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Human-cell-derived organoids as a new *ex vivo* model for drug assays in oncology

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In oncology, a 2D *in vitro* model of cancer cell lines is still widely used for large-scale drug screening. However, most promising candidates firstly identified by *in vitro* analysis tend to fail during the next steps of drug development. The generation of an *ex vivo* approach termed 'organoid' is emerging as a promising preclinical model to mimic human tumors more accurately. In this review, we focus on human-derived organoid use for anticancer drug screening. We describe the development of this new *in vitro* model, its use for anticancer agent assays and the advantages compared with the currently used 2D models. Finally, we discuss organoid limitations in the common use of this technology during preclinical studies.

Introduction

Organoid description

Recent advances in stem cell research have described new 3D culture systems of stem cells producing organotypic structures termed organoids [1]. Organoids are organ-like systems that have the essential characteristics of an organ. Similar to organogenesis *in vivo*, organoids can self-organize in a 3D architecture and have the advantage of displaying the physiology of a miniaturized organ [2,3]. Therefore, an organoid must be composed of multiple specific cell types of the organ that it reproduces. These organ-specific cells can be generated from stem cells or organ progenitors following a self-organizing process, observed during the early stages of organogenesis [4]. Two organizational processes called (i) cell sorting out and (ii) spatially restricted lineage commitment are at the origin of self-organization [1,4,5]. The 3D culture offers conditions for cell motility during expansion making the self-organization process possible [6]. More recently, Takebe *et al.* described another process named self-condensation which appears before self-organization and allows the transition from a 2D to a 3D structure [7]. The purpose of all these steps is the development of a mature structure with the main organ-specific functions (i.e., filtration for kidney organoids, albumin secretion for liver organoids) [4].

Pluripotent stem cells including embryonic stem cells (ESCs) and induced pluripotent stem cells (IPSCs) appear as the most used stem cells to produce organoids. Beyond pluripotent stem cells, several studies demonstrated adult stem cell (ASC) potential to generate organotypic architectures (Fig. 1). One of the most studied organs using the 3D *in vitro* models derived from ASCs is the gut. The NIH Intestinal Stem Cell Consortium has described several 3D structures of the intestine including enteroids, colonoids and then organoids [8]. In the case of colonoids, colon epithelial stem cells have been used to develop this model [9]. In 2009, Wnt-driven conditions were described for *in vitro* production of gut organoids from single intestinal stem cells [10,11]. Sato *et al.* associated a cocktail of growth factors with a laminin-rich matrix (Matrigel®) to establish an organoid model from leucine-rich repeat-containing G-protein-coupled receptor 5 (Lgr5)-positive stem cells [10].

Parallel to healthy tissues, organoids can be derived from resected tumors and/or cancer biopsies giving 'tumor organoids' (Fig. 1). In another study, Sato *et al.* isolated single colon cancer cells from nine patient intestinal crypts and separated fragments of Barrett's epithelium from five patients [5]. Single cells as well as epithelium fragments were cultured in a 3D environment using Matrigel® to generate tumor organoids. Further, the same team developed an organoid biobank derived from colorectal carcinoma biopsies of 20 patients [12]. In 2015, Boj *et al.* modeled a pancreatic tumor using organoids derived from resected tumorigenic pancre-

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as tissues of patients [13]. More recently, Boutier *et al*. adapted their culture conditions of a healthy liver organoid to develop 'primary liver cancer organoids' derived from patient liver tumor resection [14]. Over the years, several culture conditions have been established to generate human-derived normal or tumor organoids including kidney [15], brain [16], liver [17], pancreas [13], stomach [18], retinal tissue [4], prostate [19] and fallopian tube [20]. These miniaturized organs emerge as promising *in vitro* models to study the pathophysiology of diseases, develop the field of regenerative medicine and improve the existent preclinical models for drug screening.

Organoids as a potentially more reliable model for the development of anticancer therapies

New drug discovery has remained a complex, long and expensive process requiring 10–12 years and an investment of ~US$1 billion until FDA approval [21]. Drug tests *in vitro* present the advantage to perform large-scale assays comparing *in vivo* models. However, the majority of promising candidates first identified by *in vitro* analysis tends to fail during the next steps of drug development. This lack of success highlights the low representability of tumor complexity by simplified *in vitro* models. In oncology, a 2D *in vitro* model of cancer cell lines (CCLs) is still widely used for large-scale drug screening. Besides their accessibility and the facility of *in vitro* culture, CCLs showed their usefulness to identify genetic markers of drug resistance. Using melanoma cells with a BRAF mutation, Solit *et al*. observed a selective sensitivity to molecules that inhibit mitogen-activated protein kinase kinase (MEK) which is involved in human carcinogenesis [22]. CCL interest was confirmed by another model derived from lung cancer that also permitted the identification of pharmacological inhibitors of MEK signaling [23]. For several years, many studies correlated genetic markers with a pharmacological agent effect across a wide variety of CCLs. Further, a large-scale genomic characterization was centralized in the Cancer Cell Line Encyclopedia (CCLE), which lists >1000 CCLs [24]. The CCLE emerges as a promising database to predict genetic markers of drug sensitivity. These recent advances positioned CCLs as a more robust preclinical model. However, histological and mutational backgrounds of this model insufficiently represent human tumors [25]. In addition, 2D *in vitro* models are highly sensitive to chemotherapy making data difficult to extrapolate for clinical use.

The new generation of an *ex vivo* technology termed organoid tends to mimic the *in vivo* environment and promises to improve drug safety and efficiency assays [26] (Fig. 1). During their *in vitro* culture, organoids mimic physiological organogenesis by their ability to grow in 3D and self-organize into structures [27]. In contrast to CCLs, organoids remain genetically stable over time as shown by whole-genome sequencing after long-term course culture [28]; and they remain morphologically stable after repeated passages. Otherwise, it is important to note that an organoid model is more relevant depending on the tumor (i.e., colon cancer), whereas CCLs are of greater interest for tumors such as melanoma.

Another advantage of organoid technology is the use of human tissues. Indeed, despite the requirement of *in vivo* drug tests by regulatory agencies, animal models frequently fail because of physiological differences with humans. Olson *et al*. published a report compiling results of 150 drugs tested on animals then in humans. This comparative study pointed to the existence of variability in terms of drug response because non-rodent and rodent models failed to predict (37% and 57%, respectively) human toxicity [29]. In addition, in the field of oncology, tumor physiopathology is frequently heterogeneous depending of the patient, which makes drug discovery even more complex. Organoid technology emerges as a relevant model for the development of a patient-specific therapy called personalized therapy [3,26]. Indeed, organoids can be isolated from patient cancer biopsies preserving genetic parameters as well as histology of the disease [12,30]. Therefore, tumors can be reproduced *ex vivo* giving a disease- and patient-specific model for drug assays. Nowadays, organoid biobanks have been developed from patient tumors representing a valuable preclinical model for personalized drug screening [12,31]. Clevers and colleagues derived tumor organoids from resected colorectal neoplastic tissues obtained from 20 patients [12]. Using exome sequencing technology, RNA expression and histological analysis they evaluated the concordance between tumor organoids and primary tumors. Whole exome sequencing demonstrated a concordance between the organoid and the corresponding tumor samples. The screen of 83 anticancer agents including authorized drugs and experimental compounds demonstrated heterogeneous responses, identifying three major groups. To further customize treatment response, a heatmap of the half-maximal inhibitory concentration (IC_{50}) of tested drugs was established against each tumor organoid. Finally, the profile of gene–drug association was established for tumor organoids [12]. Others used the CRISPR/Cas9 technology to reproduce tumorigenesis *in vitro* and then to correlate genomic mutations with drug responses [32].

Presently, specific drug tests can be performed on tumor cultures *in vitro* [33]. This model presents the advantage of a large-scale screen in a relatively short time but is restricted by limited tumor proliferation. The alternative would be the use of an *in vivo* model of patient-derived tumor xenografts (PDTX) in immunodeficient mice [34]. Despite the recent increase of the use of this model that appears biologically stable and allows the maintenance of tumor heterogeneity, it presents several disadvantages. The use of immunodeficient rodents is poorly representative of the biological microenvironment of human cancer. In addition, the large-scale screening is limited by the restricted animal use and the high cost of this model. In fact, organoid cultures are more expensive to develop compared with 2D cell line cultures but remain less expensive than the PDTX model. Depending on culture conditions, an organoid model can generate drug sensitivity responses after 1 month whereas a PDTX model needs >6–8 months [32]. In this review, we focus on human-derived organoids as a promising preclinical model for drug screening in oncology. We describe the development of this new *in vitro* model, its use in several tumors and advantages compared with the currently used 2D cell lines. Finally, we discuss organoid technology limitations for anticancer drug development.

Drug assays in oncology

Hematology

Hematopoietic cancers remain frequent, severe and high-mortality tumors. Despite the development of new therapeutic

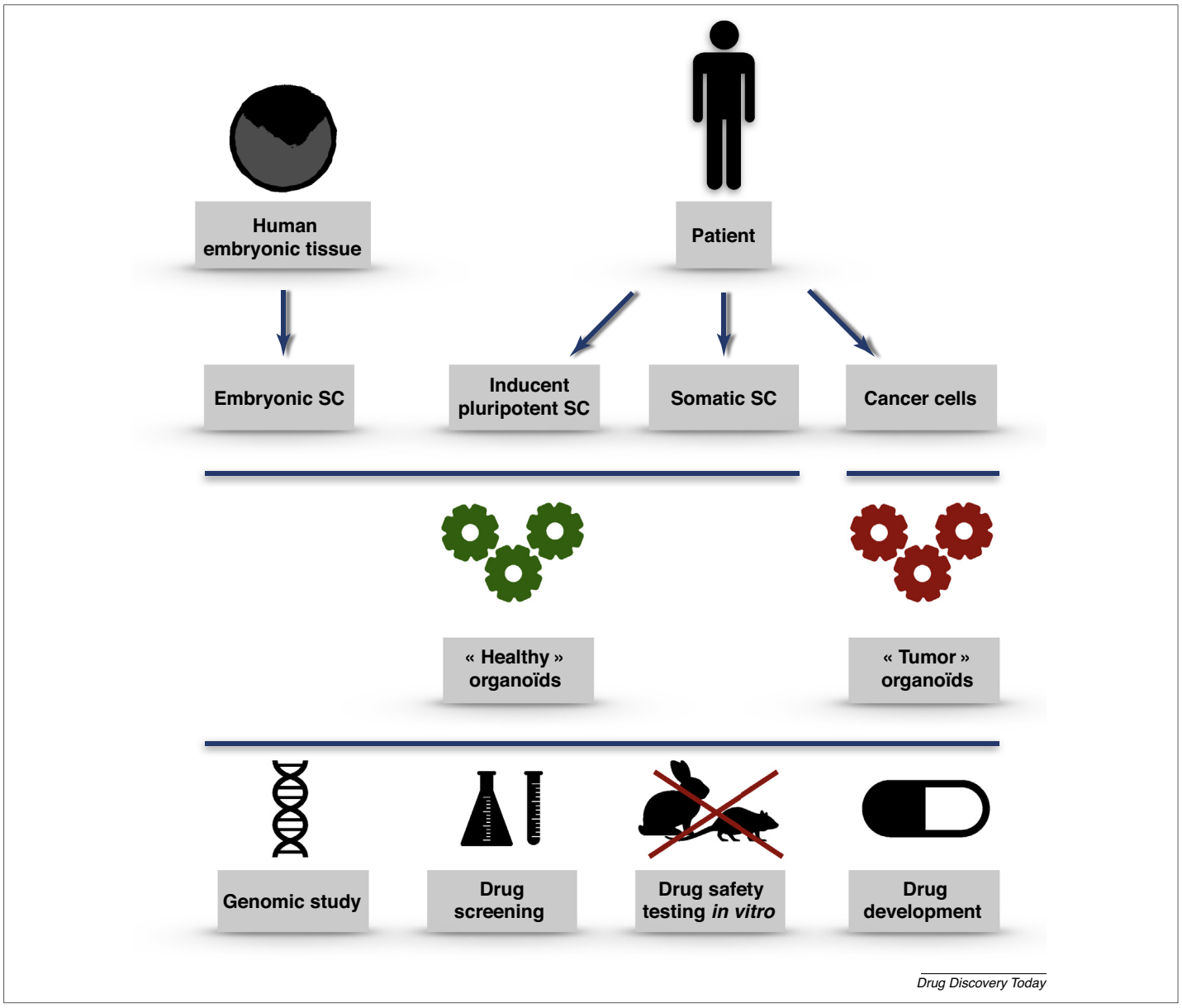

FIGURE 1

Organoid potential for drug development. The ability to develop human organoids derived from pluripotent stem cells (SCs), adult SCs or cancer cells is emerging as a promising technology to establish a new *in vitro* model for preclinical studies. In oncology, organoids offer an opportunity to test anticancer agent activity and toxicity but also to establish the genetic profile of tumors to specifically screen drugs. Organoids represent a candidate model to develop personalized therapy.

agents and the better understanding of cancer biology, heterogeneity of drug efficiency is still observed. This variability is mostly caused by the lack of our knowledge about mechanisms of drug tolerance. As mentioned previously, the absence of a reliable *ex vivo* model remains the major obstacle to developing new therapies. During multiple myeloma, it has been shown that the interactions of malignant plasma cells with their microenvironment play a key part in cell survival and drug resistance [35]. In the case of lymphomas, malignant B and T cells reside in the lymphoid niche that is not represented by standard 2D culture models [36]. In addition, it has been shown that the stromal microenvironment is involved in drug resistance [37]. Given the importance of the crosstalk between hematopoietic malignant cells and their niche, the use of a 3D model to mimic the biological microenvironment of these cells appears to be a crucial step to study a drug effect. In 2015, Tian *et al.* tested the efficiency of two chemotherapies: doxorubicin and panobinostat, on a hydrogel-based organoid model of lymphoma [38]. In brief, human malignant B and T cells were suspended in functionalized hydrogels in association with supporting dendritic cells, giving small clusters that preserve cell survival. Cell sensitivity to doxorubicin showed a significantly higher resistance of malignant B and T lymphocytes within the organoids compared with 2D cell suspension. In parallel, the effect of panobinostat, a histone

deacetylase inhibitor used in combination with bortezomib and dexamethasone to treat multiple myeloma [39], was tested in this study. Because this new treatment is currently being evaluated in several clinical trials including in patients with lymphomas, the authors have studied its efficacy on the organoid model of lymphoma. As for doxorubicin, lymphoma cell apoptosis was lower in organoids than in 2D culture [38]. Interestingly, the increase of drug resistance was not due to a limitation of compound availability within the 3D organoid model. The assessment of drug uptake showed that compound diffusion to B lymphoma cells within the organoid is not different compared to 2D culture of these cell lines. However, the authors observed an upregulation of B cell receptor (BCR) expression in the 3D microenvironment compared with the classical 2D culture model. They suggest that this mechanism could enhance malignant B cell survival and therefore decrease drug efficiency. To conclude, results from Tian *et al*. demonstrated the usefulness of the 3D organoid model in the field of hematology–oncology to assess standard and new therapeutic agent efficiency and toxicity *in vitro*.

Colorectal cancer

Colorectal carcinoma (CRC) is the third-most-common human cancer type that causes metastases when genes including APC, AXIN2 and CTNNB1 are mutated [40]. The origin of CRC would imply intestinal stem cells (ISCs) with mutations within the crypt base columnar cells [41]. Despite the large use of CCLs for CRC modeling and drug screening, this model is not representative of cancer histology and presents genetic instability after several passages of cells. Animal models are also widely used in the research on CRC but are still limited by physiological and genetic variability with humans [12]. The emergence of 3D organoid models containing normal and tumorigenic tissues is revolutionizing CRC research. The work of Hans Clevers and colleagues described a self-renewing intestinal organoid, generated from single-sorted $Lgr5^+$ cells, in the absence of a mesenchymal niche [10]. Matrigel® used as a matrix to support cell growth within this 3D model mimics the *in vivo* microenvironment of the crypt base by its laminin-rich composition [42]. CRC appears to be the most evaluated cancer using organoid models because these 3D structures can be produced from almost all CRC patients [12]. Recent studies have shown that organoid models generated from patients with CRC are representative of genetic diversity and morphology of CRC [12,43]. Thereby, a genetically varied 'living biobank' was established and appears to be useful to test anticancer agent effect [12]. Verissimo *et al*. have shown that sensitivity of a patient-derived CRC organoid to the epidermal growth factor receptor (EGFR) inhibitor afatinib depends on the genomic status [31]. Indeed, organoids derived from patients with a wild-type KRAS status were sensitive to afatinib (irreversible EGFR/HER2 inhibitor), whereas the mutant one was resistant [31]. In addition, drug resistance appears to be increased in the 3D organoid models of human CRC. Usui *et al*. confirmed these results by showing that cell death is induced at higher drug concentrations when the organoid model is used. In their study, they evaluated the toxicity of 5-fluorouracil (5-FU) and irinotecan using two *in vitro* models of CRC: 2D CCLs SW480, SW620, HCT116 and the 3D air–liquid interface organoid model derived from three human CRC tissues [43]. Their results showed that tumor organoids are more refractory to the drugs compared with 2D cell cultured cells. The authors suggested that the increase of drug resistance could be related to the stemness upregulation into organoids. Indeed, a positive correlation between expression of the stemness marker Lgr5 and the resistance of CRC specimens to 5-FU (as well as to oxaliplatin) has been shown [44,45]. Interestingly, cell death within organoid structures is associated with drug concentration increase which enables dose–effect studies. Xie and Wu showed a positive correlation between cell sensitivity and concentration of four chemotherapeutic agents including 10-hydroxycamptothecin, mitomycin C, adriamycin and paclitaxel [46]. The highest chemosensitivity of a CRC organoid model was observed with 10-hydroxycamptothecin which is structurally similar to the first-line treatment for metastatic CRC: irinotecan [47].

Although most studies compare organoid models to 2D cell culture, Schütte *et al*. have challenged an *in vitro* patient-derived organoid model versus an *in vivo* mouse xenograft model [48]. Using a biobank of 59 xenografts and 35 organoids, they evaluated responses of these two models to eight agents and classified drug sensitivity into four grades: strong response, moderate response, minor response and resistant. A high concordance of refractory to the eight studied drugs was found with both models showing that organoids would overcome the animal use to study drug resistance. However, transcriptomic analysis showed that biological signals of stromal and immune systems were not reproduced within organoid models, which could justify that PTDX remains the gold-standard model for anticancer agent assays. Interestingly, variability of genomic mutations depending of the tumor section resulted in differences of drug responses, which suggests that treatment effect could depend on the target tumorigenic region [48]. It is important to highlight that this correlation can be reproduced using organoid models unlike CCLs. Taken together, recent findings indicate that organoids derived from patient CRC can reproduce the tumor microenvironment and thus could become a novel preclinical model that is useful for examining resistance to anticancer drugs.

Liver cancer

Liver tissue has the particularity of developing either a primary tumor as hepatocellular carcinoma (HCC) or being the metastatic site of another cancer including CRC. Hepatic tumors present a reserved prognosis with a reported 5-year survival rate below 20% for HCC and 5% for CRC hepatic metastasis [49,50]. This can be explained by an extended heterogeneity of liver tumors making it difficult to identify specific drug targets and causing resistance to common anticancer treatments [51]. To improve drug development, reliable preclinical models are needed to better understand biological variability of hepatic tumors. During previous years, 3D organotypic models of the tumorigenic liver were developed. Using AlgiMatrix®, Takai *et al*. established an organoid-like spheroid model of HCC that mimics *in vivo* properties of this tumor and expresses the hepatic stem cell marker EpCAM, which is highly expressed in HCC with stemness features [52]. Around the same time, Skardal *et al*. focused on the development of a liver-tumor organoid model to study hepatic metastasis of CRC [53]. HepG2 hepatoma cells that can metabolize drugs through the enzymatic pathway of cytochrome P450 were co-cultured with a human metastatic colon carcinoma cell line: HCT-116, in 3D culture

conditions with hyaluronic acid and gelatin-based microcarriers inside bioreactors. To test organoid sensitivity to anticancer agents, the authors chose the chemotherapy 5-FU, which is commonly used to treat CRC. They noted a decrease of metabolism and viability inside the organoid model with increasing 5-FU concentrations. The cytotoxic agent effect was specific on HCT-116 cells at medium concentrations but became toxic on the surrounding HepG2 organoid tissue at high doses. The authors suggested the involvement of the Wnt pathway in organoid sensitivity to 5-FU because Wnt pathway inhibition increased drug efficacy. A better understanding of this mechanism was possible owing to the co-culture of two cell lines in a 3D organoid structure, which appears promising for drug screens and the development of new treatment in the field of liver cancer.

Prostate cancer

Despite the development of new therapeutics, prostate cancer is still the second-most-mortal cancer in men. Because of an important heterogeneity of this tumor and the lack of *in vitro* models, there is a real need to develop new models to understand mechanisms of drug resistance. To overcome disadvantages of monolayer cell line use, Gao *et al*. established six patient-derived organoid models of metastatic prostate cancer using a Matrigel® matrix to support 3D cell growth [54]. Six lines were derived from patient biopsies and one organoid line from circulating tumor cells (CTCs) with a high cell count (>100 CTCs per 8 ml of blood). Derived organoids reproduced the architectural structure and differentiation profile of the primary tumor. This 3D model was used to evaluate *ex vivo* the efficacy of three anticancer agents: the next-generation antiandrogen enzalutamide and two phosphoinositide 3 kinase (PI3K) pathway inhibitors everolimus and BKM-120, an anticancer agent used in clinical trials to treat a castration-resistant prostate cancer. To evaluate organoid sensitivity to these three agents, a cell viability and proliferation assay was used and dose–response curves were established [54]. Four days post-incubation with each drug at increasing concentration, only one organoid line presented sensitivity to all tested anticancer agents. Interestingly, this organoid line was highly sensitive to enzalutamide and presented the same results as a xenograft mouse model. Thus, findings of this study show once more that organoid models can repeat *in vivo* results [54].

Discussion

A growing and pressing need to develop new preclinical models is the new challenge of human medicine. The discovery and development of innovative anticancer therapies is still too long and is excessively expensive. The main cause is the use of preclinical models that include 2D cell cultures and animals that frequently fail when transitioned to clinical trials. The recent organoid technology emerges as a promising preclinical model to mimic human disease more accurately. Unlike animal models, organoids can be derived from humans and because of their 3D structure they can reproduce organ development and preserve the tumor microenvironment. This property has advantages over the 2D model because, besides cell–cell interactions, it reproduces cell–matrix interactions. This step is crucial for anticancer agent tests because drug efficiency is dependent not only on the target but also on the tumor environment. In addition, in contrast to cell culture, it has been shown that organoid structures stay genetically and physiologically stable for many generations. Owing to all these properties, organoids appear promising for anticancer drug screening as well as for personalized medicine. Indeed, organoids can lead to an assessment of the sensitivity of an individual patient tumor to a panel of anticancer agents. Validation for such an approach is ongoing with CTCs isolated from patients with pancreatic cancer (clinicaltrials.gov identifier NCT03033927).

Despite the advantages given above, most researchers still use 2D *in vitro* models. This could be explained by the drawbacks of organoids that have remained numerous. First, the 3D culture needs the use of a coating matrix to support cell growth and to maintain stem cells in an undifferentiated state. Matrigel® is still considered as the most efficient matrix [55] despite two major disadvantages. First, Matrigel® is a not fully defined matrix that consists of only major extracellular matrix (ECM) proteins that can be a source of heterogeneity in culture conditions [56]. In addition, this acellular membrane derives from mouse tumors resulting in a matrix of animal origin [57]. Because the use of human stem cells needs more-defined and animal-free conditions, recently new fully defined and xeno-free matrices have been developed [58]. Another important culture parameter that can impact drug response is the medium. Unlike monolayer cell lines that usually are amplified using serum (i.e., fetal bovine serum), organoid culture needs the addition of growth factors like Wnt/R-spondin, noggin and EGF to mimic the physiological cell niche [5]. The absence of standardized culture conditions results in a variability between produced organoids but can also impact drug activity. A better definition of culture conditions will help to develop a standard organoid model because a high variability between the existing models is still one of the most important limitations affecting routine use of this structure. Another limit of organoid technology is the intraculture heterogeneity that is amplified in tumor organoids [12]. Cell and tissue organization can vary between organoids, and differences in gene expression were observed within the same organoid line [12,14]. Thereby, it is difficult to establish pure organoid lines from the same patient. In addition, the long-term culture of organoid structures is a barrier to routinely using this model, especially for large-scale drug screening in a short time-period. Nevertheless, time for organoid cultures is still shorter compared with PDTX models. However, it is important to emphasize that vascular and immune systems are not yet fully reproduced in organoids whereas the PDTX model offers this advantage. Although the vascular system can impact biodistribution of a tested treatment into the organoid, drug toxicity could be caused by an indirect immunological reaction [59]. Because these parameters are crucial to evaluating drug activity, they represent the new perspective to improving organoid models [17].

Despite these physiological-like models, drug pharmacokinetics and pharmacodynamics will remain difficult to compare between a patient and an *in vitro* model. Organoids offer the opportunity to predict the treatment effect at the organ level. The method used to assess drug activity in an organoid model is therefore crucial and must be comparable to clinical data. Pharmacological parameters such as drug potency, LD_{50} and the area under curve (AUC) need to be determined in organoid models. Jabs *et al*. have developed an automated microscopy-based assay to calculate and then to compare LD_{50} and AUC values between 2D and 3D *in vitro* models.

Using a panel of clinical anticancer agents, they identified cytostatic versus cytotoxic drugs and calculated the therapeutic index within the monolayer cell model and the organoid model [60]. Using comparable tools, it will be interesting to extend the comparison of drug activity to the clinic and assess the correlation between organoid models and clinical data. For example, *in vitro* studies of potency must consider drug concentration (maximum or steady-state concentrations) in patient plasma [48]. However, despite recent progress concerning pharmacodynamics tools and clinical tests, evaluating patient responses to anticancer agents in the short- and long-term remains difficult.

Concluding remarks

Despite some disadvantages that still need to be improved, organoid technology provides an opportunity to mimic human organogenesis *in vivo*. The development of next-generation human-cell-derived organoids including cell heterogeneity, genomic mutations (e.g., using CRISPR/Cas9 technology) vascular and immune systems, as well as the use of the most appropriate matrix, would allow drug screening in the more physiological-like *in vitro* models. The decrease of production cost and the establishment of standardized templates are still limiting steps before the common use of organoids during preclinical studies. The automation of organoid production should be considered to address this problem.

Conflicts of interest

The authors report no conflicts of interest in relation to this article.

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