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Review

Tumor Organoids as a Pre-clinical Cancer Model for Drug Discovery

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Tumor organoids are 3D cultures of cancer cells that can be derived on an individual patient basis with a high success rate. This creates opportunities to build large biobanks with relevant patient material that can be used to perform drug screens and facilitate drug development. The high take rate will also allow side-by-side comparison to evaluate the translational potential of this model system to the patient. These tumors-in-a-dish can be established for a variety of tumor types including colorectal, pancreas, stomach, prostate, and breast cancers. In this review, we highlight what is currently known about tumor organoid culture, the advantages and challenges of the model system, compare it with other pre-clinical cancer models, and evaluate its value for drug development.

Challenges in Drug Development

Costs of new anti-cancer drugs have surged over the past years due to, among others, the increasing complexity of clinical trials and regulatory requirements (Hay et al., 2014; Rubin and Gilliland, 2012). Meanwhile, the likelihood that a drug will reach market approval after entering phase 1 clinical testing has remained the same, and is significantly lower for anti-cancer drugs compared with drugs in other disease areas (Hay et al., 2014; Rubin and Gilliland, 2012). When considering all indications in oncology, a mere 1 in 15 drugs that enters clinical development will reach US Food and Drug Administration approval (Hay et al., 2014). While there are several factors that contribute to the low success rate from bench to bedside, one stands out: the translatability of pre-clinical cancer models to the patient. The difficulties of using model systems to predict drug efficacy in patients hamper not only general drug development pipelines, but also the advancement of companion diagnostics that can select subgroups of patients for treatment with molecularly targeted agents. In this review, we discuss 3D tumor organoid cultures, a novel pre-clinical model system in oncology that allows *ex vivo* propagation of tumors from individual patients. We discuss the potential of this model system to facilitate drug discovery and, in comparison with cell lines and patient-derived xenograft (PDX) models, highlight its pros and cons in the perspective of drug development. Due to constraints in space, a comparison of organoids with other 3D model systems in drug development was considered outside the scope of this review.

Tumor Organoids

Establishing the Organoid Culture System

Ex vivo culture of tumor cells from patients has been hampered in the past by low culture success and a limited proliferative capacity. The ability to perform long-term culture of primary colorectal cancer (CRC) cells came from the fundamental discovery that healthy mouse intestinal stem cells could be propagated *in vitro* long term using Wnt, R-spondin1, epidermal growth factor (EGF), and Noggin (Clevers, 2016; Sato et al., 2009, 2011). Healthy intestinal stem cells formed crypt-villus-like structures in Matrigel and were able to generate all cell lineages of the gut upon withdrawal of particular medium components (Sato et al., 2009, 2011). Importantly, these cultures retained their normal genome over time (Behjati et al., 2014; Sato et al., 2009, 2011). Irrespective of its previous use to describe organogenesis experiments, the term ''organoids'' was used, mainly because of the crypt-like architecture *in vitro*, and the distinct resemblance to the *in vivo* situation (Clevers, 2016; Sato et al., 2009, 2011). Matrigel and a cocktail of essential stem cell growth factors, which were used to culture healthy mouse intestinal tissue, supplemented with a transforming growth factor β receptor inhibitor (A83-01) and p38 MAPK inhibitor (SB202190), served as the basis for growth medium of healthy human intestine/colon, and eventually also for CRC organoids (Sato et al., 2011). Subsequently, similar culture protocols were developed for healthy and malignant tissue of the pancreas (Boj et al., 2015; Huang et al., 2015), stomach (Bartfeld et al., 2015), prostate (Gao et al., 2014; Karthaus et al., 2014), and liver (Huch et al., 2013, 2015). The ability to culture patient-derived healthy and diseased cells was immediately recognized as a major breakthrough and holds potential for the transformation of biomedical research into more patient-focused approaches. Since the development of organoid culture protocols, several key papers have been published in which organoids have been used as a tool to broaden our basic understanding of cancer (Drost et al., 2015; Li et al., 2014; Matano et al., 2015; Nadauld et al., 2014). These and subsequent studies help to determine whether organoid cultures have the potential to improve drug development and clinical practice.

Culture of Diverse Tumor Types with a High Success Rate

Van de Wetering et al. (2015) were the first to describe a collection of well-characterized patient-derived organoids. They report

Table 1. Success Rates of Establishing Pre-clinical Cancer Models for Diverse Tumor Types

Tumor Type	Success Rate to Establish: PDX	Cell Lines	Organoid Cultures	References
Breast	4%–86% per subtype: ER+: 4%–7% TNBC: 30%–86% *HER2*+: 26%–33%	17%*–46%*	66%	Byrne et al. (2017) Zhang et al. (2013) Pauli et al. (2017)
Colorectal	52%–91%	10%	>90%, 71%#	Byrne et al. (2017) Dangles-Marie et al. (2007) Weeber et al. (2015) van de Wetering et al. (2015)
Pancreas	54%–100%	9%	75%–85%	Byrne et al. (2017) Rückert et al. (2012) Boj et al. (2015) Huang et al. (2015)
Prostate	39%^	7 cell lines established to date	15%–20%#	Lin et al. (2014) Gao et al. (2014)

This table illustrates the reported success rates to establish pre-clinical cancer models for four tumor types. The pre-clinical cancer models incorporated in the table include organoid cultures, cell lines, and PDX. Take rates in the table pertain to human primary tumors, unless otherwise specified. For some tumor types, culture protocols are currently further optimized. Derived from: *mouse tumors; #metastatic biopsy specimens; ^primary tumors and metastases.

the successful culture of 20 matched healthy and tumor organoids derived from treatment-naive surgical resections with a ~90% success rate (van de Wetering et al., 2015). The unique achievement to culture tumor organoids with a nearly perfect success rate from individual primary CRCs was unprecedented. Weeber et al. (2015) subsequently confirmed the feasibility to grow organoids from single 18G needle biopsy specimens of CRC metastases with a success rate of 71%, and another biobank of CRC organoids has been established with a success rate of around 60% (Schutte et al., 2017). Using a slightly modified protocol, Gao et al. (2014) succeeded in culturing organoids from metastatic prostate cancer tissue as well as from liquid biopsies. Thus far, this is the only published case in which organoid cultures from a blood sample succeeded, but may create new opportunities for minimally invasive methods to incorporate patient-derived tumor organoids in personalized medicine programs (Gao et al., 2014). Boj et al. (2015) reported the successful culture of pancreatic adenocarcinoma organoids, a tumor type for which it is especially hard to establish cell lines due to the large stromal component of the tumor. Huang et al. (2015) also reported on the successful culture of human pancreatic adenocarcinoma with a success rate of 85% (n = 20). Bartfeld and colleagues succeeded in culturing gastric organoids and used this to create a model system for *Helicobacter pylori* infection, elucidating the cascade of events that takes place after bacterial infection (Bartfeld et al., 2015; Bartfeld and Clevers, 2015). The same culture conditions can also be used to culture gastric cancer organoids. More recently, Pauli et al. (2017) cultured tumor organoids from a range of different tumor types with an overall success rate of 39%, ranging from 19% in prostate cancer to 80% in CRC. In summary, organoid cultures can be established from a range of different tumor types, which paves the way for more successful drug development and precision medicine. Table 1 summarizes the success rates of establishing the various pre-clinical cancer models (organoid cultures, cell lines, and PDX) of diverse tumor types.

Proof-of-Concept: Organoids Are a Successful Tool for Drug Development in Cystic Fibrosis

Whereas the use of tumor organoids in cancer research, drug development, and personalized medicine in oncology is still in its infancy, and validation studies to evaluate their potential as a model system are in progress, impressive proof-of-concept has already been shown for cystic fibrosis (CF) (Dekkers et al., 2013a, 2013b, 2016a, 2016b; Noordhoek et al., 2016). CF is a disease that is caused by specific mutations in the *CFTR* gene, which ultimately prevent localization of the CFTR protein to the plasma membrane (Dekkers et al., 2013b). CFTR at the plasma membrane is necessary for homeostasis of fluids and electrolytes, and its absence results in an accumulation of viscous mucus in the gastrointestinal and pulmonary tract (Dekkers et al., 2013b). Patients can suffer from persistent pulmonary infections, pancreatic insufficiency, malnutrition, and have a limited life expectancy (Dekkers et al., 2013b). The pharmaceutical industry has developed small-molecule inhibitors that can restore the function of mutant CFTR proteins by mutation-specific drug targeting. However, in clinical practice, these compounds have varying responses in patients, suggesting that more factors contribute to drug response than the genetic constitution of *CFTR* alone. Dekkers et al. (2013b) have applied organoid culture conditions to rectal tissue of patients suffering from CF and have made the observation that forskolin (a cyclic AMP agonist) leads to swelling of healthy rectal organoids, but not of organoids from CF patients. This absence of forskolin-induced swelling in CFTR-deficient organoids could be reversed by CFTR-restoring compounds and has resulted in follow-up

studies, where it was shown that this platform was very apt at predicting drug response in patients (Dekkers et al., 2016a, 2016b; Noordhoek et al., 2016). Presently, rectal organoids of individual CF patients are used for drug discovery to identify novel, promising CFTR-restoring compounds, but also to determine which pre-existent small-molecule inhibitor works best in the individual patient, especially those with rare uncharacterized *CFTR* mutations (Dekkers et al., 2016a, 2016b; Noordhoek et al., 2016). Furthermore Vidovic et al. (2016) have used CRISPR/Cas9 technology in CF mutant organoids to genetically engineer and restore CFTR function. Using rectal organoids from CF patients, Schwank et al. (2013) have also shown that the defective *CFTR* gene could be replaced by a functional copy using CRISPR/Cas9. Since the complete lining of the gastrointestinal tract is affected, as well as other organ systems, this is presently not a viable treatment strategy for CF patients, but it does demonstrate that gene therapy in (gastro)-intestinal organoids is feasible.

In the field of CF, organoids have created a unique opportunity for drug development. The challenge ahead is now to employ tumor organoids in drug development. However, whereas CF is a mono-genetic disease, cancer genetics is infinitely more complex. The practical and theoretical challenges of organoids in drug discovery for cancer is discussed in the next section.

Critical Appraisal of Tumor Organoids Compared with Other Experimental Tumor Models

Genetic and Phenotypic Representation of Original Tumor

Organoid cultures offer great promise as a pre-clinical cancer model to improve drug development. First organoid cultures closely recapitulate the genetic and morphological heterogeneous composition of the cancer cells in the original tumor (Pauli et al., 2017; van de Wetering et al., 2015; Weeber et al., 2015). Van de Wetering et al. (2015) have shown that primary tumors that were put in culture gave rise to large numbers of different primary organoids (10–1,000) suggesting that the heterogeneous composition of the original tumor was largely conserved. The heterogeneous nature of the culture was also confirmed by transcriptome analysis of single organoids, which each gave rise to different expression profiles (van de Wetering et al., 2015). Histological analysis and DNA sequencing demonstrated a high concordance in morphology and mutational profile of the tumor organoids and matched patient tumor (van de Wetering et al., 2015). The mutational profile also showed a similar distribution when compared with the mutational spectrum for CRC included in The Cancer Genome Atlas, suggesting that, in this set of 20 organoids, the genetic diversity of CRC was captured (van de Wetering et al., 2015). Furthermore, RNA sequencing demonstrated that different subtypes of CRC could be cultured as organoids (van de Wetering et al., 2015). Weeber et al. (2015) have corroborated these findings for CRC, reporting that analysis of the original tumor and matched organoid cultures demonstrated a 90% concordance of somatic mutations, and a correlation between copy-number profiles of 0.89. The most important observation, however, was that none of the discordant findings affected driver genes, or concerned actionable events (Weeber et al., 2015). A similar observation was done by Fujii et al. (2016), who also report that no discordant mutations were found in drivers. Schutte et al. (2017) have reported a high degree of genetic concordance between primary CRC and matched patient-derived CRC organoids, where only 3% of divergent mutations concerned relevant cancer genes. Transcriptome analysis and subsequent unsupervised hierarchical clustering, however, identified three main molecular groups in patient material, as opposed to two main molecular groups for matched patient-derived tumor organoids (Schutte et al., 2017). Whereas this observation was similar in PDX, patient-derived tumor organoids also displayed unique expression of the stem cell marker, ALDH1A1, and components of carbohydrate, steroid, retinoid, and fatty acid metabolism (Schutte et al., 2017). Boj et al. (2015) have found that their pancreatic adenocarcinoma organoid model reflected *in vivo* disease progression, based on transcriptomic and proteomic analyses of organoids established from tissue at different stages of malignant transformation. Huang et al. (2015) analyzed the histology and differentiation status of tumor organoids 16 days after culture initiation, and reported similar morphological and cytological features, as well as expression of differentiation markers, compared with the original tumor. A. Duarte, E. Gogola, N. Sachs, M. Barazas, S. Annunziato, J.R. de Ruiter, A. Velds, S. Blatter, M. van de Ven, H. Clevers, et al. (2017, unpublished data) have generated mammary tumor organoids from genetically modified BRCA-deficient mice. They have shown that copy-number profiles of mammary tumor organoids better resembled the parental tumor than 2D cell lines derived from the same tumor (A. Duarte, E. Gogola, N. Sachs, M. Barazas, S. Annunziato, J.R. de Ruiter, A. Velds, S. Blatter, M. van de Ven, H. Clevers, et al., 2017, unpublished data).

Maintenance of Heterogeneity in Culture

The maintenance of tumor heterogeneity in culture is a factor that is often underestimated in genotype-phenotype relationships, and likely a critical factor in the failure of many targeted agents (Russo et al., 2016; Turner and Reis-Filho, 2012). Whereas tumor organoids display genetic and phenotypic heterogeneity upon start of culture, it is not clear to what extent this heterogeneity is maintained over time (Fujii et al., 2016; van de Wetering et al., 2015; Weeber et al., 2015). It has been reported that early- and late-passage organoids displayed essentially the same mutation pattern (Pauli et al., 2017; Schutte et al., 2017). We do know, however, that particular oncogenic mutations can provide tumor cells with a competitive advantage over others *in vivo*, and this is also imaginable *in vitro* (Snippert et al., 2014; Vermeulen et al., 2013). Barcode studies investigating clonal dynamics in tumor organoids cultures have not been conducted yet, but fluorescent labeling of tumor organoid cultures demonstrated that cultures were dominated by a single color after 30–40 days, indicating a drift toward clonality (Fujii et al., 2016). As with all cancer models, this aspect has to be taken into account. Another aspect that can influence genetic composition of the organoids is the medium composition used for culture. For some CRC samples, standard culture medium appeared to be suboptimal for growth, and required specific adaptations, such as culture under hypoxic conditions or removal of p38 MAPK inhibitors from the medium (Fujii et al., 2016). Examples that actually exploited condition-dependent clonal outgrowth of oncogenic mutations *in vitro* were demonstrated by Drost et al. (2015) and Matano et al. (2015). Both research groups selected mutated clones by

retracting medium factors such as Wnt, R-spondin1, EGF, and Noggin (Drost et al., 2015; Matano et al., 2015). This suggests that deprivation of essential growth factors favors the outgrowth of mutant clones that can grow independent of one of these factors.

How is tumor heterogeneity maintained in other model systems? Gene expression analysis between solid tumors and corresponding cell lines have previously demonstrated significant differences (Stein et al., 2004; Szakacs and Gottesman, 2004). Also, cell lines often do not cover the full genetic spectrum of tumor types. Genetic analysis of both tumors and cell lines have shown that common mutations can be preserved in cell lines, while rarer mutations are frequently not represented (Iorio et al., 2016). Classical cell line culture methods can also induce significant selection pressure on tumor cells: only cells that are able to grow in common culture conditions are able to survive. This can result in a misrepresentation of the original tumor and therefore non-physiological responses. It has been demonstrated in genetic barcode studies in cell lines that complexity is progressively lost (Porter et al., 2014). This suggests that a polyclonal pool of cells will turn into a multi- or monoclonal population over time. Interestingly, Porter et al. (2014) show that barcode complexity is better preserved when cell lines are subcutaneously injected in mice. This has important implications for cell lines and *in vitro* models in general and can partly explain the limited translational value of cell line data (Begley and Ellis, 2012; Stein et al., 2004; Szakacs and Gottesman, 2004). Firstly, it implies that the genetic composition and subsequent cell behavior *in vitro* can change over time. Second, it suggests that genetic diversity can be better preserved in favorable niches, such as the subcutaneous environment for PDX. To formally investigate clonal dynamics of tumor organoids after serial passaging, barcode studies, similar to those performed for PDX and cell lines, are warranted.

Drug Response

Of course, while maintenance of genotypic and phenotypic features does inform us about the translational potential of these cancer models, correlating drug-sensitivity data and clinical or genomic data is more informative. Whereas cell lines have many practical advantages and have long been the primary source to study drug responsiveness and find novel drug targets, the question has always remained if *in vitro* cell line findings translate to the clinic (Borst, 2012). PDXs closely recapitulate the genotype and phenotype of patient tumors at establishment (Hidalgo et al., 2014). Furthermore, intra-tumor clonal architecture is largely conserved in PDX after serial passaging (Bruna et al., 2016). The PDX approach has previously shown its high degree of translatability to the patient and provided an effective means to study resistance mechanisms and design novel treatment combinations. Bertotti and colleagues have demonstrated that HER2 or insulin growth factor 2 overexpression attenuates the effect of cetuximab in CRC, resulting in novel treatment strategies to overcome unresponsiveness to cetuximab (Bertotti et al., 2011; Leto et al., 2015). Gao et al. (2015) performed high-throughput drug screening on ~1,000 PDX with 62 treatments and demonstrated reproducibility as well as associations between genotype and drug response. PDX have also helped personalized medicine programs. Hidalgo et al. (2011) generated PDXs of individual patients and tested these against a range of clinically approved compounds and combinations. PDX-guided treatment resulted in an impressive 88% response rate. Despite a small sample size, these successful efforts to directly select treatment for the individual patient underscore the translatability and potential of PDX. But, while significant successes have been achieved with PDX, this platform also has shortcoming as it does not lend itself efficiently for high-throughput screens, is costly, and it is a lengthy process to establish tumors in mice. Hidalgo et al. (2011) report that engrafted PDX require 6–8 months of propagation to be useful for treatment. This can, however, similar to take rates, differ between tumor types and tumor histology or grade. Altogether, drug response of PDX translates nicely to patient outcome. The time needed to test the platform, however, makes PDX less suited for large-scale drug discovery, but PDX do offer opportunities to confirm findings from high-throughput *in vitro* studies.

How do tumor organoids compare with cell lines and PDX models? Tumor organoids are more expensive to establish and propagate compared with cell lines, but are less costly than PDX. When derived from a single 18G histological tumor biopsy, they require approximately 2–3 months of propagation to produce drug-sensitivity data. If more starting material is available, this time frame can be reduced to 1–2 months. Studies examining drug sensitivity of organoids have thus far shown drug responsiveness in line with the molecular profile of the tumor. Van de Wetering et al. (2015) performed a drug screen with a panel of 83 compounds in CRC organoids, and demonstrated that loss-of-function *TP53* mutants were insensitive to MDM2 inhibition and *RAS* mutants resistant to EGF receptor (EGFR) inhibition. They also identified a potential treatment strategy for *RNF43* mutant CRC, as it was shown that *RNF43* mutant organoids are exquisitely sensitive to Wnt secretion inhibitors (van de Wetering et al., 2015). In another study, single-agent and combination therapy drug screens in organoids from patients with colorectal, endometrial, and uterine carcinoma revealed exquisite sensitivity to novel treatment options (Pauli et al., 2017). In the same study, sequencing of a large cohort of tumor samples identified potentially targetable cancer gene alterations in only 9.6% of patients, underscoring the value of functional models to identify promising treatment options (Pauli et al., 2017). In a study evaluating parallel organoid cultures and PDX models, the response to various drugs was generally concordant between the different model systems (Schutte et al., 2017). Gao et al. (2014) studied drug response in blood- and tissue-derived prostate cancer organoids, and also observed that it matched the expectations considering the molecular background of the tumor. An androgen-receptor (AR)-amplified organoid line was significantly more sensitive to enzalutamide (an AR inhibitor), compared with wild-type lines (Gao et al., 2014). Of course, medium components of the organoid culture model may significantly influence drug response to various compounds. For example, high concentrations of EGF may compete with EGFR inhibitors, and apoptosis inhibitors, such as the p38 MAPK inhibitor, could have a more general effect on drug response. In addition, other extracellular factors derived from the tumor micro-environment might also contribute to discrepancies between the drug response of organoids, other model systems, and the clinical response. The generation of mammary carcinoma organoids from BRCA-deficient mouse tumors, which

show differential response to PARP inhibition described by A. Duarte, E. Gogola, N. Sachs, M. Barazas, S. Annunziato, J.R. de Ruiter, A. Velds, S. Blatter, M. van de Ven, H. Clevers, et al. (2017, unpublished data). Interestingly, the authors describe a case in which organoids derived from an *in vivo* resistant tumor was sensitive *in vitro*, likely caused by a cell-extrinsic resistance mechanism (A. Duarte, E. Gogola, N. Sachs, M. Barazas, S. Annunziato, J.R. de Ruiter, A. Velds, S. Blatter, M. van de Ven, H. Clevers, et al., 2017, unpublished data). This suggests that, in some cases, the drug response of a mouse tumor cannot be captured by tumor organoids, highlighting a shortcoming of the organoid platform. Additional studies comparing the drug response of organoids *in vitro* with organoids transplanted in mice, or the clinical response of a patient from which the organoids were derived, will shed light on the importance of this issue. Verissimo et al. (2016) showed the causal relationship between $KRAS^{G12D}$ and resistance to the combination of pan-HER and MEK inhibition. This combination led to a cell-cycle arrest in $KRAS^{G12D}$ organoids, as opposed to cell death in $KRAS^{wt}$ tumor organoids (Verissimo et al., 2016). The addition of a BCL-2 inhibitor abrogated resistance to the combined pan-HER and MEK inhibition, which was also confirmed in a PDX model. This was especially interesting, as previous pre-clinical research using cell line models had concluded the opposite. More specific, the combination of MEK and pan-HER inhibition was synergistic in *KRAS* mutant CRC and non-small-cell lung carcinoma (NSCLC) cell lines (Sun et al., 2014). Based on these findings in cell lines, combined MEK and pan-HER inhibition is currently being tested in patients with colorectal, lung, and pancreatic cancer harboring a *KRAS* mutation. Results of these trials are still pending and might provide insight in the predictive value of these model systems.

Drug-Genotype Correlations

To investigate drug-genotype correlations, organoids pose a good platform for mechanistic studies. Several groups have successfully used CRISPR/Cas9 technology to investigate oncogenic transformation and model tumorigenesis (Drost et al., 2015; Li et al., 2014; Matano et al., 2015; Nadauld et al., 2014). Drost and colleagues utilized CRISPR/Cas9 to study tumorigenesis by generating various combinations of mutated backgrounds in healthy organoids harboring loss of APC, TP53, and/or SMAD4, and activating mutations in KRAS and PIK3CA (Drost et al., 2015; Matano et al., 2015). CRISPR/Cas9 is used to investigate the mechanism behind PARP inhibitor response in BRCA1-deficient mammary tumors (A. Duarte, E. Gogola, N. Sachs, M. Barazas, S. Annunziato, J.R. de Ruiter, A. Velds, S. Blatter, M. van de Ven, H. Clevers, et al., 2017, unpublished data). These and other studies illustrate the ease of using organoids as a model system to study causal relationships, because organoids can be conveniently manipulated with state-of-the art technologies. This will facilitate studies investigating the influence of mono- or polygenetic events in relevant patient material.

The Issue with Numbers

There are large collections of PDX models to support drug testing in patient-derived tumors. Similarly, tumor organoids are excellently positioned to establish "living" biobanks with large numbers of different patient-derived organoid cultures. The importance of such a living biobank is highlighted in a review by Wilding and Bodmer (2014), who state that even in studies that have used an extensive number of cell lines in their drug screens, the exact numbers for each type of cancer, let alone a subtype, remain relatively low. Taking into account the distribution of molecular characteristics within tumor types and drugs with small effect sizes, makes it nearly impossible to detect drug-sensitivity patterns (Wilding and Bodmer, 2014). Of most tumor types we do not generally possess such a large collection of cell lines, and the enormous amount of time and resources that is related to working with PDX, prevents large-scale screens using this model system (Wilding and Bodmer, 2014). Organoid libraries can be expanded to include considerable numbers of patients (>100), and can facilitate the identification of drug-sensitivity profiles for small subsets of patients with significant results. In addition, organoids can be established from healthy tissue with a nearly perfect take rate (if there is enough starting material), enabling toxicity screening (to predict potential adverse effects of treatment), as well as tumor drug-sensitivity screening for the same patient.

Variable Growth Rate

A potential caveat for the use of organoids in drug development, which is also encountered in other *in vitro* models, is their variable growth rate. Variation in growth rate can confound pharmacological parameters such as the half-maximal inhibitory concentration (IC_{50}) from dose-response curves (Hafner and Niepel, 2016; Harris et al., 2016). Although inter- and intra-organoid growth variation can mask drug effects, new metrics have been proposed that correct for this confounding factor. By performing baseline and/or synchronous measurements in time, one can obtain a more accurate picture of the relative drug response of tumor cells (Hafner and Niepel, 2016). The growth rate inhibition metric and drug-induced proliferation rate both incorporate these measurements in single metrics in order to better determine and compare drug sensitivity *in vitro* (Hafner and Niepel, 2016; Harris et al., 2016). The potential value of these corrections can be illustrated by the obvious clinical response of *HER2*-amplified breast cancer patients to EGFR/HER2 inhibition, compared with patients with other breast cancer subtypes (Hafner and Niepel, 2016). This effect cannot be distinguished *in vitro* when comparing the IC_{50} of *HER2*-amplified cell lines with the IC_{50} of other breast cancer lines upon EGFR/HER2 inhibition (Hafner and Niepel, 2016), but only becomes apparent when corrected for the different growth rate of these cell lines.

Overgrowth of Normal Epithelial Cells

A distinct challenge in the establishment of tumor organoids is the potential contamination with, and subsequent overgrowth of, normal epithelial tissue in the culture. Whereas in colorectal tumor organoids several "tricks," such as the modification of medium factors necessary for the *ex vivo* propagation of normal tissue, can be applied to remove normal organoids, in some other cultures this has proven to be more difficult. Cultures of primary prostate tumors have thus far failed to yield "pure" tumor organoids when derived from the primary prostate cancer (Karthaus et al. 2014). In lung cancer, where field cancerization of the epithelial tract is a common phenomenon, this may also prove to be a challenge. It is unclear why normal tissue has a growth advantage over tumor cells. An increased rate of apoptosis in cancer cells due to their genetically unstable nature has been proposed as a possible explanation, but other factors such as culture supplements may also play a role (Karthaus et al., 2014).

Stromal and Immune Compartment

Even though tumor organoids are a closer, heterogeneous representation of an *in vivo* tumor than tumor cell lines, it remains a model system exclusively comprised of epithelial cells but lacking other cell types present in the tumor micro-environment. The tumor micro-environment may significantly (positively or negatively) affect drug response, which is an additional explanation for discrepancies between drug sensitivity *in vitro* and *in vivo* (Junttila and de Sauvage, 2013). Co-culture of epithelial cells and stromal cells is possible and can promote growth of malignant or untransformed epithelial cells (Liu et al., 2012; Ootani et al., 2009). Furthermore, co-culture of mouse intestinal organoids with intraepithelial lymphocytes (IELs) is possible, and IELs were shown to be highly motile, dynamically entering and egressing from organoids (Nozaki et al., 2016). Stiffness of the extracellular matrix affects organoid formation, differentiation, and also drug response (Gjorevski et al., 2016; Liu et al., 2015). It will be important to further develop co-culture systems of human tumor organoids with stromal cell types to reconstitute an *in vivo* tumor. Although this could be achieved with commercially available cell lines, it is far more appealing to use cells from the same patient from whom the organoid is derived. The latter approach has the advantage of capturing heterogeneity in the stromal compartment between patients, but may suffer from limitations in access to or expansion of such cells. Of note, while attempts to reconstruct the micro-environment *in vitro* may yield valuable new test systems, in some situations an *in vivo* model, such as PDX or genetically engineered mouse models, may initially be more suitable.

Taken together, early studies have demonstrated the feasibility of growing tumor organoids from individual patients, have molecularly characterized organoid cultures at different levels, and demonstrated the preservation of numerous characteristics between patient-derived tumor organoids and the original tumor. This has ignited great enthusiasm for the potential of this new technique to detect drug-genotype correlations, and hence its use as a platform for drug discovery. What makes organoid technology very exciting, however, is the potential to adequately assess the clinical relevance of *in vitro* findings. Cell lines and PDX for most tumor types have low-to-mediocre take or growth rates, preventing large-scale comparison and calibration with the patient. Because organoids can be established on a single-patient basis, we are able to compare the drug response of this model system with the patient and, if found representative, this could be used as a filter before embarking on clinical trials.

Biobanking Efforts and Validation Studies

Biobanking Efforts

With the ability to create organoids from individual tumors, the gigantic clinical diversity of cancer can be introduced in the laboratory. Large efforts are therefore undertaken to make organoids available to the scientific community. The first is the establishment of a large collection of these cultures, the Hubrecht Organoid Technology (HUB) ''living'' biobank. The HUB is also part of the Human Cancer Models Initiative collaborating with The National Cancer Institute, Cancer Research UK, and the Wellcome Trust Sanger Institute, to develop approximately 1,000 cancer cell models that better represent the hallmarks and diversity of human cancer. The HUB biobank collects and generates organoids of tumor tissue of patients with, for example, breast, colorectal, lung, pancreatic, and prostate cancer. These cultures are then extensively analyzed by genome sequencing and expression profiling. Furthermore, baseline clinical data are also collected for every included patient, and all organoids undergo an extensive screen to analyze drug sensitivity to a vast array of anti-cancer drugs. This well-characterized library of cultures and corresponding clinical data has been created to aid basic research, find leads for new compounds, help explore novel therapeutic strategies, and is accessible to both industry and academia. However, the true value of tumor organoids in drug screening and precision medicine still needs to be determined. This will require an extensive comparison of the drug response of organoids *in vitro* with the response of the tumor in the patient.

If the use of tumor organoids as a clinical decision-making tool can be validated, two applications will be of great interest. First, to determine upfront the success of a treatment to avoid unnecessary side effects for patients and reduce costs of health care by limiting the use of expensive medication. Second, to support drug development in the setting of early clinical trials.

Comparing Treatment Response In Vitro and of the Patient

To evaluate if patient-derived tumor organoids recapitulate drug response in the patient, a large prospective observational cohort study has been initiated by our group. In this multicenter cohort study, the TUMOROID trial (NL49002.031.14), patients with metastatic colorectal, breast, or NSCLC are included before they start with standard of care treatment. The primary objective of this study is to determine whether standard of care treatment responses of organoids predict treatment responses of patients. Since metastases have genetically evolved over time, all patients undergo a pre-treatment biopsy procedure of a metastatic lesion for organoid culture. Subsequently, clinical response data are collected and correlated with the *in vitro* organoid response. This test will first focus on establishing an empirically determined *in vitro* threshold for response, using matched clinical outcome data of patients. The threshold will be set to achieve the highest achievable negative predictive value, because this addresses one of the biggest challenges in the clinical care for patients: over-treatment with anti-cancer drugs. However, mimicking chemotherapy *in vitro* is not trivial, given vast differences in pharmacokinetics and drug-dosing regimens between a patient undergoing treatment and an *in vitro* drug assay. Previous studies in cell lines have shown that timing and duration of drug exposure can be of significant influence for the measure of cytotoxicity *in vitro* (Fischel et al., 1998). Fischel et al. (1998) have shown that the type of fluorouracil exposure (continuous, mixed, or short), as well as timing of oxaliplatin (before, during, or after exposure to fluorouracil and folic acid) significantly altered the drug sensitivity of the cells. However, the fact that tumor organoids can be cultured indefinitely provides the opportunity to modify various dosing schemes *in vitro* in such a way that it generates an approach where outcome matches clinical responses of patients.

Selecting Treatment Using Patient-Derived Tumor Organoids

Patient-derived tumor organoids can also be used to select individual patients for novel targeted therapies. In the past

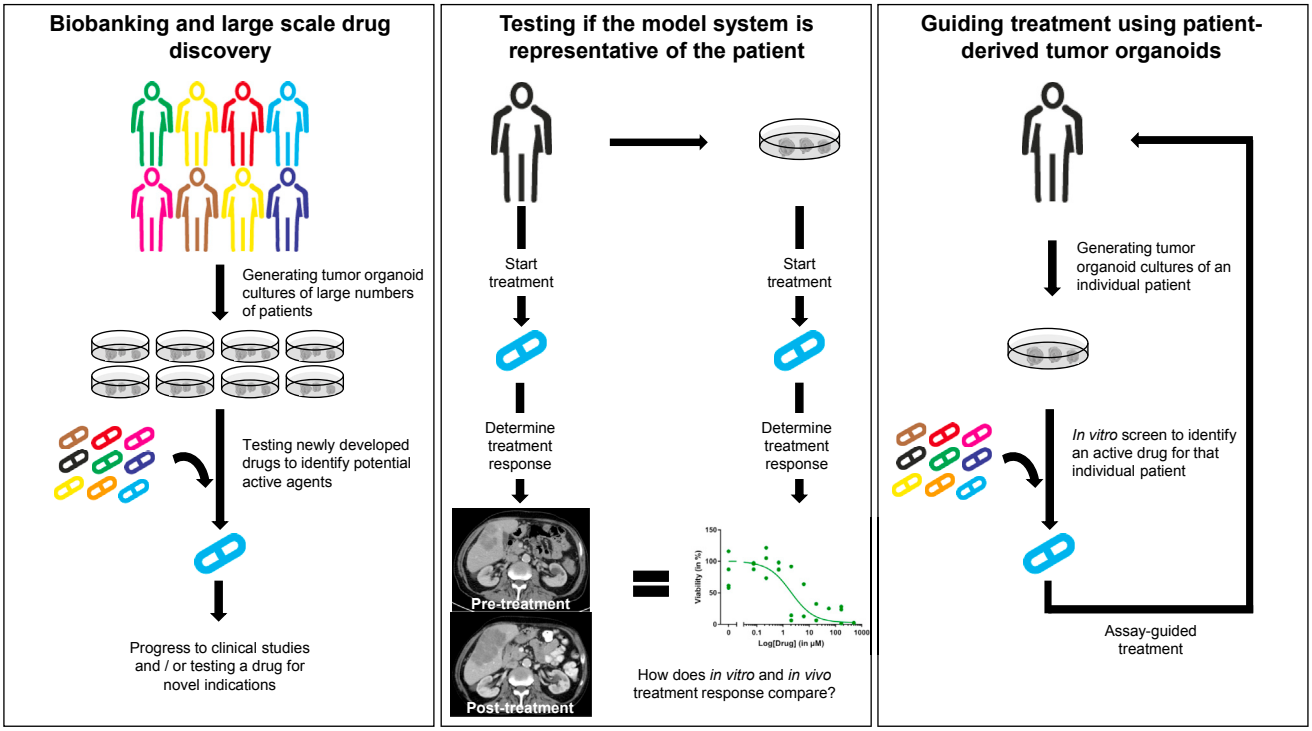

Figure 1. Patient-Derived Tumor Organoids and their Applications

This figure summarizes the potential of patient-derived tumor organoids. Because of their high culture take rate, they can be used to establish large and well-characterized biobanks that comprise the entire spectrum of molecular subtypes per tumor type. This can facilitate large-scale drug screening efforts. Furthermore, organoids lend themselves for a drug-sensitivity comparison with the individual patient responses because of their high take rates. Finally, patient-derived tumor organoids have the potential to select therapy for the individual patient if there are no regular treatment options left.

decade, the pharmaceutical industry has developed a wide array of molecularly targeted agents. However, even though the target at which a particular drug acts is known (e.g., PI3K inhibitor), this does not necessarily mean that patients with a molecular alteration in the pathway (e.g., PIK3CA mutation) will be susceptible to this treatment (Voest and Bernards, 2016). To fully elucidate which targeted agents match which molecular profile, large and costly prospective biomarker studies and basket trials are needed. In general this contributes to the low success rate of drug development in oncology. A functional assay, such as patient-derived tumor organoids, may guide and improve drug development. Therapeutic agents are often discarded due to presumed inactivity, while it could also be that the drug was not tested in the appropriate patient group, because the drug in question has a small target population. To test whether patient-derived tumor organoids can help to select patients for targeted therapy, a prospective clinical proof-of-concept study has been initiated by our group (SENSOR study, NL50400.031.14 Eudract 2014-003811-13). Patients with metastatic CRC and NSCLC are recruited before they initiate their last standard of care treatment line. Inclusion is done at an earlier time point, to bridge the culture and drug assay period. Tumor biopsies are retrieved at inclusion, cultured as organoids, and tested for eight different targeted treatments. When one of these agents is active *in vitro* the patient is offered this experimental treatment. If successful this approach may facilitate drug development. Taken together, these two studies will broaden our knowledge of this new technology, and will answer the question whether 3D organoid cultures can be translated to the patient setting. This paragraph is also summarized in Figure 1.

To conclude, tumor organoids provide new opportunities for drug development. The ability to culture organoids from every individual patient and their close resemblance to the original tumors suggest that organoids hold the promise of a more representative and clinically relevant model system compared with cell lines for drug discovery and precision medicine.

Significance

Drug development in oncology is hampered by the lack of representative model systems that can recapitulate all essential components of the patient's tumor. With the addition of a new technique, tumor organoid culture, to our repertoire of pre-clinical cancer models, it is important to evaluate the translational potential of this new model system. This model system has the potential to improve drug development by better discriminating, in an early stage, which drugs are effective, and for which indications, also serving as a selection assay in precision medicine. Here, we have reviewed current literature on organoid model systems and compared it with pre-existing models such as cell lines or PDX. We have also described the efforts that are currently ongoing to evaluate the clinical translatability of the organoid culture model and biobanking initiatives.

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AUTHOR CONTRIBUTIONS

F.W., S.O., K.D., and E.V. were involved in the design of the manuscript, review and interpretation of the literature, and writing of the manuscript.

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