Opinion

Interrogating open issues in cancer precision medicine with patient-derived xenografts

Annette T. Byrne, Denis G. Alférez, Frédéric Amant, Daniela Annibali, Joaquín Arribas, Andrew V. Biankin, Alejandra Bruna, Eva Budinská, Carlos Caldas, David K. Chang, Robert B. Clarke, Hans Clevers, George Coukos, Virginie Dangles-Marie, S. Gail Eckhardt, Eva Gonzalez-Suarez, Els Hermans, Manuel Hidalgo, Monika A. Jarzabek, Steven de Jong, Jos Jonkers, Kristel Kemper, Luisa Lanfrancone, Gunhild Mari Mælandsmo, Elisabetta Marangoni, Jean-Christophe Marine, Enzo Medico, Jens Henrik Norum, Héctor G. Palmer, Daniel S. Peeper, Pier Giuseppe Pelicci, Alejandro Piris-Gimenez, Sergio Roman-Roman, Oscar M. Rueda, Joan Seoane, Violeta Serra, Laura Soucek, Dominique Vanhecke, Alberto Villanueva, Emilie Vinolo, Andrea Bertotti and Livio Trusolino

Annette T. Byrne and Monika A. Jarzabek are members of the EurOPDX Consortium and are at the Royal College of Surgeons in Ireland, Dublin 2, Ireland.

Denis G. Alférez and Robert B. Clarke are members of the EurOPDX Consortium and are at the Breast Cancer Now Research Unit, Division of Molecular and Clinical Cancer Sciences, Manchester Cancer Research Centre, University of Manchester, Manchester M20 4QL, UK.

Frédéric Amant, Daniela Annibali and Els Hermans are members of the EurOPDX Consortium and are at the Katholieke Universiteit Leuven, 3000 Leuven, Belgium. Frédéric Amant is also at The Netherlands Cancer Institute, Plesmanlaan 121, 1066CX Amsterdam, The Netherlands.

Joaquín Arribas, Joan Seoane and Laura Soucek are members of the EurOPDX Consortium and are at the Vall d'Hebron Institute of Oncology, 08035 Barcelona, the Universitat Autònoma de Barcelona, 08193 Bellaterra, and the Institució Catalana de Recerca i Estudis Avançats (ICREA), 08010 Barcelona, Spain. Joaquín Arribas and Joan Seoane are also at CIBERONC, 08035 Barcelona, Spain.

Andrew V. Biankin and David K. Chang are members of the EurOPDX Consortium and are at the Wolfson Wohl Cancer Research Centre, Institute of Cancer Sciences, University of Glasgow, Glasgow G61 1QH, UK.

Alejandra Bruna, Carlos Caldas and Oscar M. Rueda are members of the EurOPDX Consortium and are at Cancer Research UK Cambridge Institute, Cambridge Cancer Centre, University of Cambridge, Cambridge CB2 0RE, UK.

Eva Budinská is a member of the EurOPDX Consortium and is at the Institute of Biostatistics and Analyses, Faculty of Medicine, and Research Centre for Toxic Compounds in the Environment, Faculty of Science, Masarykova Univerzita, 625 00 Brno, Czech Republic.

Hans Clevers is at the Hubrecht Institute, University Medical Centre Utrecht, and Princess Maxima Center for Pediatric Oncology, 3584CT Utrecht, The Netherlands.

George Coukos and Dominique Vanhecke are members of the EurOPDX Consortium and are at Lausanne Branch, Ludwig Institute for Cancer Research at the University of Lausanne, 1066 Lausanne, Switzerland.

Virginie Dangles-Marie is a member of the EurOPDX Consortium and is at the Institut Curie, PSL Research University, Translational Research Department, 75005 Paris, and Université Paris Descartes, Sorbonne Paris Cité, Faculté de Pharmacie de Paris, 75006 Paris, France.

S. Gail Eckhardt is at the University of Colorado Cancer Center, Aurora, Colorado 80045, USA.

Eva Gonzalez-Suarez is a member of the EurOPDX Consortium and is at the Cancer Epigenetics and Biology Program, Bellvitge Biomedical Research Institute IDIBELL, 08908 L'Hospitalet de Llobregat, Barcelona, Spain.

Manuel Hidalgo is a member of the EurOPDX Consortium and is at Beth Israel Deaconess Medical Center, Boston, Harvard Medical School, Boston,

Massachusetts 02215, USA.

Steven de Jong is a member of the EurOPDX Consortium and is at the University Medical Centre Groningen, University of Groningen, 9713GZ Groningen, The Netherlands.

Jos Jonkers, Kristel Kemper and Daniel S. Peeper are members of the EurOPDX Consortium and are at The Netherlands Cancer Institute, Plesmanlaan 121, 1066CX Amsterdam, The Netherlands.

Luisa Lanfrancone and Pier Giuseppe Pelicci are members of the EurOPDX Consortium and are at the Department of Experimental Oncology, European Institute of Oncology, 20139 Milan, Italy.

Gunhild Mari Mælandsmo and Jens Henrik Norum are members of the EurOPDX Consortium and are at Oslo University Hospital, Institute for Cancer Research, 0424 Oslo, Norway.

Elisabetta Marangoni and Sergio Roman-Roman are members of the EurOPDX Consortium and are at Institut Curie, PSL Research University, Translational Research Department, 75005 Paris, France.

Jean-Christophe Marine is a member of the EurOPDX Consortium and is at the Laboratory for Molecular Cancer Biology, Department of Oncology, Katholieke Universiteit Leuven, and the Center for Cancer Biology, VIB, 3000 Leuven, Belgium.

Enzo Medico, Andrea Bertotti and Livio Trusolino are members of the EurOPDX Consortium and are at the Candiolo Cancer Institute IRCCS and Department of Oncology, University of Torino, 10060 Candiolo, Torino, Italy.

Héctor G. Palmer, Alejandro Piris-Gimenez and Violeta Serra are members of the EurOPDX Consortium and are at the Vall d'Hebron Institute of Oncology and CIBERONC, 08035 Barcelona, Spain.

Alberto Villanueva is a member of the EurOPDX Consortium and is at the Program Against Cancer Therapeutic Resistance (ProCURE), Catalan Institute of Oncology ICO, Bellvitge Biomedical Research Institute IDIBELL, 08098 L'Hospitalet de Llobregat, Barcelona, and Xenopat S.L., Business Bioincubator, Bellvitge Health Science Campus, 08907 L'Hospitalet

de Llobregat, Barcelona, Spain.

Emilie Vinolo is at Seeding Science SAS, 75020 Paris, France.

Correspondence to A.T.B. and L.T.

annettebyrne@rcsi.ie;

livio.trusolino@ircc.it

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Abstract

Patient-derived xenografts (PDXs) have emerged as an important platform to elucidate new treatments and biomarkers in oncology. PDX models are used to address clinically relevant questions, including the contribution of tumour heterogeneity to therapeutic responsiveness, the patterns of cancer evolutionary dynamics during tumour progression and under drug pressure, and the mechanisms of resistance to treatment. The ability of PDX models to predict clinical outcomes is being improved through mouse humanization strategies and implementation of co-clinical trials, within which patients and PDXs reciprocally inform therapeutic decisions. This Opinion article discusses aspects of PDX modelling that are relevant to these questions and highlights the merits of shared PDX resources to advance cancer medicine from the perspective of EurOPDX, an international initiative devoted to PDX-based research.

Response to anticancer therapies varies owing to the substantial molecular heterogeneity of human tumours and to poorly defined mechanisms of drug efficacy and resistance[1]. Immortalized cancer cell lines, either cultured *in vitro* or grown as xenografts, cannot interrogate the complexity of human tumours, and only provide determinate insights into human disease, as they are limited in number and diversity, and have been cultured on plastic over decades[2]. This disconnection in scale and biological accuracy contributes considerably to attrition in drug development[3-5].

Surgically derived clinical tumour samples that are implanted in mice (known as patient-derived xenografts (PDXs)) are expected to better inform therapeutic development strategies. As intact tissue - in which the tumour architecture and the relative proportion of cancer cells and stromal cells are both maintained - is directly implanted into recipient animals, the alignment with human disease is enhanced. More importantly, PDXs retain the idiosyncratic characteristics of different tumours from different patients; hence, they can effectively recapitulate the intra-tumour and inter-tumour heterogeneity that typifies human cancer[6-9].

Exhaustive information on the key characteristics and the practical applications of PDXs can be found in recent reviews[10-13]. In this Opinion article, we discuss basic methodological concepts, as well as challenges and opportunities in developing “next-generation” models to improve the reach of PDXs as preclinical tools for *in vivo* studies (TABLE 1). We also elaborate on the merits of PDXs for exploring the intrinsic heterogeneity and subclonal genetic

evolution of individual tumours, and discuss how this may influence therapeutic resistance. Finally, we examine the utility of PDXs in navigating complex variables in clinical decision-making, such as the discovery of predictive and prognostic biomarkers, and the categorization of genotype-drug response correlations in high-throughput formats. Being primarily co-authored by leading members of the EurOPDX Consortium (see Further information), we provide a perspective on the value of PDX models as an important resource for the international cancer research community towards the realization of a precision medicine paradigm (BOX 1; TABLE 2).

Modelling cancer phenotypes

Interrogating intra-tumour heterogeneity and evolutionary dynamics. Cancer is increasingly being recognized as an ecosystem of cells that constantly evolves following Darwinian laws. Owing to cancer cell intrinsic mutability, an incipient tumour clone gives rise to a progeny of genetically heterogeneous subclones, some of which will thrive while others shrink, depending on their ability to cope with environmental selection pressures[14]. This is of particular relevance for cancer treatment, as most patients will eventually succumb to the disease owing to the appearance of resistant tumour subclones. Despite the considerable clinical impact of tumour heterogeneity[15], little is known about how it affects response to cancer therapy and how it may change during treatment at both the genomic and the phenotypic levels[16-20]. These issues highlight the need for preclinical models that capture the heterogeneous nature of human cancers and their on-going evolution.

For example, breast cancer is a constellation of at least 10 different genomic subtypes, each with distinct drivers and variable intra-tumour heterogeneity[15,21,22]. Recent evidence has suggested that each breast cancer comprises multiple tumour cell populations with distinct evolutionary trajectories that are likely to be affected by treatment pressure[23-25]. Genomic evolution between primary and recurrent tumours also occurs[24-28]. Such intra-tumour and inter-tumour variability affects therapeutic responses, and hence needs to be considered in the preclinical and clinical settings. Although some engraftment-associated selection has been documented[24,29], PDX models of breast cancer seemingly preserve most of the genomic clonal architecture of the original patient sample and also seem to resemble patient counterparts at the transcriptomic, epigenomic and histological levels, as well as in terms of shared signalling pathways[8,30-32]. Notably, the majority of tumour subclones that change upon engraftment do not include known breast cancer oncogenic drivers[29]. This suggests that, although engraftment pressure is observed, it is evolutionarily neutral, as it does not affect intra-tumour heterogeneity when considering the clonal representation of relevant genes. These features probably underpin the successful use of breast cancer PDXs to predict clinical drug responses[9] and mechanisms of acquired resistance[33,34].

As discussed below, an advantage of PDX models is that they can be generated with a limited amount of material; for example, using fine-needle biopsies (TABLE 1). However, these methods may be confounding when the studied tumour type is particularly heterogeneous (such as melanoma). For

example, within one tumour or metastasis, multiple melanoma subclones can exist, each harbouring different genetic and/or epigenetic alterations[35-37]. Simply taking a single biopsy sample can result in a PDX that does not represent the heterogeneity of the patient's tumour[35,38]. Notably, regional genetic variability can be exacerbated by PDX serial propagation, producing divergent responses in tumour measurements within a single cohort of treated mice[32,39]. Methods to overcome this limitation include good, standardized pre-clinical designs (those with adequate statistical power and proper randomization), as well as the mixing of heterogeneous tumour masses before implantation, such as through the use of single-cell suspension injections or rough tumour homogenates[24].

The direct derivation of PDXs from circulating tumour cells (CTCs) may represent another tool to further interrogate tumour heterogeneity. The numbers of cancer cells shed by tumours into the bloodstream may be exceedingly low, and the biological and clinical relevance of CTCs in sustaining malignant disease has been questioned[40]. However, as CTCs are shed by tumours on a stochastic rather than a deterministic basis[41], they are expected to better recapitulate the distribution of different subclonal tumour populations (TABLE 1).

Intra-tumoural heterogeneity may also be non-genetic and intrinsic to the hierarchical organization of some tumours, in which a small subpopulation of cancer stem cells (CSCs) may be responsible for long-term tumourigenicity[42-45]. CSCs are thought to be chemoresistant and the main cause of recurrence

and distant metastasis[46-48]. Much of the supporting evidence originates from PDX models that were directly derived from various clinical samples, including CTCs, ascites fluid and pleural effusion cells, and surgical biopsy samples[49-53]. PDX models have provided evidence of CSC colonization in metastatic sites and have also highlighted the role and importance of the surrounding tumour stroma, a niche that is known to influence CSC behaviour by cell-to-cell contacts and through the secretion of pro-tumorigenic ligands and cytokines[8,51,54]. An ongoing debate exists as to whether CSCs recapitulate the full characteristics of stem cells (that is, they are undifferentiated cells with limitless replicative potential, which partly self-perpetuate to maintain a tumorigenic reservoir and which partly differentiate to give rise to a diverse progeny of non-tumorigenic cells) or simply identify a more robust or proliferative population of “tumour-initiating” cells selected by engraftment. To address this quandary, it will be important to compare the results of side-by-side fate-mapping experiments and transplantation assays to analyse whether the cells endowed with tumorigenic potential after transplantation also exhibit the other typical stem-like properties, such as the ability to self-renew, asymmetric cell division and differentiation potential[55].

PDX models of treatment-resistant disease.

There are primarily two ways in which PDX models can be used to interrogate primary or acquired resistance. One strategy is to derive models from patients’ samples before initiation of therapy and again at the time of treatment resistance. Alternatively, models can be developed from pretreatment tumour samples, and resistance can be recapitulated in the PDX upon iterative cycles

of exposure to the drug, as previously observed in genetically engineered mouse (GEM) models[56]. Using cycles of drug exposure in pretreatment PDX models, paired analysis of PDX models of cisplatin-sensitive and cisplatin-resistant testicular germ cell cancer (TGCC) proposed potential alternatives for the treatment of cisplatin-refractory TGCC, including anti-angiogenic therapy[57] and blockade of the platelet-derived growth factor receptor β (PDGFRβ)–AKT pathway[58].

PDX models have also proven useful in identifying mechanisms of resistance to targeted therapies in oestrogen receptor (ER)-positive breast cancer. The analysis of four hormone-resistant PDX tumours, which were obtained from two ER-positive breast cancer PDX models by continuous treatment with tamoxifen or by oophorectomy-mediated hormone depletion, revealed that hormone resistance was associated with various forms of deregulated ER-mediated gene transcription[33]. Taking a similar approach, PDX models of ER-positive breast cancer have been used to investigate jagged 1 (JAG1)–NOTCH4 signalling as a means for attenuating sensitivity to hormonal therapy[59] and to identify mechanisms of acquired resistance to cyclin-dependent kinase 4 (CDK4) and CDK6 blockade[60].

Patients with advanced cancer who acquire resistance to several lines of treatment mostly present with multiple metastatic lesions that are not amenable to resection, and may harbour different resistance pathways. Generating PDX models that recapitulate such complex scenarios of therapy-resistant metastatic tumours has become feasible for several tumours (TABLE 1). For

example, analysis of biopsy specimens and corresponding PDXs from different drug-resistant metastases in patients with melanoma who had been treated with a BRAF inhibitor resulted in the identification of multiple resistance mechanisms both within individual lesions and among separate samples from the same patient[35,38]. The resistance mechanisms identified in PDXs were also found in the original patient samples[35], and clinically resistant tumours were also treatment-refractory when grown as PDXs[38]. These studies provide proof of principle for the heterogeneous nature of acquired resistance in individual patients with melanoma and further attest to the ability of PDX models to predict clinical outcomes. Similar results have been observed in lung adenocarcinomas[61].

Although PDXs generally retain drug-sensitivity profiles that are similar to those of the corresponding patient tumour[30,38,62,63], PDX models derived from treatment-resistant tumours can become sensitive again upon xenografting, owing to the effect of the so-called “drug holiday” in which treatment is discontinued after tumour implantation to facilitate engraftment. Some resistance mechanisms are thus reversible in the absence of drug, as shown for melanoma[64,65] and lung adenocarcinoma[66]. This suggests that treatment-resistant PDXs should be exposed to continuous treatment immediately after implantation, although this is a cost- and labour-intensive approach. However, uninterrupted therapy might also result in the further selection of a subpopulation of tumour cells, resulting in a loss of intra-tumour heterogeneity and genetic variation in the PDX tumours compared with the original tumours.

In response to the need for more sophisticated models, several groups (for example, see REF.[67]) have developed protocols and networks to generate clinical trial-associated xenografts (CTAXs) (TABLE 1). These advanced PDX models are currently being derived from image-guided biopsy samples taken at different time points during disease progression and following new lines of treatment in the context of clinical trials. Such models will be extremely valuable in evaluating how the molecular evolution of advanced tumours is associated with innate or acquired drug resistance, and will be important for studying the tumour heterogeneity and clonal selection that results from drug treatment. In principle, CTAXs may also serve as personalized cancer models to test drug combinations that aim to overcome acquired resistance, generating information that could be transferred back to the donor patient for therapeutic decisions (see below). However, this opportunity might be hindered by limitations such as the low engraftment success rates for some tumour types and the disconnection between the time needed for PDX expansion and treatment (which can be long, especially for tumours with indolent growth in mice) and the rapidity of disease progression in patients.

Finally, PDXs that are established from tumours resistant to conventional therapies delivered in the neoadjuvant setting are of special interest (TABLE 1). In triple-negative breast cancer, the establishment and molecular profiling of PDXs from residual cancer cells that persist after neoadjuvant treatment (minimal residual disease (MRD)) may lead to the identification of targetable molecular alterations in the chemotherapy-resistant component of the tumour, which may mirror micro-metastases that are destined to clinically recur[68].

Despite often being limited in size due to prior exposure to cytotoxic therapy, triple-negative breast tumours from patients treated in the neoadjuvant setting engraft much more efficiently than do treatment-naive tumours (72% and 34%, respectively) (TABLE 2). Given the high engraftment efficiency and rapid growth of PDXs from drug-tolerant MRD tissues, at least in the case of breast cancer, these models represent an unprecedented opportunity to identify genomic alterations and associated targeted therapies before tumour recurrence in patients.

Next-generation PDX models

***Humanized PDX models to evaluate cancer immunotherapies*.** The importance of the immune system in tumour progression and treatment highlights the need for PDX models to facilitate the preclinical assessment of cancer immune therapies[69]. However, to avoid immune rejection of xenotransplants by the host, PDX models are primarily generated by transplanting tumour fragments into immunodeficient mice. The absence of many components of the immune system in these mice, and the loss of endogenous human immune cells upon propagation of the human tumour tissue over multiple passages[70,71], limit the utility of such models to explore the role of the immune system in tumour progression and to test novel immune-based therapies[72].

Humanized mice (also known as human haemato-lymphoid chimeric mice or human immune system (HIS) models) are immunocompromised mice in which

selected immune components have been introduced to generate a competent human immune system with different degrees of immune reconstitution. One methodology for the generation of humanized mice involves the transplantation of total peripheral blood from healthy human donors or patients (peripheral blood lymphocyte(PBL) models) or, in particular applications, the infusion of tumour-infiltrating lymphocytes (TILs) (FIG. 1). Although these procedures are known to cause severe graft-versus-host disease (GvHD) beginning 2-5 weeks after injection[73,74], seriously limiting the useful investigative time window of these models and the translational value of these studies[75], PBL and TIL mice can be used for cost-effective short-term testing of novel immune therapeutics and for assessing short-term adverse effects.

Alternatively, HIS mice can be generated through the transplantation of CD34-positive human haematopoietic stem cells (HSCs) or precursor cells isolated from umbilical cord blood, bone marrow and peripheral blood, either alone or in combination with additional human immune tissues (bone ossicles or human thymic tissue)[76] into immunodeficient mice (FIG. 1). Compared with PBL- and TIL-derived models, transplantation with HSCs results in a more complete haematopoietic reconstitution, as HSCs give rise to various lineages of human blood cells throughout the life of the animal. Methods for transplantation depend on the source of HSCs, the co-transplantation of immune tissues, the mouse strain and the age of the recipient mice[75-78]. In order to avoid the immune reactions caused by human leukocyte antigen (HLA) mismatch, the ideal source of HSCs is the same patient from whom the PDX has been established. However, isolating HSCs from cancer patients may prove daunting: on the one

hand, bone marrow biopsies are difficult in debilitated individuals; on the other hand, growth factor-stimulated bone marrow mobilization for HSC collection from peripheral blood might foster tumour progression[79] Moreover, even when applicable, the low yield of HSCs obtainable from cancer patients severely limits the number of mice than can be humanized. An attractive alternative is the *in vitro* expansion of HSCs[80], although this procedure could introduce biological perturbations affecting stemness and differentiation potential.

Whereas various strains of immunodeficient mice are used to transplant solid tumour tissue, not all of these strains are suitable for generating HIS models. The survival of human immune cells is highly dependent on the compatibility of the "do-not-eat-me" signals (CD47–signal-regulatory protein α (SIRPα)) on phagocytes in the host[81]. The most commonly used mice to generate compatible HIS models are those derived from the non-obese-diabetic (NOD)-severe combined immune deficiency (SCID)-interleukin-2 receptor common γ-chain (IL2-Rγ)-deficient (NSG; also known as NOD.Cg-*Prkdc*^{scid} *Il2rg*^{tm1Wjl}/SzJ) strain and the NOD/Sci-SCID/IL-2Rγ strain (NOG; also known as NOD-Cg-*Prkdc*^{scid} *Il2rg*^{tm1Sug}/JicTac). Substantial efforts are thus being made to develop novel GEM strains that not only express human-specific do-not-eat-me signals but also express human-specific cytokines or HLAs. These mouse strains differ upon transplantation in durability and quality of engraftment of the human immune system[78]. Some key examples of how humanized models are currently evolving to support PDX transplantation towards application in the immune-oncology space are presented as online supplementary information (see Supplementary information S1 (table)).

Modelling metastatic disease. Subcutaneous transplantation usually fails to reproduce the organ-specific tropism of distant metastases that is observed in patients. Therefore, models of metastatic disease are typically generated through orthotopic procedures. These include the transplantation of fragments of the primary tumour into the same location in the mouse, which is usually followed by the development of spontaneous metastases, or the direct transfer of metastatic lesions into the same organ in the host (TABLE 1). Patient-derived orthotopic xenografts (PDOXs; also known as orthoxenografts) of primary tumours can reproducibly lead to local invasive growth and metastases, often identical to those observed in the patient[82-84]. PDOX models for most cancer indications have typically been developed from surgical specimens. More recently, however, they have been successfully derived from biopsy samples, despite the limited quantity and quality of tissue available[85].

Advantages of orthotopic models include the ability to investigate tumour-host interactions at the relevant site of primary and secondary tumour growth, the development of patient-like metastases, the ability to interrogate site-specific dependence of therapy, and the potential to conduct clinically relevant studies, such as monitoring the effects of adjuvant therapy on occult metastases (TABLE 1). Nevertheless, orthotopic models remain relatively rare, probably owing to the non-trivial microsurgical procedures that are required for organ-specific transplantation. Furthermore, the incorporation of clinically relevant imaging modalities and appropriate *in vivo* imaging probes is necessary to visualize tumour orthotopic implants and metastatic progression in deep

tissues and to ensure timely therapeutic intervention when animals develop disease symptoms[86].

PDOX models of breast cancer are particularly amenable for modelling metastasis. They primarily rely on mammary fat pad injection of primary tumour samples, which successfully recapitulate the entire metastatic process from the appropriate primary anatomic site[8,87]. PDOX models of brain metastases and primary brain tumours are challenging. To prevent the default seeding of intravenously injected tumour cells in the lung and to ensure colonization of the central nervous system, intra-cardiac left ventricular inoculation of tumour cells is required[88]. Cells may also be implanted intracranially to overcome the blood-brain barrier[89]. Orthotopic homing and the metastatic potential of cancer cells can be boosted by genetic modification; for example, colorectal cancer PDX cells engineered to express C-C motif chemokine receptor 9 (CCR9) efficiently localize to the mouse colon after tail-vein injection, attracted by the abundance of the CCR9 ligand C-C motif chemokine ligand 25 (CCL25) in the intestine, and then spontaneously metastasize to the liver[90]. Genetic manipulation is useful to develop models of spontaneous metastasis for mechanistic studies *in vivo*; however, the introduction of exogenous molecules to patient-derived material may affect some properties of the original tumour, thus reducing translational relevance.

Whether PDOX models more accurately recapitulate clinical response to anticancer drugs compared with conventional subcutaneous PDX models remains to be established. One report showed that the antitumoural effects of

a microtubule-stabilizing drug on PDX models of brain metastases from non-small-cell lung cancer were different in orthotopic versus subcutaneous implants[85], but results remain anecdotal. It is conceivable that therapies that target components of the tumour microenvironment, such as endothelial cells and immune cells, would be better evaluated in an orthotopic context. Conversely, the therapeutic response of “oncogene-addicted tumours”, which intrinsically rely on activating mutations for their growth and survival, is likely to be less dependent on anatomical location and more influenced by the underlying cancer genetic makeup. Indeed, despite their heterotopic location, subcutaneous PDXs from *BRAF*-mutant melanoma[9,91] and *HER2* (also known as *ERBB2*)-amplified colorectal cancer[6,92,93] mimic the therapeutic response observed in patients. Sharing results from different experimental models within the EurOPDX consortium will allow us to shed some light on this critical question.

CTC-derived PDX models. As mentioned above, a step forwards for minimally invasive tumour sampling is the isolation and characterization of CTCs, detected at low concentrations in the peripheral blood of patients with different solid tumours[40]. Although the role of CTCs in metastasis development is still uncertain[40], their levels ostensibly correlate with patient survival and response to therapy[94-96]. These features mean that CTCs are promising tools to monitor cancer burden and drug susceptibility in metastatic and late-stage disease, when repetitive biopsies are not indicated. Technological advances now allow the isolation of viable CTCs, which maintain tumorigenicity when xenografted in immunocompromised mice[97-99] (TABLE 1).

Several reports have demonstrated the feasibility of establishing CTC-derived PDX models by directly injecting freshly isolated and enriched CTCs from patients with different cancers into immunocompromised mice. Using various CTC-capture techniques (such as epithelial cell adhesion molecule (EPCAM) or cytokeratin-based selection of cancer cells derived from epithelial tissues or microfluidic-based leukocyte depletion[100,101]), CTC-derived xenografts are now practicable for breast cancer[97], prostate cancer[102], gastric cancer[103], small-cell lung cancer (SCLC)[98] and melanoma[91]. Moreover, it has also been shown that *ex vivo* cultivated and fully molecularly characterized breast[104] and colorectal[105] CTCs maintain their tumorigenic potential. Notably, both freshly isolated CTCs and CTC-derived PDXs genetically and histologically mirror the original tumour and retain analogous drug sensitivities[91,97,98,100,102-105]. As an example, PDXs that are established from chemotherapy-naive circulating SCLC cells recapitulate donor patients' response to both platinum and etoposide[98]. In patients with ER-positive breast cancer, CTCs have also proven to be a useful model to study the genetic evolution of the tumour and to identify novel drug susceptibilities[104].

Although technically challenging, the use of CTC-derived PDX models opens new possibilities for translational research. In addition to being a source of information regarding disease prognosis[106], tumour heterogeneity[107,108], evolution[109] and dissemination[110,111], CTC-derived PDXs hold promise for precision medicine applications (TABLE 1). For instance, CTCs from women with treatment-refractory ER-positive breast tumours have been recently

analysed to investigate the functional and phenotypic consequences of prolonged anti-hormonal therapies, and xenografts from such CTCs have been used to design new therapies to overcome resistance[112]. Similarly, the next-generation sequencing of tumours, complemented with genomic analysis of CTCs and CTC-derived PDX mouse models, has proven to be a powerful platform for developing precision medicine strategies in patients with melanoma[91]. This approach has, in specific cases, facilitated the clinical implementation of alternative therapeutic strategies informed by the preclinical models[91].

PDXs for clinical decision-making

PDX population xenopatient trials. Across tumours of the same origin, genetic lesions that sustain tumorigenesis (and that therefore associate with response to targeted drugs) often involve many different oncogenes, each of which is mutated at low frequency[113]. Furthermore, genotype-based prediction of drug response is not unequivocal. Despite harbouring the genetic lesion known to correlate with drug response, many tumours do not regress due to the presence of signals that compensate for target inhibition[114]. Collectively, this information indicates that the genetic selection of tumours for the application of targeted therapies requires representative study populations and suitable pharmacogenomic platforms.

Provided that they are generated in high numbers and extensively characterized at the molecular level, PDXs can act as a powerful resource for

large-scale genotype-response correlations and therapeutic studies in genetically defined tumour subsets. Several recent studies testify to this potential; in late-stage colorectal cancer, for example, a systematic assessment of response to antibodies targeting epidermal growth factor receptor (EGFR) using PDX models ("xenopatients") derived from hundreds of individual tumours was coupled to candidate-gene or whole-exome sequencing analyses. Through this effort, several genetic determinants of resistance to EGFR blockade were discovered, including amplifications or mutations in genes encoding druggable kinases[6,7,115,116]. Similarly, more dynamic features such as expression changes in pro-survival genes and the activation of compensatory feedback loops during treatment were identified as mechanisms of tumour adaptation to EGFR family[117,118] or MEK[119] inhibition in colorectal cancer. The flexibility of PDXs also enabled preclinical testing of drug combinations in models displaying some of these resistance traits, with a permutation capability that was clearly beyond the number of testable hypotheses in humans (FIG. 2).

An analogous population-based drug screen has been recently performed in more than 1,000 PDX models representing a wide range of solid cancers (the 'PDX Encyclopaedia')[9]. Some genetic hypotheses and biomarkers of drug sensitivity, which emerged from cultured cancer cell lines, were successfully validated in this large panel of PDX models (FIG. 2). Notably, experiments in PDXs also enabled the identification of therapeutic candidates that *in vitro* model systems failed to capture[9]. In all these studies, responses obtained in mice were highly consistent with responses in patients. For example, the distribution of tumour regression, disease stabilization and progression in

colorectal cancer xenopatients receiving EGFR antibodies was similar to that found in the clinic, and treatment-refractory tumour grafts were enriched for known genetic predictors of therapeutic resistance in patients[6] (TABLE 3); moreover, in analogy with clinical studies[120], the addition of an EGFR small-molecule inhibitor to the EGFR antibody increased tumour regression[118]. Similarly, PDXs from *BRAF*-mutant melanomas underwent substantial shrinkage when treated with BRAF inhibitors, a response that was further magnified – as in patients – by the addition of a MEK inhibitor[9,121]. PDX platforms have recently been used for the systematic identification of cancer vulnerabilities through RNA interference-based genetic screens in tumour grafts, which have revealed new oncogenic drivers in melanoma[122] and pancreatic tumours[123].

PDX population trials may be highly informative, but they are also expensive and technically cumbersome, and the trade-off between sufficient sample size to ensure adequate coverage of inter-patient heterogeneity and experimental feasibility requires careful study design. To reduce the number of animal replicates while preserving statistical power, reproducibility studies have been conducted to compare response calls made on a single mouse with majority responses in reference cohorts composed of many animals. Thus, a strong concordance between single-mouse responses and majority responses has been found, with a prediction accuracy varying from 75%[124] to 95%[9]. Accordingly, 'one animal per model per treatment' (1 x 1 x 1) approaches have been recently advocated[9,125].

Alternative strategies to reduce experimental burden could rely on step-wise approaches, whereby large-scale pharmacogenomic screens are performed using less laborious formats (such as cancer cell lines) followed by *in vivo* validation in selected, molecularly relevant PDX models. In this regard, it is noteworthy that patient-derived material from human tumours, such as colorectal, pancreas and prostate cancers[126-132], can be grown and nearly indefinitely expanded as three-dimensional (3D) organoids. These can be easily transplanted to establish PDXs, and vice versa, and are amenable to drug screens in a semi-high-throughput manner[130]. Albeit more difficult to establish and propagate, two-dimensional (2D) primary cultures of dissociated cancer cells from both patient samples and PDXs are also being attempted with similar rationale and objectives[133]. In this vein, a platform for drug testing in short-term cultured breast cancer cells from PDXs has been recently developed and shown to predict *in vivo* drug response[29].

PDX co-clinical 'Avatar' trials. The term co-clinical trial refers to simultaneous clinical and preclinical trials with anticancer agents in patients with a tumour type of a defined genetic makeup and a mouse model with similar genetic abnormalities[134]. The underpinning idea is that comparison of responses between the patients and the preclinical model will help to define the mechanism of action of a given drug, as well as biomarkers of response. Originally implemented with GEM models, the co-clinical trial concept has been expanded to include PDX models ("avatars"), which are generated from cancer patients enrolled in clinical trials and, in parallel, treated with the same drug or drugs that the patient is receiving[10] (FIG. 2). In general, these studies aim to

develop a PDX model from newly diagnosed patients and use it to explore therapies that can be administered to the patient at the time of disease progression. Ongoing trials cover different tumour settings, including sarcomas (NCT02720796)[135], head and neck carcinomas (NCT02752932)[136], ovarian cancer (NCT02312245)[137] and pancreatic cancer (NCT02795650)[138]. Although a cogent argument exists for implementing avatar trials, and several case reports have provided data to support the concept[139-141], the logistical difficulties and technical hurdles are likely to limit the broad applicability of this approach (see above).

PDX models in biomarker development. The validation of mechanisms that link specific biomarkers to treatment efficacy will have direct clinical effects, allowing patient stratification for tailored treatment protocols. Large-scale PDX trial formats, such as the PDX Encyclopaedia[9] mentioned above, represent a more accurate approach to identify predictive biomarkers compared with the use of cell lines (TABLE 1). A transcriptional profiling study on 85 PDX models of nine different cancer types treated with nine separate cancer drugs identified 1,578 genes, the expression of which correlated with sensitivity to at least one drug; 333 of these genes showed significant association with sensitivity to two or more drugs, and 32 genes predicted response to six or seven drugs[142]. This type of study provides an initial set of biomarkers that require further evaluation in clinical material to determine translatability into a clinically useful assay.

Epigenetic biomarkers, such as DNA methylation, can also be assessed in PDXs as possible response predictors. A study including 28 glioblastoma

PDOXs showed that the poly(ADP-ribose) polymerase (PARP) inhibitor veliparib significantly enhances the efficacy of temozolomide (TMZ) chemotherapy only in models with *O*-6-methylguanine-DNA methyltransferase (*MGMT*) promoter hyper-methylation[143]. On the basis of these data, *MGMT* promoter hyper-methylation was included as an eligibility criterion for TMZ and veliparib combination treatment in an ongoing] phase II/III glioblastoma clinical trial (NCT02152982)[144].

Determinants of therapeutic sensitivity can be identified at the protein level using pathway analysis in PDXs: a proteomic survey of 20 PDX models of glioblastoma and their parental tumours identified a subset of cases with comparable proteomic profiles displaying high levels of expression and phosphorylation of EGFR and its downstream signalling proteins[145]. The expression and phosphorylation status of EGFR and downstream targets might be used as a predictive biomarker of response to EGFR inhibition in preclinical trials and, if successful, included in future clinical trials aiming at inhibiting EGFR signalling in patients with glioblastoma.

PDX models are also useful for the preclinical identification of metabolic biomarkers using magnetic resonance spectroscopy (MRS). This technique has recently been used to demonstrate differences in metabolic characteristics between molecular subtypes of breast cancer[146,147]. Elevated phosphocholine levels and low glycerophosphocholine levels have been proposed to be metabolic markers of aggressive disease in breast cancer based on *in vitro* studies[148]. However, MRS on intact tissue from PDX models of poor-prognosis

basal-like breast cancer displays an inverted metabolic profile, with high glycerophosphocholine rather than high phosphocholine concentration[146,147]. These observations suggest that proper tumour architecture, as maintained in PDXs, influences choline metabolism. Accordingly, a strong correlation between PDX models and clinical material was observed in the expression of genes that are involved in key metabolic pathways[146]. MRS technology also holds potential for *in vivo* non-invasive detection of metabolic biomarkers through tailored techniques such as 31P MRS or hyperpolarized 13C MRS[149,150]. Recently, a proof-of-principle study demonstrated the ability of *in vivo* MRS to distinguish basal-like from luminal-like breast cancer PDXs non-invasively using 31P MRS imaging[151].

For some cancer types, the ability of tumours to successfully engraft in mice can be considered per se a surrogate biomarker of risk for disease progression. For example, in mammary tumours, the ability to generate stable tumour grafts significantly predicted reduced survival[8,152], and gene expression signatures associated with successful PDX engraftment correlated with worse survival outcome when tested in prognostically annotated data sets of triple-negative breast cancer[153]. Similarly, tumour grafts of pancreatic ductal adenocarcinoma displayed higher expression of metastasis-associated genes compared with samples that failed engraftment, and patient donors of successfully engrafted tumours had shorter survival[154].

It is now well established that human tumour stromal cells are replaced by mouse counterparts following engraftment[155]. As a consequence of this substitution, species-specific RNA sequencing-based expression profiling of

PDXs offers a unique opportunity to distinguish mouse stroma-derived transcripts from human cancer cell-derived transcripts without the need to physically separate the two components before RNA extraction. Such analyses led to the identification of stromal-associated transcriptional signatures in colorectal cancer associated with poor prognosis and treatment resistance[156]. The negative prognostic significance of tumour stromal transcriptional signatures and their value for therapeutic decision-making and patient follow-up have also been described in other reports[157,158].

Challenges and opportunities

Ideal animal models for preclinical experimentation in oncology should fulfil several criteria: reflecting the diversity of cancer patients at the epidemiological and molecular levels; retaining, to the highest possible extent, the functional, phenotypic and genotypic characteristics of human tumours; faithfully predicting response to therapies, and recapitulating mechanisms of innate and acquired resistance, and allowing for experimental flexibility.

Although PDXs fulfil several of these criteria and can be further improved to meet additional requirements, certain inherent limitations remain difficult to address. A major obstacle is the necessity of using immunocompromised mice to circumvent xenograft rejection. This requirement hampers the use of current PDX models to assess immunotherapeutics. Although emerging humanization procedures are now expected to overcome some of the most important concerns (see Supplementary information S1 (table)), issues still remain with

the incorporation of particular immune cell types, immune responses and lymphoid structures in these humanized models and with the eradication of xenogeneic GvHD. It is expected that the development of novel immune-deficient mice will take advantage of emerging technologies based on engineered nuclease enzymes for genome editing (such as transcription activator-like effector nuclease (TALEN) and CRISPR–Cas9). These modifications will include the replacement or introduction of combinations of human-specific cytokine receptors and adhesion molecules, as well as more comprehensive sets of HLA class I and class II molecules.

As mentioned above, serial passaging of tumours leads to substitution of human stroma by murine components, and mouse-derived cytokines and growth factors in some cases do not crossreact with receptors that are expressed by human (cancer) cells[159-162]. This makes the contribution of the tumour microenvironment to drug response difficult to assess in PDXs. Moreover, the lack of a species-compatible tumour stroma complicates the identification of pharmacodynamic markers of target inactivation for drugs that intercept cancer-related microenvironmental processes, such as angiogenesis and inflammation. While mouse humanization procedures seek to reconstitute the human immune system, the replacement of stromal elements such as endothelial cells and fibroblasts with their human counterparts is currently daunting, if not unfeasible.

PDX-based efforts for cancer precision medicine also require adequate logistics, from proper archival biobanking to continuous propagation of live

biospecimens, intensive animal experimentation and systematic integration of therapeutic results with high-content molecular annotation. The perception of this complexity and the awareness that resource sustainability cannot be maintained by individual academic laboratories have fuelled initiatives for creating and implementing shared large-scale PDX platforms, including the European EurOPDX resource, the US National Cancer Institute (NCI) repository of patient-derived models, the Public Repository of Xenografts (PRoXe), the Children's Oncology Group (COG) cell culture and xenograft repository, and the Pediatric Preclinical Testing Consortium (PPTC) (BOX 1).

When dealing with such large multi-institutional platforms, standardized methodological procedures should be carried out to ensure reproducibility and to streamline readouts so that they are interpretable across different laboratories (BOX 1). Further, therapeutic outcomes should be univocally deciphered and stringently interpreted. Retardation of tumour growth during therapy typically results in tumours that are smaller than controls at end point, but larger than they were before starting treatment; while this may well suggest that the therapy is biologically active (because it affects cancer cell proliferation), it is not an indication that the therapy is clinically effective; indeed, this kind of response would be clinically defined as ‘disease progression’ or, at best, ‘disease stabilization’. In the EurOPDX experience, even manifest effects of tumour growth inhibition – as observed, for example, after blockade of MEK in PDXs of *KRAS*-mutant colorectal cancer[125] – did not translate into clinical benefit when analogous therapies were applied to patients[163]. By contrast, overt regression in PDXs predicted positive results in the clinic: the finding that an

antibody and small molecule combination against HER2 induced massive regressions in *HER2*-amplified colorectal tumour grafts[6,117] has been recently translated into a successful clinical trial, with the vast majority of patients achieving tumour shrinkage when treated with the same regimen[93]. It has also become increasingly clear that the use of quantitative metrics to classify response (equivalent to clinical Response Evaluation Criteria in Solid Tumours (RECIST)) should be implemented to more precisely assess therapeutic effects in PDX trials. Modified RECIST criteria for mouse xenograft applications have recently been described[9]. 'Best response' is defined as the minimum value of percentage tumour volume change, compared with tumour volume at baseline, for treatment durations equal to or longer than 10 days, and 'best average response' is the minimum value of the mean percentage of tumour volume change, as measured at each evaluation time point along treatment, compared with baseline[9]. Such definitions, coupled with specific tumour volume cut-offs, have been applied to categorize complete response, partial response, stable disease and progressive disease in tumour-bearing mice. These modified RECIST criteria capture response kinetics, robustness and durability, and thus improve the ability of preclinical studies to accurately predict patient outcome.

Extended and detailed molecular annotation is a prerequisite for precision oncology paradigms. However, the accumulation of multiple layers of genomic information requires the development of computational systems with common or interoperable standards for normalization, correction and retrieval of complex data sets. The issue of big data collection, harmonization and storage is particularly critical when working with large PDX collections, in which one

original tumour from a single patient gives rise, upon serial passages, to many descendants that expand at an exponential rate (BOX 2). In EurOPDX, efforts are ongoing to aggregate cancer genomic profiles obtained through different technologies in different laboratories and to implement a user-friendly, open-source portal that showcases the molecular characteristics of the participating collections (BOX 1). Importantly, besides the detection of individual variants with clinically actionable potential, multi-dimensional molecular information from existing PDX models can be subjected to systems-based bioinformatics analysis to extract algorithms that identify key biological parameters[164]. Preliminary evidence suggests that such algorithms can be subsequently used to identify one or more 'biofacsimile' or 'proxy' PDX models for individual patients, and PDX-associated information may be leveraged to instruct treatment options and/or to derive predictive indicators in the clinic[164] (FIG. 2).

All these considerations underscore the opportunities offered by PDX models to illuminate new angles of translational cancer research, but they also put forward the challenges that are intrinsic to this approach, and the need for finding new ways to maximize PDX potential. Industry-led PDX ventures rely on common and extensively tested operating procedures, backed by considerable funding, which ensures scalable, homogeneous and reproducible experimental schemes; however, pharmaceutical initiatives are typically bound to preclinical testing of proprietary compounds and may face obstacles in publishing results, especially when data relate to sensitive commercial or patenting issues. Conversely, due to their multi-institutional nature, scholarly consortia usually suffer from heterogeneous characterization of their PDX

collections, a flaw that is hardly corrected by the relatively limited resources provided by government or charity grants; however, PDX academic efforts enjoy flexibility in drug testing and unfettered scientific reporting (including reporting of negative results, which avoids the duplication of effort and reduces costs). As EurOPDX members working in academia, we share with our colleagues of PRoXe the concern that “academic centers are ill suited to bear the burden of housing, expanding, archiving, characterizing, and disseminating PDXs to investigators (academic and industrial) across the world”(REF. [165]). Meanwhile, we believe that joining forces, incorporating models, coordinating methodologies, and improving the public shareability and visibility of molecular data in an academic-oriented rather than industry-scale format are viable objectives that will foster not only a stronger collaborative spirit in cancer medicine, but also a change of mind-set within institutional authorities and industrial stakeholders. EurOPDX has started as a crowd-funded initiative of scientists with common goals, complementary skills and similar needs, and is now growing in a more structured fashion thanks to enterprise-wide development plans. Ultimately, we envision a virtuous circle in which new knowledge from bottom-up efforts like ours and others will inform clinical decision making, which in turn will orient public and private financial interests to secure further sustainability of PDX-based activities. Successful examples in other contexts of biomedical research, such as TRANSAUTOPHAGY (see Further information; a European consortium for multidisciplinary research and translation of knowledge on autophagy) and GENiE (see Further information; a network of scientists using *Caenorhabditis elegans* as a model organism), bode well to achieve this ambition.

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Competing Interests Statement

The authors declare competing interests: see Web version for details.

DATABASES

Children's Oncology Group (COG) cell culture and xenograft repository: http://www.cogcell.org/xenografts.php
Public Repository of Xenografts (PRoXe): http://www.proxe.org
US National Cancer Institute (NCI) repository of patient-derived models: https://dtp.cancer.gov/repositories/

FURTHER INFORMATION

EurOPDX: http://www.europdx.eu
GENiE: http://worm-genie.eu/
TRANSAUTOPHAGY: http://cost-transautophagy.eu/
US Pediatric Preclinical Testing Consortium (PPTC): http://www.ncipptc.org/

SUPPLEMENTARY INFORMATION

See online article: S1 (table)

ALL LINKS ARE ACTIVE IN THE ONLINE PDF

BOX 1 - The EurOPDX Consortium and other related initiatives

EurOPDX (see Further information; established in 2013) is a collaborative network of 16 European academic institutions with expertise in basic, preclinical, translational and clinical oncology. Participating laboratories are affiliated with comprehensive cancer centres within which preclinical experimentation is closely associated with clinical activities. This allows for the efficient sharing of patient specimens – together with fully annotated clinical information – and facilitates the collection of tumours with unique characteristics (for example, rare types, exceptional responders and therapy-refractory cases). Currently sustained by membership fees organized by a consortium agreement, EurOPDX aims to obtain competitive infrastructural funding to further implement collaborative research projects and formalize external access procedures to models. The consortium agreement also sets forth general rules for confidentiality and intellectual property issues to regulate activities among EurOPDX members (co-ownership of results) and between EurOPDX and potential partners, including other patient-derived xenograft (PDX) consortia and industry.

MAIN OBJECTIVES:

- To create a uniquely extensive collection of characterized PDX models. The collection consists of more than 1,500 subcutaneous and orthotopic models from over 30 different pathologies (see TABLE 2). The models and their molecular annotation are currently being made publicly available through the cBioPortal, and are accessible for collaboration

upon signature of a material transfer agreement. Systematic derivation of primary cultures and organoids for *in vitro* studies is planned.

- To provide a platform for population-scale studies to discover low-prevalence genetic alterations with clinically actionable potential; to explore mechanisms of therapeutic resistance in molecularly defined tumour subtypes; and to develop predictive biomarkers for personalized cancer treatment.
- To harmonize working practices. This entails several aspects: first, standardization of biobanking procedures, including systematic assessment of genetic identity by single nucleotide polymorphism (SNP) DNA fingerprinting. Second, the implementation of common rules for PDX expansion and archiving; discussions are ongoing to limit PDX propagation to a maximum of 5 passages, but exceptions will be considered for tumour types known to deteriorate after freezing-thawing steps and for models characterized by very indolent growth, for which expansions up to 5 passages would take exceedingly long. Third, optimization of inter-laboratory reproducibility through proof-of-concept studies by which models from the same source are tested independently. And finally, the definition of a set of minimal information to be linked to each PDX.

OTHER MAJOR PDX INITIATIVES:

- US National Cancer Institute (NCI) repository of patient-derived models (see Further information).

- US Pediatric Preclinical Testing Consortium (PPTC; see Further information), a US National Cancer Institute (NCI)-centralized and NCI-funded collection for *in vivo* testing of paediatric anticancer drug candidates.
- Children's Oncology Group (COG) cell culture and xenograft repository (see Further information), a COG-based resource that provides validated cell lines and PDXs from paediatric cancers.
- Public Repository of Xenografts (PRoXe; see Further information), an open-source repository of leukemia and lymphoma PDXs[165]. Many of the models are being licensed to the Jackson Laboratories for industry-scale purposes, including distribution on a fee-for-service basis
- Novartis Institutes for Biomedical Research PDX Encyclopedia (NIBR PDXE), an industry-led initiative that includes approximately 1,000 models[9]. Clinical, pathological and PDX-level data from this collection are currently being incorporated into PRoXe[165].

BOX 2 - Data management and integration

By combining the flexibility of preclinical analysis with the instructive value of population-based studies, patient-derived xenografts (PDXs) offer unprecedented opportunities for drawing statistically robust correlations between genetic or functional traits and sensitivity to anticancer drugs. However, the advantages of high-throughput studies with PDX-based approaches may become major hurdles when dealing with large-scale data management, analysis and utilization. The deployment of PDX models for

translational studies often requires their stratification into existing predictive or prognostic molecular classes and subgroups as derived on tumours from patients. The portability of the stratification criteria from human to mice, and vice versa, is not trivial, owing to multiple sources of biological and genomic variation, which may be introduced in the process of engrafting and propagating patient tumour material into murine hosts.

DATA MANAGEMENT ISSUES:

- *Data complexity and dynamics.* The representation of cancer data in classical oncogenomic portals is normally static: the results obtained by analysing such public resources are not fed back to refine, update or complement the original information. The possibility to incrementally stratify and integrate multiple layers of information generated from the same original sample by diverse laboratories at different times represents one of the key added values of PDX-based approaches. This implies the need for further dimensions of complexity to interrogate an almost infinite number of variables and to implement decision-making algorithms in case of data inconsistency across experiments[166].

- *Data normalization and annotations.* The joint utilization of human and PDX data requires the standardization of sample metadata such as clinical and molecular ontologies. Through this effort, data derived from different experiments, technologies and platforms can be normalized against common categories and used to interrogate samples with integrative queries exploring heterogeneous data domains.

DATA ANALYSIS ISSUES:

- *Population selection bias.* Owing to the different engraftment efficacies inherent to each tumour sample, the population of xenografts might not recapitulate the full distribution of tumour phenotypic or molecular variables observed in patients. Any prior-dependent statistical models should be adapted to the new distribution of subclasses within the PDX population. This implies the necessity to identify the missing or underrepresented subgroups through analytical investigation of multidimensional parameters (genomics, transcriptomics, histopathology, and so on).
- *Loss of human immune and stromal cells.* Athough both stromal and immune components are replaced over time by murine analogues, the haematopoietic elements show important differences in their spatial distribution[167] or may be missing overall[156,168]. This affects the signal received from molecular profiling, and could require application of specific algorithms for signal correction to avoid or reduce artefacts and biases[156,169].

Table 1: Modelling cancer phenotypes with PDX models

PDX model	Open clinical question	Advantages	Challenges
Primary tumour specimens implanted s.c.	• Interrogation of primary or acquired resistance mechanisms • Discovery of prognostic and predictive biomarkers • Drug response • Identification of targetable molecular alterations • Characterization of intra-tumour clonal evolution	• Intact primary tumour tissue that maintains tumour architecture • Captures clonal diversity • Easy to measure tumour responses • Intravital tumour imaging	• Lack of proper anatomical niche • Not all grades of tumour engraft s.c. Generally, higher grade, more aggressive tumours engraft more easily.
Primary tumour specimens implanted orthotopically (PDOX)	• Mechanisms of metastasis • Study site-specific dependence of therapy • Monitoring the effects of adjuvant therapy on occult metastasis • Stromal contribution to response	• Intact primary tumour tissue that maintains primary tumour architecture • Local growth of primary tumour in proper anatomical context • Spontaneous distant metastases from primary tumour • Presence of primary and metastatic tumour niche • Recapitulates the entire metastatic process from the appropriate anatomical site • Ability to mimic clinical scenarios, for example, surgical removal of primary tumour or adjuvant therapy	• Access to imaging technologies to visualize tumour in longitudinal studies • Microsurgical skills • Large collections and high-throughput screens difficult to implement
Metastatic tumour specimens implanted s.c.	• Interrogation of primary or acquired resistance mechanisms	• Intact metastatic tumour tissue that maintains tumour architecture	• Lack of tumour metastatic niche

	• Discovery of prognostic and predictive biomarkers • Drug response • Identification of targetable molecular alterations • Characterization of intra-tumour clonal evolution		
Metastatic tumour specimens implanted orthotopically at the metastatic site	• Mechanisms of metastasis • Drug resistance • Genetic and cellular mechanisms of tumour growth • Drug response in the setting of metastatic disease • Stromal contribution to response	• Intact metastatic tumour tissue that maintains tumour architecture	• Access to imaging technologies to visualize tumour in longitudinal studies • Microsurgical skills • Large collections and high-throughput screens difficult to implement
PDX models of MRD	• Drug resistance • Discovery of prognostic and predictive biomarkers • Biological and pharmacological studies • Identification of targetable molecular alterations	• Studies can help us to understand the molecular bases of and optimize therapies for MRD • Higher tumour take rate when compared with untreated cancers • Enables study of clonal evolution and cancer stem cell behaviour	• PDXs are never therapy naive.
Clinical trial-associated xenografts (CTAXs)	• Discovery of prognostic and predictive biomarkers • Drug resistance • Drug response • Identification of targetable molecular alterations • Mechanisms of metastasis	• Possibility to establish xenografts at different clinical stages during patient tumour progression • Permits the parallel testing of novel drug combinations	• Limited quantity and quality of tissue • Limited number of successfully generated PDXs • A PDX derived from a single biopsy sample may not represent the patient's tumour.

CTC-derived PDX models	• Molecular tumour heterogeneity • Discovery of prognostic and predictive biomarkers • Study of the genetic evolution of the tumour • Identification of targetable molecular alterations	• Minimally invasive sampling • Ability to monitor cancer burden and drug susceptibility in metastatic and late-stage settings • Recapitulates donor patient's response to treatment • Facilitates investigation of the biology of otherwise inaccessible tumour specimens	• Low concentration in peripheral blood of patients with different solid tumours • Access to technologies to isolate all CTCs (both epithelial and mesenchymal) • Technically challenging
Humanized PDX models	• Investigation of immune therapeutics	• Recapitulates human immune system in mice	• Requires lengthy mouse humanization procedures • Hurdles to achieve complete human immune system reconstitution • See Supplementary information S1 (table) for further details

CTC, circulating tumour cell; MRD, minimal residual disease; PDX, patient-derived xenograft; PDOX, patient-derived orthotopic xenograft; s.c., subcutaneously

Table 2: Facts and figures about the EurOPDX collection*

				Average engraftment rate: treatment naive and adjuvant samples (%)		Engraftment rates: neoadjuvant samples (if relevant) (%)	
Tumour type or Organ	**Subtype**	**Primary tumour or metastasis**	**Total number of established models**	**Subcutaneous**	**Orthotopic**	**Subcutaneous**	**Orthotopic**
CRC	All subtypes included	Primary	291	52-75	80	NA	NA
		Liver metastasis	444	73-91	90	84	NA
Pancreas (PDAC)	All subtypes included	Primary	211	54-71	70	NA	NA
		Liver metastasis	24	60-100	90	NA	NA
Breast	ER+ (incl. ER+HER2+)	Primary	24	4-7	7	20	NA
		Metastasis	20	25-49	33-47	NA	NA
	TNBC	Primary	78	30-34	60-86	72	86
		Metastasis	26	60	50-66	NA	NA
	HER2+ only	Primary	16	26	NA	13	NA
		Metastasis	5	NA	33	NA	NA
Skin melanoma	All subtypes included	Primary	8	67-90	29	NA	NA
		Metastasis (cutaneous, liver, lung)	161	72-90	83-85	NA	NA
Ovary	All subtypes included	Primary	123	40-85‡	68	62‡	NA
		Metastasis	19	47-85‡	80	NA	NA
Gastric	All subtypes included	Primary	87	41-50	70	34	NA

Endometrial	All subtypes included	Primary Metastasis	67 10	43-55 10-60[§]	74 95	NA NA	NA NA
Lung	NSCLC	Primary and metastasis	59	50-70 (primary)	52	NA	NA
	SCLC	Primary and metastasis	12	50	75	Not applicable	Not applicable
HNSCC	All subtypes included	Primary Metastasis	50 13	45 83	65	NA NA	NA NA
Glioblastoma	All subtypes included	Primary	52	Not applicable	95-100	Not applicable	NA
Uveal melanoma	All subtypes included	Primary	12	32	NA	Not applicable	Not applicable
		Liver metastasis	14	65	NA	Not applicable	Not applicable
Testicular	All subtypes included	Primary and metastasis (lymph node, lung and brain)	18	NA	35	NA	NA
Uterine Sarcoma	High grade	Primary	3	75	NA	Not applicable	Not applicable
		Metastasis	9	100	NA	Not applicable	Not applicable
Renal	All subtypes included	Primary	8	30	NA	NA	NA

CRC, colorectal cancer; ER, oestrogen receptor; HNSCC; head and neck squamous cell carcinoma; NA, not available; NSCLC, non-small-cell lung cancer; PDAC, pancreatic ductal adenocarcinoma; SCLC, small-cell lung cancer; TNBC, triple-negative breast cancer. *The data presented represent the range of implantation rates obtained across EurOPDX partner laboratories as of October 2016. ‡Highest take rates obtained with the high-grade serous ovarian cancer subtype. §Take rates of 10–15% for abdominal, pelvic lymph node and peritoneal metastases, 60% for vaginal metastases.

Table 3: Comparative quantitative data of response rates in PDXs versus human patients

Tumour type	Clinical question	Comparative response rates	
CRC*	**Response to EGFR antibody monotherapy in genetically unselected CRC PDXs[6], or unselected chemorefractory patients with CRC[178]**	PDXs: • **PR: 5 of 47 (10.6%)** • **SD: 14 of 47 (29.8%)** • **PD: 28 of 47 (59.6%)**	Patients: • **PR: 12 of 111 (10.8%)** • **SD: 24 of 111 (21.6%)** • **PD: 59 of 111 (53.2%)** • **Not evaluated: 16 of 111 (14.4%)**
CRC*	• **PDXs[118]: Response to EGFR antibody monotherapy in KRAS, NRAS and BRAF wild-type models** • **Patients[179]: Response to EGFR antibody plus chemotherapy in chemorefractory patients with KRAS, NRAS and BRAF wild-type CRC**	PDXs: • **PR: 31 of 125 (24.8%)** • **SD: 60 of 125 (48%)** • **PD: 34 of 125 (27.2%)**	Patients: • **PR: 15 of 56 (26.8%)** • **SD: 29 of 56 (51.8%)** • **PD: 12 of 56 (21.4%)**
NSCLC	**Co-clinical trial, PDX versus donor patient[66]: response to EGFR small-molecule inhibitors in four representative cases out of six established PDXs**	**1 PR (both patient and PDX)** **1 SD (both patient and PDX)** **2 PD (both patient and PDX)**	
Breast cancer	**Co-clinical trial, PDX versus donor patient[63]: response to several therapies**	• **Doxorubicin:** **4 PD (both patient and PDX)** • **Docetaxel:** **1 PR (both patient and PDX)** **6 PD (both patient and PDX)** • **Anti-HER2 combination therapy (trastuzumab**	

	and lapatinib): 1 PR (both patient and PDX)

CRC, colorectal cancer; EGFR, epidermal growth factor receptor; NSCLC, non-small cell lung cancer; **PD: progressive disease**; PDX, patient-derived xenograft; PR, partial response; SD, **stable disease.** *Data represent separate PDX and patient population studies.

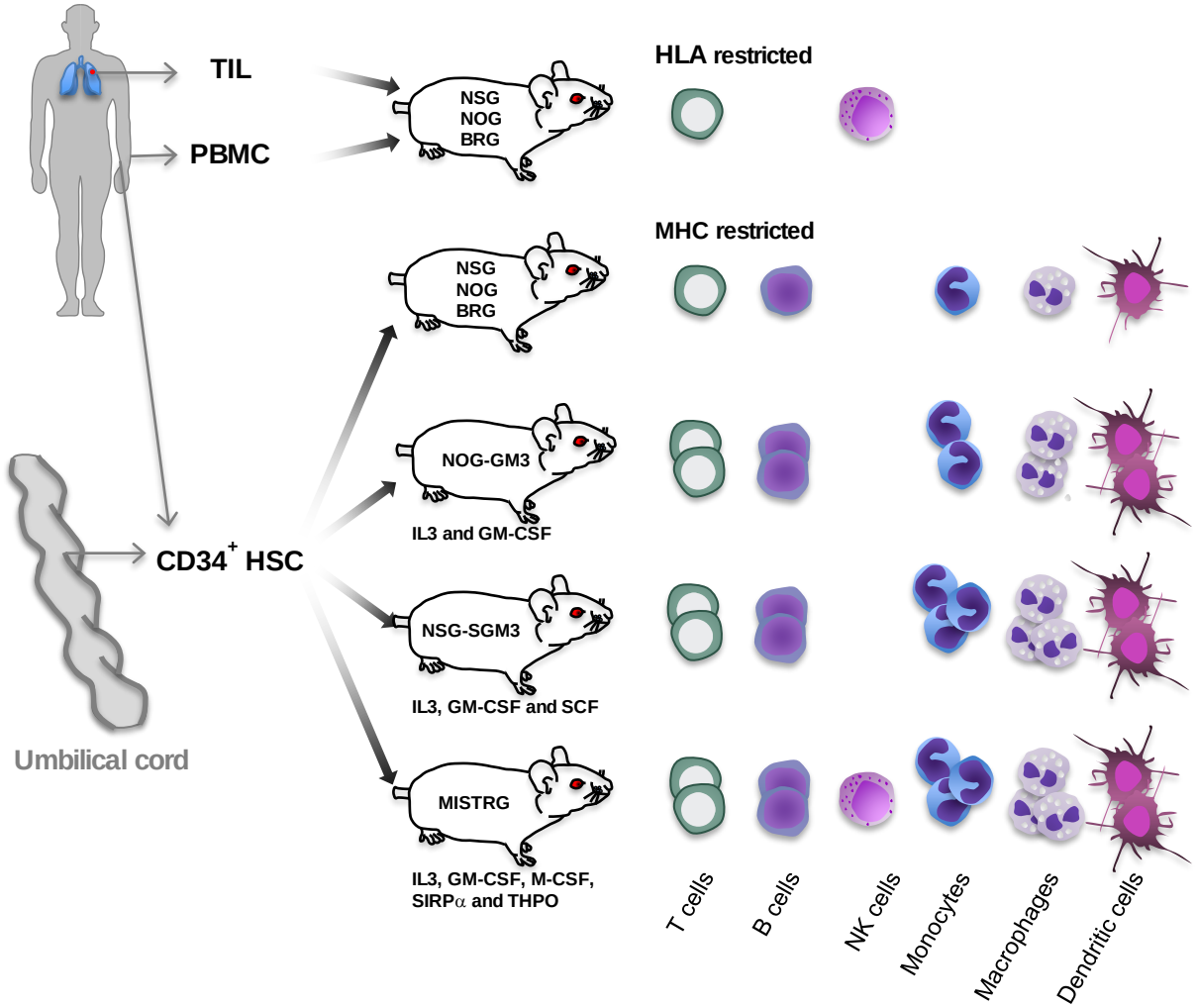

Figure 1: Strategies to generate humanized PDXs. Sources of immune cells include tumour-infiltrating lymphocytes (TILs), peripheral blood mononuclear cells (PBMCs) and CD34-positive haematopoietic stem cells (HSCs); HSCs may be purified from

mobilized adult peripheral blood, bone marrow or umbilical cord blood. Engrafted TILs or PBMCs generate mainly circulating human leukocyte antigen (HLA)-restricted T cells and natural killer (NK) cells (top row). This system is characterized by a vigorous graft-versus-host reaction that narrows the experimental window to approximately 2-5 weeks. Despite this limitation, the system is useful for certain analyses, such as monitoring the recruitment of T lymphocytes to tumours by therapeutic antibodies[170]. Fully humanized systems (bottom four rows) use severely immunodeficient mouse strains such as NOG (NOD-Cg-*Prkdc*^{scid} *Il2rg*^{tm1Sug}/JicTac)[171], NSG (NOD.Cg-*Prkdc*^{scid} *Il2rg*^{tm1Wjl}/SzJ)[172] and BRG (C.Cg-Rag1^{tm1Mom} IL2rg^{tm1Wjl}/SzJ)[173,174]. Mice with a NOD (non-obese **diabetic) background have functionally deficient NK cells. SCID (severe combined immunodeficiency) is a loss-of-function mutation that affects DNA-dependent protein kinase (DNA-PK), a DNA repair enzyme involved in V(D)J recombination during T cell and B cell development. As a consequence, SCID mice have reduced levels of T cells and B cells. Inactivation of the interleukin-2 (IL-2) receptor γ-chain leads to impaired T cell and B cell development and prevents the generation of NK cells. Recombination-activating gene 1 (RAG1) is necessary for V(D)J recombination; thus, RAG1 inactivating mutations affect T cell and B cell development. All these different strains show subtle differences to support** engraftment of functional human immune cells[173]. Injection of human CD34-positive HSCs into these mice leads to the generation of major histocompatibility complex (MHC)-restricted T cells and B cells, as well as to limited amounts of monocytes, macrophages, neutrophils and dendritic cells. In addition, these mouse strains have been improved by genetic modifications for the production of a variety of human cytokines that stimulate the differentiation of additional

haematopoietic lineages. For example, strains such as NOG-GM3 (which expresses human IL-3 and granulocyte-macrophage colony-stimulating factor (GM-CSF; also known as CSF2)[175], NSG-SGM3 (which expresses human IL-3, GM-CSF and SCF (also known as KIT ligand))[176] and MISTRG (which expresses human IL-3, GM-CSF, macrophage CSF (M-CSF, also known as CSF1), signal regulatory protein-α (SIRPα) and thrombopoietin (THPO))[177] produce increased numbers of human myeloid and mast cells, regulatory T cells and NK cells (see Supplementary information S1 (table)). PDXs, patient-derived xenografts.

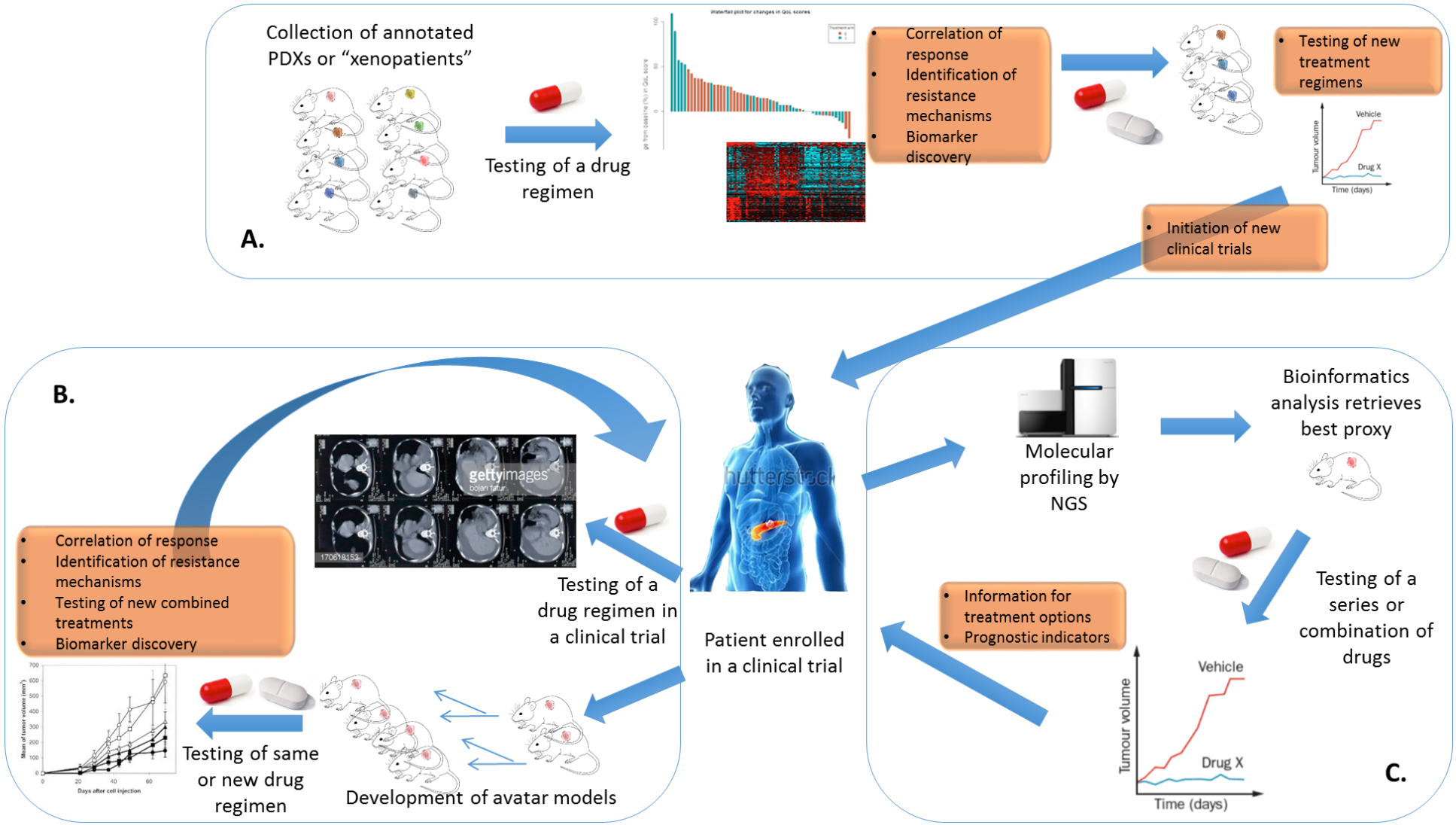
A.
Collection of annotated PDXs or "xenopatients"
Testing of a drug regimen
Correlation of response
Identification of resistance mechanisms
Biomarker discovery
Testing of new treatment regimens
Vehicle
Drug X
Tumour volume
Time (days)
Initiation of new clinical trials
B.
Correlation of response
Identification of resistance mechanisms
Testing of new combined treatments
Biomarker discovery
Testing of a drug regimen in a clinical trial
Patient enrolled in a clinical trial
Molecular profiling by NGS
Bioinformatics analysis retrieves best proxy
Information for treatment options
Prognostic indicators
Testing of a series or combination of drugs
Testing of same or new drug regimen
Development of avatar models
C.

Figure 2: PDX preclinical study designs. a. Large collections of patient-derived xenograft (PDX) models (‘xenopatients’) now allow population-based studies to be carried out, which better mimic the inter-tumour heterogeneity that is seen in patients and are more predictive of clinical efficacy than conventional xenografts of immortalized cancer cell lines. PDX molecular characterization and correlation with therapeutic response also facilitates biomarker discovery, as well as identification of primary (and acquired) resistance mechanisms. These studies can lead to new hypotheses and support the initiation of new clinical trials. b. For some cancer types for which avatar models can be developed, co-clinical avatar studies allow for simultaneous drug testing in mice and patients for real-time adaptive therapeutic decisions. c. In the “biofacsimile” or “proxy” study format, integrative systems-based bioinformatics analysis can be used to pinpoint the best-matched PDX for a given patient from a collection of molecularly profiled models. PDX-associated information is then leveraged to instruct clinical treatment options and/or derive prognostic indicators. NGS, next generation sequencing.

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Online Only information

Annette Byrne is Senior Lecturer at the Royal College of Surgeons in Ireland (RCSI), Department of Physiology and Medical Physics, Principal Investigator at the RCSI Centre for Systems Medicine and Director of the RCSI Laboratory for Tumour Biology and Molecular Imaging. She received her BSc in Physiology in 1996 and was awarded a PhD in Cell Biology from the University of York, UK in 1999. Her work supports the preclinical development of novel targeted anticancer therapeutics and biomarkers applying multi-modality molecular imaging. Her laboratory focuses on colorectal, brain and breast malignancies implementing patient-derived xenograft (PDX) population and bio-facsimile trials.

Livio Trusolino is an associate professor of histology at the University of Turin Medical School, Candiolo, Turin, Italy, and co-directs, together with Andrea Bertotti, the Laboratory of Translational Cancer Medicine at the Candiolo Cancer Institute, Candiolo, Turin, Italy. He received his M.D. in 1993, a Ph.D. in human oncology in 1997 and a specialization in pathology in 2003. The work in his laboratory concentrates on the characterization of the genetic makeup and intracellular signalling networks underlying sensitivity and resistance to anticancer targeted therapies, with a focus on colorectal cancer, using patient-derived xenograft (PDXs) as the main preclinical models.

Competing interests statement

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ToC summary

This Opinion article discusses progress and challenges in using patient-derived tumour xenograft (PDX) models in cancer precision medicine. It is primarily co-authored by members of the EurOPDX Consortium and as such highlights the merits of shared PDX resources.

Supplementary Information S1 | **Examples of improvements in humanised mouse models for PDX studies**

Pending issues	Details	Circumvention or solutions	Models (Supplier or Developer)
Graft versus Host Disease (GvHD)	Engraftment with mature human T cells leads to xenogeneic GvHD due to mismatch between murine MHC and human HLA in engrafted cells or tissues.	• Use of genetically modified mouse strains that develop reduced or no GvHD • GvHD reported to depend on HLA haplotype of HSC donor[1]	• Immune-deficient strains lacking *B2m* (*NSG-B2m*), MHC-I (*NSG-(K^bD^b)null* or MHC-II (*NSG-(H2-Ab)null* (Jackson Laboratory[2,3]) • *B6RG-Cd47*: C57BL/6 mice lacking *Rag2* and *Il2rg*, and deficient for CD47[4]. Absence of GvHD due to improper "education" and functionality of mouse myeloid cells. However, functionality of human myeloid cells still to be validated.
HLA restriction of T cells and compatibility with tumours	Absence of human HLA molecules on thymic epithelial cells generates human T cells unable to recognize de novo antigens (eg. Tumor specific antigens) in a HLA-restricted manner in HSC transplanted mice[5].	• Transplantation of human thymus tissue (BLT/ Bone Liver Thymus mice)[6] • Use of human HLA class I and/or class II transgenic immune deficient mice[7-11]	• E.g. *NOG-Dr4* mice (Taconic); *NSG-Dr1*, *NSG-Dr4* or *NSG-HLA-A2* mice (Jackson Laboratory).
Species-specific cytokines and factors	Some human cytokines or factors are species specific, preventing generation or maintenance of specific human immune cell types.	• New mouse strains expressing human cytokines or receptors to obtain a more complete human immune system[12] • Onset of anaemia described as a limitation for many of the current models with improved myeloid reconstitution. Efforts being made to avoid anaemia for example by introducing human CD47.	Immune deficient mouse strains transgenic for human cytokines to promote myeloid and NK lineage commitment include: • *NOG-GM3*: NOG mice expressing human GM-CSF and IL-3 (CIEA, Japan)[13]; • *NSG- SGM3*: NSG mice expressing human IL-3, GM-CSF and SCF (Jackson Laboratory[14]); • *MISTRG*: BALB/c x 129S4 *Rag2;Il2rg* double ko mice expressing human M-CSF, GM-CSF, IL-3, THPO and a human *SIRPA* allele[15]; • *NOG-hIL6*: NOG mice expressing human IL6 (Taconic), featuring increased human monocytes and macrophages (in particular M2 type) • *NSG-W41*: NSG mice with mutated mouse *Kit*. Reduced mouse haematopoiesis results in higher human reconstitution levels without the need to

			precondition mice. Display improved human myeloid reconstitution as compared to NSG mice[16]. • *NOGh-IL2*: NOG mice expressing human IL2, featuring higher numbers of human NK cells (Taconic[17])
Remaining mouse innate immunity	Despite multiple gene modifications to eliminate mouse immune cells, commonly used NSG and NOD mice still have mouse myeloid cells (macrophages, dendritic cells and granulocytes) which can play a role in tumour biology[18].	• Models with reduced mouse innate immune cells. These include strains transgenic for human myeloid specific cytokines mentioned earlier. • Immune-deficient mice further modified to functionally incapacitate remaining innate cells.	• *NSG-Tlr4-/-*: facilitates monitoring of human TLR4 responses only (mentioned in[19]) • *BRGF*: *Rag2*-deficient, *Il2rg*-deficient BALB/c (BRG) mice lacking mouse *Flt3*, resulting in loss of mouse dendritic cells, increased numbers of human dendritic cells, NK and T cells[20] • *B6RG-Cd47* mice (as above)
Impaired humoral immune responses, low Ig levels and impaired Ig class switching	B cells in humanised NSG or NOG mice do not undergo sufficient maturation to become memory and antibody-producing cells[21].	Development of new mouse strains: • e.g. human HLA class II transgenic mice, with improved humoral responses due to increased CD4-mediated help[22]; • Mice strains transgenic for human cytokines[23]; • Improved lymphoid organ development resulting in increased B cell development and Ig class switching (see below).	• *SRG-IL6*: *Rag2*;*Il2rg* double ko mice with a human *SIRPA* allele and expressing human IL6 show increase in development of CD45+ cells, in particular B cells and class switched B cells (discussed in[24]) • Human HLA class II transgenic NSG or NOG mice[7,9]
Impaired lymph node development, poorly developed germinal centres	• Defects in cytokine signalling in immune-deficient strains results in poorly developed secondary lymphoid tissues (NSG, NOG and BRG are all *Il2rg*-deficient[25]). This includes poor germinal centre formation, ineffective class switching of B cells and antigen presentation to naïve T cells, impeding robust adaptive immune responses upon humanisation[26].	• Mouse models with restored secondary lymphoid tissue development. • Discussed in[24]	• *NOG-pRorgt-IL2Rg:* transgenic NOG mice in which IL2Rγ expression is driven by the promoter of Rorγt, an isoform of Rorc whose expression is restricted to the thymus. This leads to specific IL2Rγ expression in lymphoid tissue inducer cells resulting in normal lymph node development and improved B cell maturation and Ig class switching.
Impaired	Immunodeficient strains based on NOD	• Use immune-deficient mice	• *NSG-C5a*: NSG-C5a mice have the intact *Hc* gene,

complement system	background lack hemolytic complement due to a mutation in the C5 complement gene, preventing the formation of the C5b-9 membrane attack complex[18]. These mice lack complement dependent cytotoxicity in e.g. antibody dependent therapies.	strains that do not have the NOD background such as SRG mice. • Genetically modified NOD based mice that have a functional C5 gene.	restoring the complement system[27].

B2m, beta2-microglobulin; DR, Antigen D-related; *Flt3*, Fms-like tyrosine kinase 3; GM-CSF, granulocyte-macrophage colony stimulating factor; GvHD, Graft versus Host Disease; Hc, hemolytic complement; HSC, haematopoietic stem cell; Ig, immunoglobulin; *Il2rg*, interleukin 2 receptor common gamma chain; IL2, interleukin 2; IL3, interleukin 3; IL6, interleukin 6; ko, knockout; M-CSF, macrophage colony-stimulating factor; NK, natural killer; *Rag2*, recombination activating gene 2; SCF, stem cell factor; *SIRPA*, signal-regulatory protein α; TLR4, toll-like receptor 4; THPO, thrombopoietin.

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