ANNUAL REVIEWS

Annual Review of Pathology: Mechanisms of Disease

Human Organoids: Tools for Understanding Biology and Treating Diseases

Frans Schutgens[1,2] and Hans Clevers[1,3]

[1]Hubrecht Institute, Royal Netherlands Academy of Arts and Sciences, 3584 CT Utrecht, The Netherlands; email: h.clevers@hubrecht.eu

[2]Department of Pathology, Amsterdam University Medical Centers, Location VU Medical Center, 1081 HV Amsterdam, The Netherlands

[3]Princess Máxima Center for Pediatric Oncology, 3584 CS Utrecht, The Netherlands

Annu. Rev. Pathol. Mech. Dis. 2020. 15:211–34

First published as a Review in Advance on September 24, 2019

The *Annual Review of Pathology: Mechanisms of Disease* is online at pathol.annualreviews.org

https://doi.org/10.1146/annurev-pathmechdis-012419-032611

Keywords

organoids, adult stem cell, model system, personalized medicine, biobank

Abstract

Organoids are in vitro–cultured three-dimensional structures that recapitulate key aspects of in vivo organs. They can be established from pluripotent stem cells and from adult stem cells, the latter being the subject of this review. Organoids derived from adult stem cells exploit the tissue regeneration process that is driven by these cells, and they can be established directly from the healthy or diseased epithelium of many organs. Organoids are amenable to any experimental approach that has been developed for cell lines. Applications in experimental biology involve the modeling of tissue physiology and disease, including malignant, hereditary, and infectious diseases. Biobanks of patient-derived tumor organoids are used in drug development research, and they hold promise for developing personalized and regenerative medicine. In this review, we discuss the applications of adult stem cell–derived organoids in the laboratory and the clinic.

1. INTRODUCTION

Most research on human cells during the past decades has focused on two-dimensional (2D) cultured cell lines. Classical cell lines are relatively cheap, easy to handle, and amenable to a multitude of experimental techniques. However, their initial establishment is highly inefficient and involves extensive genetic and phenotypic adaptation to culture conditions. Thus, cell lines are almost invariably derived from tumors or have acquired oncogenic potential in vitro. Importantly, when used to represent diseased (tumor) cells, matching normal cells are lacking. Another drawback is the typical absence of most, if not all, of the differentiated cell types present in the original tissue. Together, these issues limit the use of cell lines in personalized medicine and make them less suited to studying tissue physiology that involves multiple differentiated cell types.

In cancer research, an alternative model system is used: patient-derived xenografts (PDXs). In this model, primary tumor tissue is transplanted into immunodeficient mice. PDXs better retain the complexity and heterogeneity of the parental tumor than do cell lines, but establishment is still relatively inefficient, and aggressive tumors are the easiest to establish (1). In addition, high-throughput analyses are expensive and are encumbered by complex logistics.

In recent years, organoid cultures have been developed that avoid many of the disadvantages associated with cell lines. An organoid is defined as a 3D structure grown from stem cells that consists of organ-specific cell types that self-organize through cell sorting and spatially restricted lineage commitment (2). Organoid cultures can be established from embryonic stem cells or induced pluripotent stem cells (together referred to as pluripotent stem cells, or PSCs) and from adult stem cells (ASCs) (2, 3).

Both PSC- and ASC-derived organoids require a source of extracellular matrix to serve as the basal lamina for the cells in culture. In most cases, Basement Membrane Extract (R&D Systems) or Matrigel (Corning), which are rich in laminins and collagens, are used. Both types of organoids have proved amenable to all standard laboratory techniques, as well as to clustered regularly interspaced short palindromic repeats-Cas9 (CRISPR-Cas9)–based genetic modification (4–6). Yet fundamental differences between PSC- and ASC-derived organoids exist, making them complementary model systems. PSC-derived organoids form structures through processes that occur only during embryonic development (2), thus recapitulating in vivo development. Typically, PSCs are expanded and subsequently differentiated through a multistep protocol that moves toward a fully differentiated structure, and specific cocktails of growth factors are needed for each step (**Figure 1**). The duration of this differentiation depends on the type of tissue, but generally it takes approximately 2–3 months (7). PSC-derived organoids are structurally complex (or organ-like) and may contain mesenchymal as well as epithelial and, sometimes, endothelial components. Because differentiation protocols recapitulate development ex vivo, PSC-derived organoids are excellent models for studying development (8) and genetic diseases (9), notably for organs that once established show little, if any, regenerative capacity, such as the brain or the renal glomerulus. This type of organoid has also been used to study infectious disease, including infections that occur during development, such as Zika virus infection (10). PSC-derived organoids were first developed for brain (11) and later for other organs, including stomach, liver, intestine, lung, and kidney (7, 12–15). Of note, each germ layer (endoderm, mesoderm, and ectoderm) is represented among this set of organs.

When induced PSCs need to be derived as the first step, the generation of PSC-derived organoids typically takes months. Patient-derived cells (usually derived from skin fibroblasts) are first reprogrammed into PSCs, then expanded and subsequently differentiated into the desired tissue. The resulting, fully differentiated organoid often cannot be further expanded. Since cancer organoids necessarily need to be derived from cancer cells, the indirect PSC route appears less

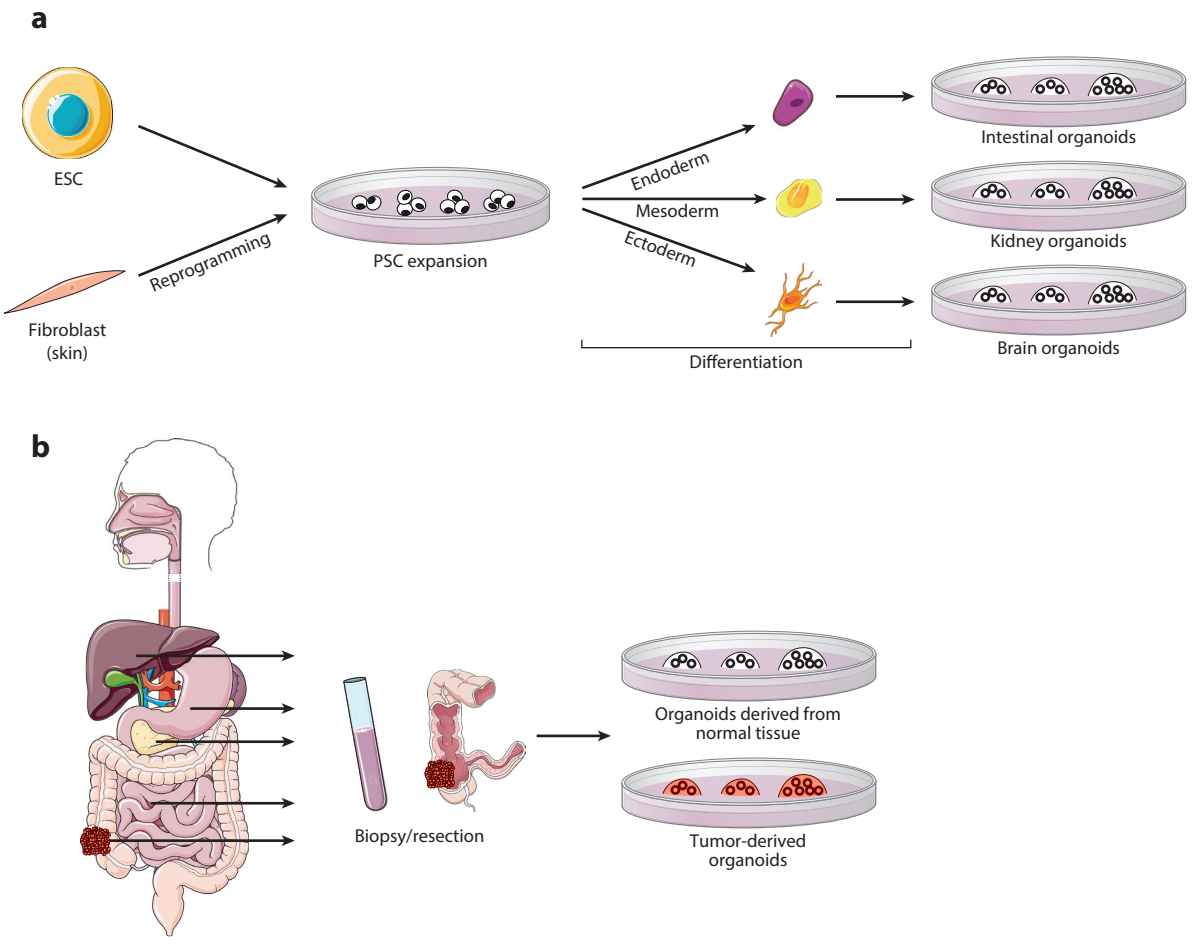
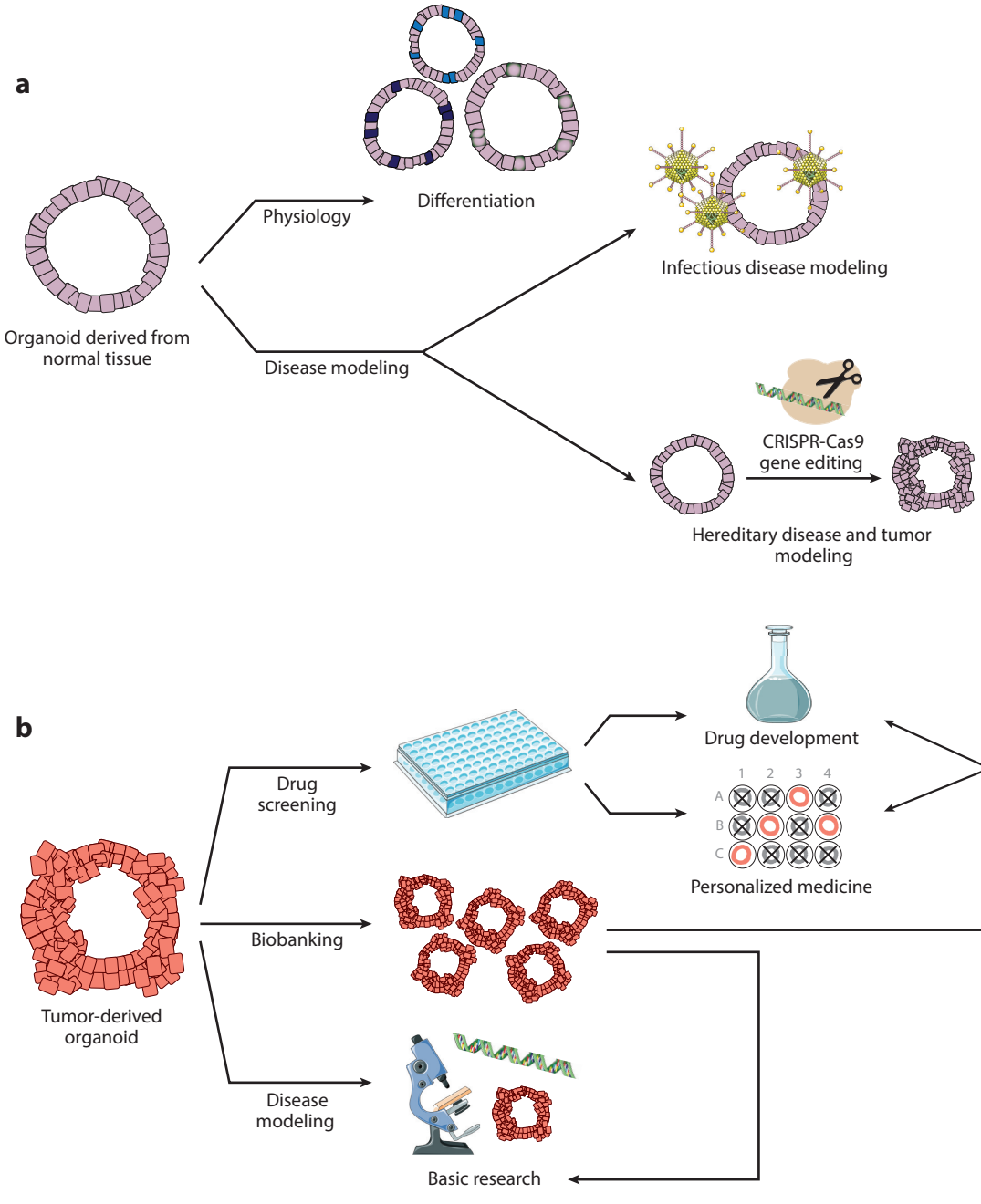


Figure 1

Different strategies used to obtain organoids. (*a*) Pluripotent stem cell (PSC)–derived organoids use induced PSCs derived from cells such as reprogrammed skin fibroblasts or embryonic stem cells (ESCs), and these are then differentiated toward each of the three germ layers (endoderm, mesoderm, and ectoderm). Specific differentiation protocols are used to obtain the tissue of interest, such as intestine, kidney, and brain. (*b*) Adult stem cell–derived organoids use samples from biopsies or resections from many organs, including normal liver, pancreas, and intestine, or from cancers that occur in these organs, and these samples are then used to obtain, respectively, organoid cultures derived from normal epithelial tissue and tumor tissue–derived organoid cultures. This figure was created using Servier Medical Art and licensed under a Creative Commons Attribution 3.0 Unported License.

appropriate than direct culturing of the tumor cells as organoids, particularly if personalized cancer modeling is the objective. Of note, the differentiation process of PSCs is not 100% efficient and unintended (although sometimes interesting) cell types may arise: Differentiation toward kidney organoids using optimal protocols yielded 10–20% nonrenal cells, including a neuronal lineage (16). PSC-derived organoids and their applications have been reviewed elsewhere (2, 17). In the remainder of this review, we discuss how ASC-derived organoids are applied in experimental biology and personalized medicine (**Figure 2**).

In contrast to PSC-derived organoids that model development, ASC-derived epithelial organoids recapitulate adult tissue repair (2). Consequently, ASC-derived organoids can be established only from tissue compartments with regenerative capacity. At present, essentially all



(*Caption appears on following page*)

Figure 2 (*Figure appears on preceding page*)

Applications of adult stem cell–derived organoids. (*a*) Organoids derived from normal tissue are useful for studying physiology, for example, for determining differentiation processes in the gut. For disease modeling, normal organoids can be infected with different types of pathogens. In addition, by using CRISPR-Cas9, organoids can be genetically engineered to model genetic and malignant diseases. (*b*) Tumor-derived organoids can be used for drug screening studies, both for personalized medicine (to determine the most effective treatment for a specific patient) and drug development (to test a compound library on a specific set of tumor organoids). In addition, tumor-derived organoids can be used directly in basic cancer biology research. More broadly, collections of diseased organoids can be stored in biobanks and used subsequently in basic or applied research. This figure was created using Servier Medical Art and licensed under a Creative Commons Attribution 3.0 Unported License.

ASC-derived organoid types represent only the epithelial parts of organs, and there is an absence of stroma, nerves, and vasculature. Hence, ASC-derived organoids are structurally of lower complexity than PSC-derived organoids. Human ASC-derived organoids are typically cystic, polarized epithelial structures (**Figure 3*a***), recapitulating the general architecture and functional aspects of the original epithelium (18–21).

In principle, ASC-derived organoids can be derived from any individual and from normal epithelial tissues as well as from malignant (22) or otherwise diseased (23) epithelium within approximately 7 days after seeding of the cells of the parental tissues (**Figure 1**). The resulting structures can be expanded long term while remaining genetically stable (2). Thus, this type of organoid culture allows the direct parallel expansion of diseased cells and matched normal cells from individual patients, which facilitates its potential application in personalized therapy.

ASC organoid technology was first developed for the intestine (20, 24) after the identification of *Lgr5* (25) as a marker of Wnt-driven adult gut stem cells. By providing the essential factors of the in vivo intestinal stem cell niche [the Wnt agonist R-spondin, epidermal growth factor (EGF),

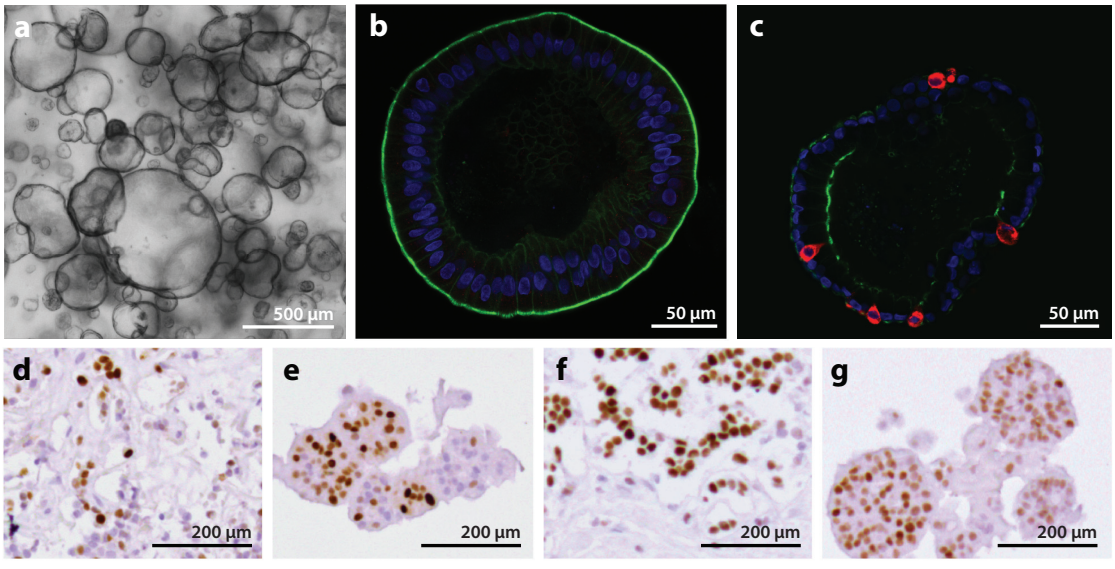


Figure 3

Morphology of several types of human adult stem cell–derived organoids. (*a*) Bright-field image of a typical cystic kidney organoid culture. (*b*) Confocal image of an intestinal organoid in expansion medium (*blue* indicates DAPI staining and *green* indicates F-actin staining) and (*c*) in enteroendocrine differentiation medium (*blue* indicates DAPI, *green* indicates F-actin, and *red* indicates chromogranin A). (*d*) Breast cancer tissue stained for progesterone receptor (PR) and (*f*) estrogen receptor (ER) positivity and the corresponding breast cancer organoid line with similar (*e*) PR and (*g*) ER positivity.

and the bone morphogenetic protein (BMP) inhibitor Noggin], Sato and colleagues (20, 24) were able to culture adult Lgr5-positive stem cells into self-organizing, ever-expanding crypt–villus-like organoids.

By modifying cocktails of growth factors and cell isolation procedures, similar protocols were used to develop normal and cancerous organoids from other organs, including the stomach (18), esophagus (20), salivary gland (26), fallopian tube (27), ovary (28), liver (21, 29, 30), prostate (31), pancreas (19), breast (32), airway (33), colon (22), taste buds (34), endometrium (35), and kidney (36).

Recently, a striking new organoid type (37) was established: trophoblast organoids resembling first trimester human placenta. These could be differentiated into the two main trophoblast subpopulations: extravillous trophoblasts (for attachment to the maternal endometrium, or decidua) and syncytiotrophoblasts (for hormone production and nutrient exchange). Histologically, the organoids resembled the villus structures of the placenta. Several hormones were produced, including sufficient human chorionic gonadotropin to produce a positive pregnancy test with conditioned media from a dish containing trophoblast organoids.

In most cases, including in human intestine (38, 39) and liver (21), a distinct growth factor cocktail is required to obtain more differentiated cells in the organoids, thus resulting in the use of separate expansion and differentiation media. For example, inhibition of Notch and mitogen-activated protein kinase kinase in human intestinal organoids led to enteroendocrine cell differentiation (38, 39) (**Figure 3*b*,*c***). However, because ASCs are already specified (i.e., are lineage committed), this process of differentiation usually takes 3–5 days at most.

Culturing tumor organoids is often complicated by an overgrowth of normal epithelium, which, in some instances, can be prevented by using cancer-specific selection methods. An overgrowth of normal cells seems counterintuitive, yet it is consistently observed. It should be kept in mind that healthy tissue proliferates only when lost tissue needs to be replaced, in contrast to tumors that continuously expand. Thus, while normal cells are superior proliferators compared with tumor cells, they proliferate only when required—that is, when they are prompted by growth factors released as a damage response. The organoid growth factor cocktail removes the selective advantages that tumor cells have in vivo. This applies to organoids derived both from tissues that have a high turnover and from those with a low turnover. For example, colorectal tumors often carry mutations that activate the Wnt pathway and allow cell proliferation in vivo without an actual Wnt signal being present, whereas in vitro, a Wnt signal is provided in the organoid culture medium. Indeed, removing Wnt from the culture medium allows for the expansion of colon cancer cells, while normal epithelial cells arrest (22). Thus, it is feasible to enable the selective outgrowth of tumor cells in vitro by exploiting the genetic background of tumor cells and withdrawing growth factors that are essential for the proliferation of healthy cells. A second method to selectively culture tumor cells is based on adding the MDM2 inhibitor Nutlin-3, which stabilizes wild-type P53 (5). Tumor cells with a loss of *TP53* (e.g., occurring in 47% of sporadic ovary tumors and 43% of colorectal cancers, but only in 5% of cervical cancers) (40) are not affected by Nutlin-induced stabilization of P53. However, in normal cells in culture, this leads to cell cycle arrest and death, allowing for the selective outgrowth of tumor cells.

2. ORGANOIDS AS EXPERIMENTAL TOOLS

2.1. Tissue Physiology

Organoid technology has already expanded the study of physiology ex vivo for diverse reasons: Organoid culture allows for the generation of specific cell types that were previously impossible to culture; organoids contain multiple differentiated cell types; and organoids are genetically stable.

As an example, organoid culture has allowed the in vitro culture and proliferation of hepatocytes (30, 41). Hitherto, attempts at the long-term in vitro culture of human hepatocytes in 2D cultures have failed. Based on a previous study that enabled the culture of bipotent biliary tree–derived progenitor organoids (21), culture conditions were developed that supported the growth of mouse and human hepatocyte organoids (fetal as well as adult) that could be expanded for more than 20 passages (30). The resulting hepatocytes contained cytoplasmic glycogen particles, formed bile canaliculi, and expressed albumin and cytochrome P450 enzymes at physiological levels, demonstrating the mature hepatocyte nature of the cultures. Peng and colleagues (41) were able to similarly culture mouse hepatocytes. In their system, the authors described a unique effect of tumor necrosis factor-α, a cytokine essential for liver regeneration in vivo (42). More generally, this study implicated the addition of regeneration-enhancing cytokines in facilitating the in vitro expansion of cell types that are otherwise difficult to culture.

The study of physiological phenomena requires the coculture of multiple cell types. For example, to study the effect of growth factors on the differentiation of stem cells, a culture system containing stem cells that can be differentiated toward different fates is essential. After establishing a protocol to obtain enteroendocrine cells in organoids, Beumer and colleagues (38, 39) used organoids to study the effect of growth factors on hormone expression in enteroendocrine cells. In organoids, hormones in enteroendocrine cells were differentially expressed based on the presence or absence of BMP4. This finding was further studied in a mouse model, and it was found that the BMP gradient along the crypt–villus axis in vivo dictates a switch in expressed hormones in enteroendocrine cells that migrate up this BMP gradient.

The genetic stability of organoids and the fact that organoids can be established from a single cell make it possible to study the mutational status of single stem cells, as organoid lines established from a single stem cell yield sufficient DNA for whole-genome sequencing. In this way, Blokzijl and colleagues (43) were able to unveil the mutations that accumulate in single human stem cells throughout life (with peripheral blood as a reference for germline mutations). Interestingly, the mutation rate, with approximately 40 novel mutations per year per stem cell, was similar in liver, small intestine, and colon stem cells. However, the types of mutations detected and the resulting mutational signatures in colon and small intestine cells were different from those in liver cells.

A more comprehensive review on using organoids to model tissue physiology can be found elsewhere (2).

2.2. Disease Models

2.2.1. Infectious disease. ASC-derived organoids have been used to model parasitic, bacterial, and viral infectious diseases, including diseases caused by pathogens that previously could not be studied in vitro. These models recapitulate features of in vivo infection.

The protozoan parasite *Cryptosporidium* causes life-threatening diarrhea in immunocompromised individuals (e.g., people living with HIV and malnourished children) (44), and infection may spread to the lungs. *Cryptosporidium*'s complex life cycle, comprising sexual and asexual parts, could not be easily studied in in vitro models, hampering research into its pathophysiology. In contrast, intestinal and lung airway organoids allowed the asexual and sexual life cycle of *Cryptosporidium* to be completed for multiple rounds (45).

Organoids can be cocultured with bacteria. This facilitates the study of mechanisms of infection as well as, for instance, the bacterial contribution to carcinogenesis. *Helicobacter pylori*, a cause of chronic gastritis, peptic ulcers, and stomach cancer (46), was injected into stomach organoids. Upon luminal injection, which ensured the appropriate apical localization of *H. pylori*, a potent nuclear factor κB–mediated inflammatory response occurred (18). In a follow-up study in organoids,

the mechanism of how *H. pylori* finds its gastric niche was elucidated: The gastric epithelium produces urea, and the bacteria possess a sensitive system for detecting urea, which functions as a chemoattractant (47).

Chronic *Salmonella* infection of the gall bladder is associated with gall bladder carcinoma (48). Scanu and colleagues (49) showed that infection with this bacterium has a direct oncogenic effect in both $APC^{+/-}$ mice and pretransformed murine gall bladder organoids. Gall bladder organoids that lack functional *TP53* were infected with *Salmonella enterica* serovar Typhimurium at the single-cell stage. The resulting infected organoids displayed tumor-like characteristics. First, infected organoids were able to grow in the absence of factors that were essential for the growth of uninfected controls. Second, infected organoids showed histopathological signs of transformation, such as a loss of polarity and polymorphic nuclei.

Viral infection can also be studied in organoids, including infection with viruses that do not replicate in classical, transformed 2D cell lines. The study of human noroviruses, the most common cause of foodborne acute gastroenteritis (50), has been limited by the lack of a model that allows in vitro viral replication. Intestinal organoids that were cultured as monolayers (another strategy used to ensure infection on the apical side) allowed for extensive replication of multiple strains of noroviruses. For some strains, the addition of bile to the culture medium was required for replication (51), indicating that not only are in vivo–like host cells required for productive infection but also an in vivo–like environment is relevant as well.

BK virus is a polyomavirus that infects 1–10% of transplanted kidneys, leading in 10–80% of these infected kidneys to the loss of the donor organ (52). Infection of kidney tubuloids (kidney-derived organoids in which only the tubular epithelium of the kidney is represented and glomeruli are lacking) with BK virus yielded a patchy infection with nuclei that increased in diameter (due to intranuclear basophilic viral inclusions), similar to what is observed in kidney biopsies from patients with BK virus nephropathy (53).

Respiratory syncytial virus (RSV) causes an estimated 66,000–199,000 deaths annually among children, mostly in developing countries (54). Infection of airway organoids with RSV recapitulated typical in vivo disease phenomena, including syncytia formation, cytoskeletal changes, and shedding of epithelial cells (55). RSV-infected organoids attracted neutrophils more than did mock-infected control organoids, making this the first organoid model suitable for studying neutrophil–epithelium interactions. Intriguingly, RSV infection strongly increased organoid motility and ultimately resulted in organoid fusion.

Influenza viruses pose a major public health problem worldwide, and emerging viruses may cause highly lethal disease, as evidenced by the poultry-derived H7N9 virus infection that has had a 39% mortality rate since 2013 (56). An in vitro tool to assess the infectivity of emerging viruses is currently lacking: Cell lines do not recapitulate airway histology, while bronchus explant culture, which is used as a parameter in the World Health Organization's Tool for Influenza Pandemic Risk Assessment (57), is restricted by the limited availability and viability of these tissues. These latter drawbacks would be mitigated by easily expandable airway organoids. The infection of differentiated airway organoids—cultured in 3D or as a 2D monolayer—with distinct strains of influenza virus can discriminate between poorly infective and highly infective strains (58). Importantly, in a direct comparison with ex vivo cultured human bronchus explant cultures (59), the infection of airway organoids yielded similar results regarding virus replication and cytokine response. Taken together, these studies highlight the potential of airway organoids for modeling influenza virus infection and predicting the infectivity of emerging viruses.

2.2.2. Genetic disease. Organoids derived from patients with genetic disease have also been shown to recapitulate disease phenotypes.

Liver organoids have been established from patients with α1-antitrypsin (A1AT) deficiency. A1AT deficiency results in insufficient protection of the lungs against neutrophil elastase, which leads to the destruction of lung parenchyma. In parallel, accumulation of mutant A1AT in the endoplasmic reticulum in the liver leads to fibrosis or cirrhosis. Indeed, liver organoids from patients contained A1AT aggregates and showed increased apoptosis, which might ultimately result in fibrosis and cirrhosis (21). Alagille syndrome is caused by loss-of-function mutations in *JAG1* or *NOTCH2* and leads to partial or complete biliary atresia. Accordingly, in organoids derived from an Alagille patient, the differentiation of organoids toward the biliary fate was not possible, whereas in expansion conditions, no differences with healthy controls were observed (21).

Intestinal organoids derived from a patient with microvillus inclusion disease caused by a syntaxin-3 mutation (60) showed partial loss of brush border microvilli and subapical accumulation of vesicles, which are typical histological phenomena of the disease. In addition, patients with multiple intestinal atresia caused by mutations in *TTC7A* (leading to the loss of the protein) display disrupted intestinal epithelial barriers. Analogously, organoids derived from these patients showed loss of apical–basal cell polarity that could be prevented by the addition of Rho kinase inhibitors (61).

Cystic fibrosis (CF) is a monogenic channelopathy caused by inactivating mutations in the CF transmembrane conductance regulator (*CFTR*) gene. Reduced CFTR function affects multiple organs (see the sidebar titled Organoid-Based Forskolin-Induced Swelling Assay to Assess Treatment Efficacy in Cystic Fibrosis) and results in reduced chloride transport through CFTR toward the extracellular space, leading to a reduced water flow by osmosis and, consequently, an increased density of mucus. In the lung, this results in recurrent pneumonia, which, in turn, leads to fibrosis. Early work with rectal organoids from CF patients revealed their usefulness for demonstrating CFTR function: Wild-type organoids rapidly swell upon opening the CFTR channel through the addition of forskolin (FSK) (as explained in the sidebar) (62). This response does not occur in organoids from CF patients, but it is restored upon preincubation with recently developed

ORGANOID-BASED FORSKOLIN-INDUCED SWELLING ASSAY TO ASSESS TREATMENT EFFICACY IN CYSTIC FIBROSIS

Cystic fibrosis (CF) is a monogenic channelopathy caused by the loss or decreased function of the chloride channel cystic fibrosis transmembrane conductance regulator (CFTR) as a result of a wide range of mutations in *CFTR*. The effects are altered chloride transport, namely a reduction in chloride transport from the cytoplasm into the extracellular compartment in the lungs and pancreas, and an increase in chloride transport from the extracellular compartment into the cytoplasm in the sweat glands. In the sweat glands, loss of CFTR function leads to a high saline concentration in sweat, whereas in the lungs and gastrointestinal organs, it leads to reduced water flow by osmosis and, hence, increased mucus viscosity.

The forskolin (FSK)-induced swelling assay (62) involves intestinal organoids for which lines are established from rectal biopsies. It is based on the fact that FSK opens CFTR in a cyclic adenosine monophosphate (cAMP)-dependent manner, while in the intestine, CFTR is the only channel that opens in a cAMP-dependent manner. As a consequence of CFTR opening on the apical membrane, chloride moves from the cytoplasm into the lumen of the organoid. Fluid follows, presumably by osmosis, leading to rapid swelling of the organoid. Without functional CFTR, no or less swelling occurs. Subsequently, drugs can be tested using the restoration of swelling as a straightforward readout to determine the most effective drug for restoring CFTR function. This assay correlates well with the patient's clinical response. As a result of this high-throughput, robust swelling assay, the use of organoids in personalized medicine has advanced further for CF than for any other disease.

CF drugs (62) or upon CRISPR-Cas9 correction of the *CFTR* mutation (4). The rectal organoid swelling test has proven to be an excellent predictor of drug response (63). CF organoids were also established from bronchoalveolar lavage fluids (no biopsies were required). CF airway organoids had an increased mucus layer, recapitulating the disease phenotype. FSK-induced swelling in airway organoids was reduced compared with organoids from normal controls and could be restored with CFTR-restoring compounds. However, in contrast to rectal organoids, FSK-induced swelling in lung organoids did not depend on CFTR alone, but it was also influenced by the chloride transporter TMEM16A, which is a potential therapeutic target in CF (64). As such, airway organoids may function as an additional platform for evaluating drug efficacy in CF, particularly for drugs acting on TMEM16A, as this protein is not expressed in rectal epithelium. For a discussion of the use of rectal organoids for personalized medicine, see Section 4.4.

2.2.3. Cancer. To study cancer, organoids have been applied in two different ways. First, tumor organoids have been directly derived from patients' tumors, as first established in 2011 (20). Second, normal epithelial organoids have been genetically engineered to study the role of specific mutations in tumorigenesis. In order to model metastatic disease, both engineered and patient-derived organoids have been orthotopically transplanted into mice.

The high culture efficiency of organoids enables the expansion of single cancer cells. Genetic analyses of the resulting organoid lines allow for the study of tumor heterogeneity. In a study published in 2018, clonal organoid lines were established from between four and six regions of three colon tumors (65). It was found that the number and type of mutations varied per region of the tumor. Heterogeneity was also observed at the level of the transcriptome and methylome. Of note, drug screening experiments showed that cells resistant to each of the tested drugs were present in the three tumors analyzed, even though the patients did not undergo any neoadjuvant treatment.

Organoids can be genetically engineered using CRISPR-Cas9. The technique has been extensively applied to study the effects of specific oncogenic mutations using isogenic controls. In wild-type intestinal organoids, the sequential introduction of mutations in *KRAS*, *APC*, *SMAD4*, and *TP53* (genes commonly mutated in colon cancer) resulted in an in vitro model of colorectal cancer (5, 66). Mutant cells were functionally selected based on the loss of growth factor dependencies: The activating mutation in *KRAS* results in EGF independence and, therefore, could be selected for by EGF withdrawal. Similarly, *APC* mutant cells could be selected for by withdrawal of Wnt and R-spondin, and *SMAD4* mutant cells could be selected for by the withdrawal of the BMP inhibitor Noggin. P53 mutant cells were selected for by the addition of Nutlin-3 (see Section 1). This sequential introduction of mutations allowed for the study of the effect of single mutations in an isogenic background, and it would not have been possible with tumor-derived organoids, as these do not contain premalignant cells with only a single mutation. Using this strategy, it was found that the loss of both *APC* and *TP53* was sufficient for aneuploidy and chromosome instability and that all four mutations were essential for a full-blown cancer phenotype upon xenotransplantation.

The loss of DNA mismatch repair enzymes, such as MutL homolog 1 (MLH1), is commonly seen in colorectal cancers, resulting in tumors with an extremely high mutational load. These tumors show microsatellite instability (MSI), as repetitive sequences in the genome, termed microsatellites, undergo changes in copy number following the loss of mismatch repair enzymes. Drost and colleagues (6) knocked out *MLH1* using CRISPR-Cas9 and cultured organoids for 2 months to allow mutations to accumulate. Subsequent DNA analyses of organoid lines derived from individual stem cells revealed an increase in mutational load compared with controls. The detected mutational profile was similar to that of MSI colorectal tumors. Using the same

strategy, the authors also studied the role of the endonuclease III-like protein 1 (*NTHL1*) gene, which is involved in base excision repair (67). Again, inactivation of this DNA repair gene resulted in increased mutational load. In the *NTHL1*-mutated organoids, a particular mutational signature (signature 30) was predominantly observed, which is a signature previously identified in breast cancer (68). Retrospectively, this same signature was identified in a breast cancer occurring in a patient with a germline *NTLH1* mutation. This study shows that organoids faithfully recapitulate in vivo mutagenesis and allow for the identification of mechanisms of tumor development.

In addition to point mutations and small indels, gene fusion has also been introduced in organoids using CRISPR-Cas9. Fusions between the androgen receptor (AR)-responsive transmembrane protease serine 2 (*TMPRSS2*) gene and E26 transformation-specific (ETS) gene family members occur in up to 80% of prostate cancers (69). *TMPRSS2* and *ERG* (the ETS-related gene most commonly involved in these fusions) are both located on chromosome 21 and separated by approximately 3 million base pairs. Using CRISPR-Cas9, a *TMPRSS2–ERG* fusion was successfully introduced into mouse prostate organoids using a template that brought these two DNA regions together. This genetic alteration resulted in AR-driven overexpression of *ERG*, an effect that was prevented by androgen-inhibiting agents, features matching those seen in vivo (70).

Xenotransplantation of engineered colorectal tumor organoids enabled the in vivo study of cancer stem cells (71, 72) and led to metastatic disease, thus designating organoids as a useful platform for studying mechanisms of metastasis (73, 74).

Engineered colorectal cancer mouse organoids expressing diphtheria toxin receptor fused to an enhanced green fluorescent protein from the *Lgr5* locus allowed visualization and specific elimination of Lgr5-positive stem cells (71). Using this model, it was found that in the absence of cancer stem cells, liver metastases did not occur, whereas primary tumors did not regress. This study indicates that Lgr5-positive cancer stem cells are required for metastatic disease and that targeting cancer stem cells in unresectable liver metastases may be a therapeutic option. In a similar study, human colorectal cancer organoids engineered to carry an inducible caspase-9 in the *LGR5* locus as a strategy to ablate LGR5-positive cancer stem cells (caspase-9 expression leads to apoptosis) were used to study in vivo tumor growth (72). Killing LGR5-positive cancer stem cells ultimately resulted in reversion of differentiated cancer cells into stem cells, refilling the cancer stem cell niche. Such plasticity also occurs in damaged normal intestinal epithelium (75). This study indicates that targeting cancer stem cells as a therapeutic option may not be sufficient to obtain durable regression.

Fumagalli and colleagues (73) orthotopically transplanted *KRAS*, *APC*, *TP53*, and *SMAD4* comutated human colon organoids into mice and showed that these metastasized to the liver and lungs in 44% of the mice. Rates of metastasis were negligible when transplantations were performed with organoids carrying mutations in only three of these four genes; however, the lack of the fourth mutation could be overcome by providing the niche factor upstream of the absent mutation: Triple mutants lacking *SMAD4* inactivation metastasized when Noggin was provided to the cells. These findings indicate that metastatic potential is directly related to the loss of niche factor dependency.

3. ORGANOID BIOBANKING: AS A LAB TOOL AND FOR TREATING PATIENTS

3.1. Living Organoid Biobanks

As described above, organoids can be directly derived from diseased tissue. This approach can be taken one step further by establishing living organoid biobanks. These are sets of organoids, histologically and genetically characterized, with matched normal organoids from large numbers

Table 1 List of biobanks

Organ of origin	Number of lines[a]	Histological subtypes	Reference
Colon	22	Adenocarcinomas	22
Colorectum	55	Premalignant lesions (tubular and tubulovillous adenomas, sessile serrated lesions, and a hyperplastic polyp) Adenocarcinomas (well differentiated, moderately differentiated, poorly differentiated, mucinous, not specified) Metastases of adenocarcinomas Neuroendocrine carcinomas	79
Colorectum	10	Colorectal metastases	78
Rectum (for cystic fibrosis)	71	Not applicable	23
Pancreas	8	Ductal adenocarcinomas	19
Pancreas	39	Ductal adenocarcinomas	80
Pancreas	114	Ductal adenocarcinomas	82
Liver	8	Hepatocellular carcinomas Cholangiocarcinomas Combined hepatocellular cholangiocarcinomas	86
Bladder	20	Urothelial carcinomas Squamous cell carcinomas	
Prostate	7	Adenocarcinoma metastases and circulating tumor cells	76
Ovary	33	High-grade serous carcinoma	28
Ovary	56	Borderline tumors (both mucinous and serous) Clear cell carcinomas Endometrioid carcinomas Mucinous carcinomas Low-grade serous carcinomas High-grade serous carcinomas	85
Breast	95	Ductal carcinoma Lobular carcinoma	32
Mixed[b]	56	Tumors from prostate, breast, colorectal, esophagus, brain, pancreas, lung, small intestine, ovary, uterus, soft tissue (not further specified), bladder, ureter, kidney	87
Mixed	[c]	Metastatic colorectal cancer Metastatic gastroesophageal cancer	83

[a]Refers to the number of organoid lines reported, not the number of patients (for some patients, multiple lines were established).
[b]Histological types were not comprehensively reported.
[c]The number of lines was not specifically mentioned (83).

of individuals. Living biobanks contribute to basic as well as to clinical, personalized medicine–related research.

A number of organoid biobanks have been described since 2014 (**Table 1**). Information about only one non-tumor organoid biobank has been published: This is an intestinal organoid biobank derived from CF patients (see Section 4.4).

A collection of metastatic prostate adenocarcinoma organoids (76) captured the most commonly found genetic aberrations in prostate cancer, including the *TMPRSS2–ERG* fusion, homozygous deletions of the phosphatase and tensin homolog (*PTEN*) gene, homozygous deletions of the chromodomain helicase DNA binding protein 1 (*CHD1*) gene, as well as typical

copy number variations. Both AR-positive and -negative organoids are present in this biobank. Of note, one line was established from circulating tumor cells, showing that at least in some cases, organoids can be derived from less invasive blood samples.

A collection of bladder tumoroids ($N = 20$ lines derived from 16 patients) contained urothelial carcinomas ($n = 15$) and one squamous cell carcinoma (77). Also in this biobank, the mutational spectrum of the tumor type was recapitulated by the organoids, including activating mutations in the fibroblast growth factor receptor 3 (*FGFR3*) gene and mutations in epigenetic regulators such as *ARID1A*. Gene expression analyses showed differences between the primary tissues compared with the corresponding organoids. This may be due to non-epithelial tumor components (such as stromal or immune cells) that are present in the tissue but not in organoids. This observation will likely be seen in other organoid cultures and is relevant when categorizing organoids using gene expression–based subclassifications that were originally established for tissue samples.

The first biobank ($N = 22$ lines), which was a colon carcinoma–derived biobank (22), showcased important principles of patient-derived organoids. First, it included samples of all five gene expression–based subtypes of colon carcinomas, showing that organoid culture was not limited to specific subtypes. Second, proof-of-principle drug screening experiments showed differences in drug sensitivity among the organoid lines that in some cases correlated with specific mutations in the tumor organoids. This indicated that organoids could be used for physiologically relevant drug screening. Third, the mutations in the organoids and the matched biopsies were largely concordant, demonstrating that the organoids reflected the patient's tumor. This last principle was confirmed in a set of organoids established from colorectal metastases. Despite the genomic instability that often occurs in metastases, organoids also accurately reflected the genetics of metastatic disease (78).

Fujii and colleagues (79) described an additional, more extensive colorectal organoid biobank from premalignant (e.g., tubular and tubulovillous adenomas) and malignant lesions. This biobank included neuroendocrine tumors and rectal carcinomas that were not included in the biobank described earlier (22) (for a complete overview of the types of tumors included, see **Table 1**).

Boj and colleagues (19) were the first to show for pancreatic cancer that organoids can be developed from patient-derived pancreatic ductal adenocarcinomas (PDACs). Recently, Seino and colleagues (80) followed up on this study by generating a larger biobank of PDACs that was characterized in more detail. The two gene expression–based subtypes (classical and basal) (81) were detectable in the organoids. Subsequently, tumor organoids were classified based on their dependency on the niche factor Wnt. A subset of tumor organoids was independent of Wnt and remained proliferative in the absence of external Wnt signals (that had been provided in the culture medium), which for normal pancreas organoids is never observed (similar to colon cancer organoids; see Section 1). The level of Wnt independence correlated with the subtype of PDAC: In general, the stronger the basal-type gene expression phenotype, the more independent of Wnt signaling the organoids were. Accordingly, clinically the basal subtype is more invasive and aggressive, which are characteristics associated with niche factor independence (73). In addition, for PDAC, organoid culture allows for detailed analysis of copy number variations. Hitherto, such analyses had been complicated by the low percentage of tumor cells in surgically resected PDACs. However, in organoid culture, a pure tumor cell population can be obtained, thereby enriching for tumor cells carrying genetic alterations and enabling the detailed determination of copy number variations that are relevant in PDACs.

In most biobank studies, drug screens were performed that revealed variability in responses among different organoid lines. In some cases, these differences could be attributed to specific genetic alterations in the tumors. However, organoids lack many components that influence drug response in vivo, such as drug metabolism and tissue penetration. Hence, it is essential to prove that

the in vitro organoid response correlates with the in vivo response (e.g., in mice and, ultimately, in the patients). Here, we discuss the first studies (32, 82, 83) that indicated such correlations exist.

A set of breast cancer organoids ($N = 95$ lines) has been described that included the major histological (invasive ductal carcinoma and invasive lobular carcinoma) and gene expression–based subtypes (32). Important functional characteristics of breast cancer were retained in these organoids: hormone status [estrogen receptor (ER) and progesterone receptor (PR) responsiveness] and HER2 expression. These are routinely determined in the clinic, as they predict sensitivity to certain treatments [e.g., tamoxifen and trastuzumab (Genentech)], and in the organoids, the expression of these markers correlated with their status in the tumors of origin (**Figure 3*d–g***). Indeed, Sachs and colleagues (32) orthotopically transplanted two breast cancer–derived organoid lines into mice and obtained anecdotal evidence for response prediction when comparing the responses to the HER2 inhibitor afatinib and to tamoxifen between organoids in culture and in PDXs (32). In these two instances, organoid sensitivity correlated with the clinical responses in the corresponding patients.

Tiriac and colleagues (82) generated a PDAC biobank ($N = 114$ lines derived from 101 patients) and exposed a subset of these organoid lines to therapies. Specifically, these tumor organoids were classified as resistant, intermediate, or sensitive to the five most commonly used chemotherapies, and their sensitivities were then correlated retrospectively with responses in patients. Notably, of six patients with above average disease-free survival, five were treated with a chemotherapeutic agent that was scored as "sensitive" in the corresponding organoid lines. In contrast, two of the three patients with below average disease-free survival were treated with a therapy that was scored as "resistant" in the corresponding organoids.

A clear statistical correlation between drug response in organoids and clinical response was also observed in a clinical study of metastatic gastroesophageal and colorectal cancer ($N = 21$) (83). There was a strong correlation (100% sensitivity, 93% specificity, 88% positive predictive value, and 100% negative predictive value) between the in vitro organoid response to a set of targeted therapies and chemotherapies and the response of the tumor in patients. Together, these studies indicate the potential of tumor-derived organoids to predict patients' responses. In addition, organoids might serve as tools to screen for alternative therapies for cancers that are resistant to standard therapy.

Hill and colleagues (28) established an organoid biobank of high-grade serous ovarian cancer ($N = 33$ organoid lines from 22 patients) and used this set of organoids to address a clinical problem. Approximately half of all patients with this type of ovarian cancer have mutations in DNA repair genes or pathways (84), typically *BRCA1* or *BRCA2*. These patients were thought to benefit from treatment with poly (ADP-ribose) polymerase (PARP) inhibitors that prevent PARP from repairing single-strand breaks, which, after DNA replication, result in double-strand breaks. The lack of functional BRCA1 or BRCA2 to repair the double-strand breaks leads to death of the tumor cells. However, in the clinical setting, mutation analysis alone is not sufficient to adequately predict drug sensitivity. The authors showed that functional assays in organoids are a better predictor than the genomic analysis currently performed in the clinic. Thus, this study implies that functional assays in organoids may improve the prediction of drug sensitivity beyond what can be achieved with genomic analysis alone.

Kopper and colleagues (85) established a second ovarian cancer biobank that captured all of the main histological subtypes (**Table 1**). Notably, the organoids could be expanded over the long term, and a novel single-cell DNA sequencing method was used to demonstrate that tumor heterogeneity was preserved in organoids when compared with the parental tumor.

Organoid biobanks have also contributed to the identification of new therapeutic targets. Huch and colleagues (29) described a liver tumor biobank containing the two most common primary

liver tumors (86), hepatocellular carcinoma and cholangiocarcinoma, as well as the rarer combined hepatocellular cholangiocarcinoma. In drug screening experiments with 29 compounds, the extracellular signal-regulated kinase (ERK) inhibitor SCH772984 was found to effectively inhibit the growth of tumor organoids. This effect was subsequently validated in vivo using xenotransplanted organoid lines in mice, highlighting SCH772984 as a possible therapeutic agent. This study showed that organoids can be used to find new compounds for treating disease.

Lastly, a diverse biobank established by Pauli and colleagues (87) exemplifies that organoids can be derived from tumors of many organs. This biobank included tumors derived from prostate ($n = 10$), breast ($n = 6$), colorectum ($n = 8$), esophagus ($n = 1$), brain ($n = 5$), pancreas ($n = 5$), lung ($n = 1$), small intestine ($n = 2$), ovary ($n = 1$), uterus ($n = 2$), soft tissue ($n = 3$ not further specified), bladder and ureter ($n = 8$), and kidney ($n = 6$).

In summary, organoid biobanks have been established for many tumor types (**Table 1**), mostly carcinomas, and several principles can be deduced from this collection of biobanks. The tumor organoids can be established from very small biological samples (e.g., prostate tumor organoids were derived from circulating tumor cells) for many types of carcinomas, and these are tumorigenic upon xenotransplantation (e.g., bladder cancer organoids) (77). The organoids in these biobanks recapitulate genetic and histological aspects of the parental tumors (e.g., in breast cancer organoids, they recapitulate ER and PR status). This holds even for a set of metastatic colorectal tumors (78), in which due to genomic instability variations might have been expected. Gene expression comparisons may be complicated by the lack of stromal cells in organoid cultures (77). Organoids are tools for obtaining a pure population of tumor cells for DNA sequencing analysis (e.g., in the case of PDAC in which there is a low percentage of tumor cells in resected specimens) (80). Drug screening experiments in organoids correlate with the response in patients (83) and have led to the identification of new therapeutic targets (29).

3.2. Ethics of Organoid Biobanking

The development of a model based on living human tissues that can be stored and expanded in biobanks—potentially forever—has raised a set of ethical issues regarding informed consent and ownership (88). For organoid biobanks, patient consent is required because complete anonymization is not desirable. If anonymization is used, clinically relevant results from organoid research cannot be used in the clinic to treat the specific patient. The most common type of patient consent restricts the use of a patient's material to only a specific research aim. However, biobanks are useful for researchers in multiple fields, and the use of biobanks in a combination of fields may provide potentially synergistic data. Therefore, Bredenoord and colleagues have suggested that a broad consent be used for governance (88, 89). This broad consent would allow donors to make informed decisions about how their samples are used after they have been provided with relevant information about the establishment and regulation of the biobank, such as the public or commercial parties involved, how incidental findings will be dealt with, and how donors can withdraw consent.

Another issue that has arisen with the development of living biobanks is ownership. Organoids are increasingly used by commercial parties as tools for drug development or in validation studies. Such uses will inevitably result in patentable compounds. It may be helpful to include regulations covering the distribution of any financial gains from intellectual property among stakeholders (such as the donor, the institute or researcher who established the organoid line, and industry) in the governance of the biobanks.

Apart from biobanking, organoids raise other ethical concerns. Organoid culture might change the role of animal experimentation, change the use of human fetal material in science (particularly

in the case of PSC-derived organoids), and allow for the culture of human brain tissues (resulting in issues related to consciousness). These and other ethical issues are more extensively discussed elsewhere (88, 90).

4. ORGANOIDS: TOWARD TREATING PATIENTS

4.1. Proof of Principle for Genetic Repair in Organoids

Schwank and colleagues (4) were the first to demonstrate that it is feasible to repair genetic defects in cultured human stem cells. Intestinal organoids derived from two CF patients with a homozygous F508 deletion were repaired using CRISPR-Cas9. After repair, FSK-induced swelling was restored (see the sidebar), functionally demonstrating CFTR activity.

Of note, *CFTR* gene repair in organoids and subsequent transplantation (see Section 4.2) into patients will most likely not be the first application in the clinic. First, the loss of CFTR functions results in disease in multiple organ systems, which would require the transplantation of organoids into multiple tissue sites. Second, a high percentage of repaired cells per organ would be required for functional restoration. However, for metabolic diseases, for which only a fraction of normal enzyme levels suffices to prevent severe symptoms, the transplantation of repaired organoids is a more obvious early strategy.

4.2. Proof of Principle for Organoid Transplantation as Therapy

Human and murine organoids have been (xeno)transplanted into mice for several reasons: to be used as a disease model of in vivo tumor dynamics (71, 72) and metastasis (73, 74) (see Section 2.2.3) or to show tumorigenic potential (32) (see Section 3). Below, we discuss studies that used organoid transplantation as a proof of principle for therapy.

Colon organoids, derived from a single murine colon stem cell, have been transplanted orthotopically into mice with acute colitis (91). Organoid-transplanted mice showed reduced weight loss when compared with sham-transplanted controls. Additionally, this was the first proof of principle that organoids can functionally integrate in vivo for more than 6 months, highlighting the potential use of organoids for therapy and regenerative medicine.

Orthotopic transplantation of liver organoids has also shown promising results. In a mouse model of toxicity-induced acute liver failure, transplantation of mouse biliary duct organoids yielded detectable organoid-derived nodules in 20–40% of cases, depending on the transplanted organoid line. Although engraftment was low (approximately 1%), transplantation increased the life span of the diseased mice (92). In follow-up studies using the same injury model, the transplantation of mouse (41) and human fetal (30) hepatocyte organoids, rather than organoids with a biliary duct phenotype (92), yielded much more extensive engraftment. These studies show that the efficiency of engraftment may be enhanced by transplantation of the most physiologically relevant cell type.

Together, these studies indicate the potential use of organoids in regenerative medicine. However, many hurdles are yet to be cleared: for example, integration upon transplantation requires optimization, and animal-based extracellular matrices used for organoid culture need to be replaced with a synthetic matrix (see Section 5).

4.3. Tumor-Derived Organoids as Tools for Immunotherapy

One of the hallmarks of cancer is immune evasion (93), and organoids can be used to study the interaction of the immune system with tumor cells. Batlle and colleagues (94) used orthotopically

transplanted mouse colorectal tumor organoids to demonstrate the contribution of TGF-β to immune evasion. The tumor organoids displayed high TGF-β expression, which corresponded with reduced intratumor T cell presence. Inhibition of TGF-β resulted in a decreased tumor size, and liver metastases failed to develop. This study shows that organoids can be used to facilitate research on tumor immune evasion, which may ultimately enhance immunotherapy.

Recently, a culture technique (95) was described that maintains the tumor immune microenvironment in organoids. Specifically, culture of organoids on an air–liquid interface retained macrophages, natural killer cells, and B and T lymphocytes. The T cell repertoire in the organoids was comparable to that in the parental tumor. In the future, such organoid cultures may facilitate personalized immunotherapy testing, which is highly relevant, given its potentially severe side effects.

One promising immunotherapy strategy is adoptive cell therapy—that is, the expansion of autologous immune cells in vitro followed by the subsequent transplantation of these cells back into the patient, thereby enhancing the immune response against a tumor. Using this strategy, durable regression of melanoma was achieved by in vitro expansion of autologous tumor-infiltrating lymphocytes (96). However, this approach requires resection specimens from which tumor-infiltrating lymphocytes can be obtained. A strategy to circumvent resection is to isolate peripheral blood lymphocytes, activate these cells in vitro by coculture with tumor cells, and subsequently transplant these activated T lymphocytes back into the patient. For this strategy, tumor-derived organoids are a highly useful source of tumor cells for coculture: Tumor organoid cultures can be efficiently established from a small tissue sample, and tumor-derived organoids are heterogeneous (78) (i.e., the risk that only a minor subclone of the tumor grows out efficiently is limited) and retain the genetic and histological characteristics of the parental tumors. Dijkstra and colleagues (97) were thus able to obtain tumor-reactive T lymphocytes from peripheral blood lymphocytes after 2 weeks of coculture with tumor organoids derived from non–small cell lung cancer and MSI colon carcinoma. Before coculture, organoids were stimulated with IFN-γ to enhance antigen presentation. To enhance T cell activation, PD-1 blocking antibody, IL-2, and anti-CD28 were added. After coculture, T lymphocytes were activated, as demonstrated by marker expression (IFN-γ and CD107a). Accordingly, after an additional 3 days of coculture of activated T lymphocytes with tumor organoids, the survival of the tumor organoids was reduced. Importantly, matched normal organoids were unaffected.

In short, this study is an important step toward delivering personalized immunotherapy by transplantation of in vitro–activated, autologous, tumor-reactive lymphocytes. It also shows the potential contribution of organoids to the field of immunotherapy, illustrating the versatile role of organoids in personalized medicine.

4.4. Organoids for Treating Patients: Cystic Fibrosis Treatment in the Netherlands

The development of the FSK-induced organoid swelling assay (see the sidebar) for testing CF therapeutics has resulted in the first personalized therapy approach based on organoids. Nearly 2,000 CF-causing mutations in *CFTR* have been described, and drug efficacy varies among the different genotypes (98). However, even in patients with the same genotype, drug responses vary, likely as a consequence of differences in genetic background (23). Furthermore, some genotypes are rare (23), making clinical trials impossible and requiring drug efficacy testing to be conducted on an individual basis. Thus, there is a need for a personalized medicine approach to predict treatment response, and the fact that CFTR-targeting therapies are expensive further emphasizes this need.

Beekman and colleagues (23) developed and validated a personalized medicine approach by establishing and characterizing a biobank of intestinal organoids from 71 CF patients with 28 different *CFTR* genotypes. The correlation of in vitro organoid data with clinical data demonstrated that the FSK-induced swelling assay can be used to select clinical responders to CF modulators. Of note, two patients with the rare and uncharacterized F508del/G1249R genotype responded in vitro to a specific CF modulator [ivacaftor (KALYDECO), Vertex Pharmaceuticals]. During subsequent in vivo treatment, the patients showed a clinical response as well: Results of pulmonary function and sweat chloride tests improved. In a prospective follow-up study involving 24 participants (63), the predictive power of the FSK assay was further substantiated, as the in vitro assay correlated with changes in pulmonary function and sweat chloride tests conducted in vivo. These findings hold promise for patients with rare, uncharacterized mutations for which clinical trials are not possible. Currently, in the Netherlands the licensing of ORKAMBI (lumacaftor/ivacaftor, Vertex Pharmaceuticals) allows treatment of CF patients solely on the basis of a positive organoid swelling response, demonstrating the potential of organoid-based assays for delivering personalized medicine.

5. LIMITATIONS

There are hurdles and limitations associated with using organoids. First, organoid culture requires the use of an animal-based matrix extract, usually in the form of Matrigel or Basement Membrane Extract. These extracts suffer from batch-to-batch variability in their composition, which may affect the reproducibility of experiments. In addition, they may carry unknown pathogens and are potentially immunogenic when transplanted to humans, limiting the use of organoids in a clinical transplantation setting (e.g., for cancer immunotherapies) (97). This may be solved by culturing with clinical grade collagen, which has been successfully used for the expansion of colon organoids (91). Steps toward fully defined culture conditions have been made with the development of a synthetic polyethylene glycol–based gel that sustains the short-term growth (four passages) of mouse ASC-derived intestinal organoids (99). However, this matrix remains to be optimized for the long-term expansion of intestinal organoids and for the expansion of nonintestinal organoids.

Second, the costs of organoid culture are higher than those of standard 2D cell culture as a result of the costly growth factor cocktails and the animal-based matrix extracts. The latter costs may be reduced in the future when synthetic gels can be efficiently produced.

Third, for ASC-derived organoids, only the epithelial compartment of organs is represented; blood vessels, immune cells, stroma, and nerves are lacking. This also holds for tumors, with thus far one exception: In Wilms' tumor, a stromal compartment can be maintained and expanded for at least 10 passages (36).

In the future, the lack of non-epithelial compartments may be overcome by establishing cocultures, analogous to what has already been done with immune cells, such as by adding neutrophils to RSV-infected airway organoids (33) and autologous lymphocytes to colorectal tumor organoids (97).

6. PERSPECTIVES

In conclusion, ASC-derived organoids have demonstrated their potential for modeling infectious, hereditary, and malignant diseases, as well as for drug screening for personalized medicine. The scope and clinical impact of these applications are expected to increase in the future.

Regarding infectious diseases, *Mycobacterium tuberculosis* is a major public health problem worldwide, and antibiotic resistance is on the rise (100). Mycobacteria are notoriously slow

growing and, hence, long-term culture of airway organoids, possibly cocultured with immune cells, may prove useful for modeling the infection. The mechanisms of virus-induced malignant transformation, for example, by Epstein–Barr virus in stomach and nasopharynx cancers, may be studied in long-term cocultures of the virus with normal epithelium from the respective organ. In epithelial genetic diseases, organoids have particular potential for the study of rare diseases because of their high expansion capacity and genetic stability. In the realm of malignancies, culture conditions have yet to be developed for many types of carcinomas and for sarcomas and melanomas.

In the area of regenerative medicine, the transplantation of organoids as therapy is still some time away: Several hurdles, including the development of a non-animal-based alternative for Matrigel and efficient delivery procedures, remain to be overcome. However, the success of the FSK-induced swelling assay for personalized medicine in CF demonstrates that clinical translation is feasible. Analogously, for other monogenic channelopathies or transporter diseases, for example, for Gitelman and Bartter syndromes, drug screening assays with straightforward read outs, such as organoid swelling, can be envisioned.

Large-scale biobanks will facilitate drug development by enabling many compounds to be screened for a specific disease or a specific compound to be screened for many forms of a given disease. Particularly for rare genetic diseases—for which large-scale clinical trials are not cost effective or feasible due to low numbers of patients—organoid technology may offer solutions for therapy development.

SUMMARY POINTS

1. Organoids are 3D epithelial in vitro–cultured structures that can be established from adult stem cells (ASCs) or from pluripotent stem cells (PSCs).
2. ASC-derived organoids model epithelial tissue regeneration, whereas PSC-derived organoids model organ development. These systems are complementary, and each has specific applications.
3. ASC-derived organoids can routinely be derived from normal and diseased tissue, and they are genetically stable and can be expanded, cloned, and genetically modified (e.g., with CRISPR-Cas9).
4. In the lab, ASC-derived organoids have been used to study tissue physiology. Moreover, models of infectious, hereditary, and oncological diseases that recapitulate essential features of in vivo disease have been developed using organoids.
5. Living organoid biobanks with histologically and genetically characterized tumor and matching normal organoids have been established for multiple organs and tumor types. These biobanks are resources for studying cancer biology and for drug development.
6. The response of organoids to drugs correlates with the patient's response (e.g., for cystic fibrosis). This holds promise for rare diseases for which clinical trials are not cost effective.
7. In the Netherlands, diagnostic decisions for patients with cystic fibrosis are already partially based on a functional organoid assay (the forskolin-induced swelling assay).
8. The limitations of ASC-derived organoids include that they represent only the epithelial compartment of organs, they require an animal-based extracellular matrix, and they have a relatively high cost.

DISCLOSURE STATEMENT

H.C. holds several patents related to organoid technology. F.S. is not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

ACKNOWLEDGMENTS

Thanks to Else Driehuis for critically reading and commenting on the manuscript as well as for designing **Figures 1** and **2**. Thanks to Joep Beumer for providing the confocal images of intestinal organoids (**Figure 3*b*,*c***). Thanks also to Norman Sachs for providing the breast cancer tissue and organoid staining for ERs and PRs (**Figure 3*d–g***). And thanks also to Carola Ammerlaan for providing the kidney organoid bright-field image (**Figure 3*a***).

LITERATURE CITED

1. John T, Kohler D, Pintilie M, Yanagawa N, Pham N-A, et al. 2011. The ability to form primary tumor xenografts is predictive of increased risk of disease recurrence in early-stage non–small cell lung cancer. *Clin. Cancer Res.* 17:134–41
2. Clevers H. 2016. Modeling development and disease with organoids. *Cell* 165:1586–97
3. Rookmaaker MB, Schutgens F, Verhaar MC, Clevers H. 2015. Development and application of human adult stem or progenitor cell organoids. *Nat. Rev. Nephrol.* 11:546–54
4. Schwank G, Koo BK, Sasselli V, Dekkers JF, Heo I, et al. 2013. Functional repair of CFTR by CRISPR/Cas9 in intestinal stem cell organoids of cystic fibrosis patients. *Cell Stem Cell* 13:653–58
5. Drost J, Van Jaarsveld RH, Ponsioen B, Zimberlin C, Van Boxtel R, et al. 2015. Sequential cancer mutations in cultured human intestinal stem cells. *Nature* 521:43–47
6. Drost J, van Boxtel R, Blokzijl F, Mizutani T, Sasaki N, et al. 2017. Use of CRISPR-modified human stem cell organoids to study the origin of mutational signatures in cancer. *Science* 358:234–38
7. McCracken KW, Howell JC, Wells JM, Spence JR. 2011. Generating human intestinal tissue from pluripotent stem cells in vitro. *Nat. Protoc.* 6:1920–28
8. Takasato M, Er PX, Chiu HS, Maier B, Baillie GJ, et al. 2015. Kidney organoids from human iPS cells contain multiple lineages and model human nephrogenesis. *Nature* 526:564–68
9. Freedman BS, Brooks CR, Lam AQ, Fu H, Morizane R, et al. 2015. Modelling kidney disease with CRISPR-mutant kidney organoids derived from human pluripotent epiblast spheroids. *Nat. Commun.* 6:8715
10. Garcez PP, Loiola EC, da Costa RM, Higa LM, Trindade P, et al. 2016. Zika virus impairs growth in human neurospheres and brain organoids. *Science* 352:816–18
11. Eiraku M, Watanabe K, Matsuo-Takasaki M, Kawada M, Yonemura S, et al. 2008. Self-organized formation of polarized cortical tissues from ESCs and its active manipulation by extrinsic signals. *Cell Stem Cell* 3:519–32
12. McCracken KW, Catá EM, Crawford CM, Sinagoga KL, Schumacher M, et al. 2014. Modeling human development and disease in pluripotent stem cell–derived gastric organoids. *Nature* 516:400–4
13. Takasato M, Er PX, Chiu HS, Maier B, Baillie GJ, et al. 2015. Kidney organoids from human iPS cells contain multiple lineages and model human nephrogenesis. *Nature* 526:564–68
14. Chen Y-W, Huang SX, De Carvalho ALRT, Ho S-H, Islam MN, et al. 2017. A three-dimensional model of human lung development and disease from pluripotent stem cells. *Nat. Cell Biol.* 19:542–49
15. Takebe T, Sekine K, Enomura M, Koike H, Kimura M, et al. 2013. Vascularized and functional human liver from an iPSC-derived organ bud transplant. *Nature* 499:481–84
16. Wu H, Uchimura K, Donnelly EL, Kirita Y, Morris SA, Humphreys BD. 2018. Comparative analysis and refinement of human PSC-derived kidney organoid differentiation with single-cell transcriptomics. *Cell Stem Cell* 23:869–81.e8

17. McCauley HA, Wells JM. 2017. Pluripotent stem cell–derived organoids: using principles of developmental biology to grow human tissues in a dish. *Development* 144:958–62
18. Bartfeld S, Bayram T, van de Wetering M, Huch M, Begthel H, et al. 2015. In vitro expansion of human gastric epithelial stem cells and their responses to bacterial infection. *Gastroenterology* 148:126–36.e6
19. Boj SF, Hwang CI, Baker LA, Chio II, Engle DD, et al. 2015. Organoid models of human and mouse ductal pancreatic cancer. *Cell* 160:324–38
20. Sato T, Stange DE, Ferrante M, Vries RG, van Es JH, et al. 2011. Long-term expansion of epithelial organoids from human colon, adenoma, adenocarcinoma, and Barrett's epithelium. *Gastroenterology* 141:1762–72
21. Huch M, Gehart H, van Boxtel R, Hamer K, Blokzijl F, et al. 2015. Long-term culture of genome-stable bipotent stem cells from adult human liver. *Cell* 160:299–312
22. van de Wetering M, Francies HE, Francis JM, Bounova G, Iorio F, et al. 2015. Prospective derivation of a living organoid biobank of colorectal cancer patients. *Cell* 161:933–45
23. Dekkers JF, Berkers G, Kruisselbrink E, Vonk A, De Jonge HR, et al. 2016. Characterizing responses to CFTR-modulating drugs using rectal organoids derived from subjects with cystic fibrosis. *Sci. Transl. Med.* 8:344ra84
24. Sato T, Vries RG, Snippert HJ, van de Wetering M, Barker N, et al. 2009. Single Lgr5 stem cells build crypt–villus structures in vitro without a mesenchymal niche. *Nature* 459:262–65
25. Barker N, van Es JH, Kuipers J, Kujala P, van den Born M, et al. 2007. Identification of stem cells in small intestine and colon by marker gene *Lgr5*. *Nature* 449:1003–7
26. Nanduri LSY, Baanstra M, Faber H, Rocchi C, Zwart E, et al. 2014. Purification and ex vivo expansion of fully functional salivary gland stem cells. *Stem Cell Rep.* 3:957–64
27. Kessler M, Hoffmann K, Brinkmann V, Thieck O, Jackisch S, et al. 2015. The Notch and Wnt pathways regulate stemness and differentiation in human fallopian tube organoids. *Nat. Commun.* 6:8989
28. Hill SJ, Decker B, Roberts EA, Horowitz NS, Muto MG, et al. 2018. Prediction of DNA repair inhibitor response in short-term patient-derived ovarian cancer organoids. *Cancer Discov.* 8:1404–21
29. Broutier L, Mastrogiovanni G, Verstegen MMA, Francies HE, Gavarró LM, et al. 2017. Human primary liver cancer–derived organoid cultures for disease modeling and drug screening. *Nat. Med.* 23:1424–35
30. Hu H, Gehart H, Artegiani B, López-Iglesias C, Dekkers F, et al. 2018. Long-term expansion of functional mouse and human hepatocytes as 3D organoids. *Cell* 175:1591–606
31. Karthaus WR, Iaquinta PJ, Drost J, Gracanin A, van Boxtel R, et al. 2014. Identification of multipotent luminal progenitor cells in human prostate organoid cultures. *Cell* 159:163–75
32. Sachs N, de Ligt J, Kopper O, Gogola E, Bounova G, et al. 2018. A living biobank of breast cancer organoids captures disease heterogeneity. *Cell* 172:373–86.e10
33. Sachs N, Papaspyropoulos A, Zomer-van Ommen DD, Heo I, Böttinger L, et al. 2018. Long-term expanding human airway organoids for disease modeling. *EMBO J.* 38:e100300
34. Ren W, Lewandowski BC, Watson J, Aihara E, Iwatsuki K, et al. 2014. Single Lgr5- or Lgr6-expressing taste stem/progenitor cells generate taste bud cells ex vivo. *PNAS* 111:16401–6
35. Turco MY, Gardner L, Hughes J, Cindrova-Davies T, Gomez MJ, et al. 2017. Long-term, hormone-responsive organoid cultures of human endometrium in a chemically defined medium. *Nat. Cell Biol.* 19:568–77
36. Schutgens F, Rookmaaker MB, Margaritis T, Rios A, Ammerlaan C, Jansen J, et al. 2019. Tubuloids derived from human adult kidney and urine for personalized disease modeling. *Nat. Biotechnol.* 37:303–13
37. Turco MY, Gardner L, Kay RG, Hamilton RS, Prater M, et al. 2018. Trophoblast organoids as a model for maternal–fetal interactions during human placentation. *Nature* 564:263–67
38. Basak O, Beumer J, Wiebrands K, Seno H, van Oudenaarden A, Clevers H. 2017. Induced quiescence of $Lgr5^+$ stem cells in intestinal organoids enables differentiation of hormone-producing enteroendocrine cells. *Cell Stem Cell* 20:177–90
39. Beumer J, Artegiani B, Post Y, Reimann F, Gribble F, et al. 2018. Enteroendocrine cells switch hormone expression along the crypt-to-villus BMP signalling gradient. *Nat. Cell Biol.* 20:909–16

40. Olivier M, Hollstein M, Hainaut P. 2010. *TP53* mutations in human cancers: origins, consequences, and clinical use. *Cold Spring Harb. Perspect. Biol.* 2:a001008
41. Peng WC, Logan CY, Fish M, Anbarchian T, Aguisanda F, et al. 2018. Inflammatory cytokine TNFα promotes the long-term expansion of primary hepatocytes in 3D culture. *Cell* 175:1607–19
42. Yamada Y, Kirillova I, Peschon JJ, Fausto N. 1997. Initiation of liver growth by tumor necrosis factor: deficient liver regeneration in mice lacking type I tumor necrosis factor receptor. *PNAS* 94:1441–46
43. Blokzijl F, De Ligt J, Jager M, Sasselli V, Roerink S, et al. 2016. Tissue-specific mutation accumulation in human adult stem cells during life. *Nature* 538:260–64
44. Checkley W, White AC Jr., Jaganath D, Arrowood MJ, Chalmers RM, et al. 2015. A review of the global burden, novel diagnostics, therapeutics, and vaccine targets for *Cryptosporidium*. *Lancet Infect. Dis.* 15:85–94
45. Heo I, Dutta D, Schaefer DA, Iakobachvili N, Artegiani B, et al. 2018. Modelling *Cryptosporidium* infection in human small intestinal and lung organoids. *Nat. Microbiol.* 3:814–23
46. Salama NR, Hartung ML, Müller A. 2013. Life in the human stomach: persistence strategies of the bacterial pathogen *Helicobacter pylori*. *Nat. Rev. Microbiol.* 11:385–99
47. Huang JY, Sweeney EG, Sigal M, Zhang HC, Remington SJ, et al. 2015. Chemodetection and destruction of host urea allows *Helicobacter pylori* to locate the epithelium. *Cell Host Microbe* 18:147–56
48. Shukla VK, Singh H, Pandey M, Upadhyay SK, Nath G. 2000. Carcinoma of the gallbladder—Is it a sequel of typhoid? *Dig. Dis. Sci.* 45:900–3
49. Scanu T, Spaapen RM, Bakker JM, Pratap CB, Wu L-E, et al. 2015. *Salmonella* manipulation of host signaling pathways provokes cellular transformation associated with gallbladder carcinoma. *Cell Host Microbe* 17:763–74
50. Ramani S, Atmar RL, Estes MK. 2014. Epidemiology of human noroviruses and updates on vaccine development. *Curr. Opin. Gastroenterol.* 30:25–33
51. Ettayebi K, Crawford SE, Murakami K, Broughman JR, Karandikar U, et al. 2016. Replication of human noroviruses in stem cell–derived human enteroids. *Science* 353:1387–93
52. Hirsch HH, Brennan DC, Drachenberg CB, Ginevri F, Gordon J, et al. 2005. Polyomavirus-associated nephropathy in renal transplantation: interdisciplinary analyses and recommendations. *Transplantation* 79:1277–86
53. Bohl DL, Brennan DC. 2007. BK virus nephropathy and kidney transplantation. *Clin. J. Am. Soc. Nephrol.* 2(Suppl. 1):S36–46
54. Nair H, Nokes DJ, Gessner BD, Dherani M, Madhi SA, et al. 2010. Global burden of acute lower respiratory infections due to respiratory syncytial virus in young children: a systematic review and meta-analysis. *Lancet* 375:1545–55
55. Mueller NJ, Kuwaki K, Knosalla C, Dor FJ, Gollackner B, et al. 2005. Early weaning of piglets fails to exclude porcine lymphotropic herpesvirus. *Xenotransplantation* 12:59–62
56. WHO (World Health Organ.). 2018. Human infection with avian influenza A(H7N9) virus—China: update. *WHO*. **https://www.who.int/csr/don/05-september-2018-ah7n9-china/en/**
57. WHO (World Health Organ.). 2016. *Tool for Influenza Pandemic Risk Assessment (TIPRA)*. Geneva: WHO
58. Zhou J, Li C, Sachs N, Chiu MC, Wong BH-Y, et al. 2018. Differentiated human airway organoids to assess infectivity of emerging influenza virus. *PNAS* 115:6822–27
59. Hui KPY, Ching RHH, Chan SKH, Nicholls JM, Sachs N, et al. 2018. Tropism, replication competence, and innate immune responses of influenza virus: an analysis of human airway organoids and ex-vivo bronchus cultures. *Lancet Respir. Med.* 6:846–54
60. Wiegerinck CL, Janecke AR, Schneeberger K, Vogel GF, van Haaften-Visser DY, et al. 2014. Loss of syntaxin 3 causes variant microvillus inclusion disease. *Gastroenterology* 147:65–68.e10
61. Bigorgne AE, Farin HF, Lemoine R, Mahlaoui N, Lambert N, et al. 2014. *TTC7A* mutations disrupt intestinal epithelial apicobasal polarity. *J. Clin. Investig.* 124:328–37
62. Dekkers JF, Wiegerinck CL, de Jonge HR, Bronsveld I, Janssens HM, et al. 2013. A functional CFTR assay using primary cystic fibrosis intestinal organoids. *Nat. Med.* 19:939–45
63. Berkers G, van Mourik P, Vonk AM, Kruisselbrink E, Dekkers JF, et al. 2019. Rectal organoids enable personalized treatment of cystic fibrosis. *Cell Rep.* 26:1701–8.e3

64. Sondo E, Caci E, Galietta LJV. 2014. The TMEM16A chloride channel as an alternative therapeutic target in cystic fibrosis. *Int. J. Biochem. Cell Biol.* 52:73–76
65. Roerink SF, Sasaki N, Lee-Six H, Young MD, Alexandrov LB, et al. 2018. Intra-tumour diversification in colorectal cancer at the single-cell level. *Nature* 556:457–62
66. Matano M, Date S, Shimokawa M, Takano A, Fujii M, et al. 2015. Modeling colorectal cancer using CRISPR-Cas9–mediated engineering of human intestinal organoids. *Nat. Med.* 21:256–62
67. Weren RDA, Ligtenberg MJL, Kets CM, de Voer RM, Verwiel ETP, et al. 2015. A germline homozygous mutation in the base-excision repair gene *NTHL1* causes adenomatous polyposis and colorectal cancer. *Nat. Genet.* 47:668–71
68. Nik-Zainal S, Davies H, Staaf J, Ramakrishna M, Glodzik D, et al. 2016. Landscape of somatic mutations in 560 breast cancer whole-genome sequences. *Nature* 534:47–54
69. Tomlins SA, Rhodes DR, Perner S, Dhanasekaran SM, Mehra R, et al. 2005. Recurrent fusion of *TMPRSS2* and ETS transcription factor genes in prostate cancer. *Science* 310:644–48
70. Driehuis E, Clevers H. 2017. CRISPR-induced *TMPRSS2*–ERG gene fusions in mouse prostate organoids. *JSM Biotechnol. Biomed. Eng.* 4:1076
71. de Sousa e Melo F, Kurtova AV, Harnoss JM, Kljavin N, Hoeck JD, et al. 2017. A distinct role for $Lgr5^+$ stem cells in primary and metastatic colon cancer. *Nature* 543:676–80
72. Shimokawa M, Ohta Y, Nishikori S, Matano M, Takano A, et al. 2017. Visualization and targeting of $LGR5^+$ human colon cancer stem cells. *Nature* 545:187–92
73. Fumagalli A, Drost J, Suijkerbuijk SJE, van Boxtel R, De Ligt J, et al. 2017. Genetic dissection of colorectal cancer progression by orthotopic transplantation of engineered cancer organoids. *PNAS* 114:E2357–64
74. Roper J, Tammela T, Cetinbas NM, Akkad A, Roghanian A, et al. 2017. In vivo genome editing and organoid transplantation models of colorectal cancer and metastasis. *Nat. Biotechnol.* 35:569–76
75. Tetteh PW, Basak O, Farin HF, Wiebrands K, Kretzschmar K, et al. 2016. Replacement of lost Lgr5-positive stem cells through plasticity of their enterocyte-lineage daughters. *Cell Stem Cell* 18:203–13
76. Gao D, Vela I, Sboner A, Iaquinta PJ, Karthaus WR, et al. 2014. Organoid cultures derived from patients with advanced prostate cancer. *Cell* 159:176–87
77. Lee SH, Hu W, Matulay JT, Silva MV, Owczarek TB, et al. 2018. Tumor evolution and drug response in patient-derived organoid models of bladder cancer. *Cell* 173:515–28
78. Weeber F, van de Wetering M, Hoogstraat M, Dijkstra KK, Krijgsman O, et al. 2015. Preserved genetic diversity in organoids cultured from biopsies of human colorectal cancer metastases. *PNAS* 112:13308–11
79. Fujii M, Shimokawa M, Date S, Takano A, Matano M, et al. 2016. A colorectal tumor organoid library demonstrates progressive loss of niche factor requirements during tumorigenesis. *Cell Stem Cell* 18:827–38
80. Seino T, Kawasaki S, Shimokawa M, Tamagawa H, Toshimitsu K, et al. 2018. Human pancreatic tumor organoids reveal loss of stem cell niche factor dependence during disease progression. *Cell Stem Cell* 22:454–67
81. Bailey P, Chang DK, Nones K, Johns AL, Patch A-M, et al. 2016. Genomic analyses identify molecular subtypes of pancreatic cancer. *Nature* 531:47–52
82. Tiriac H, Belleau P, Engle DD, Plenker D, Deschênes A, et al. 2018. Organoid profiling identifies common responders to chemotherapy in pancreatic cancer. *Cancer Discov.* 8:1112–29
83. Vlachogiannis G, Hedayat S, Vatsiou A, Jamin Y, Fernández-Mateos J, et al. 2018. Patient-derived organoids model treatment response of metastatic gastrointestinal cancers. *Science* 359:920–26
84. Cancer Genome Atlas Res. Netw. 2011. Integrated genomic analyses of ovarian carcinoma. *Nature* 474:609–15
85. Kopper O, de Witte CJ, Lõhmussaar K, Valle-Inclan JE, Hami N, et al. 2019. An organoid platform for ovarian cancer captures intra- and interpatient heterogeneity. *Nat. Med.* 25:838–49
86. Srivatanakul P, Sriplung H, Deerasamee S. 2004. Epidemiology of liver cancer: an overview. *Asian Pac. J. Cancer Prev.* 5:118–25

87. Pauli C, Hopkins BD, Prandi D, Shaw R, Fedrizzi T, et al. 2017. Personalized in vitro and in vivo cancer models to guide precision medicine. *Cancer Discov*. 7:462–77
88. Bredenoord AL, Clevers H, Knoblich JA. 2017. Human tissues in a dish: the research and ethical implications of organoid technology. *Science* 355:eaaf9414
89. Boers SN, van Delden JJM, Bredenoord AL. 2015. Broad consent is consent for governance. *Am. J. Bioeth*. 15:53–55
90. Boers SN, van Delden JJM, Clevers H, Bredenoord AL. 2016. Organoid biobanking: identifying the ethics. Organoids revive old and raise new ethical challenges for basic research and therapeutic use. *EMBO Rep*. 17:938–41
91. Yui S, Nakamura T, Sato T, Nemoto Y, Mizutani T, et al. 2012. Functional engraftment of colon epithelium expanded in vitro from a single adult $Lgr5^+$ stem cell. *Nat. Med.* 18:618–23
92. Huch M, Dorrell C, Boj SF, van Es JH, Li VS, et al. 2013. In vitro expansion of single $Lgr5^+$ liver stem cells induced by Wnt-driven regeneration. *Nature* 494:247–50
93. Hanahan D, Weinberg RA. 2011. Hallmarks of cancer: the next generation. *Cell* 144:646–74
94. Tauriello DVF, Palomo-Ponce S, Stork D, Berenguer-Llergo A, Badia-Ramentol J, et al. 2018. TGFβ drives immune evasion in genetically reconstituted colon cancer metastasis. *Nature* 554:538–43
95. Neal JT, Li X, Zhu J, Giangarra V, Grzeskowiak CL, et al. 2018. Organoid modeling of the tumor immune microenvironment. *Cell* 175:1972–88
96. Rosenberg SA, Restifo NP. 2015. Adoptive cell transfer as personalized immunotherapy for human cancer. *Science* 348:62–68
97. Dijkstra KK, Cattaneo CM, Weeber F, Chalabi M, van de Haar J, et al. 2018. Generation of tumor-reactive T cells by co-culture of peripheral blood lymphocytes and tumor organoids. *Cell* 174:1586–98
98. Cutting GR. 2015. Cystic fibrosis genetics: from molecular understanding to clinical application. *Nat. Rev. Genet*. 16:45–56
99. Gjorevski N, Sachs N, Manfrin A, Giger S, Bragina ME, et al. 2016. Designer matrices for intestinal stem cell and organoid culture. *Nature* 539:560–64
100. Gandhi NR, Nunn P, Dheda K, Schaaf HS, Zignol M, et al. 2010. Multidrug-resistant and extensively drug-resistant tuberculosis: a threat to global control of tuberculosis. *Lancet* 375:1830–43