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Patient-derived xenografts (PDXs) as model systems for human cancer

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Patient-derived xenografts (PDXs) are obtained by transplanting fragments of a patient's tumour into immunodeficient mice. Growth and propagation of PDXs allows correlating therapeutic response *in vivo* with extensive, multi-dimensional molecular annotation, leading to identification of predictive biomarkers. PDXs are increasingly recognised as clinically relevant models of cancer for several reasons, of which the main is the possibility of studying the behaviour of cancer cells in a natural microenvironment, where they interact with stromal components accrued from the mouse host. PDXs maintain close similarities with the tumour of origin, in terms of tissue architecture, molecular features and response to treatments. Indeed, preclinical trials in PDXs have been shown to match and also anticipate data obtained in patients. Exploration of more complex processes like metastatic evolution and antitumour immune responses is actively pursued with PDXs, as new generations of host models emerge on the horizon.

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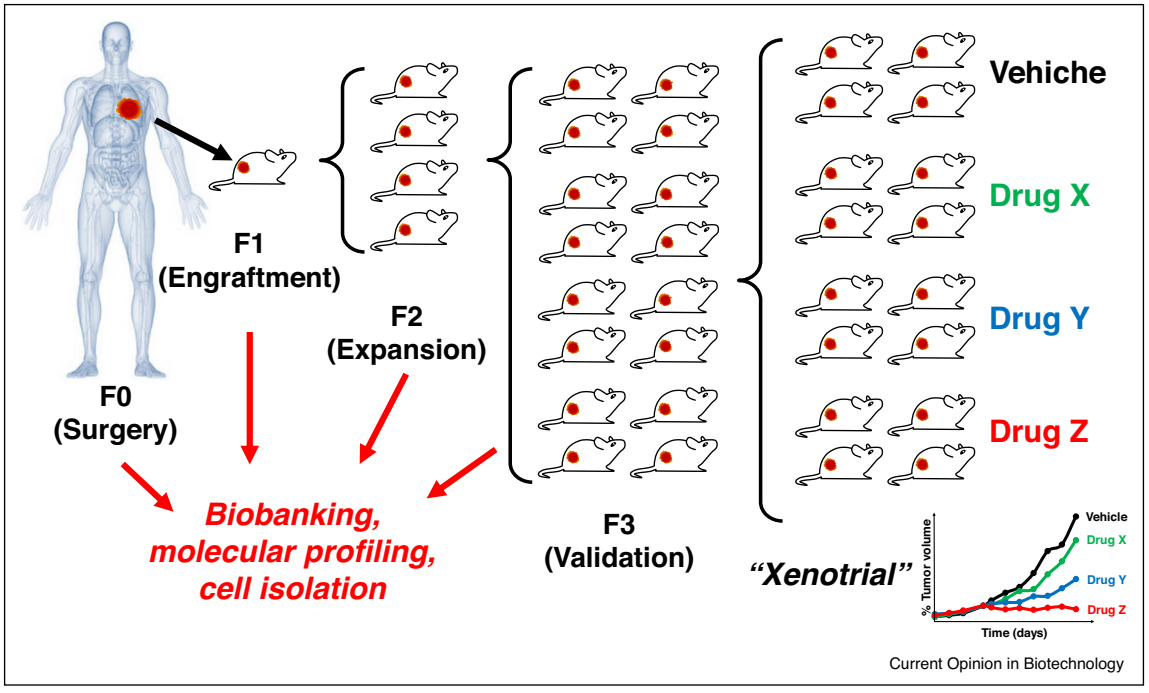
Introduction

Development of experimental models that correctly recapitulate tumour biology, genetic heterogeneity and drug response has been a key objective of cancer research since its inception. PDXs are generated by implanting patient-derived tumour tissue into host animal models, typically immunocompromised mice. After a first step of engraftment and adaptation to the new host, PDX tumours can be grown, explanted, aliquoted and implanted in further animals, a process known as 'passage', to generate cohorts of animals hosting the same tumour, for preclinical treatment efficacy studies (Figure 1). PDX-derived tumour tissue can also be cryopreserved, to generate of a 'living tissue biobank' for virtually unlimited tissue availability for experiments and molecular profiling. The main advantage of PDXs versus *in vitro* cancer cell cultures is the possibility of studying cancer cells in their microenvironment and assessing the involvement of fibroblasts, endothelial cells and leukocytes in tumour biology and response to treatments. Over many years, PDXs have been used to study multiple aspects of the neoplastic disease [1]. The PDX field is in continuous expansion, and large consortia like EurOPDX in Europe (URL: http://www.europdx.eu/) and PDXNet in the USA (URL: https://www.pdxnetwork.org/) have been established to standardise procedures and make PDXs available to the cancer research community. In this review, we summarise the most recent findings derived from the use of PDXs as cancer models.

Modelling drug response and resistance

Patient-derived xenografts (PDXs) are widely used to recapitulate how the complexity of tumour biology affects drug response. In the last few years, several studies have been conducted to define pharmacological vulnerabilities. In this context, a large-scale *in vivo* screen in ~1000 PDX models assessed the value of such approach in term of reproducibility and clinical translatability, to identify associations between genotypes and drug responses [2]. As an alternative to using large PDX cohorts to establish genotype-response correlations, a more empirical strategy is based on the 'Avatar' approach: an individual patient's tumour tissue is used to derive PDXs, on which multiple treatments are tested, so that the most effective can be administered to the patient of origin. PDXs can be used to investigate personalised treatments in real-time (co-clinical trials), as well as to find and validate new targets [3,4•]. PDX-based studies allowed optimisation of clinical trial designs, providing strong rationale for new combinatorial regimens in patients refractory to conventional treatments [5,6•]. PDX models have been employed to recapitulate mechanisms of primary and acquired drug resistance [7,8•]. *In vivo* studies highlighted that tumour resistance mechanisms identified in PDXs are also found in the original patient samples resistant to target therapy. This provided potential treatment options in resistant tumours [9•] and the proof of concept for the heterogeneous nature of

Figure 1



Generation, expansion and use of PDXs. The patient-derived tumour is implanted into an immunodeficient mouse and, after the engraftment phase, it is explanted and expanded in multiple passages, enabling the generation of cohorts of PDXs, suitable for preclinical «xenotrials» to evaluate drug efficacy. PDX-derived tumour samples can be collected at every passage to create a frozen, vital tissue biobank and to perform molecular profiles and *ex vivo* experiments.

acquired resistance [10]. A limitation of the PDX approach is the considerable effort and time required to test *in vivo* the response to multiple drugs. To overcome this issue, *in vivo* implantable devices allow testing simultaneously different drugs in adjacent regions of the same PDX tumour [11]. Moreover, loss-of-function genetics screenings have been exploited to identify new therapeutic targets, employing pooled short hairpin RNA (shRNA) libraries, adapted for *in vivo* screens in cell line xenografts and PDXs [12].

PDX-derived *in vitro* models

In vivo experiments in PDXs are limited by cost, size, time and resources. Reaching the adequate sample size to explore inter-patient heterogeneity is therefore quite challenging. The use of *in vitro* tests on PDX-derived cells followed by *in vivo* validation is a valid alternative and can be achieved in different ways. Short-term 2D cultures can be used for rapid drug tests, that displayed good concordance with *in vivo* experiments [13••]. Alternatively, long-term cultures may be employed to establish cell lines retaining the properties of the PDX of origin [14•]. Patient-derived or PDX-derived cells can be grown as 3D organoids [15••], that retain certain architectural features and can be used for drug efficacy studies and also to further generate PDXs when direct *in vivo* propagation is limiting [16•,17]. Cancer-initiating stem cells can be derived from PDXs and maintained in suspension as spheroids, a good model for studying the biology and drug sensitivity of cancer stem cells [18•].

Modelling tumour-stroma interactions

It is widely recognised that the biology of solid tumours depends on the ability of cancer cells to recruit and educate vascular, mesenchymal and immune cells, that collectively form the tumour microenvironment, or tumour stroma. During engraftment in a PDX, cancer cells retain this ability, so that while the tumour grows human stromal cells are replaced by mouse counterparts, thus providing an ideal experimental model to characterise tumour-stroma interactions [19•]. Indeed, the fact that stromal cells in a PDX are of mouse origin has been considered a limit, potentially affecting tumour biology and evolution [20]. However, the histological architecture of the tumour tissue is maintained during PDX propagation, suggesting that the key mediators of tumor-stroma interactions are functional [21]. Moreover, proteomic analyses revealed high correspondence of matched tumour and PDX stromal profiles, as well as adaptation of the stromal proteome upon PDX engraftment, with distinct profiles for different PDX samples [22•,23].

Tumor-stroma interactions in PDX have been extensively explored as potential therapeutic targets, we report here the most recent findings. In colorectal cancer (CRC) PDXs harbouring APC mutations, blockade of the RSPO3 signal from cancer-associated fibroblasts (CAFs) to cancer cells enhanced the activity of paclitaxel-based chemotherapy [24••]. Prolonged treatment of lung cancer xenografts with a kinase inhibitor induced a metabolic shift toward increased lactate production in cancer cells, that in turn induced a paracrine supply of HGF from CAFs, promoting resistance. This adaptive resistance mechanism was also observed in patients, which showed clinical relevance [25]. In CRC PDXs, non-canonical TGF-beta signalling from cancer cells was found to activate CAFs. Inhibition of this axis led to reduction of metastatic dissemination and increased sensitivity to therapy [26••]. Finally, in breast cancer PDXs, microvesicle-mediated transfer of miR-221 from CAFs to cancer cells was found to promote resistance to hormonal therapy, highlighting a new therapeutic avenue [27].

Modelling metastasis

The possibility of modelling metastatic progression using subcutaneous PDX implants is quite limited. However, a much better mimic of this process is obtained by implanting PDXs in the same tissue from which the patient's tumour was explanted [28]. This type of implant, called orthotopic, in most cases requires microsurgical competences and *in vivo* imaging, for the implant [29] and for subsequent evaluation of primary tumour and metastases development. A notable exception is breast cancer, for which the standard PDX implant in the mammary fat pad is intrinsically orthotopic and allows modelling metastasis [30•].

Several approaches have been developed and exploited to study the metastatic process and its therapeutically actionable dependencies in PDXs. The most straightforward is the implant of the primary tumour in the corresponding site of the mouse. Metastatic behaviour can be further enhanced when stromal cells are added to cancer cells [31]. When the source material is limited (e.g. metastasis biopsies), a first generation of PDXs can be derived subcutaneously, and then implanted orthotopically, retaining the features of the tumour of origin, including metastatic ability [17]. Human metastases have been implanted orthotopically in the primary tumour site, to model at the same time tumour growth at the primary site and metastatic propagation [32•]. Finally, human metastases can be implanted in the primary metastasis site, as in the case of liver-metastatic CRC, to recapitulate the behaviour of the disease within its metastatic microenvironment [33]. It is worth noting that in all these cases morphological, molecular and pharmacological features of the orthotopic PDXs correctly recapitulated those of the tumour of origin.

Modelling anticancer immunotherapy

To avoid rejection of human tumour implants, PDXs are invariably generated in immunocompromised mice. Various mouse strains have been developed over several decades with progressively increasing immunosuppression, from athymic nude mice in 1966 to non-obese diabetic-severe combined immunodeficiency (NOD-SCID) mice in 1995, to NOD-SCID-Gamma-null (NSG) in 2002. Higher levels of immunodeficiency allow better engrafting [34], but also progressively reduce the possibility to explore the role of the immune system in tumour biology and response to treatments [35•]. This drawback is particularly critical for modelling immunotherapy based on checkpoint inhibitors, as it requires an intact human immune system. A way to overcome this limitation is the generation of 'humanized' mouse models, by engrafting NSG mice with human leukocytes or hematopoietic stem cells. Patient tumour tissues are then engrafted into the humanized mice and used for studying the efficacy of immunomodulatory treatments. However, this approach has a high risk of graft-versus-host disease and therefore allows only short term experiments [36,37]. Mouse 'humanization' has been improved by genetically inserting human cytokine genes (M-CSF, IL-3,GM-CSF thrombopoietin) at their respective mouse loci, plus transgenic expression of human SIRPα28 which enables mouse phagocytes to tolerate human cells [38]. Indeed, recent studies with humanized mouse PDXs have provided significant advances in the comprehension of cancer immunotherapy [39•,40•].

Conventional PDXs can still have a good use for modelling another type of immunotherapy, called 'adoptive immunotherapy', in which cytotoxic T-lymphocytes, natural killer cells or engineered killer cells are administered to test their direct anticancer activity. As an example, Jespersen and colleagues inserted melanoma tumour cells and T cells from the same patient into NSG mice and showed tumour inhibition [41•]. Alternatively, cytokine-induced killer (CIK) cells can be generated from the patient or from donors and tested for anticancer efficacy in PDX, alone or in combination with cancer-targeting antibodies [42]. An interesting research frontier in adoptive immunotherapy is the engineering of killer cells with a chimeric antigen receptor, to direct them against a specific antigen expressed by cancer cells. Also this approach has been successfully validated in PDX models [43•,44].

PDXs for biomarker development

The fact that PDXs provide a virtually unlimited source of tumour tissue for multi-dimensional molecular profiles and allow testing response to multiple drugs in the same model opened a new avenue for biomarker discovery. Moreover, the fact that DNA and RNA from stromal cells are of mouse origin enables distinguishing cancer cell-intrinsic (human) from microenvironmental (mouse) molecular profiles without the need for microdissection

or single-cell sequencing [45]. Recent examples of PDX-based biomarker discovery include: (i) identification of lung cancer cell-specific downregulation of histocompatibility antigens, further confirmed in clinical samples and associated to resistance to immunotherapy [46]; (ii) lack of GATA4 expression as a predictor of sensitivity to TGFBR1 inhibition [47]; (iii) identification of p38-alpha inhibition as sensitizer to taxanes in breast cancer [48]. PDXs have also been used to study circulating tumour cells (CTCs). In breast cancer, a molecular signature associated with the presence of CTC clusters in PDXs was associated with worse outcome in patients [49]. The possibility of performing species-specific RNA sequencing of PDXs to distinguish cancer cell from stromal transcriptomes has been extensively exploited in CRC, leading to the identification on one side of a poor prognosis stromal signature [45], and on the other of cancer-cell-intrinsic molecular subtypes of CRC (CRIS subtypes) with unprecedented predictive and prognostic power [50,51•]. Similarly, a cancer cell-intrinsic microsatellite instability transcriptional signature defined in gastric cancer PDXs was found to identify patients with worse prognosis [52].

Conclusion and outlook

PDXs offer unprecedented opportunities for developing precision medicine approaches to cancer treatment. Vast PDX collections have been generated globally, summing up to thousands of cases and enabling population-scale preclinical trials that capture inter-tumour heterogeneity. Yet, each individual model recapitulates within itself the complexity of a single human tumour, allowing in-depth, longitudinal exploration of adaptive and evolutive dynamics that typically confer resistance to treatments. The PDX community is facing important challenges to improve the efficacy of these models. In particular, the time, effort and costs necessary for *in vivo* experiments are urging researchers to develop and use *ex vivo* systems, exploiting PDX-derived cells for simpler *in vitro* experiments before embarking in PDX tests. Improved ways of modelling metastatic progression and interactions between cancer and its microenvironment are continuously being developed, as well as in-depth molecular profiling of all tumour components. The time in which newly diagnosed cancer patients will find existing PDXs with matching features for treatment personalization is not far.

Conflict of interest statement

Nothing declared.

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