besides several other known correlations between drug responses and the presence of mutations, several compounds were identified with differential cytotoxicities among patient-derived tumour organoids without an apparent genetic marker[28]. Increasing the number of biobanked organoids will be necessary to increase the statistical power to that required to correlate genetic markers with differences in drug sensitivity. In a separate study, a subset of CRC organoids from this same biobank were used for proteomic analyses and comparative transcriptomic analysis[43]. Substantially different protein profiles were observed between tumour organoids and matching healthy organoids as well as among tumour organoids from individual patients displaying distinct personalized profiles[43]. This finding points towards an important role for proteomic profiling in personalized medicine. However, a direct comparison should be made between the proteomic profiles of tumour organoids and the primary tumour tissue to ensure preservation of protein expression profiles following expansion in vitro.

In line with the previous study[28], Sato and colleagues[29] generated an organoid biobank derived from 55 patients with CRC. For certain CRC subtypes, organoids could be established only when cultured under specific conditions. For instance, some organoid lines were maintained in medium lacking an inhibitor of p38 MAPK, a component of the original human colon organoid culture medium, or under hypoxic conditions[29]. These dependencies may reflect differences in the mutational backgrounds of tumours. As the genetic background of tumours is in many cases not determined when establishing organoid cultures from fresh patient-derived tissues, high efficiencies may result from culturing under various media conditions that differ in their combinations of growth factors. Importantly, it was shown that tumour organoids preserve the histopathological features of the original tumours not only in vitro but also following xenotransplantation under the kidney capsule of immunodeficient mice[29]. This finding suggests that organoid transplantations can be used to validate in vitro drug responses in a more complex in vivo environment. Another study exploited a biobank consisting of both CRC-derived PDTXs and organoids to identify biomarkers to predict sensitivity towards EGFR inhibitors[44].

A collection of organoid lines was established from the primary pancreatic ductal adenocarcinoma (PDAC) tissue of 17 patients[30]. Although mutation analysis to validate tumour origin was not performed, organoids maintained similar histological features to the parental tumours both in vitro as well as following xenotransplantation. Organoids could not be established from poorly differentiated to moderately differentiated PDACs and from intraductal papillary mucinous cystic neoplasms, suggesting that — as for different CRC subtypes[29] — different tumour subtypes require specific compositions of culture media. Similarly, Seino et al.[45] generated a genetically characterized organoid biobank derived from 39 patients with PDAC. Three functional PDAC organoid subtypes were identified as having distinct dependencies on WNT signalling, which were also associated with different gene expression subtypes[45].

Recently, a biobank of breast cancer organoids was generated from >100 patients representing all major breast cancer subtypes[24]. Besides preservation of histopathological and genetic features, the majority of the breast cancer organoids also retained expression of the breast cancer biomarkers oestrogen receptor (ER), progesterone receptor (PR) and human EGFR2 (HER2; also known as ERBB2). The receptor status of the parental tumour was lost in only a small number of organoid cases, yet this highlights the need to adequately characterize tumour-derived organoids before their utilization in downstream experimental procedures and drug screening platforms. A proof-of-principle drug screen using various drugs that target the HER signalling pathway family demonstrated that drug sensitivity levels generally correlated with HER2 status[24].

Besides these living biobanks, smaller tumour organoid collections from advanced prostate cancer (seven lines)[33], CRC metastases[32] (eight lines), primary pancreatic intraepithelial neoplasms (eight lines)[14] and primary liver cancer (seven lines)[31] have been generated (TABLE 2). Notably, prostate cancer organoids carrying an androgen receptor (AR) amplification showed higher sensitivity towards the AR signalling inhibitor enzalutamide than AR^- prostate cancer organoids[33], again demonstrating that tumour organoid biobanks enable physiologically relevant drug screens.

Currently, a combined effort of the US National Cancer Institute (NCI), Cancer Research UK, the UK Wellcome Trust Sanger Institute and the foundation Hubrecht Organoid Technology, Netherlands — which is known as the Human Cancer Models Initiative (HCMI) — is ongoing to generate a large, globally accessible bank of new cancer cell culture models, including organoids, available for the research community.

Drug development and personalized cancer treatment. Although drug screens on large 2D cancer cell line collections have provided major insights into genetic predictions of drug response[46], their fairly poor reflection of native tumour tissue may have contributed to the high failure rate of newly discovered drugs in clinical trials[2,3]. Patient-derived tumour organoids better recapitulate native tumours and may be superior models to identify and test novel anticancer drugs. The development of high-throughput drug screening methods in patient-derived organoids is just beginning to be explored. As discussed above, small-scale drug screens on organoid biobanks performed so far have yielded promising results[24,28,33].

Table 2 | **Overview of the currently available tumour organoid collections**

Cancer type	Source	*n*	Efficiency	Refs
Colorectal cancer	Primary tumour	20	~90%	28
		55	~100%	29
	Metastases	8	~70%	32
	Primary tumour and metastases	35	~60%	44
Gastrointestinal cancer	Metastases	23[a]	~70%	47
Pancreatic cancer	Primary tumour	8	~80%	14
		17	~85%	30
	Primary tumour and ascites specimens	39	~80%[b]	45
Prostate cancer	Metastases and circulating tumour cells	7	~15–20%[c]	33
Breast cancer	Primary tumour	>100	~80%	24
Liver cancer	Primary tumour	7	~100%[d]	31
Multiple cancer types	Primary tumour	56	~37% (average)	88
Glioblastoma	Primary tumour and metastases	NR	NR	115

NR, not reported. [a]Number of organoid lines that were genetically characterized. The total amount of established organoid cultures was not explicitly mentioned[47]. [b]The residual ~20% of cultures were considered normal-like organoid cultures[45]. [c]The lower efficiency is possibly due to the small amount of input material or suboptimal culture conditions[33,34]. [d]Results achieved when establishing organoid cultures from tissue samples derived from tumours that contained >5% proliferating cells[31].

Profiling of patient-derived organoids may reveal causal epigenetic and/or genetic changes that underlie drug resistance, which could be used to stratify individual patients to specific treatment regimens. To test the potential of organoids to evaluate drug responses in a preclinical setting, Verissimo et al.[36] utilized a CRC organoid biobank to investigate the effect of different RAS pathway inhibitors, either as single agents or in combinations, that are currently used in the clinic. Using this strategy, the authors confirm earlier observations in cancer cell lines that combinatorial targeting of MEK and ERK, or of the HER family and MEK in *RAS*-mutant CRCs, effectively suppressed growth. These treatments merely forced organoids into cell cycle arrest rather than apoptosis, and cells rapidly re-initiated growth when the treatment was stopped[36]. These results therefore question the effectiveness of this combination therapy in the treatment of *RAS*-mutant CRCs. However, combinatorial EGFR pathway inhibition sensitized *RAS*-mutant CRCs to low levels of anti-apoptosis inhibitors[36], possibly providing an alternative treatment strategy. More recently, patient-derived organoid models of metastatic gastrointestinal cancer (CRC and gastroesophageal cancer) were generated and used to determine whether organoids can predict patient treatment response[47]. Using a compound library of drugs either used in the clinic or currently in clinical trials, organoid drug sensitivities were tested and compared with the responses of patients. The authors report a positive predictive value (predicting that a particular drug works) of 88% and a negative predictive value (predicting that a particular drug does not work) of 100%, suggesting that patient-derived organoids recapitulate patient responses in clinical trials and can be used for personalized medicine programmes[47].

By performing drug screens on human primary liver cancer organoids, Huch and colleagues[31] identified ERK inhibition as a potential therapeutic approach for the treatment of primary liver cancers. As another example, patient-derived castration-resistant prostate cancer organoids (either WT or deficient for chromodomain helicase DNA-binding protein 1 (*CHD1*)) were used to demonstrate that *CHD1* deletions sensitize to DNA-damaging agents[48]. One patient with prostate cancer demonstrated rapidly progressing disease. Following tumour organoid development, *CHD1*-deficiency was identified as resulting in an improved response to carboplatin. This patient was subsequently successfully treated with this DNA-damaging agent[48]. Lastly, Chen and colleagues[49] developed colonic organoids from iPSCs from patients with familial adenomatous coli (FAP), which is caused by mutations in the adenomatous polyposis coli (*APC*) gene, a negative regulator of the WNT pathway. They used these organoids as a platform for drug testing and found that cells with hyperactive WNT signalling were more sensitive to the ribosome-binding antibiotic geneticin. They additionally identified two compounds that decreased proliferation in organoids derived from patients with FAP but also affected cell proliferation in WT organoids, thereby highlighting the value of organoids for drug testing before clinical therapeutic application[49].

Another major advantage of exploiting organoid technology for drug development is that organoids from both healthy and tumour tissue can be generated, which allows for screening for drugs that specifically target tumour cells while leaving healthy cells unharmed. This approach may result in reduced toxicities in patients. Drug-induced liver failure caused by hepatotoxicity is a major reason for drug failure in clinical trials[50]. Recently established hepatic organoid cultures could provide a relevant model for preclinical testing of the hepatotoxicity of experimental compounds[51,52] (FIG. 1). The most common mechanisms of drug-induced hepatotoxicity are mediated through cytochrome P450 enzymes, and

it is encouraging that hepatic organoids express these enzymes at near physiological levels upon induced differentiation[13,52]. On the same note, iPSC-derived cardiac organoids could be used for testing cardiotoxicity[53,54], and iPSC-derived kidney organoids were recently exploited for toxicological studies[55]. For patients with cystic fibrosis (CF; a disease caused by a spectrum of mutations in the gene encoding the cystic fibrosis transmembrane conductance regulator (CFTR) chloride channel), intestinal organoids are already successfully used to predict patient drug response. Readout is based on in vitro assays to test CFTR functionalities in patient-derived rectal organoids[56]. Beekman and colleagues[57] exploited this assay to test different CFTR potentiators on rectal organoids derived from patients with CF that differ in *CFTR* genotypes. This work revealed that in vitro measurement of CFTR function in such organoids is useful to identify patients who would benefit from a particular type of drug treatment[57]. On the basis of these promising results, this organoid application is currently being implemented in the diagnostic pipeline in the Netherlands.

Immunotherapy. The emergence of resistance against single-agent targeted therapies is another important rate-limiting factor in the successful development of new anticancer drugs. Therefore, combination strategies have to be considered to minimize development of resistance to single compounds[58,59]. A very promising strategy is to exploit the patient's own immune system to eradicate malignant cells. Since its initial successes in the clinic, cancer immunotherapy has seen a major revival[60,61]. However, it is essential that tumour cells display sufficient immunogenicity to elicit a proper immune response[62–64]. For tumour cells, this is — at least partially — determined by the mutational load of a tumour, as immune responses are dependent on antigens that result from these mutations (so-called neo-antigens)[62,63,65]. In many cases, the potency of the immune response elicited by the neo-antigens of the tumour is inadequate. In vitro activation and expansion of immune cells could provide a way to generate sufficient numbers of cytotoxic immune cells for in vivo use in patients.

Indeed, several recent studies successfully established culture systems in which haematopoietic cells are expanded in vitro in co-cultures with organoids. Nakamura and colleagues[66] demonstrated short-term maintenance of intraepithelial lymphocytes (IELs) in a co-culture with mouse intestinal organoids. When the culture medium was supplemented with interleukin-2 (IL-2), IL-7 and IL-15, IELs were maintained and expanded over several weeks while retaining their functionality[66]. Finnberg et al.[67] demonstrated that $CD45^+$ lymphocytes can be maintained up to 8 days in co-culture with human air–liquid interface tumour organoids, whereas other haematopoietic cell populations were rapidly lost. Moreover, co-cultures of organoids together with a specific T cell subset, $V\delta2^+$ T lymphocytes, can be directly established from primary human breast epithelia[68]. Interestingly, these T lymphocytes from the breast organoids can effectively kill triple-negative breast carcinoma cells[68]. It was recently demonstrated that naive T lymphocytes derived from healthy blood donors provide a source of T cells that recognize neo-antigens and target and kill melanoma cells harbouring such neo-antigens[69]. This finding reveals the exciting possibility of testing the cytotoxic potential of healthy donor-derived T cells on patient-derived tumour organoids. T cells displaying immune reactivity could be expanded in organoid co-cultures and subsequently used to treat patients. The recent development of thymus organoids could possibly provide a more physiological in vitro environment to efficiently maintain and expand tumour-specific T lymphocytes[70].

Organoids for basic research

Organoids to explore the link between infectious agents and cancer development. An estimated one out of five cancer cases have been linked to infectious agents[71]. However, in many cases, it is not known how a particular pathogen contributes to malignant transformation. As several studies have succeeded in establishing organoid co-culture systems with different types of pathogens, organoid technology could potentially be used to study these processes. Of particular interest for cancer researchers are organoid co-cultures with pathogens as potential risk factors for cancer development. For example, stomach organoids[17] may be used to study the relationship between chronic *Helicobacter pylori* infections and gastric cancer[72]. Stomach organoids provoke strong primary inflammatory responses when micro-injected with *H. pylori*[17] and have been used to demonstrate how *H. pylori* locates and colonizes the gastric epithelium[73]. Moreover, iPSC-derived stomach organoids have been used to model the acute response of the gastric epithelium to *H. pylori* infections[74]. However, to study the effects of chronic infection and its possible causal role in gastric tumorigenesis, a long-term co-culture system may have to be established and might need to involve immune elements as well. Scanu et al.[75] used organoids of pre-transformed gallbladders derived from different transgenic mice as a model to study the contribution of *Salmonella enterica* infections to the development of gallbladder carcinoma. The authors demonstrated that *S. enterica* infection of gallbladder organoids with *TP53* mutations and *MYC* amplifications induces growth factor-independent cell growth and neoplastic transformation through activation of AKT and MAPK signalling[75]. Moreover, viral infections can also be modelled in organoids[76], suggesting that organoid–virus co-culture models can be exploited for cancer research as well. Liver or hepatocyte organoids could potentially be used to study the contribution of hepatitis infections to the development of hepatocellular carcinoma, whereas stomach organoids could be exploited to study the connection between Epstein–Barr virus infections and gastric cancer development.

Organoids to interrogate mutational processes underlying tumorigenesis. Cancer is caused by the gradual accumulation of mutations in disease-driving genes[77]. It is therefore important to gain insight into the mutational processes that are active during both tissue homeostasis and tumorigenesis. The high genetic

Intraepithelial lymphocytes (IELs). Lymphocytes residing in the epithelial layer of mammalian mucosal linings, such as the small and large intestines, lungs, upper respiratory tract, reproductive tract and skin.

Mutation signatures
Unique combinations of mutation types caused by different mutational processes.

stability of healthy organoid cultures over long periods of time[11,13,78] enables the study of mutagenic processes in detail. Blokzijl et al.[79] used single stem cell-derived (that is, clonal) organoid cultures to determine genome-wide mutation patterns in healthy stem cells of the small intestine, colon and liver over the course of life. The authors performed whole genome sequencing on clonal organoids derived from donors of various ages (FIG. 2). Surprisingly, although mutation rates did not differ among the different types of stem cell, the types of mutations or mutation signatures[80] were distinct in small intestinal and colon stem cells from those of liver

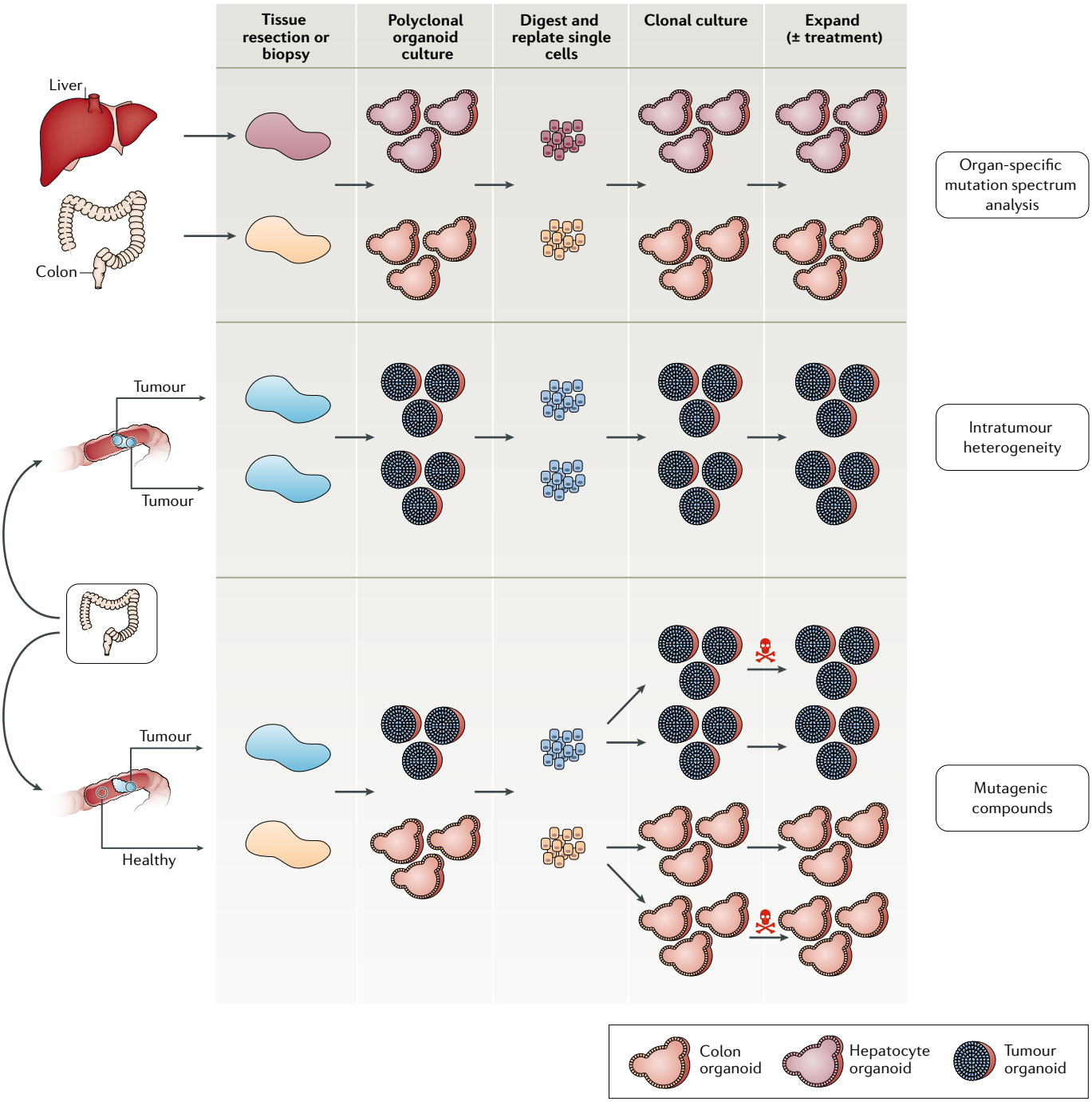

Fig. 2 | **Organoid cultures to study mutational processes underlying tumorigenesis.** Growing clonal organoid cultures from different healthy organs followed by whole genome sequencing of the cultures enables analysis of the organ-specific mutation spectrum[79] (upper panel). Organoids can be used to study intratumour heterogeneity by growing clonal organoid cultures from different regions of the same tumour (middle panel). Region-specific mutation spectra can be revealed by whole genome sequencing of the clonal cultures[89]. Using approaches similar to those described above, organoid cultures can be exploited to study the effects of particular compounds on the mutation profiles of both healthy and tumour cells (lower panel).

stem cells. The authors suggest that the rapid cycling of intestinal and colonic stem cells causes deamination-induced mutagenesis, resulting in the acquisition of a specific mutation signature, with frequent C>G to T>A transitions at CpG dinucleotides. Remarkably, this type of mutation is also found in common CRC driver genes, while driver mutations in the same genes arise through a different mechanism in liver cancer[79]. Overall, these findings suggest that organ-specific mutagenic processes result in the accumulation of specific types of somatic point mutations during malignant transformation. In an earlier study, genome sequencing data of clonally expanded mouse organoids derived from stomach, small intestine, colon and prostate were used to reconstruct developmental lineages on the basis of the presence of somatic point mutations[78].

The importance of understanding the origin of mutational signatures in cancer was recently demonstrated by Davies et al.[81], who showed that BRCA1 or BRCA2 deficiency can be predicted based on the mutational signature present in a tumour. Using this prediction, BRCA1-deficient or BRCA2-deficient tumour cells that lack *BRCA1* or *BRCA2* gene mutations can be identified and, akin to *BRCA1*-mutant or *BRCA2*-mutant tumours[82–84], could benefit from poly(ADP-ribose) polymerase (PARP) inhibitors[81]. In cancer cells, many different mutational processes are active, which makes it challenging to study the origin of mutational signatures. The genetic stability of healthy organoid cultures provides a platform to causally attribute specific mutational processes to mutation signatures. We recently knocked out DNA repair genes in healthy human colon organoids using CRISPR–Cas9 genome editing; whole genome sequencing of these clonal knockout organoids demonstrated that a high contribution of signature 30 mutations in a tumour is indicative of (germline) mutations in the base excision repair gene nth like DNA glycosylase 1 (*NTHL1*), which could indicate a familial cause of the cancer[85]. Using a similar approach, it will be interesting to exploit organoids to study the effects of, for instance, chemotherapeutics and irradiation on the mutation rates and profiles of both healthy and tumour cells (FIG. 2). Such experiments may provide novel insights into the deleterious side effects of chemotherapeutics and the increased risk of secondary cancers later in life. Moreover, sporadic clones that develop resistance towards the treatment could be characterized to identify mutations as the cause of the acquired therapy resistance.

The majority of tumours harbour unstable genomes, and consequently, individual tumour cells within a tumour contain different sets of genetic alterations[77,86]. Although this intratumour heterogeneity is believed to play a key role in cancer progression and therapy resistance, knowledge of how tumour heterogeneity arises and contributes to disease progression is still limited[87]. The heterogeneous genetic composition of a tumour is retained in organoids that are derived from it[14,28,29,32] and is maintained in culture over time[31,44,88]. However, clonal dynamics within tumour organoid cultures can occur[29], suggesting that the genetic composition of a tumour-derived organoid culture changes over time. In a comprehensive genetic analysis of multiple patient-derived clonal organoids derived from different regions of the same treatment-naive tumour, Roerink et al.[89] recently exploited organoid technology to study intratumour heterogeneity of CRC in great detail (FIG. 2). This strategy allowed an in-depth analysis of the genetic, epigenetic and transcriptomic profiles of different regions from the same tumour and to determine drug sensitivity of different tumour subclones. The authors observed a striking increase in mutation rates and the acquisition of novel mutational signatures during the development of a tumour. A similar observation was found when performing exome sequencing on organoids derived from normal tissue and adenomas of $Apc^{min/+}$ mice[90]. Thus, during the conversion of a normal cell into a malignant cell, the probability of accumulating additional driver mutations substantially increases and disease progression accelerates. In addition, Roerink et al.[89] demonstrated that the contribution of mutational processes varies in different parts of the tumour. Another surprising observation came from drug treatment of the individual tumour clones with different anticancer drugs that are commonly used in clinical practice. This work revealed that within all three tumours examined, resistant cells already existed for each of the tested drugs before treatment[89].

Genetic cancer modelling in organoids. Organoids can be used to model and study cancer initiation and progression in specific organs. Starting from healthy human intestinal organoids, two reports exploited CRISPR–Cas9 genome editing to introduce combinations of common CRC driver mutations to generate CRC progression models[35,91]. Using these models, it was shown that CRC driver mutations result in growth that is independent of intestinal stem cell niche factors, as organoids harbouring activating mutations in *KRAS* ($KRAS^{G12D}$) and inactivating mutations in *APC*, *TP53* and *SMAD4* grow independently of the niche factors EGF, WNT, R-spondin 1 and noggin[35,91]. We additionally demonstrated that loss of *APC* and *TP53* are key drivers of chromosome instability and aneuploidy[35], which are hallmarks of CRC[92]. Upon subcutaneous xenotransplantation into mice, the quadruple-mutant organoids grow as invasive adenocarcinomas[35]. Surprisingly, although organoids efficiently grew in vivo as invasive tumours, the developed tumours did not spontaneously metastasize, most likely owing to the lack of a native microenvironment. Indeed, when the same tumour organoids were orthotopically transplanted into the caecum of mice, spontaneous metastases developed in the liver and lungs[93,94]. This orthotopic organoid transplantation strategy was further used to demonstrate that a key contributor to the colonization of a metastatic site is the loss of dependency on stem cell niche signals[93], thereby confirming earlier observations in patient-derived CRC organoids[29].

Similar CRC progression models were generated in air–liquid interface mouse small intestinal and colonic organoids using RNAi-based strategies[95]. Remarkably, mouse small intestinal organoids showed a much more promiscuous transformation capacity than colonic

Base excision repair
A DNA repair mechanism that removes damaged bases (oxidized, alkylated or deaminated) that could otherwise cause mutations.

Caecum
A pouch located between the small and large intestine that is considered to be the beginning of the large intestine and is thus part of the gastrointestinal tract.

organoids, as high-grade dysplasia developed with only two driver mutation combinations, *Kras*^{G12D} and *Apc*$^{-/-}$ or *Apc*$^{-/-}$ and *Trp53*$^{-/-}$, in small intestinal organoids[95]. This finding implies that mouse small intestinal organoids may not be a representative model to study multi-hit CRC tumorigenesis. The importance of transplanting tissue into its orthotopic environment was also demonstrated in a study by De Sauvage and colleagues[96], where mouse small intestinal organoids carrying different combinations of CRC mutations were directly injected into the colonic epithelium. Mutations in *Apc*, *Kras*, *Trp53* and *Smad4* were required for progressive tumour growth and metastasis upon transplantation of organoids into their relevant physiological niche, while subcutaneous transplantation of the same organoids did not result in development of metastases, thereby confirming earlier studies[35,93]. Two other recent studies further underscore the need for an orthotopic transplantation environment for studying the metastatic cascade of CRC[97,98].

Organoids derived from mice harbouring the diphtheria toxin receptor knocked into the *Lgr5* locus to enable targeted cell ablation were used to investigate the contribution of cancer stem cells (CSCs) to CRC growth. Remarkably, specific killing of CSCs did not result in regression of the primary tumour, but CSCs were required for the formation and maintenance of CRC-derived liver metastases[96]. In line with this, Shimokawa et al.[99] performed renal capsule transplantations of human CRC organoids, in which an inducible caspase 9 (which is involved in the activation of apoptosis) was knocked into the *LGR5* locus, thereby enabling the ablation of CSCs. Although elimination of CSCs initially resulted in reduction of the primary tumours, these eventually regrew owing to the reappearance of LGR5^{+} CSCs, demonstrating the extensive cellular plasticity within tumours[99]. Finally, human pancreatic organoids were recently used to model PDAC. Oncogenic mutations in cyclin-dependent kinase inhibitor 2A (*CDKN2A*), *KRAS*, *TP53* and *SMAD4* resulted in transformation of pancreatic cells into cancer cells that, upon xenotransplantation, grew as tumours with characteristics of pancreatic intraepithelial neoplasia and invasive PDAC[45].

Organoid technology can also be utilized to study cancer-related processes and signalling pathways. For instance, mouse small intestinal organoids were exploited to show that expression of recurrent hotspot mutations in *RHOA*, which were identified in gastric cancer, induces resistance to anoikis[100]. Moreover, serrated colon adenomas are thought to be driven by oncogenic *BRAF*^{V600E} mutations[101]. CRISPR–Cas9-engineered *BRAF*^{V600E}-expressing human colon organoids were therefore used as a model system to demonstrate that transforming growth factor-β (TGFβ) signalling drives serrated colon adenoma to CRCs of the mesenchymal subtype, which have a very poor prognosis[102]. Leung and colleagues exploited human serrated colon adenoma-derived organoids to demonstrate that *RNF43* mutations, as identified in serrated neoplasia, activate WNT–β-catenin signalling[103], thereby confirming earlier observations in mouse intestinal organoids[104]. The presence of *RNF43* mutations in a subset of serrated colon neoplasias potentially provides therapeutic opportunities, as it was previously demonstrated that tumours harbouring mutations in *RNF43* are sensitive to inhibitors of the *O*-acyltransferase porcupine, which inhibit WNT secretion[28,105].

The establishment of organoids from transgenic mouse strains with particular oncogenic transgenes enables rapid testing of the effects of a particular mutation in the context of a specific genetic background. Kuo and colleagues demonstrated that TGFβ receptor 2 (*Tgfbr2*) loss in the context of inactivated cadherin 1 (*Cdh1*) and *Trp53* induces metastatic diffuse gastric cancer by knocking down *Tgfbr2* in stomach organoids derived from *Cdh1*$^{-/-}$;*Trp53*$^{-/-}$ mice[106]. Transgenic mouse-derived organoids were also used to study the genetic interaction between loss of the transcription factor caudal type homeobox 2 (*CDX2*) and *BRAF*^{V600E} mutations, alterations that frequently co-occur in human serrated CRCs[38]. By histologically and genetically analysing organoids derived from WT, *Cdx2*$^{-/-}$, *Braf*^{V600E} and *Cdx2*$^{-/-}$;*Braf*^{V600E} mice, the authors showed that these mutations cooperate to promote development of serrated CRC[38].

The recent revolution in genome-editing technologies such as CRISPR–Cas9 provides a platform to investigate the effects of 'repairing' disease-causing genes. Organoids could presumably be used to test whether the repair of a particular oncogenic mutation reverts the tumorigenic phenotype. Previously, a mutated, dysfunctional *CFTR* allele was successfully repaired in rectal organoids derived from patients with CF[104]. Although cancer is genetically much more complex, with tumours typically harbouring hundreds of mutations, it was recently shown that restoration of APC expression recovers crypt homeostasis in a mouse CRC model and in organoids derived thereof[97,107]. Although restoration of a single driver mutation will likely result in the emergence of resistant tumour clones, it would be interesting to test whether restoring the activity of several driver genes simultaneously in patient-derived tumour organoids results in tumour regression upon xenotransplantation.

Limitations and perspectives

In this Review, we have discussed a spectrum of applications of organoid technology for cancer research. Many of these are just beginning to be explored, and although the first indications are promising, organoids have limitations as well. For example, compared with cancer cell lines, organoid culture consumes both time and resources (TABLE 1). One of the intrinsic limitations of organoid culture is the lack of stroma, blood vessels and immune cells. Future studies will address the possibility of developing co-culture systems incorporating additional cellular (and microbial) elements[108]. In a recent effort using iPSC-derived tissue engineering, intestinal organoid cultures with a functional enteric nervous system were developed[109], demonstrating the potential of developing more complex organoid-based structures. Additionally, the Tuveson laboratory[110] successfully established co-cultures of mouse pancreatic stellate cells (which differentiate into cancer-associated fibroblasts (CAFs)) with PDAC organoids. In line with this, Sato and colleagues[45] demonstrated that

Anoikis
A process of programmed cell death initiated by loss of cell–matrix interactions in anchorage-dependent cells.

Serrated colon adenomas
A precursor colorectal cancer (CRC) subtype that is characterized by a serrated histopathological morphology. Serrated CRCs are genetically distinct from the classical adenocarcinomas. Whereas classical adenocarcinomas are typically initiated by mutations in the WNT pathway (for example, adenomatous polyposis coli (*APC*)), serrated CRCs are likely initiated by *BRAF* mutations.

patient-derived CAFs provide WNT ligands for PDACs using a co-culture of CAFs and PDAC organoids.

The requirement for mouse-derived extracellular matrix (ECM) substitutes (for example, Matrigel or basement membrane extract) and, in some organoid cultures, for fetal calf serum (required for the production of WNT-conditioned medium) incorporates undefined extrinsic factors that may influence the outcome of experiments, such as drug screens. For instance, the presence of serum was shown to be unfavourable for long-term growth of human pancreatic organoids[45]. Recently developed synthetic matrices that support the growth of mouse and human intestinal organoids could possibly replace mouse-derived ECM substitutes[111]. However, these matrices will have to be further optimized to increase efficiency and to sustain the growth of organoids derived from other organs as well. Garcia and colleagues[112] developed water-soluble Frizzled (FZD)–low-density lipoprotein receptor-related protein 5 (LRP5) or LRP6 heterodimerizers that function as surrogate WNT agonists. These surrogate WNT agonists were shown to efficiently support the growth of a range of human organoid cultures[112], thereby providing a serum-free alternative for WNT-conditioned medium. Similarly, Mihara et al.[113] demonstrated that water-soluble WNT can be produced when co-expressed with afamin, a component of fetal calf serum. Furthermore, lipid-mediated stabilization of WNT proteins may provide another means for serum-free growth of organoids[114].

Another possible limitation may be that organoids derived from advanced cancers often grow more slowly than organoids derived from normal epithelium, possibly resulting in overgrowth of tumour organoids by organoids derived from contaminating normal epithelium. This finding is most likely due to a much higher rate of mitotic failure and subsequent cell death in tumour organoids. Future studies will provide additional insights into this counterintuitive observation. Finally, all organoids discussed above were grown from tumours of epithelial origin, that is, (adeno)carcinomas. It is currently unclear whether the organoid approach can be adapted for organoid culture systems from non-epithelial tumours. The recent advances in growing organoids from primary glioblastoma tissue[115] may set the stage for growing other non-epithelial tumours.

Despite these limitations, organoids have emerged as a physiologically relevant in vitro model to study cancer. Organoids can be established with high efficiency from individual-patient-derived tumour tissue, making them a highly relevant model for translational applications and the development of personalized cancer treatments. It will be essential that patient-derived organoids can be generated and expanded efficiently to enable drug screening in a clinically meaningful time window. While anecdotal validation for the use of organoids as a diagnostic for CF already exists, solid validation of the predictive value of organoids for tumour drug responses will have to come from ongoing trials. Results of a first study comparing drug responses of patient-derived gastrointestinal organoids with the responses of the patients in the clinic are very encouraging[47]. The optimization of drug screening platforms in terms of sensitivity and robustness will be key before organoid-based personalized medicine can be implemented in the clinic.

Published online 24 April 2018

1. Torre, L. A. et al. Global cancer statistics, 2012. *CA Cancer J. Clin.* **65**, 87–108 (2015).
2. Kamb, A. What's wrong with our cancer models? *Nat. Rev. Drug Discov.* **4**, 161–165 (2005).
3. Caponigro, G. & Sellers, W. R. Advances in the preclinical testing of cancer therapeutic hypotheses. *Nat. Rev. Drug Discov.* **10**, 179–187 (2011).
4. Cheon, D. J. & Orsulic, S. Mouse models of cancer. *Annu. Rev. Pathol.* **6**, 95–119 (2011).
5. Liu, X. et al. ROCK inhibitor and feeder cells induce the conditional reprogramming of epithelial cells. *Am. J. Pathol.* **180**, 599–607 (2012).
6. Ben-David, U. et al. Patient-derived xenografts undergo mouse-specific tumor evolution. *Nat. Genet.* **49**, 1567–1575 (2017).
7. Byrne, A. T. et al. Interrogating open issues in cancer medicine with patient-derived xenografts. *Nat. Rev. Cancer.* **17**, 254–268 (2017).
8. Sato, T. et al. Single Lgr5 stem cells build crypt-villus structures in vitro without a mesenchymal niche. *Nature* **459**, 262–265 (2009).
Sato and colleagues describe the generation of organoids from mouse intestinal stem cells, which initiated the development of many other adult stem cell-derived organoid culture protocols.
9. Carmon, K. S., Gong, X., Lin, Q., Thomas, A. & Liu, Q. R-Spondins function as ligands of the orphan receptors LGR4 and LGR5 to regulate Wnt/beta-catenin signaling. *Proc. Natl Acad. Sci. USA* **108**, 11452–11457 (2011).
10. de Lau, W. et al. Lgr5 homologues associate with Wnt receptors and mediate R-spondin signalling. *Nature* **476**, 293–297 (2011).
11. Sato, T. et al. Long-term expansion of epithelial organoids from human colon, adenoma, adenocarcinoma, and Barrett's epithelium. *Gastroenterology* **141**, 1762–1772 (2011).
This paper describes the generation and long-term expansion of patient-derived organoids from normal and cancerous colon tissue.
12. Jung, P. et al. Isolation and in vitro expansion of human colonic stem cells. *Nat. Med.* **17**, 1225–1227 (2011).
This paper describes the establishment of human colon organoids from a single cell.
13. Huch, M. et al. Long-term culture of genome-stable bipotent stem cells from adult human liver. *Cell* **160**, 299–312 (2015).
14. Boj, S. F. et al. Organoid models of human and mouse ductal pancreatic cancer. *Cell* **160**, 324–338 (2015).
15. Karthaus, W. R. et al. Identification of multipotent luminal progenitor cells in human prostate organoid cultures. *Cell* **159**, 163–175 (2014).
16. Chua, C. W. et al. Single luminal epithelial progenitors can generate prostate organoids in culture. *Nat. Cell Biol.* **16**, 951–961 (2014).
17. Bartfeld, S. et al. In vitro expansion of human gastric epithelial stem cells and their responses to bacterial infection. *Gastroenterology* **148**, 126–136 e126 (2015).
18. Kessler, M. et al. The Notch and Wnt pathways regulate stemness and differentiation in human fallopian tube organoids. *Nat. Commun.* **6**, 8989 (2015).
19. Ren, W. et al. Single Lgr5- or Lgr6-expressing taste stem/progenitor cells generate taste bud cells ex vivo. *Proc. Natl Acad. Sci. USA* **111**, 16401–16406 (2014).
20. Maimets, M. et al. Long-term in vitro expansion of salivary gland stem cells driven by wnt signals. *Stem Cell Rep.* **6**, 150–162 (2016).
21. DeWard, A. D., Cramer, J. & Lagasse, E. Cellular heterogeneity in the mouse esophagus implicates the presence of a nonquiescent epithelial stem cell population. *Cell Rep.* **9**, 701–711 (2014).
22. Rock, J. R. et al. Basal cells as stem cells of the mouse trachea and human airway epithelium. *Proc. Natl Acad. Sci. USA* **106**, 12771–12775 (2009).
23. Turco, M. Y. et al. Long-term, hormone-responsive organoid cultures of human endometrium in a chemically defined medium. *Nat. Cell Biol.* **19**, 568–577 (2017).
24. Sachs, N. et al. A living biobank of breast cancer organoids captures disease heterogeneity. *Cell* **172**, 373–386.e10 (2017).
25. Clevers, H. Modeling development and disease with organoids. *Cell* **165**, 1586–1597 (2016).
26. Ootani, A. et al. Sustained in vitro intestinal epithelial culture within a Wnt-dependent stem cell niche. *Nat. Med.* **15**, 701–706 (2009).
Ootani and colleagues develop an organoid culture system for intestinal epithelium using an air–liquid interface and underlying stromal elements.
27. Papapetrou, E. P. Patient-derived induced pluripotent stem cells in cancer research and precision oncology. *Nat. Med.* **22**, 1392–1401 (2016).
28. van de Wetering, M. et al. Prospective derivation of a living organoid biobank of colorectal cancer patients. *Cell* **161**, 933–945 (2015).
This paper describes the generation of the first organoid biobank consisting of primary tumour and matching healthy organoids from patients with CRC.
29. Fujii, M. et al. A colorectal tumor organoid library demonstrates progressive loss of niche factor requirements during tumorigenesis. *Cell Stem Cell* **18**, 827–838 (2016).
30. Huang, L. et al. Ductal pancreatic cancer modeling and drug screening using human pluripotent stem cell- and patient-derived tumor organoids. *Nat. Med.* **21**, 1364–1371 (2015).
31. Broutier, L. et al. Human primary liver cancer-derived organoid cultures for disease modeling and drug screening. *Nat. Med.* **23**, 1424–1435 (2017).
32. Weeber, F. et al. Preserved genetic diversity in organoids cultured from biopsies of human colorectal cancer metastases. *Proc. Natl Acad. Sci. USA* **112**, 13308–13311 (2015).

33. Gao, D. et al. Organoid cultures derived from patients with advanced prostate cancer. *Cell* **159**, 176–187 (2014).
34. Drost, J. et al. Organoid culture systems for prostate epithelial and cancer tissue. *Nat. Protoc.* **11**, 347–358 (2016).
35. Drost, J. et al. Sequential cancer mutations in cultured human intestinal stem cells. *Nature* **521**, 43–47 (2015).
Starting from healthy colon organoids, Drost and colleagues use CRISPR technology to introduce common CRC mutations and study tumour progression and chromosome instability.
36. Verissimo, C. S. et al. Targeting mutant RAS in patient-derived colorectal cancer organoids by combinatorial drug screening. *eLife* **5**, pii: e18489 (2016).
37. Cancer Genome Atlas, N. Comprehensive molecular characterization of human colon and rectal cancer. *Nature* **487**, 330–337 (2012).
38. Sakamoto, N. et al. BRAFV600E cooperates with CDX2 inactivation to promote serrated colorectal tumorigenesis. *eLife* **6**, pii: e20331 (2017).
39. Kondo, J. et al. Retaining cell-cell contact enables preparation and culture of spheroids composed of pure primary cancer cells from colorectal cancer. *Proc. Natl Acad. Sci. USA* **108**, 6235–6240 (2011).
40. Chen, B. et al. Small molecule-mediated disruption of Wnt-dependent signaling in tissue regeneration and cancer. *Nat. Chem. Biol.* **5**, 100–107 (2009).
41. Hao, H. X. et al. ZNRF3 promotes Wnt receptor turnover in an R-spondin-sensitive manner. *Nature* **485**, 195–200 (2012).
42. Koo, B. K. et al. Tumour suppressor RNF43 is a stem-cell E3 ligase that induces endocytosis of Wnt receptors. *Nature* **488**, 665–669 (2012).
43. Cristobal, A. et al. Personalized proteome profiles of healthy and tumor human colon organoids reveal both individual diversity and basic features of colorectal cancer. *Cell Rep.* **18**, 263–274 (2017).
44. Schutte, M. et al. Molecular dissection of colorectal cancer in pre-clinical models identifies biomarkers predicting sensitivity to EGFR inhibitors. *Nat. Commun.* **8**, 14262 (2017).
45. Seino, T. et al. Human pancreatic tumor organoids reveal loss of stem cell niche factor dependence during disease progression. *Cell Stem Cell* **22**, 454–467.e6 (2018).
46. Barretina, J. et al. The cancer cell line encyclopedia enables predictive modelling of anticancer drug sensitivity. *Nature* **483**, 603–607 (2012).
47. Vlachogiannis, G. et al. Patient-derived organoids model treatment response of metastatic gastrointestinal cancers. *Science* **359**, 920–926 (2018).
In this paper, Vlachogiannis and colleagues describe for the first time that drug responses in patient-derived tumour organoids recapitulate patient responses in the clinic.
48. Shenoy, T. R. et al. CHD1 loss sensitizes prostate cancer to DNA damaging therapy by promoting error-prone double-strand break repair. *Ann. Oncol.* **28**, 1495–1507 (2017).
49. Crespo, M. et al. Colonic organoids derived from human induced pluripotent stem cells for modeling colorectal cancer and drug testing. *Nat. Med.* **23**, 878–884 (2017).
50. Ballet, F. Hepatotoxicity in drug development: detection, significance and solutions. *J. Hepatol.* **26** (Suppl. 2), 26–36 (1997).
51. Meng, Q. Three-dimensional culture of hepatocytes for prediction of drug-induced hepatotoxicity. *Expert Opin. Drug Metab. Toxicol.* **6**, 733–746 (2010).
52. Katsuda, T. et al. Conversion of terminally committed hepatocytes to culturable bipotent progenitor cells with regenerative capacity. *Cell Stem Cell* **20**, 41–55 (2016).
53. Eder, A., Vollert, I., Hansen, A. & Eschenhagen, T. Human engineered heart tissue as a model system for drug testing. *Adv. Drug Deliv. Rev.* **96**, 214–224 (2016).
54. Voges, H. K. et al. Development of a human cardiac organoid injury model reveals innate regenerative potential. *Development* **144**, 1118–1127 (2017).
55. Takasato, M. et al. Kidney organoids from human iPS cells contain multiple lineages and model human nephrogenesis. *Nature* **526**, 564–568 (2015).
56. Dekkers, J. F. et al. A functional CFTR assay using primary cystic fibrosis intestinal organoids. *Nat. Med.* **19**, 939–945 (2013).
57. Dekkers, J. F. et al. Characterizing responses to CFTR-modulating drugs using rectal organoids derived from subjects with cystic fibrosis. *Sci Transl Med* **8**, 344ra384 (2016).
58. Groenendijk, F. H. & Bernards, R. Drug resistance to targeted therapies: deja vu all over again. *Mol. Oncol.* **8**, 1067–1083 (2014).
59. Bernards, R. A missing link in genotype-directed cancer therapy. *Cell* **151**, 465–468 (2012).
60. Sharma, P. & Allison, J. P. The future of immune checkpoint therapy. *Science* **348**, 56–61 (2015).
61. Chen, D. S. & Mellman, I. Elements of cancer immunity and the cancer-immune set point. *Nature* **541**, 321–330 (2017).
62. Van Allen, E. M. et al. Genomic correlates of response to CTLA-4 blockade in metastatic melanoma. *Science* **350**, 207–211 (2015).
63. Rizvi, N. A. et al. Cancer immunology. mutational landscape determines sensitivity to PD-1 blockade in non-small cell lung cancer. *Science* **348**, 124–128 (2015).
64. Sato, T. & Clevers, H. Snapshot: growing organoids from stem cells. *Cell* **161**, 1700–1700 e1 (2015).
65. Le, D. T. et al. PD-1 Blockade in tumors with mismatch-repair deficiency. *N. Engl. J. Med.* **372**, 2509–2520 (2015).
66. Nozaki, K. et al. Co-culture with intestinal epithelial organoids allows efficient expansion and motility analysis of intraepithelial lymphocytes. *J. Gastroenterol.* **51**, 206–213 (2016).
67. Finnberg, N. K. et al. Application of 3D tumoroid systems to define immune and cytotoxic therapeutic responses based on tumoroid and tissue slice culture molecular signatures. *Oncotarget* **8**, 66747–66757 (2017).
68. Zumwalde, N. A. et al. Analysis of immune cells from human mammary ductal epithelial organoids reveals Vdelta2+ T Cells that efficiently target breast carcinoma cells in the presence of bisphosphonate. *Cancer Prev. Res.* **9**, 305–316 (2016).
69. Stronen, E. et al. Targeting of cancer neoantigens with donor-derived T cell receptor repertoires. *Science* **352**, 1337–1341 (2016).
70. Tajima, A., Pradhan, I., Trucco, M. & Fan, Y. Restoration of thymus function with bioengineered thymus organoids. *Curr. Stem Cell Rep.* **2**, 128–139 (2016).
71. De Flora, S. & Bonanni, P. The prevention of infection-associated cancers. *Carcinogenesis* **32**, 787–795 (2011).
72. Salama, N. R., Hartung, M. L. & Muller, A. Life in the human stomach: persistence strategies of the bacterial pathogen *Helicobacter pylori*. *Nat. Rev. Microbiol.* **11**, 385–399 (2013).
73. Huang, J. Y. et al. Chemodetection and destruction of host urea allows *Helicobacter pylori* to locate the epithelium. *Cell Host Microbe* **18**, 147–156 (2015).
74. McCracken, K. W. et al. Modelling human development and disease in pluripotent stem-cell-derived gastric organoids. *Nature* **516**, 400–404 (2014).
75. Scanu, T. et al. *Salmonella* manipulation of host signaling pathways provokes cellular transformation associated with gallbladder carcinoma. *Cell Host Microbe* **17**, 763–774 (2015).
76. Yin, Y. et al. Modeling rotavirus infection and antiviral therapy using primary intestinal organoids. *Antiviral Res.* **123**, 120–131 (2015).
77. Stratton, M. R., Campbell, P. J. & Futreal, P. A. The cancer genome. *Nature* **458**, 719–724 (2009).
78. Behjati, S. et al. Genome sequencing of normal cells reveals developmental lineages and mutational processes. *Nature* **513**, 422–425 (2014).
This study is the first to exploit genome sequencing of clonal organoids as a tool to study mutational processes.
79. Blokzijl, F. et al. Tissue-specific mutation accumulation in human adult stem cells during life. *Nature* **538**, 260–264 (2016).
80. Alexandrov, L. B. et al. Signatures of mutational processes in human cancer. *Nature* **500**, 415–421 (2013).
81. Davies, H. et al. HRDetect is a predictor of BRCA1 and BRCA2 deficiency based on mutational signatures. *Nat. Med.* **23**, 517–525 (2017).
82. Bryant, H. E. et al. Specific killing of BRCA2-deficient tumours with inhibitors of poly(ADP-ribose) polymerase. *Nature* **434**, 913–917 (2005).
83. Fong, P. C. et al. Inhibition of poly(ADP-ribose) polymerase in tumors from BRCA mutation carriers. *N. Engl. J. Med.* **361**, 123–134 (2009).
84. Farmer, H. et al. Targeting the DNA repair defect in BRCA mutant cells as a therapeutic strategy. *Nature* **434**, 917–921 (2005).
85. Drost, J. et al. Use of CRISPR-modified human stem cell organoids to study the origin of mutational signatures in cancer. *Science* **358**, 234–238 (2017).
86. Greenman, C. et al. Patterns of somatic mutation in human cancer genomes. *Nature* **446**, 153–158 (2007).
87. McGranahan, N. & Swanton, C. Clonal heterogeneity and tumor evolution: past, present, and the future. *Cell* **168**, 613–628 (2017).
88. Pauli, C. et al. Personalized in vitro and in vivo cancer models to guide precision medicine. *Cancer Discov.* **7**, 462–477 (2017).
89. Roerink, S. et al. A high-resolution molecular history of intra-cancer diversification. *Nature* https://doi.org/10.1038/s41586-018-0024-3 (2018).
90. Lugli, N. et al. Enhanced rate of acquisition of point mutations in mouse intestinal adenomas compared to normal tissue. *Cell Rep.* **19**, 2185–2192 (2017).
91. Matano, M. et al. Modeling colorectal cancer using CRISPR-Cas9-mediated engineering of human intestinal organoids. *Nat. Med.* **21**, 256–262 (2015).
92. Rajagopalan, H., Nowak, M. A., Vogelstein, B. & Lengauer, C. The significance of unstable chromosomes in colorectal cancer. *Nat. Rev. Cancer* **3**, 695–701 (2003).
93. Fumagalli, A. et al. Genetic dissection of colorectal cancer progression by orthotopic transplantation of engineered cancer organoids. *Proc. Natl Acad. Sci. USA* **114**, E2357–E2364 (2017).
94. Fumagalli, A. et al. A surgical orthotopic organoid transplantation approach in mice to visualize and study colorectal cancer progression. *Nat. Protoc.* **13**, 235–247 (2018).
95. Li, X. et al. Oncogenic transformation of diverse gastrointestinal tissues in primary organoid culture. *Nat. Med.* **20**, 769–777 (2014).
96. Melo, F. S. et al. A distinct role for Lgr5+ stem cells in primary and metastatic colon cancer. *Nature* **543**, 676–680 (2017).
97. O'Rourke, K. P. et al. Transplantation of engineered organoids enables rapid generation of metastatic mouse models of colorectal cancer. *Nat. Biotechnol.* **35**, 577–582 (2017).
98. Roper, J. et al. In vivo genome editing and organoid transplantation models of colorectal cancer and metastasis. *Nat. Biotechnol.* **35**, 569–576 (2017).
99. Shimokawa, M. et al. Visualization and targeting of LGR5+ human colon cancer stem cells. *Nature* **545**, 187–192 (2017).
References 96 and 99 describe the use of intestinal organoids to study the contribution of CSCs to primary and metastatic CRC.
100. Wang, K. et al. Whole-genome sequencing and comprehensive molecular profiling identify new driver mutations in gastric cancer. *Nat. Genet.* **46**, 573–582 (2014).
101. Leggett, B. & Whitehall, V. Role of the serrated pathway in colorectal cancer pathogenesis. *Gastroenterology* **138**, 2088–2100 (2010).
102. Fessler, E. et al. TGFbeta signaling directs serrated adenomas to the mesenchymal colorectal cancer subtype. *EMBO Mol. Med.* **8**, 745–760 (2016).
103. Yan, H. H. et al. RNF43 germline and somatic mutation in serrated neoplasia pathway and its association with BRAF mutation. *Gut* **66**, 1645–1656 (2016).
104. Schwank, G. et al. Functional repair of CFTR by CRISPR/Cas9 in intestinal stem cell organoids of cystic fibrosis patients. *Cell Stem Cell* **13**, 653–658 (2013).
105. Koo, B. K., van Es, J. H., van den Born, M. & Clevers, H. Porcupine inhibitor suppresses paracrine Wnt-driven growth of Rnf43; Znrf3-mutant neoplasia. *Proc. Natl Acad. Sci. USA* **112**, 7548–7550 (2015).
106. Nadauld, L. D. et al. Metastatic tumor evolution and organoid modeling implicate TGFBR2 as a cancer driver in diffuse gastric cancer. *Genome Biol.* **15**, 428 (2014).
107. Dow, L. E. et al. Apc restoration promotes cellular differentiation and reestablishes crypt homeostasis in colorectal cancer. *Cell* **161**, 1539–1552 (2015).
108. Yin, X. et al. Engineering stem cell organoids. *Cell Stem Cell* **18**, 25–38 (2016).
109. Workman, M. J. et al. Engineered human pluripotent-stem-cell-derived intestinal tissues with a functional enteric nervous system. *Nat. Med.* **23**, 49–59 (2017).

110. Ohlund, D. et al. Distinct populations of inflammatory fibroblasts and myofibroblasts in pancreatic cancer. *J. Exp. Med.* **214**, 579–596 (2017).
111. Gjorevski, N. et al. Designer matrices for intestinal stem cell and organoid culture. *Nature* **539**, 560–564 (2016).
In this paper, Gjorevski and colleagues engineer synthetic matrices supporting expansion of intestinal organoids, which hold great potential for the applications of organoids in regenerative medicine.
112. Janda, C. Y. et al. Surrogate Wnt agonists that phenocopy canonical Wnt and beta-catenin signalling. *Nature* **545**, 234–237 (2017).
113. Mihara, E. et al. Active and water-soluble form of lipidated Wnt protein is maintained by a serum glycoprotein afamin/alpha-albumin. *eLife* **5**, pii: e11621 (2016).
114. Tuysuz, N. et al. Lipid-mediated Wnt protein stabilization enables serum-free culture of human organ stem cells. *Nat. Commun.* **8**, 14578 (2017).
115. Hubert, C. G. et al. A three-dimensional organoid culture system derived from human glioblastomas recapitulates the hypoxic gradients and cancer stem cell heterogeneity of tumors found in vivo. *Cancer Res.* **76**, 2465–2477 (2016).
116. Sachs, N. & Clevers, H. Organoid cultures for the analysis of cancer phenotypes. *Curr. Opin. Genet. Dev.* **24**, 68–73 (2014).

Acknowledgements
The authors thank K. Kretzschmar and J. Meijerink for critical reading of the manuscript. We are grateful for support from the Dutch Cancer Society (KWF) and the Alpe d'HuZes Bas Mulder Award to J.D. (KWF/Alpe d'HuZes, 10218) and for the support of Oncode Institute to H.C.

Author contributions
J.D. and H.C. researched data for the article, wrote the article and reviewed and/or edited the manuscript before submission.

Competing interests
J.D. and H.C. are named as inventors on several patents related to leucine-rich repeat-containing G protein-coupled receptor 5 (LGR5)$^+$ stem cell-based organoid technology.

Publisher's note
Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.