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(Article begins on next page)

Preclinical models for precision oncology

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ABSTRACT

Precision medicine approaches have revolutionized oncology. Personalised treatments require not only identification of the driving molecular alterations, but also development of targeted therapies and diagnostic tests to identify the appropriate patient populations for clinical trials and subsequent therapeutic implementation. Preclinical *in vitro* and *in vivo* models are widely used to predict efficacy of newly developed treatments. Here we discuss whether, and to what extent, preclinical models including cell lines, organoids and tumorgrafts recapitulate key features of human tumors. The potential of preclinical models to anticipate treatment efficacy and clinical benefit is also presented, using examples in different tumor types.

KEYWORDS

Preclinical models – Patient-derived xenografts (PDX) – Organoids

1. INTRODUCTION

Genomic driven precision medicine approaches are transforming the field of oncology [1-4]. The advent of high throughput DNA sequencing techniques led to the identification of hundreds of recurrent somatically altered genes through the analysis of tens of thousands of cancer samples [5-8]. Thanks to these efforts, a new generation of biomarkers has become available with the discovery of the genetic alterations that are responsible for the initiation and progression of human cancers [7, 9-12]. Personalized treatments require not only the identification of the driver molecular alterations, but also the development of targeted therapies and diagnostic tests to identify the appropriate patient populations for clinical trials and eventual new therapeutic implementation [13-15].

Successful investigations encompass the use of trastuzumab, a monoclonal antibody against HER2, for *HER2* amplified breast tumors [16], and the drug imatinib, an ABL kinase inhibitor, used in a subset of leukemia driven by *BRC-ABL* gene fusions [17]. This early positive research has been replicated in numerous cancers [1-4]. Different tumor types, such as colorectal, breast and non-small cell lung cancer, are routinely genomically profiled, and patients are treated with therapies directed against the specific molecular alterations driving their tumors. However, translational research has demonstrated individual diversity, as well as intratumoral heterogeneity within a patient's tumor. Precision medicine aims to exploit patient-specific molecular alterations present in tumors to identify treatments with the greatest probability of clinical benefit. Therefore, precision medicine strategies that encompass tumor heterogeneity and patient diversity are urgently needed.

Preclinical models that recapitulate key features of human tumors are widely used to confirm the therapeutic efficacy of a compound or to identify additional options beneficial to both clinicians and patients. Both the discovery and preclinical testing of novel therapeutic strategies require the use of *in vitro* and also *ex-* and *in vivo* models, such as cell lines, conditionally reprogrammed cells, organoids and/or patient-derived xenografts (PDX). The selection of the appropriate preclinical model has the potential to ensure higher predictability of preclinical research. However, of all the candidate-targeted therapies according to preclinical data, only approximately 5% demonstrate efficacy within clinical trials [18]. Advances in methods to generate patient-derived *in vitro* and *in vivo* tumor models provide an opportunity to directly test the sensibility of patient tumor cells to a large number of drugs (Figure 1).

Herein, we will discuss successful approaches performed using different preclinical models, with a focus on the models that led to new treatment hypotheses which, when tested in the clinic, established new therapeutic strategies.

2. CANCER MODELS: FROM PAST TO PRESENT

The use of preclinical models is crucial for translational cancer research and precision medicine, from the biologic aspects of the disease to the development of new treatments. The identification and validation of targets requires models which represent the disease, the patient population and the healthy reference. The use of cancer models for drug screening began in the 1970s after a three-decade period of murine models used for drug screening [19]. They are selected based on similarity to human biology and disease genotype and phenotype.

Cell culture has been widely used in preclinical research. Numerous immortalized cell lines derived from different human tumors have been published, though many histological subtypes are underrepresented. While they are suitable for molecular and genetic research, as well as for biochemical and mechanistic analysis, they may fail in predicting how a drug will perform in a patient population. Unfortunately, they do not adequately model the biology of the native tumor. There are also murine cell lines available, although there are far fewer murine lines and they are not as well characterized as human lines. In addition, less common cancer mutations are generally unavailable among these cell lines. Depending on the cultivation method, there are several models. Primary cultures are short-term cultures derived from freshly isolated tumor cells or small pieces of tissues or organs. Although they mimic the pathological and physiological environment, they have several limitations, such as slow growth capacity, limited overall lifespan, cell selection over passages and loss of tissue histology and microenvironmental factors. Primary cultures are still imperfect models and cannot be derived from matching normal tissue (Table 1).

Nevertheless, there are different ways of creating *in vivo* conditions for drug discovery. Cell line xenografts are generated by the injection of human cells subcutaneously into an immunodeficient mouse. This technique is relatively easy to perform and tumor formation can often be easily obtained. On the other hand, they do not fully recapitulate tumor heterogeneity and histological features, do not encompass the role of host immune system, and the interactions between cancer and stromal cells and the injection in the subcutaneous tissue is a very different microenvironment than its original onset (Table 2). More sophisticated and advanced models, such as three-dimensional (3D) organoid cell cultures, PDXs or organoid xenografts using primary human samples, have significantly improved drug studies. These latest models overcome some of the limitations of cell line culture and xenografts and they are thought to better represent the *in vivo* situation with regard to cell shape, molecular heterogeneity, host immune system and extracellular environment. Allograft mouse tumor models, also known as syngeneic models, are tumor tissues derived from the same genetic background as a given mouse strain. Mouse cancer cell lines or solid tumors are engrafted back into inbred immunocompetent mouse strain, creating an immunocompetent model for immunotherapy assessment. Finally, three-dimensional culture systems allow the recombination of epithelial and stromal cells, simulating the cell interactions that exist *in vivo*. Both organoids and PDXs models will be further discussed in details. More recently, strategies for orthotopic

transplant models or genome editing lead to the development of innovative cell lines and mouse models that could significantly improve drug discovery and precision medicine.

As mentioned above, we will discuss successful approaches using different preclinical models that led to new hypotheses that in turn established new therapeutic approaches.

2.1. Cell line models

The use of human cell lines as an *in vitro* model has been the gold standard for elucidating signaling pathways in cancer since the derivation of the HeLa cervical cancer line in 1951 [20]. They have several advantages. Cell lines are relatively easy to handle and inexpensive to use and provide rapid experimental results. In addition, they provide an unlimited self-replicating source that can be grown in large quantities. Standard cell lines are relatively molecularly homogeneous and most of the commonly used cell lines have been genomically profiled and found to properly recapitulate the genetic landscape of different human cancers. Of special interest are large collections of cell lines often referred to as human cancer cell encyclopedias, which have been extensively characterized at the genomic and pharmacologic levels. For example, the Broad-Novartis Cancer Cell Line Encyclopedia (CCLE) provides public access to genomic and drug sensitivity features for about 1000 cell lines (<https://portals.broadinstitute.org/ccle>). Most notably, cell lines can be genetically manipulated through different mechanisms such as homologous recombination, short hairpin RNA (shRNA) gene knockdown, or CRISPR-Cas9 gene editing. Finally, multiple agents can be concurrently tested against a range of cell lines.

While they can be easily expanded, pharmacologically tested and genetically manipulated, cancer cell lines also have several intrinsic limitations. They often only represent a clonal population of the initial tumor mass. The inability to properly recapitulate 'molecular heterogeneity' of human tumors is often considered a major limitation of cancer cell lines. However several cell lines were shown to at least in part maintain molecular heterogeneity. For example lung or colorectal cancer cells are known to carry preexisting drug resistance mutations (such as the KRAS or EGFR variants), which are present at low frequency in human cancer tissues and emerge upon treatment with targeted therapies such as EGFR inhibitors. [21, 22]. Thus, genomic heterogeneity is also a feature of standard cancer cell lines

Cancer cell lines are selected to grow in culture plates and predefined media and continuous passaging in culture may result in genotypic and phenotypic drift. Therefore, subpopulations may arise causing phenotypic changes over time. In addition, they do not entirely recapitulate the functional and genetic heterogeneity of human cancers, which goes towards explaining resistance to targeted therapies [23]. Importantly, cross-contamination among cell lines has been confirmed [24], authentication techniques and the use of cells from repositories have minimized the risk of cross contamination. Finally, cell lines are difficult to establish directly from individual patient tumors. Despite these limitations results obtained in cells models have been successfully translational in the clinical settings often with remarkable results (Table 1).

For example Prahallad and colleagues discovered the molecular mechanism responsible for resistance to BRAF inhibitors in BRAF mutant colorectal cancers (CRC) using a series of melanoma and CRC cell models [25]. Activating V600E mutation in the *BRAF* gene is seen in 70% of primary melanomas, 10% of CRC and between 30-70% of papillary thyroid carcinomas [26-28]. Clinical responses to vemurafenib, a highly selective inhibitor of the BRAF (V600E) oncoprotein, differ extensively between different tumors. It is highly effective in the treatment of melanoma, while having limited response and poor prognosis in colorectal cancer patients [29, 30]. Clinical experiences were replicated in both short- and long-term proliferation *in vitro* assays, with melanoma cells being more sensitive than CRC cells to vemurafenib. The authors performed an RNA-interference (RNAi) based genetic screen of 518 human kinases and 17 additional kinase-related genes to identify enzymes whose knockdown synergizes with BRAF (V600E) inhibition [25, 31]. They reported that blockade of the epidermal growth factor receptor (EGFR) by cetuximab, gefitinib or erlotinib synergized with BRAF (V600E) inhibition. It was discovered that inhibiting BRAF (V600E) in CRC cell lines leads to feedback EGFR activation, which supports continued proliferation. On the contrary, melanoma cells express low EGFR levels and therefore do not experience this feedback activation. The study led to a new hypothesis: BRAF (V600E) mutant colorectal patients might benefit from a combinational therapy consisting of BRAF and EGFR inhibitors. This hypothesis was successfully clinically tested and approximately 8-10% of all colon cancers that harbor the *BRAF* gene V600E mutation benefit from this dual EGFR-BRAF targeted treatment [29, 30, 32].

Recently, McDermott and colleagues (2017) exploited cancer cell models to propose that combining **oestrogen receptor** (ER) IGF1R and HER2 targeted therapies may be an alternative for HER2/ER/IGF1R positive breast cancer patients [33]. The prognosis of *HER2* amplified breast cancer patients has improved significantly with the use of trastuzumab. However, patients that co-express ER have poorer response rates to HER2 targeted therapies. McDermott and colleagues explored publically available gene expression repositories and found that high expression of IGF1R is associated with shorter disease-free survival in HER2 and ER positive breast cancer patients. Therefore, they evaluated the therapeutic response of targeting ER and IGF1R in HER2/ER/IGF1R-positive breast cancer cell lines. Cells were treated with tamoxifen and two IGF1R targeted inhibitors (NVP-AEW541 and BMS-536924) and results indicated that dual **blockade** of ER and IGF1R enhanced **growth inhibition in the HER2 positive cell lines**. Furthermore, combined treatment with trastuzumab, tamoxifen and IGF1R inhibitors enhanced **tumor response**. This hypothesis should be tested in future clinical trials.

Finally, the use of cancer cell models for synthetic lethality studies has led to remarkable findings. The genetic concept of synthetic lethality was proposed over a century ago. A defect in one or two genes has little effect on the cell, but the combination of both defects results in cell death. In 2005, two groups used cell lines to demonstrate that tumors from patients carrying germline mutations in either *BRCA1* or *BRCA2* are sensitive to poly (ADP-ribose) polymerase (PARP) inhibitors [34, 35]. BRCA1 and BRCA2 proteins are critical to repair double-strand DNA breaks by a process called homologous recombination repair. PARP1 and

PARP2 enzymes are also key components of the DNA damage response. These BRCA-mutant tumors are characterized by a specific DNA repair, and these publications suggested a novel therapeutic strategy. PARP inhibitors (PARPi) [36, 37] were successfully tested in clinical trials [38-42]. Thus, a new therapeutic window was defined by the BRCA-mutant biomarker and patients can be stratified accordingly. This preclinical studies based on standard cell lines led to the first clinically approved drug designed to exploit synthetic lethality. Different tumors with deficiencies in other tumor suppressor genes involved in homologous recombination are candidates to test PARPi efficacy [43, 44].

2.2. Patient-derived xenograft models

Patient-derived xenograft models (PDXs) have emerged as an important platform for translational research and preclinical testing, to elucidate new treatments and biomarkers in oncology. They are used to address the therapeutic efficacy of new treatment targets, novel combinations of therapies, optimal schedules according to tolerability and sensitivity and primary and secondary resistance. Several studies suggest that PDX predict clinical response to therapy better than traditional xenografts [45, 46]. Currently, there are several collections of well-characterized PDX models from different tumor types in use for different personalized medicine strategies. These collections have been used for drug screening purposes, simulating a phase II trial in animals. PDXs capture more comprehensively than cell lines the epi/genetic landscapes, the cancer evolutionary dynamics during tumor progression and/or drug pressure, the tumor heterogeneity that exists both within a single patient tumor and across a population of patient tumors, and the mechanisms of resistance to treatment. Furthermore, genetic and histopathologic features of PDXs are thought to recapitulate the original tumor and to maintain their molecular landscape across passages [47, 48]. Gene expression profiles have shown that the donor tumors and their corresponding PDX are often well maintained.

Despite their advantages, some limitations are also associated with this preclinical model. Most notably, not all tumors transplanted in mice lead to the establishment of a stably growing PDX, and engraftment rates vary between 20-80% depending on tumor type and stage [49-52]. Successful implantation depends on various factors such as the site of implantation, mouse strains, tumor type and the aggressiveness of the tumor growth. Notably, many researchers embed the tumor fragment in matrigel before transplantation as this has been shown to improve engraftment's rates [48, 49]. As already mentioned, PDX models are generated by injecting small pieces of human-derived tumor samples subcutaneously into immunocompromised mice, which lacks the adaptive immune system. Accordingly, while PDX are well suited to interrogate cell autonomous processes they cannot assess the impact of T cell based phenotypes. Several strategies are in principle available to counteract this issue and humanized mouse models are being developed to study human xenografts in the context of a functional immune system. One possibility is humanizing the mouse immune system through replacing mouse genes with their human versions; alternatively it is possible to reconstitute an immune system in severely immunodeficient mice through engraftment of human peripheral

blood mononuclear cells, hematopoietic stem cells or fetal tissue [53, 54]. However these models are still being optimized and several hurdles need to be overcome. Notably, mice that have been genetically humanized or proteins involved in drug metabolism and toxicity and mice engrafted with human hepatocytes are emerging as promising *in vivo* models for an improved prediction of the pharmacokinetic, drug-drug interaction and safety characteristics of compounds in humans. [55].

Additional limitations of PDX are associated with fact that during the transplantation process, the stromal components of the tumor tissue are replaced by mouse stroma. However, although the human tumor microenvironment is lost during the engraftment, and the initial passages, which makes the evaluation of compounds targeting this compartment or crosstalk between stromal compartment and tumor cells still potentially possible [56].

While PDX have become widely used only recently, initial studies based on PDX date back to the 1980s when Fiebig and colleagues published a high degree of correlation between clinical response and cytotoxic agents in lung cancer patients and in their corresponding PDX models [57]. Employing PDX for individual treatment stratification remains a major challenge and expensive for clinical purposes. The engraftment time takes between 2-4 months and the evaluation of different treatment options takes even longer, a waiting time patients cannot afford before treatment initiation. Additionally, genomic changes occurring during tumor evolution in the patient are not reflected in the PDX, which might delineate comparability. Recently, Ben-David and colleagues analyzed 1110 PDX samples from 24 different tumor types in order to monitor the dynamics of copy number alterations (CNA). They observed a rapid accumulation of CNA during PDX passaging, and these new CNA differed from those acquired during tumor evolution in their corresponding patients [58] (Table 2).

Bertotti et al. have repeatedly demonstrated the value of large PDX cohorts in exploring mechanisms of resistance to cetuximab in CRC, evaluating alternative targeted-treatments and thereby creating an evidence-based rationale for clinical trials [59]. In 2011 Bertotti and colleagues published a large molecularly characterized PDX cohort from 85 metastatic CRC patients (xenopatients) [33]. PDX tumors retained the morphology and genomic features of their original counterparts and responded to cetuximab (anti-EGFR antibody) in the same way as was observed in the clinic. Of the non-responders, a fraction harbored activating mutations in *KRAS*, *NRAS*, *BRAF* and *PIK3CA*, known to contribute to primary resistance against EGFR inhibitors. Among wild-type cases *HER2* amplification was frequent, and confirmed clinically non-responsive *KRAS* wild-type tumors. *HER2* amplified PDXs were found to respond effectively to a combination of trastuzumab and lapatinib but not when the two drugs were used alone. The remarkable tumor regressions observed in PDX upon *HER2* blockade highlighted a therapeutic opportunity in cetuximab-resistant metastatic CRC patients. Accordingly a clinical trial named HERACLES was rapidly initiated. The HERACLES trial enrolled 914 CRC patients, identified 27 *HER2* amplified cases that were treated leading to a disease control of 59% [60].

2.3. Syngeneic mouse models

Syngeneic mouse tumor models are allografts immortalized from mouse cancer cell lines, which are then engrafted back into the same inbred immunocompetent mouse strain and they were initially developed over 50 years ago and have been used for *in vivo* pharmacological studies [61, 62]. These preclinical models have experienced resurgence due to the importance of immunotherapy assessment, as they endure an effective immune system [63, 64]. The allograft transplant is not rejected by the host immune system because the cancer tissue and the recipient share ancestry. Therefore, therapeutic interventions can be performed and syngeneic mice represent valuable preclinical models also for immune-oncology studies.

Recently, immunotherapy has evolved into a mainstream therapeutic option for many cancer patients. Clinical evidences of remarkable responses by immuno checkpoint inhibitors lead to the quest of additional immunotherapy targets as well as to models to understand the mechanisms of action of current drugs. Accordingly, *in vivo* models that recapitulate both phenotypic and genotypic features and the immune system across different tumor types are needed to validate the efficacy and safety of immunotherapy agents before their transition into clinical trials.

A particularly noteworthy advantage of the syngeneic model is that they also encompass the tumor microenvironment. In addition, their use is relative simple compared with other immunocompetent models such as humanized mice. Many syngeneic models have been extensively studied; they have been genomically and immunologically characterized. Finally, they are a suitable model for drug administration experiments and to evaluate the efficacy of drugs acting in cell autonomous and non-autonomous fashion.

A major disadvantage of syngeneic tumor models is that the transplanted mouse tissue likely does not represent the complexity of human tumors under clinical situations, since many of the mouse tumor cells are generated from carcinogen-induced models carrying complex and unstable genetic alterations. Another limitation is the number of cell lines available; which do not represent all tumor types.

Of special interest are genetically engineered mouse models (GEMMs), sophisticated models developed through the introduction of genetic mutations either in oncogenes or tumor suppressors that are associated with specific tumor types. They exist for several cancer types, including prostate, lung, breast, colon and pancreatic cancers [65]. Genetic engineering technologies have significantly improved over the last decade and the generation of GEMMs has been intensified. The introduction of conditional GEMMs, which are more sophisticated models, allowed controlling tumor onset and progression, in an inducible and cell lineage-/tissue-specific manner and have further expanded the applicability of GEMMs. Their application has enabled numerous mechanistic findings on tumor onset, progression, metastasis and responses to therapy [62] as they are designed to mimic human cancer in terms of genetic composition, interactions of cancer cells with their tumor microenvironment, drug response and resistance.

The major advantage of GEMMs is that these models have defined target genes involved in specific signaling pathways, known to be an important cellular component for cancer

progression and/or metastasis and with intact immune competent mice [66-68]. Therefore, novel target-drugs and/or drug combinations can be tested to improve therapeutic efficacy and to test the appearance of resistance mechanisms. Moreover, the generation of GEMMs in immune competent mice allows testing novel immunotherapies. Finally, they are a suitable model for translation research and biomarker studies, as they can be exploited for stratification of patients by specific mutations.

A significant caveat of the traditional GEMMs is that they do not properly recapitulate tumor heterogeneity as their molecular landscape is typically rather homogeneous. Most of the different GEMMs have been created from single germline mutations through the use of distinct technologies, and involve transgenes, targeted knock-out or knock-in among others. Furthermore, GEMM tend to harbor fewer mutations (lower mutation load) as compared to the corresponding human cancers. This, may compromise their utility in therapies based on the immune system, due to their low immunogenicity.

A major future goal of the GEMM field will be the development of models that completely recapitulate the human disease progression. In particular the availability of additional mouse cancer models capable of developing spontaneous metastasis would tremendously add to the field.

2.4. Organoid models: the combination of old and new strategies

Three-dimensional (3D) cancer cell cultures, represent an intermediate model between cancer cell lines *in vitro* and xenografts *in vivo* [69]. Multicellular tumour spheroids (MCSs) are spherical aggregates of malignant cells with reduced dimensions (less than 1 mm) that simulate some features of solid tumours. They get their spherical form by mechanical rotation or by other methods such as hanging droplets [70]. MCSs have been used since the second half of the 20th century [71]. MCSs models were further improved by using organ-specific stem/progenitor cells, henceforth called organoids. Organoids can be derived either from epithelium-only systems or with the addition of nonepithelial components such as stroma, immune cells, microbiota and so forth. These cells are embedded in a biomimetic hydrogel porous scaffold that creates an artificial niche allowing its self-renewing. First used in 2009 by Sato and colleagues using LGR5+ intestinal stem cells under selective culture conditions and embedded in matrigel produced an organotypic growth with highly polarized epithelial structures and proliferative crypts with differentiated villus compartments retaining in this way their tissue identity [72]. Organoids allow more accurate simulation of the native cancer tissue, as it is possible to preserve cellular morphology and heterogeneity as well as cell-environment crosstalk [73-75]. In addition, the complex structure of the 3D cultures induces the formation of concentric layers with different phenotypes, simulating the *in vivo* situation where the inner part of the tumor usually receives fewer nutrients and is less vascularized. Accordingly 3D model mimic the diffusion of chemicals inside the tumor, making this model suitable for drug efficacy studies [71, 76, 77]. Another advantage aforementioned of this preclinical model is the possibility to co-

culture different cell types, tumor and non-malignant cells, in order to explore the crosstalk between them.

Organoids can be derived from an individual patient, which creates the opportunity to build biobanks, to perform drug screens and/or facilitate drug development [74]. In addition, organoids have the advantage to reflect the original tumor heterogeneity. Therefore, in line with this thoughts and naming just a few, Sachs et al., established a collection of human mammary epithelial organoids that broadly recapitulate the genetic diversity of the disease, matching histopathology, hormone receptor status, Her2 status even gross genomic organization of the original tumor allowing *in vitro* drug screens consistent *in vivo* xeno-transplantations and with patient response [78]. Similarly, a study using an organoid biobank from colorectal cancer patients appeared to recapitulate the genetic properties of the original tumor, thus they were able to confirm sensibility to inhibitors of Wnt because one organoid carried a mutation in the gene RNF43, negative regulator of the path [74] Finally, in a study with organoids derived from human colorectal cancer metastases was confirmed the high similarity with the tissues from which they were derived, and interestingly, they also resemble the original tumor [75].

As discussed above organoids are supposedly derived from the tissue 'stem cell compartments' and according to the original definition should derive from a single (or a few) 'progenitor/stem cells. However there is confusion in the literature and several authors appear to consider 'spheroids', which are indeed just 'balls of cells' as equivalent to organoids. This is important, as 'patients derived organoids' are often simply a fragment of tumor tissue grown in 3D matrix.

Despite the noteworthy advantages of organoids, further work is needed to overcome the limitations caused by the long growth time compared with 2D cultures, and the high cost of the 3D matrices. Another limitation is the fact that vital tissue to generate organoids is not always available, particularly in advanced disease. Organoids have been obtained from different tumor types including colon, prostate, breast, lung and gastric cancers [77-79]. The application of organoids to precision oncology is still at an early stage and further optimizations and standardizations are needed (Table 1).

Finally, organ-on-a-chip devices that recapitulate 3D tissue architecture and physiological fluid flow conditions have been recently developed [80]. Bioengineered technologies have evolved and can now model organs that can be maintained for long periods of time. These model "organs" mimic the structure, cell-cell and cell-matrix interactions, cellular heterogeneity and function of *in vivo* tissues. Cancer-on-a-chip systems can be used for drug development and toxicology testing, although further research is needed to establish their relevance as preclinical cancer models [81-83].

3. CONCLUSION AND FUTURE PERSPECTIVES

Precision medicine in oncology is rapidly evolving. Tumors are often genomically profiled to determine treatment strategy in clinical practice. This approach represents a significant advance in translational cancer research, although further advances are required due

to intratumoral heterogeneity and individual diversity. We have reviewed how a group of patients treated with the same target-therapy could respond differently to a specific drug in the face of a molecular alteration. Cell lines have been extensively studied as *in vitro* and *in vivo* models and will likely continue to play an important role in drug discovery research. However, novel approaches such as PDX, syngeneic mouse, organoid or organ-on-a-chip models represent advancement in this area and better reflect tumor heterogeneity and more comprehensively recapitulate the landscape of human tumors. These models are more suitable for personalized medicine because tumor and normal tissues can be derived from individual patients for genetic, functional and drug response studies. As we have tried to summarize in this review, different preclinical models have distinct features which are accompanied by advantages and shortcomings. Since human tumors are composed of many cellular constituents and are under continuous immune editing it is likely that the development of 'optimal' preclinical models will remain a highly active area of research.

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FIGURE LEGENDS

Figure 1: Precision medicine approach.

Table 1 : Advantages and disadvantages of *in vitro* preclinical models.

Model type	Precinical model	Advantages	Disadvantages
<i>In vitro</i>	Cell lines	<ul style="list-style-type: none"> • Easy to culture • Inexpensive to use • Rapid experimental results • Unlimited self-replicating source • High degree homogeneity • Most of the commercially available are molecularly characterized • Represent the landscape of different human cancers • Can be genetically manipulated • Multiple agents can concurrently be tested 	<ul style="list-style-type: none"> • Clonal subpopulations may arise • Genotypic and phenotypic drift during culture • Architecture of tumor is lost • Do not recapitulate functional and genetic heterogeneity of all tumors • Microenvironment and crosstalk between tumor is lost • Problems of cross-contamination • Difficult to create from individual patients • Cannot be derived from matching normal tissue
	Organoids	<ul style="list-style-type: none"> • Intermediate model between cell lines and xenografts • More acute simulation of native cancer tissue. Preserve morphology and heterogeneity and cell-environment crosstalk • Mimics the diffusion of chemicals inside the tumor • Co-culturing different cell types (tumor and non-malignant cells) • Retains genome over time • Large biobanks • Possibility for personalized treatment • Easy to model multiple genetic mutations 	<ul style="list-style-type: none"> • High-quality tissue is not always possible • Long time to grow • High cost of 3D matrices • Drug screening depends on the tumor type • Early stage research. Further work is needed
	Three-dimensional cell culture		

Table 2: Advantages and disadvantages of *in vivo* preclinical models.

Model type	Preclinical model	Advantages	Disadvantages
<i>In vivo</i>	Cell lines xenografts	<ul style="list-style-type: none"> • Easy to establish, often rapid tumor onset • Recapitulate genomic abnormalities of human tumors • Properly cell autonomous cancer properties 	<ul style="list-style-type: none"> • Require immunodeficient mice to grow • Loss tissue histology and micro-environmental factors • Rather homogeneous do not fully recapitulate tumor heterogeneity
	Patient-derived xenograft (PDX)	<ul style="list-style-type: none"> • Capture epigenetic character and cancer evolutionary dynamics • Partially recreate tumor heterogeneity • Genetic and histopathologic features are largely matched with original tumor • There are several collections of well-characterized PDX models from different tumor types • Personalized therapy is allowed and multiple therapies can be tested 	<ul style="list-style-type: none"> • Require immunodeficient mice to grow • Presently cannot be used to study the impact of immune system on tumor growth • Human tumor microenvironment is lost during engraftment • Not all tumors transplanted lead to stably growing PDX • Long engraftment time for individual treatment evaluation • Genomic changes in the tumor during evolution are not reflected in the PDX
	Syngeneic mouse models	<ul style="list-style-type: none"> • Have a fully functional immune system • The role of microenvironment can be studied • Many syngeneic models have been genomically and immunologically characterized • Relatively simple to use; suitable for drug administration studies 	<ul style="list-style-type: none"> • High cost, labor-intensive and technically challenging • Transplanted mouse tissue may not represent the complexity of human tumors • Many syngeneic models are generated from carcinogen-induced models and carry genetic alterations that do not entirely recapitulate human tumors of the same histology • The number of cell lines available is relatively low • Not available for all tumor types • They are mouse tumors with mouse targets
	Genetically engineered mouse models (GEMMs)	<ul style="list-style-type: none"> • Have a fully functional immune system • The role of microenvironment can be studied • Designed to recapitulate molecular landscape of human tumor • Novel target-drugs and/or combinations can be tested, including immunotherapy. 	<ul style="list-style-type: none"> • Time consuming and expensive to model multiple mutations • Variability in tumor formation and development • Homogeneous genomic landscape • Limited tumor mutational load, which can compromise immune oncology studies

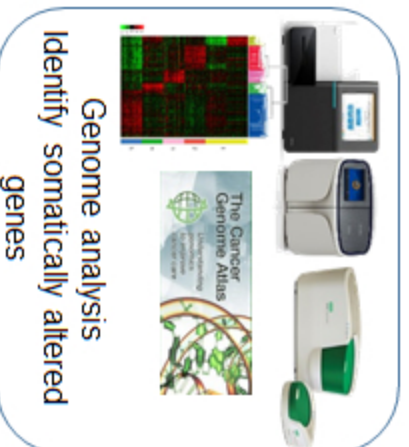
Precision medicine



Treatment on patients
Monitor the effects
Establishing new treatment
guidelines

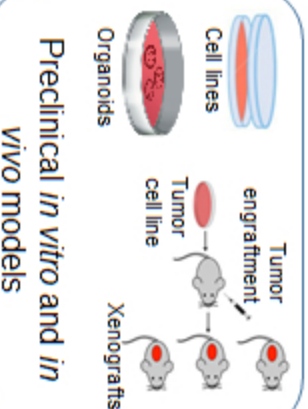


Sample collection

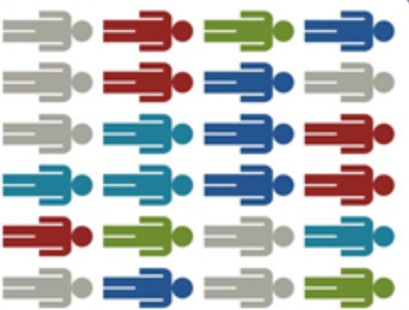


Genome analysis
Identify somatically altered
genes

Confirm therapeutic efficacy
of a compound
Identify addition options

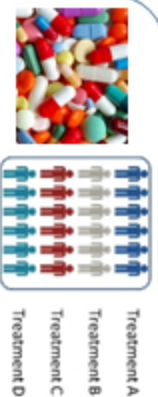


Preclinical *in vitro* and *in vivo* models



Patient population

Development of
new targeted
therapies



Identify the appropriate
patient population