

Patient-derived tumour models as predictor of treatment response in cancer patients: How precise is precision medicine?

Review Article

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Abstract

Standard cut, poison and burn approaches to cancer treatment fail to save around 10 million patients every year across the globe. Precision medicine cancer treatment attempts to improve treatment by molecularly profiling a patient through (epi)genomics and transcriptomics to identify biomarkers in the patient's tumour. Personalized treatment is hence targeted towards these biomarkers for optimal patient care. While targeted treatment is more effective than non-targeted treatment, due to inter- and intratumour heterogeneity and clonal dynamics in a tumour, biomarker-targeted treatment often cannot treat a tumour completely. Combining drug candidates with patient-derived tumour models allow for sensitivity screening to assess drug efficacy before deciding on a treatment plan for an individual patient. In this literature review, five patient-derived tumour models applicable to precision medicine are discussed and compared. These include cell culture, tumour explants, mice or zebrafish xenograft transplantation and tumour organoids. The models are scored on several features regarding patient tumour representation, practicality of the model and drug screen effectiveness. Within these criteria, patient-derived organoids make a good case for suitability in a precision medicine context. Future improvements in co-culturing protocols and drug screen automation will further strengthen its position. Additionally, important considerations for drug screen study design, such as clear design and reporting guidelines and protocol standardization, will further improve the precision medicine approach to cancer treatment and allow for optimal individualized therapy.

Layman's summary

Annually, cancer claims around 10 million victims worldwide. Continued development and improvement of cancer treatment approaches is necessary to address this problem. A promising approach to cancer treatment is precision medicine. In this approach, an individual patient and its tumour are extensively analysed to determine the characteristics of the cancer type, such as its DNA and RNA. With this data, potential drug targets in the tumour can be identified. In order to test whether the potentially effective drugs indeed work, a patient model is constructed. This patient model is derived from patient material obtained after initial surgery of the tumour. The patient model is then subjected to the possibly effective drugs that were identified before. The tumour model will be analysed to see what drugs had the best effect on battling the tumour cells, in order to inform the doctors on what treatment is best suited for the specific patient. In this literature review, the different tumour models that can be used in this approach are discussed and compared. Patient-derived tumour models are scored on three categories: (1) patient tumour representation, as the tumour model needs to recapitulate the characteristics of the patient's tumour it was derived from. (2) The practicality of the model, since application of the model needs to be expanded for many patients, which comes with financial considerations. Also, the model needs to provide drug screen results quickly, since the patient needs to receive the targeted treatment as soon as possible. (3) Drug screen effectiveness discusses the applicability of the model in drug screens and the results of previously published studies on their capabilities to predict patient response.

Five different tumour model approaches have potential in precision medicine. Firstly, the patient's tumour can be removed and cut into very small fragments or slices. These fragments, called patient-derived explants (PDEs), are then cultured and subjected to the anti-cancer drugs. While these fragments resemble the tumour architecture closely and quickly produce results, the explants are not expandable and difficult to maintain. Secondly, patient tumour cells (PDCs) can be dissociated and cultured in dishes to form a two dimensional model of the tumour cells. These dishes are then subjected to drug screens to determine efficacy. These PDCs lose important characteristics of the original tumour, yet they are cost-effective and quick to expand for large-scale drug screens. Alternatively, patient-derived tumour cells can be transplanted into a mouse or zebrafish animal model. By treating the animal with drugs and monitoring the response of the tumour inside the animal, drug efficacy is observed in a living organism. These transplant models give good tumour representation, but they are expensive, slow to produce and ethically questionable. Finally, tumour cells can also be cultured three-dimensionally in culture dishes, resulting in a model called patient-derived organoids (PDOs). Organoids resemble the patient's tumour better than PDCs, yet remain relatively easy to handle.

Taken together, patient-derived organoids form a good combination between the other culturing models and transplant models, and hold great promise as an ideal model for precision medicine in cancer treatment. Future improvements to the PDO model regarding tumour representation, growth efficiency and drug screen automation will further strengthen its position. Other considerations for drug screen study design, including clear guidelines and standardization, will further improve the precision medicine approach to cancer treatment.

Introduction

Cancer remains a main cause of death worldwide, with a reported 9.5 million victims in 2018 [1]. This number is estimated to increase up to 22.2 million by 2030 [2]. As such, it is important to continue development and improvement of cancer treatment approaches. Currently, surgery, chemotherapy, radiotherapy, and immunotherapy are the four standard cancer treatment approaches. Although these approaches are highly successful for many types of cancer, they are not successful in all cases. Tumour responses to these treatment strategies differ according to the tumour subtype, clinical stage, and associated risk factors. If the response is insufficient, recurrence of disease or metastasis can occur, which are associated with high resistance to further therapy. Additionally, chemotherapy side effects often significantly affect quality of life for the patient, indicating a need for improving the efficiency of cancer therapy approaches to optimize patient care.

Advances in high-throughput sequencing methods expanded the fields of genomics, epigenomics and transcriptomics, and these datasets turned out to be particularly useful for treatment of cancer. The (epi)genome and transcriptome data of the patient and its tumour can be analysed to determine possible patient-specific molecular targets for anticancer agents, an approach dubbed “personalized medicine” [3]. In personalized medicine (also described as precision medicine or targeted therapy), the molecular information is used to inform diagnosis and treatment of individual patient malignancies. Early successes of precision medicine have been shown in large-scale settings. A meta-analysis study of 346 phase I clinical trials, involving more than 13,000 patients, showed an average tumour shrinkage rate of 30.6% in biomarker-directed precision medicine arms, while undirected treatment only managed 4.9% [4]. Additionally, patients receiving precision medicine treatment had longer progression-free survival times. However, a large part of patient tumours will not lend themselves to a biomarker-based treatment, and for most anticancer agents no genetic markers are available [5]. Therefore, current biomarker-directed precision medicine is not accurate and broadly applicable enough to efficiently inform the therapeutic approach for cancer patients. Furthermore, extensive inter- and intratumour heterogeneity is observed in patient tumours [6]. Identification of a potent biomarker often provides a solution for only a part of the patient tumour. In line with this, biomarkers identified after initial sequencing of the tumour might not represent the patient tumour at the time of treatment, as tumour evolution will alter clonal composition. Additional assessment of the tumour response is needed to make sure that the biomarker-directed anticancer agents will tackle the patient tumour effectively. In order to prevent further discomfort for the patient, these assessments can be done in tumour models.

Over the past decades, many tumour models have been developed to both study the fundamentals of cancer and the efficacy of anticancer agents. Several of these models could lend themselves to a role in precision medicine, where anti-cancer agents identified as potentially effective can be tested for sensitivity in a tumour model prior to therapeutic decision making for the patient. Given the patient-centered nature of precision medicine, the model should replicate the tumour of an individual patient as closely as possible. As a result, the model response would provide the most guidance on whether an effective drug in the model will concordantly be effective in the patient. Models of this nature have been developed both *in vitro* and *in vivo*, ranging from patient-derived cell cultures and mini-tumours, to xenotransplantation of tumour tissue in immunodeficient mice or zebrafish. These models all have differing characteristics and features. In this literature review, the suitability of these models for

precision medicine cancer treatment is the main focus. Firstly, the general precision oncology methodology will be presented. Next, the models, their advantages, disadvantages and applicability for precision medicine will be discussed. Finally, the most promising models, as well as important developments and considerations for precision oncology studies, will be laid out.

Main

Precision Oncology

Precision medicine approaches in cancer treatment generally follow a similar workflow, which is illustrated in *figure 1*. The process starts with resection surgery treatment, where the cancerous tissue is removed, and a patient biopsy of the tumour is collected. The patient biopsy is collected for two uses; molecular profiling and establishment of a patient-derived tumour model. Firstly, a molecular profile is constructed of both the patient and its cancerous tissue, in order to identify any molecular tumour markers. Different sequencing and analysis steps are performed on the patient material, and through these steps, information is gained on the genomic, epigenomic, pharmacogenomic and/or transcriptomic profiles of a patient and its tumour. Subsequently, these datasets are analysed for identification of possible biomarkers derived from clinical, molecular and drug knowledge databases. Potentially effective anti-cancer agents are listed and tested in sensitivity assays on patient-derived tumour models. This patient-derived tumour model is the second destination for the patient biopsy obtained after surgery, as these models are established from the tumour cells of the patient. Drug efficacy is determined by measuring variables which are correlated with sensitivity, such as tumour size or cell viability. The amount and type of compounds which are included in drug screens can vary, and inclusion criteria rely on molecular and clinical suggestions of a drug's efficacy. Where molecular profiling provides a theoretical base for possible drug efficacies, functional testing of the agents will provide practical evidence of drug efficacies. Combined, this information guides therapeutic decision making to provide optimal cancer therapy for an individual patient [7].

The effectiveness of precision oncology relies heavily on the predictive power of the tumour model. Suitability of patient-derived tumour models for this pipeline depends on several characteristics that can be grouped into three categories. (1) Patient tumour representation will dictate whether the response of the model will portray the response of the patient. (2) The practicality of the model describes its ease of use for precision medicine purposes. This includes aspects such as model establishment efficiency, scalability or automation, and the costs of maintaining the model. Additionally, speed of process is important, as results need to be obtained quickly to maintain clinically relevant for the patient. (3) Drug screen effectiveness determines whether the model can be implemented in low- or high-throughput drug screens and whether the obtained sensitivities hold predictive value for respective patient responses. With these important features in mind, the different patient-derived tumour models that were developed over the past decades will be discussed next.

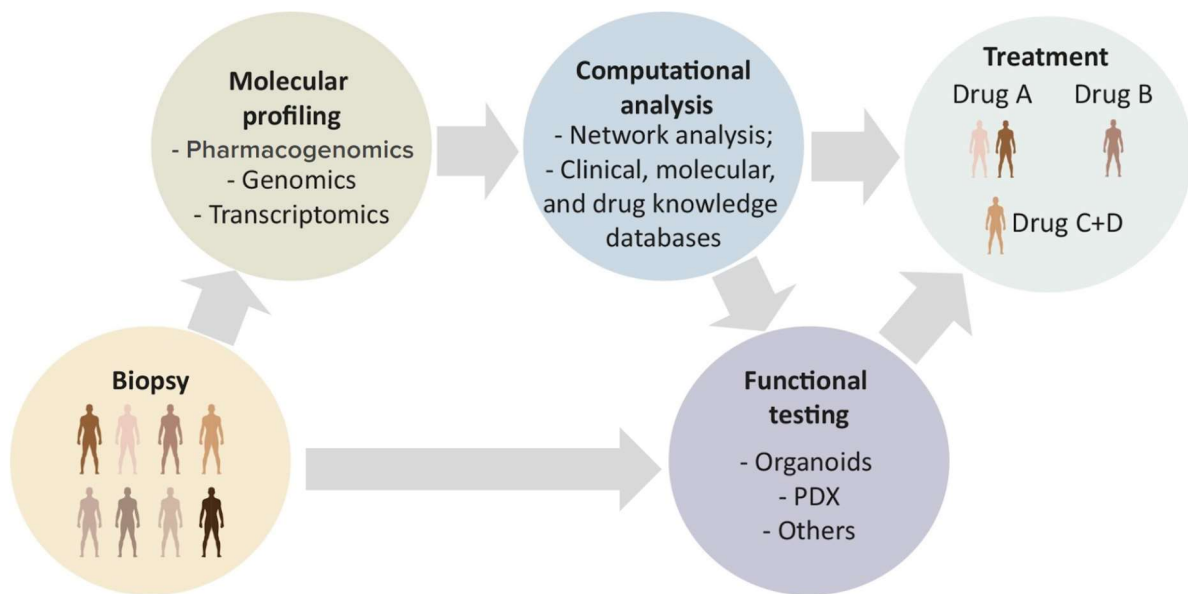


Figure 1: The general precision oncology workflow. Firstly, a biopsy is taken from patients in surgery. Based on molecular profiling and computational analysis, biomarkers for targeted therapy are identified. Additionally, biopsy material is used to establish a patient-derived tumour model. Drug screening of relevant compounds will reveal drug sensitivities specific for the patient’s tumour. Information from both the biomarker targets and functional testing will guide decision making on treatment strategies. Image adapted from Senft *et al.* [7]

Patient-derived tumour models

Patient-derived Explants

The most direct method for drug screening of a patient-derived model is the explant (PDE) model. This model describes the approach to directly use the tumour resection in drug screens, without resuspending the tumour cells [8]. The surgically removed tumour is fragmented in small explants or thin slices and cultured *ex vivo*, while retaining the histological features of the original patient tumour. A good example of such a slice culture approach is the CANScript technology and the associated company Mitra Biotech [9][10]. Here, patient tumour slices are cultured in wells coated with tumour-stromal matrix proteins and supplied with serum derived from the same patient. Thereafter, the recreated tumour ecosystem can be subjected to drug sensitivity tests. In the CANScript platform, the functional response of the patient-derived explants to anticancer drugs, combined with the corresponding clinical outcome is used to train an algorithm. This machine learning model is then able to derive the clinical outcome of independent patients to the anticancer drugs from the explant screen data [9]. A different explant model, culturing tumour fragments on the air-liquid interface of membranes, was also effective to assess drug responses for several types of cancer [11][12]. However, clinical predictability data from this approach is still lacking.

The main advantage of the PDE model is the ability to preserve the original architecture of the patient tumour, including tumour–stroma interactions and intra-/inter-tumoural heterogeneity. Additionally, PDEs can be cultured and screened within 72 hours of surgical resection, allowing rapid sensitivity assessment and treatment strategy advice. On the flip side, the explants will only remain intact in

culture for a maximum of 72 hours. Not only does this impact the study design and practicality of the model, tumour mechanisms such as acquired drug resistance, invasion and metastasis cannot be assessed. On top of that, tumour tissue cannot be expanded, which severely limits the maximum scale of drug screens.

Patient-derived Cells

A more versatile and expendable approach to personalized drug screens is the patient-derived tumour cell (PDC) model. In this approach, the tumour cells of the patient's resection are dissociated and cultured in 384-well plates. Some studies explored this method for drug screening of glioblastoma patient material [13][14]. Recently, a large-scale PDC-based drug sensitivity screen was performed across multiple cancer types. In this screen, researchers assessed the clinical response and predictive power of PDCs in retrospective clinical studies [15]. Lee *et al.* screened PDCs from 31 patients across four major cancer types (gastric cancer (n=17), glioblastoma multiforme (n=8), lung adenocarcinoma (n=5), and atypical meningioma (n=1)). The *in vitro* PDC drug sensitivity demonstrated high concordance with the clinical response of the patients. For example, some patients showed promising genomic indications for sensitivity to certain drugs. Subsequently, these drugs gave no response in the PDC model, and clinical response in the patient was similar to the model. Even though these initial results are promising and show the value of the PDC model, additional large-scale clinical studies are required to confirm the predictive power of PDCs.

Over the past years, a new technique was developed to improve the stability of PDCs. This new protocol allows for expandable and stable long-term culture of patient-derived cell lines -dubbed "conditional reprogramming" (CR) [16][17]. The technique uses a coculture of irradiated mouse fibroblast feeder cells with patient tumour cells in the presence of a Rho kinase inhibitor (Y-27632) to establish a stable tumour model. This approach combats the lack of stability in the original PDC model and allows for long-term culture of PDCs. Even minimal primary tissue biopsies can be expanded up to 1 million cells within a week, making the technique ideal for drug screen purposes [17]. A recent study created 28 CR cell (CRC) patient-derived tumour models of pancreatic cancer and conducted anticancer drug response analysis [18]. The CRC models showed similar characteristics to the original tumour samples, and CRC model drug sensitivity was concordant with patient prognosis.

The main disadvantage of PDCs and CRCs is that the model will not mimic the tumour architecture, such as the tumour microenvironment, since the patient tumour is dissociated to single cells before culture. However, cell line culture is very scalable and conditionally reprogrammed cells are additionally easier to maintain, which makes the model suitable for high-throughput drug screens. Furthermore, cell line culture is cost-effective and drug screen results can be obtained within weeks after surgery.

Patient-derived Xenograft Mice

The patient-derived xenograft (PDX) *in vivo* mouse model has been widely used as a platform for cancer research and anticancer agent development for many years [19]. In the xenograft approach, the patient's tumour biopsy is engrafted into an immuno-deficient mouse host. The tumour remains viable for weeks to months under proper immunosuppression to prevent tissue rejection. The graft acceptance efficiency is sufficient, although it varies heavily. More aggressive cancer types are easier to establish, compared to lower graft efficiency rates evident in less aggressive malignancies [20][21][22]. Studies in many cancer types have confirmed the resemblance of PDXs to patient tumours for genomic, epigenetic and histopathological characteristics [23][24][25][26][27][28][29].

Interestingly, gene expression profiles were reported to differ between patient-derived cell lines and PDXs grown from the same patient's primary small cell lung cancer biopsy [30]. While the PDX model showed gene expression resemblance to the original tumour, in vitro culturing of PDCs seemed to have caused a gene expression drift from the original tumour. More recently, another direct comparison between PDXs and PDCs was conducted for 20 liposarcoma patients [31]. In this study, the genomic profile of both models was similar, and also resembled the profile of the patient. Again, the PDX model reproduced the histological characteristics of the patient tumour.

Although the PDX model can represent the patient tumour to an impressive extent, evidence for the predictive power of the model for clinical response in the patient is still lacking. Some studies have explored the concordance between the PDX model response and patient response to the same anticancer agents, and these results are promising [32][33][34][22]. A good example of such results is the study by Hidalgo et al., where PDX models were established from fourteen patients with refractory advanced cancers, and a 63-agent drug screen was conducted [35]. 12 patient models showed significant sensitivity to one or more treatment regimens, and 11 patients received one or more prospectively guided treatments. Of the total of 17 targeted treatments that were carried out, 15 resulted in durable partial remissions in the patient. While such results are circumstantial and more large-scale clinical studies are needed, the predictive power of PDX models could certainly inform therapeutic decision making in future.

Still, several limitations are apparent when assessing the suitability of PDX models in precision medicine drug screen approaches. In terms of establishing the patient-derived model, engraftment efficiency varies for different cancer types, and it takes around 9 months before the engraftment is stable and large enough for screening to commence. Furthermore, while PDX tumours generally resemble the patient tumour sufficiently, it has been shown that clonal selection occurs upon engraftment of the tumour, which will affect clonal dynamics of the PDX and therefore might affect the predictive power of the model [36]. Also, the murine host will affect the tumour microenvironment. After 3 to 5 passages, which is also when PDX models are sizable and viable enough to be used for drug screens, the tumour-associated stroma of the graft are almost completely replaced by murine-derived extracellular matrix and fibroblasts. Not only can this influence intratumoural heterogeneity and clonal dynamics, the new mouse stroma likely causes drastic changes in the paracrine regulation and the physical properties of the tumour such as the interstitial fluid pressure [37]. These factors will influence both patient tumour representation and anti-cancer agent distribution through the model. Additionally, high throughput screens are extremely difficult in this model, as limited patient tissue and engraftment failure will restrict the amount of PDX mice eligible for screening. On top of that, high costs are associated with the PDX mouse model, as large facilities are needed to maintain high amounts of immunodeficient mice for long periods of time.

Patient-derived Xenograft Zebrafish

Zebrafish have been a well-established model in many veins of research for decades. With regards to personalized medicine, recent studies provide arguments for using zebrafish as hosts for xenotransplantation of the patient's tumour. Both zebrafish larvae and adult zebrafish are worth considering in this context, as both models provide different opportunities which will now be discussed. Xenotransplantation efficiency will rely on circumventing the immune system of the host to prevent tissue rejection. The adaptive and innate immune systems in zebrafish are highly conserved with humans and mice [38]. While innate immunity development starts immediately after fertilization, the adaptive immune system only matures 2 to 3 weeks later [39]. This discordance leaves a window of

opportunity where the zebrafish larvae immune system is compromised, which allows for efficient transplantation and short-term survival of xenotransplants [40].

Zebrafish Larvae

Initial attempts of xenotransplantation in zebrafish larvae included transplanting human cancer cell lines, as described in 2006–2007 [41][42][43]. A large number of similar studies have reported cancer cell line xenotransplantation into zebrafish larvae since. A decade later, three proof-of-concept studies developed patient-derived xenografts (PDXs) in zebrafish larvae from both blood malignancies and solid tumours [44][45][46]. Additionally, studies showing tumour subpopulations cooperation and drug response tests in zebrafish larvae were published [47][48].

A large advantage of the larval zebrafish model is that no steps for immunosuppression of the host need to be taken. Not only does this improve ease of use in drug response studies, it additionally improves standardization and prevents interaction of immunosuppression agents with the tumour or the drug screens. Furthermore, the zebrafish larva model has the advantage of a natural optical clarity, as pigmentation of the zebrafish only develops later on. This lends the model to easy *in vivo* imaging, analysis and quantification of the transplant.

A good example of a proof-of-concept study analysing the predictive power of sensitivity of larvae models by Fior *et al.* [48]. FOLFOX chemotherapy and cetuximab treatment were assessed in five colorectal cancer zebrafish larvae PDXs. PDX responses were concordant to the patient responses for both FOLFOX treatment (4 out of 5 patients) and cetuximab treatment (3 out of 3 patients). An ongoing trial (NCT03668418) is currently recruiting 120 cancer patients to quantitatively determine the predictive power of larval PDXs [49].

Zebrafish larvae are a quick model to produce drug screen data for the patient. Drug assays can provide a read-out of drug sensitivity in 4 to 7 days. However, zebrafish larvae would not provide accurate information on secondary resistance development in the tumour, as the small graft does not represent many tumour subclones, and the window of graft acceptance is too short to monitor acquired resistance. Furthermore, several limitations regarding zebrafish larva model practicality may obstruct its use in precision medicine. Firstly, while the immune system discordance creates a window for rejection-free transplantation, the engrafted cells will be rejected as soon as the adaptive immune system matures after one week [48]. Secondly, another significant limitation of a zebrafish larva is that its small body size only allows engraftment of about 100-200 cells. The small size of the transplant will therefore likely not contain cancer stem cells or emulate the heterogeneity of the patient tumour [48][50]. After transplantation, the development of the tumour and its subclones will therefore likely not follow a pattern representative of the patient. Thirdly, the zebrafish larvae are usually best kept in temperatures around 34°C. This relatively low temperature will affect proliferation rates and behaviour of the tumour graft [51]. These limitations significantly impact the viability of zebrafish larvae as patient avatars, and recent advancements in adult zebrafish sought to overcome these hurdles.

Adult Zebrafish

A good example of an adult zebrafish model for xenotransplantation is the immunodeficient zebrafish strain that was developed and published in 2019 by the Langenau group [50]. Via homozygous inactivating mutations of two immune system proteins in a transparent zebrafish strain, an optically clear and immunodeficient model was created. Their studies show how this model accepts both different cancer cell lines and patient-derived xenografts for over 28 days. On top of that, the adult

zebrafish will accept grafts of up to two million cells. Finally, the adult zebrafish can tolerate a habitat of 37°C if slowly acclimatised. Efficiency of the engraftment is also sufficient, as several proof-of-principle studies reported successful engraftment in around 40-90% of larvae and in 30-80% of adults across multiple cancer types [48][52][53][54][55]. Taken together, these characteristics overcome the previously discussed limitations of zebrafish larvae, rendering adult zebrafish as a viable consideration in precision medicine. However, although an initial model was developed by the Langenau group, establishment of a robust patient-derived tumour model in adult zebrafish that lends itself to drug screening is needed. Due to this lack of proper models, no co-clinical studies assessing the predictive power of adult zebrafish PDX models have been published.

The zebrafish PDX model, both larval and adult, makes a strong case regarding its practicality in drug screens. Drug treatment is carried out by either adding agents directly to the water, via intraperitoneal injection, or oral gavage, making the treatment method accessible and open for automation [56][57][58]. The optically clear nature of the model allows for facile *in vivo* real time imaging of the tumour response. With apt labelling of the patient-derived cells, tumour response can be analysed down to single-cell resolution on a high-throughput or even automated basis [59][60][61]. Lastly, maintenance and husbandry costs are relatively low. Given their size, small facilities can hold thousands of zebrafish and rough estimates indicate a cost of around US\$0.01 per adult zebrafish versus a cost of US\$1.05 per mouse [62]. While the predictive power of the zebrafish PDX model still requires extensive and diligent testing, the apparent practical advantages and automation opportunities render the model worthwhile to pursue.

Patient-derived Organoids

The organoid technology continues to have a growing impact in oncology research. On top of the role of organoids in improving understanding of disease development and drug discovery, patient-derived organoids (PDOs) are more and more being used in proof-of-concept precision medicine studies [63]. One of the first reports of organoids derived from cancerous tissue was published in 2011 by Sato *et al.*, where small intestine and colon organoids were grown in culture conditions initially established for mouse-derived organoids [64]. Since then, the organoid field has expanded even more, and organoids for many different types of tissue and tumours have been developed [65].

In general, PDOs are generated by plating a single-cell suspension in medium resembling an extracellular support matrix, usually Matrigel® or Basement Membrane Extract [66]. The single-cell suspension is obtained by dissecting the patient biopsy via enzymal or mechanical digestion. Upon proper nutrient provision, the patient-derived cells will grow into spheroid shapes that retain the genetic landscape and histological properties of the original tumour. At the time of writing, currently established and published patient-derived cancer organoids include PDOs of liver, prostate, breast, colon and pancreas tumours [67][68].

Several characteristics show that PDOs are able to resemble the patient's tumour closely. Firstly, gene expression profiles remain stable, even after long periods of culturing. In a study on patient-derived prostate cancer organoids, the organoid lines showed mutational landscapes identical to the original patient tumour after three months of *in vitro* cell culture [69].

Furthermore, intertumour heterogeneity is preserved in the organoid culture, and sub-clonal populations from the original tumour re-establish in the PDO, as shown by a colorectal cancer organoid study from the Clevers lab [70]. Advances in automated readout techniques, such as imaging and

sequencing [71], made analysis of PDOs on a larger scale possible, leading to proof-of-concept drug response studies in PDOs.

The first report of a PDO-based drug response study appeared in 2018, when Vlachogiannis *et al.* analysed 23 metastatic gastrointestinal cancer organoids [72]. As reported before, the phenotypic and genotypic profiles of the PDOs showed high similarity to the original patient tumours. Drug responses of the PDOs were compared to those of the patients, revealing the predictive power of the organoids. 100% of the patient organoids were sensitive to at least one anticancer agent, with 88% of organoids exhibiting a positive predictive value and 100% showing a negative predictive value for the patient response. On the basis of these promising results, similar studies were conducted to assess the predictive power of PDOs for various cancer types.

Recently, Wensink *et al.* published a detailed overview of the total of 17 PDO predictive power studies that have appeared since the Vlachogiannis paper [73]. By critically evaluating the data and methodology of these studies, an overview is created of the power of PDOs as a predictor of patient response to anticancer agents in various types of cancer. It must be taken into account that it is difficult to compare these different studies, as aspects such as cancer types, culturing approaches, end-point readouts differed extensively between the studies. Nonetheless, Wensink and colleagues reported interesting observations from these studies. In general, the patient cohorts were small, with a median of 7 patients per study. Five of the 20 cohorts from the 17 studies showed a significant correlation and/or predictive value between the PDO model drug response and the patient's clinical response. In 11 of the 20 groups a trend for correlation and/or predictive value was concluded, against 3 groups where no correlation was found, while one study was not able to determine association. Reproducibility of PDO responses was shown in two studies, indicating that drug responses remain consistent between passages and replicates [74][75]. Additionally, Wensink *et al.* evaluated initiation efficiency of the patient-derived organoid cultures across these studies. The establishment rate was reported in 12 studies and ranged from 30% to 90%. Via a random effects pooled analysis, establishment rates were pooled and determined at around 68%. Initiation efficiency is mainly dependent on both cancer type and the tumour cell percentage in the patient biopsy, as is the growth period for PDOs, which averages around 20-30 days. Taken together, the collected data from the clinical studies illustrate the viability of PDOs in precision medicine.

However, several limitations of PDOs will need to be overcome for the model to claim a strong position as a precision medicine model. As discussed, initiation efficiency and culturing time differ greatly, and have only been tested in a limited amount of cancer types. Furthermore, structural characteristics of the patient tumour are lost when PDOs are generated via single-cell solutions. Aspects such as tumour vascularization, the tumour microenvironment and the surrounding immune system are not represented in the model [76].

Comparison of models

As discussed before, suitability of a patient-derived tumour model for precision medicine therapy can be scored on several features regarding (1) patient tumour representation, (2) practicality of the model and (3) drug screen effectiveness. A comparison of the performance of the different models in these categories is laid out in *table 1*. (1) Patient tumour representation is divided into important factors for genome and clonal dynamics, namely genetic stability and heterogeneity, and tumour architecture, defined as tumour-stroma interactions and conservation of the tumour microenvironment (TME). These factors heavily influence therapeutic resistance of a tumour and therefore need to be

represented in the model to achieve a high predictive power [77]. (2) The practicality of the model is determined by ease of use, scalability and costs, but also efficiency of establishment of the model. Furthermore, the time that it takes for drug screen results to come through is significant for the patient. Speed is therefore also an essential consideration for the applicability of the model. (3) Drug screen effectiveness is scored on the ability to scale and implement the model into low- and high-throughput drug screens, and more importantly whether results from clinical studies showed a predictive value of the drug responses.

When scoring the different models for these features, several patterns are observed. Patient-derived explants represent the patient tumour best, as the tumour architecture remains intact during processing. However, tissue cannot be expanded or maintained and therefore practicality and drug-screen potential is very limited. PD Cell lines lose important characteristics of the patient tumour, but are very suitable for large scale screening purposes. Organoids can more sufficiently resemble the patient tumour and remain relatively easy to handle. The mouse and zebrafish xenograft models allow for *in vivo* modelling of the patient tumour, ensuring good tumour representation. However, they are difficult to scale up and maintain, limiting their viability for drug screens.

Table 1. Characteristics of patient-derived tumour model systems. Features are categorised for patient tumour representation (orange rows), practicality of the model (green rows) and drug screen effectiveness (blue rows). Features are rated as best (++), suitable (+), possible (-/+) and unsuitable (-) (adapted from Sachs & Clevers, 2014 [78], and Bleijs et al., 2019 [79])

	Feature	PD Explants	PD Cells	PD Organoids	PDX Mice	PDX Zebrafish
Patient tumour representation	Genetic stability	++	+	++	+	+
	Intra-tumour heterogeneity	++	+/-	+	+	+
	Tumour–stroma interaction	++	-	+/-	+/-	+
	Tumour microenvironment conservation	++	-	-	+/-	+/-
Practicality	Ease of use	-	++	++	+	+
	Initiation efficiency	+	++	+	+/-	+/-
	Scalability	-	++	++	+/-	+
	Speed	++	++	+	-	+/-
	Costs	++	++	+	+/-	+
Drug screening	Low-throughput drug screens	+/-	++	++	+	+
	High-throughput drug screens	-	++	+	-	+
	Prediction of clinical response	+	+	++	++	+

Taken together, the comparison of the patient-derived tumour models identifies suitable and unsuitable models for precision medicine approaches. The PDX Mouse model suffers from high costs, low scalability and low speed, rendering it inefficient as a model for clinical drug screening. The PDX zebrafish models improve all these factors significantly, but clinical evidence is lacklustre and more proof-of-concept studies need to be conducted to validate predictive power and tumour representation of the model. Patient-derived explants have excellent tumour representation capabilities, and can produce drug sensitivity results extremely quickly. However, explants cannot be maintained or expanded, which excludes the model for application in large-scale drug screens. Patient-derived cells are inexpensive and easy to implement in large drug screens, yet their limited tumour representation questions the predictive power of the model. Patient-derived organoids form an intermediate between the *in vivo* and *in vitro* models. Their tumour representation is impressive, and the model remains relatively easy to handle and scale up. Given the clear potential of organoids as a model for patient tumours in drug screen applications, the ongoing improvements of the model for this context will later be discussed.

Discussion

Additional considerations on patient-derived tumour models

Importantly, all models share characteristics that need to be discussed. Firstly, none of the patient-derived tumour models is able to replicate an active immune system. This is very relevant since the development and potential of immunotherapy cancer treatment. Immunotherapy relies on the immune system of the host to help combat cancerous tissue [80]. The current models are not able to predict efficacy of immunotherapy agents, severely limiting guidance in clinical decision making of these therapies. Additionally, ethical considerations need to be taken into account when comparing these models. Currently, many guidelines and ethical committees watch over ethical standards in research, and aspects such as animal welfare, patient consent, public opinion, privacy and handling of patient material or data are closely monitored [81]. However, should individualized therapy expand to become more central to cancer care, these ethical aspects become even more important. *In vivo* models already stir a public debate, making it undesirable to opt for *in vivo* model implementation in large-scale precision medicine programs.

Future of patient-derived organoids as tumour model

Regarding the improvement of tumour representation in the PDO model, many promising approaches are emerging. As discussed, the tumour microenvironment (TME) is very influential in tumour dynamics, and is difficult to recapitulate in a tumour model. Attempts to resemble the TME in organoids led to the development of protocols for co-cultures of organoids with stromal and immune cells [82]. Not only might co-cultures improve tumour representation in PDOs, differentiation yield and initiation efficiency could also be improved by co-culturing organoids with human-induced pluripotent stem cells or stromal cells [83][84]. Dijkstra *et al.*, even showed how co-culture of PDOs from colorectal cancer or non-small-cell lung cancer with peripheral lymphocytes leads to the expansion and enrichment of T-cells [85]. These T-cells are able to both recognise and kill tumour organoids but, remarkably, ignore healthy organoids. With this type of model, investigations into immunotherapy on PDOs are becoming a possibility.

The tumour environment can also be tailored to specific types of cancer. Usui *et al.*, showed how the luminal surface in colorectal cancers can be resembled by building an air-liquid interface system for the

organoids to grow in [86]. Here, the basal surface of stem cells is in contact with growth media while the apical surface is exposed to air, which can more accurately resemble tumour microenvironment conditions. Interestingly, the air-liquid system organoids demonstrated a more resistant response to anti-cancer agents compared to classic colorectal organoids. All these factors will further improve tumour representation of the patient-derived organoid, potentially leading to a higher predictive power of the model.

Several recent studies are tackling practical limitations of PDOs for precision medicine purposes. Currently, most organoid studies use animal-derived extracellular matrices, such as Matrigel® or Basement Membrane Extract, which are biologically variable and contain animal-derived growth factors [87]. Given the variable nature of these extracts, the animal-derived matrices could reduce the reproducibility of drug screen results and influence PDO stability and dynamics in culture, reducing patient tumour representation. Synthetic hydrogel matrices are fully defined and free of growth factors. These hydrogels can establish human PDOs from cell lines in single cell suspensions and are subsequently amenable to drug screens [88].

An interesting alternative was shown by Mollica *et al.*, who produced a self-generating hydrogel consisting of extracellular matrix derived from human mammary tissue instead of Matrigel® [89]. Future studies will determine whether synthetic or human-derived hydrogels can also be used to establish PDOs directly from patient-derived tissue and whether use of synthetic hydrogels improves the reproducibility of drug screens compared to animal-derived matrices. Similarly, Wnt growth factor supplements required for adequate growth of PDOs are currently serum-based. Serum-free supplements are becoming increasingly available, which will allow for serum-free organoid culture medium [90].

Automation of organoid-based drug screens is also developing at a high pace. Recent studies show high throughput drug screen improvements such as protocol optimization or an automated microfluidic platform for PDOs, which enables addition of agents at different time points [91][92]. This better resembles the combination treatment regimens given to patients in clinics. To improve on end point analysis methods such as tumour volume or cell viability, automated image-based analysis was developed [93]. This allows researchers to assess multiple end points, which can better resemble the full drug response of PDOs.

Considerations for precision medicine study design

Regardless of what model is used in the precision oncology pipeline, several considerations for study design need to be taken for further improvement and implementation of the approach. Mainly, studies need to be properly reported and more standardized. A complete report of a drug screen study includes clear mentioning of the establishment rate of the model and detailed defining of clinical and model drug response. Establishment efficiency limits many of the different models, and several factors influence this rate. In order for labs to produce patient-derived models efficiently, clear reporting and communication of aspects such as culturing techniques or transplantation protocols is essential. Similarly, factors such as the origin and type of tissue obtained, initiation efficiency per patient and per sample, patient profile characteristics associated with successful initiation, and timespan between surgery and drug screen results, could be reported. Additionally, transparent reporting of how a successful initiation was defined and verified needs to be included. Lastly, a clear definition for clinical response and *in vitro* response needs to be disclosed. As indicated by the comparison study of Wensink

et al. [73], drug response analysis and definition varies between studies. In order to interpret the clinical applicability of a model, and to allow for reproducibility, this response analysis and definition needs to be discussed. In essence, methodological standards should dictate quality assessment, result interpretation and reporting. A good example of such standardization is the REporting recommendations for tumour MARKer prognostic studies (REMARK) guidelines. These can be used for reporting of studies regarding predictive biomarkers in cancer [94]. Standardized experimental design will allow for facile comparison of study results, which provides several advantages. Validation of new experimental designs or models can be done much faster, as previously tested and reported model studies can be used to validate results. Furthermore, previously published drug screen results can be consulted to determine whether a patient's or patient's model drug screen response is relatively resistant or sensitive.

Another important aspect of the precision medicine methodology is the size of the drug screen. While adding many drugs to the screen might identify potentially efficacious compounds, a larger screen also requires more tumour models to be expanded. Since expansion is difficult and time-consuming, drug screen size should be limited but sufficient. Compound inclusion can be limited to drugs that are hypothesized to be effective from literature and biomarkers, but are also available to the patient either on market or in trial. While this might limit compound choices and chances for identifying an effective agent, drug screening can start earlier, providing guidance on therapy decisions as early as possible.

Finally, an interesting addition to precision medicine studies is the opportunity to establish a patient-derived model of healthy patient tissue. During surgery, often an extra layer of healthy tissue is taken to ensure that all cancerous tissue is removed. Alternatively, during surgery it is usually possible to additionally obtain small biopsies from normal tissue without great discomfort for the patient. With the healthy patient cells, healthy patient-derived models can be established next to the patient-derived tumour model for various uses. First of all, the healthy models can be subjected to potential drugs in order to assess toxicity of the anti-cancer agent to the healthy patient tissue. This allows for additional information on drug concentrations and therapeutic decision making on a personalized basis.

Concluding remarks

Precision medicine holds great promise as an approach to improve drug treatment efficacy in cancer. Patient-derived tumour models can be used to screen anti-cancer agents and guide clinical decision making. Upon review of the different models, patient-derived organoids emerge as front-runners for a practical and patient resembling model that allows for large scale drug screens. However, the other models all fulfil a different combination of characteristics that merit additional research and development. More clinical studies into the predictive power of these models could point out more potential and significance. On top of that, precision medicine programs do not necessarily need to stick to a single model for all patients. Patient-specific considerations such as cancer type or tumour tissue availability might favour certain models, or possibly even a combination of models. Again, more clinical data on the models and their predictiveness for different cancer types is needed. In conclusion, future development and engineering of the patient-derived tumour models will tailor them towards optimal precision medicine implementation. When models can be established efficiently for the majority of patients within a feasible time frame, this potential predictive biomarker model can facilitate personalized treatment guidance to patients for whom there is a great need for valid predictive biomarkers.

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