Towards precision oncology with patient-derived xenografts

Eugenia R. Zanella[1,3], *Elena Grassi*[1,2,3] *and Livio Trusolino*[1,2]✉

Abstract | Under the selective pressure of therapy, tumours dynamically evolve multiple adaptive mechanisms that make static interrogation of genomic alterations insufficient to guide treatment decisions. Clinical research does not enable the assessment of how various regulatory circuits in tumours are affected by therapeutic insults over time and space. Likewise, testing different precision oncology approaches informed by composite and ever-changing molecular information is hard to achieve in patients. Therefore, preclinical models that incorporate the biology and genetics of human cancers, facilitate analyses of complex variables and enable adequate population throughput are needed to pinpoint randomly distributed response predictors. Patient-derived xenograft (PDX) models are dynamic entities in which cancer evolution can be monitored through serial propagation in mice. PDX models can also recapitulate interpatient diversity, thus enabling the identification of response biomarkers and therapeutic targets for molecularly defined tumour subgroups. In this Review, we discuss examples from the past decade of the use of PDX models for precision oncology, from translational research to drug discovery. We elaborate on how and to what extent preclinical observations in PDX models have confirmed and/or anticipated findings in patients. Finally, we illustrate emerging methodological efforts that could broaden the application of PDX models by honing their predictive accuracy or improving their versatility.

The characterization of cancer genomes has provided a catalogue of oncogenic mutations across tumours and ignited the development of therapeutic strategies targeting individual, tumour-specific genetic aberrations with clinical actionability[1,2]. Several issues, however, complicate the routine implementation of treatments based on tumour genotyping. First, the response of tumours to targeted therapies might transcend their genomic constitution[3]. For example, inhibiting the product of a single dominant oncogene could trigger compensatory signalling feedback mechanisms, switches in cancer cell plasticity and/or deviations in evolutionary trajectories, all leading to the acquisition of new dependencies that substitute for those conferred by the inhibited target[4,5]. Owing to this complexity, tumour genomic profiling is not always sufficient to inform therapeutic decisions and predict treatment outcomes, and in many cancers a presumed bona fide oncogenic driver has been proven to be a non-viable therapeutic target[6]. Second, although some genetic aberrations occur at high frequency in specific tumours types, many cancers exhibit a 'long tail' distribution of rare alterations that are difficult to identify in modestly sized patient cohorts and are hard to categorize as driver genes rather than random passenger mutations[7]. These considerations consolidate what we believe to be the notion of precision oncology, which consists of finding co-dependencies and connectivities that attenuate responses to targeted therapies in genetically defined contexts, and identifying combinatorial ways to tame such collateral liabilities pharmacologically.

For precision oncology to manifest its full potential, platforms and datasets are needed that maximally capture the molecular diversity of tumours. Patient-derived xenografts (PDXs) are tumour fragments from surgical resections or biopsies that are propagated in mice through a series of implants and explants. By enabling genomic profiling, biological annotation and therapeutic investigation of a virtually unlimited number of samples, PDX models have been demonstrated to be powerful tools to pursue the inventory and experimental interrogation of cancer genes and to test their value as drug targets. A detailed discussion of the technical aspects of PDX engraftment and expansion is available elsewhere[8,9]. In this Review, we describe relevant functional and clinical insights that have emerged from PDX-based research in the past decade and how these studies have generated important knowledge to advance precision oncology.

[1]*Candiolo Cancer Institute – FPO IRCCS, Candiolo, Italy.*

[2]*Department of Oncology, University of Torino, Candiolo, Italy.*

[3]*These authors contributed equally: Eugenia R. Zanella, Elena Grassi.*

✉*e-mail: livio.trusolino@ircc.it*

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Key points

- The generation of patient-derived xenograft (PDX) models involves a selection bottleneck imposed by tumour engraftment, and subsequent propagation influences the evolutionary trajectories of cancer cells; therefore, these models can be used to investigate tumour clonal composition and competition during spontaneous tumour progression and under the selective pressure of treatment.
- Work in which PDX models were studied at the moment of maximal tumour shrinkage during exposure to a given therapy has provided insights into lineage-specific phenotypic adaptations, which underlie the acquisition of drug tolerance and are responsible for sustaining residual disease.
- The substitution of human stromal cells by mouse stromal cells that occurs after tumour implantation has enabled the identification of transcriptional signatures related to either cancer or stromal cells with predictive and prognostic value.
- Large collections of PDX models have contributed to the discovery and validation of novel biomarkers of response to treatment and have aided the design of new therapeutic options, some of which have entered the clinic.
- Next-generation models with higher tissue complexity (humanized mice) or easier manageability (non-mammal organisms, ex vivo cultures) are being developed that complement conventional PDX models.

Tumour heterogeneity and evolution

In solid tumours, cancer cells are embedded in a supportive tumour microenvironment (TME) that comprises stromal cells, including fibroblasts and endothelial cells, and innate and adaptive immune cells[10]. Moreover, cancer cells encompass a multitude of distinct subpopulations with different phenotypic and genotypic features that are constantly moulded over time, spontaneous attrition dynamics and therapy-imposed selective pressures[11]. Owing to the varied nature of tumour cell composition and the heterogeneous intratumour distribution of genetic alterations, the establishment of a PDX involves the concatenation of random events (FIG. 1): (1) tumour subsampling to generate implantable tissue fragments introduces a certain degree of anatomical bias; (2) once injected into mice, only a fraction of cancer cells are competent to engraft, which reduces genetic diversity owing to out-competition by the fittest and most rapidly proliferating clone(s); and (3) serial PDX propagation can further exacerbate PDX divergence owing to mutational evolution and phenotypic adaptation over time and space.

When pondering the accuracy of these models in fully phenocopying tumours from donor patients, these features could be deemed as limitations of the methodology for generating PDXs. Nevertheless, the analysis of tumour molecular deviation through repeated passages in mice has provided considerable insight into the mechanisms that drive cancer evolutionary trajectories, clonal competition and non-genetic adaptation[12]. Likewise, assessment of the dynamic effect of treatment on the clonal architecture and transcriptional features of PDX models has advanced our understanding of the mechanisms underlying drug resistance[13].

Preservation of genetic identity

Current evidence. Whether the architecture of copy number alterations (CNAs) evolves or whether it remains stable throughout PDX propagation in mice is a subject of ongoing and lively debate. In a study using gene expression microarray data to infer CNA profiles in >500 PDX models across 24 cancer types, ~60% of the models acquired at least one large chromosomal abnormality within a single passage, and 90% after the first four passages[14]. As expected for a founder effect (whereby model initiation imposes a strong selection pressure), genomic diversity was more evident in first-passage and early-passage (up to seven passages) PDXs than in those at later passages (≥19 passages).

A joint initiative of the National Cancer Institute PDXNet consortium and the EurOPDX consortium, involving a collection of >1,400 samples from 509 PDX models across 16 tumour types, questioned the assumption that copy number divergence between tumours of origin and PDXs is extensive[15]. The controversy mainly stemmed from technical issues, specifically from the consideration that expression-based CNA assessment has lower segmental resolution than DNA-based methods, enabling CNA enumeration only at the scale of entire chromosomal arms. Moreover, in DNA microarray analyses using bulk pre-implantation tumours, but not in those using PDXs, the intensities of signals generated by cancer cells are diluted by those from human stromal cells, which results in variability in the expression signals that can be erroneously interpreted as CNAs. In the PDXNet–EurOPDX study[15], copy number estimation by DNA-based measurements did not support the occurrence of mouse-induced copy number evolution as had been previously proposed. Moreover, the study showed that CNAs involving cancer-related genes are not positively selected in PDX models[15]. Notably, CNA variations between original and mouse-passaged tumours were comparable to the differences between multiregion tumour samples or samples of multiple tumours from the same patient, suggesting that spatial heterogeneity has a greater influence on PDX-associated genetic drift than genetic instability.

A preliminary consensus across studies is emerging, whereby ~90% of the genome seems to be concordant between PDX models and original tumours. According to a reassessment of the PDXNet and EurOPDX datasets, a median of 10% of the genome of matched tumours and PDXs is differentially affected by CNAs[16]. Similarly, an analysis of another collection of 536 matched samples across 25 cancer types revealed a 10% divergence between PDX models and their tumours of origin at the level of single-nucleotide alterations in key driver genes, along with occasional examples of PDX-associated CNA evolution consolidated along serial passages[17]. Whether these differences should be considered relevant in a context of general concordance remains a matter of opinion.

Clinical considerations. Even in the scenario of a molecular fidelity of ≥90% between PDX models and their tumours of origin, the bottlenecks associated with model derivation and propagation, coupled with the inherent genomic instability of cancer, inevitably introduce some extent of genetic deviation. This divergence might not affect the dominant prevalence of clonal (or 'trunk') mutations, which are expected to be equally represented in the original and propagated tumours. By contrast, the pattern of subclonal (or 'branch') mutations is likely to be different between passaged and pre-implantation

samples owing to neutral evolution and selective pressures. Evidence continues to emerge that most cancers contain a minority of cells with innate resistance to drugs owing to subclonal mutations[18], and thus responses to therapy might differ between PDX models and the matched original tumours.

These caveats notwithstanding, potential genomic evolution does not seem to critically affect the accuracy of therapeutic response prediction in tumours of origin and their derived PDX models. In a retrospective analysis, data on the treatment outcomes in 92 patients with advanced-stage solid tumours were compared with sensitivity to the same drugs in the corresponding PDXs, revealing a sensitivity and specificity of 96% and 70%, respectively, for the PDX drug screens, with positive and negative predictive values of 85% and 91%, respectively[19]. In principle, these results bode well for the implementation of prospective co-clinical trials that exploit PDX models as 'avatars' for drug efficacy studies, with the aim of using pharmacological information from these avatars to inform direct therapeutic interventions in the donor patients. Although initial efforts in this direction have been successfully attempted in patients with pancreatic cancer[20], the value of PDX models as predictive tools for real-time clinical decision-making is limited by the laboriousness of PDX-based experimentation, which hardly aligns with the faster time scales of clinical practice.

Clonal dynamics

Current evidence. PDX engraftment and expansion are accompanied by changes in the clonal organization of the tumours. This divergence makes PDX models versatile tools to trace the intratumour architecture of genomic diversity and to correlate clonal competition with fitness effects, which is instrumental in analysing the principles of drug resistance in heterogeneous cancer cell populations.

Breast cancer and colorectal cancer (CRC) are the tumour types for which knowledge of clonal dynamics in PDX models is most advanced. In a seminal study, genomic clonal analysis at single-cell resolution in 15 breast cancer PDXs revealed strong variability in the patterns of initial clonal selection, ranging from moderate reshaping of clonal prevalence to extreme selective engraftment (and subsequent dominance) of minor subclones representing only a small fraction of the original population[21]. Over serial passages, the spectrum of clonal expansions and declines was wider for tumours subject to weak initial selective pressure than for those

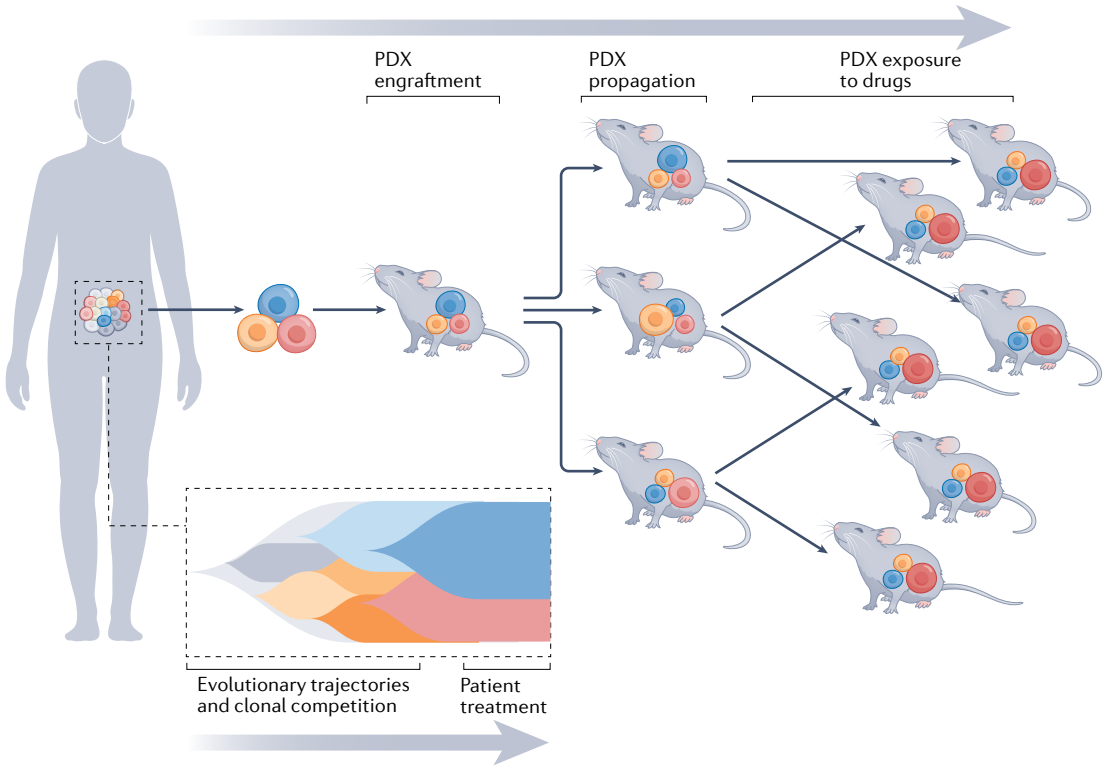

Fig. 1 | **PDX models as dynamic tools to trace cancer clonal evolution.** Cancers display extensive intratumour genetic diversity, with founding clones giving rise to genetically heterogeneous subclones endowed with different biological fitness, spatial distribution and evolutionary trajectories. Clonal competition dynamically shapes the genomic architecture of tumours during cancer progression and under treatment-imposed selective pressure. Implantation of tumour fragments into mice to generate patient-derived xenograft (PDX) models involves an initial anatomical bias related to sampling, followed by a strong selection bottleneck owing to the successful engraftment of only a fraction of cancer cells. Serial passaging in PDX models also contributes to clonal evolution, which can be further exacerbated by drug exposure in recipient mice. Thus, PDX models can be exploited to investigate how the clonal fitness landscape and the ensuing phenotypic divergence of individual tumours are influenced by space, time and drug insults under controlled experimental conditions.

subject to strong early selective pressure. Of note, similar clonal dynamics were observed when cell subpopulations from the same tumour were transplanted into multiple mice, with reproducible outgrowth of initially minor subclones[21]. This observation suggests that, in the models tested, directional clonal dynamics over time are not defined by stochastic processes (such as random genetic drift) but are deterministically associated with favourable mutational landscapes that confer a predictable fitness advantage. In a study published in 2021, clonal fitness was mapped over time in three PDX models of triple-negative breast cancer (TNBC) using single-cell CNAs as heritable genotypes to trace clonal trajectories during exposure to standard-of-care chemotherapy[22]. Prolonged exposure to cisplatin (leading to the development of resistance) suppressed clones with high fitness that had dominated in the absence of therapy; conversely, chemotherapy selected for phylogenetic lineages initially endowed with low fitness[22].

Engraftment-related subclonal skewing has also been documented in PDX models of CRC. In four of nine tumours analysed, dominant parental clones were less represented and minor parental subclones had become dominant in PDXs compared with their tumours of origin, with a general reduction in clonal heterogeneity and a decreased prevalence of regionally confined subclonal mutations[23]. In another study, spontaneous and drug-induced evolutionary dynamics were monitored by combining CNA analysis and deep sequencing of mutational hotspots with lentiviral lineage tracing in ten PDX models. After the usual clonal selection during engraftment, all initial clones remained present and genetically stable after serial passages[24]. Despite this stability, when genetically homogeneous clones were marked with lentiviral vectors to track the progeny of single CRC cells, the ensuing lineages showed marked functional heterogeneity, with idiosyncratic variabilities in growth rates, a tendency to persist or the propensity to decline[24]. Consistent with the fitness mapping conducted in models of breast cancer[22], exposure of mice bearing CRC PDXs to oxaliplatin preferentially eliminated persistent, high-fitness progeny and increased the proportion of previously dormant lineages[24]. Unlike in breast cancers, however, variations in the fitness landscape under the selective pressure of treatment were not dictated by clonal selection of heritable genomic traits; rather, fitness was shaped by non-genetic mechanisms affecting cell phenotypes[24].

Clinical considerations. The finding that cancer cell subpopulations poised to become chemorefractory have reduced competitive ability in the absence of therapy[22,24] indicates that drug resistance has an evolutionary fitness cost, which, in principle, could be increased by therapeutic intervention. Intriguingly, the biological characteristics of clones with low fitness in breast cancer PDX models and of dormant lineages in CRC PDX models echo those of slow-growing 'persister' cells, which have been repeatedly identified in cell cultures after prolonged exposure to tyrosine kinase inhibitors (TKIs)[25–27]. These persister cells display consistent hallmarks, such as an altered chromatin organization[25], diminished apoptotic thresholds[26] and a metabolic shift towards fatty acid oxidation[27]. Targeting such hallmarks — for example, with histone deacetylase (HDAC) inhibitors to modulate chromatin state, BH3 mimetics to promote apoptosis, and antagonists of fatty acid catabolism to counteract metabolic adaptation — reduced the fraction of persister cells in cell culture-based experiments[25–27].

The clinical information on patients treated with approaches analogous to those described earlier is, at present, fragmentary. Among 38 patients with chemorefractory metastatic CRC treated with a combination of the HDAC inhibitor vorinostat and the antimetabolite chemotherapeutic agent 5-fluorouracil plus leucovorin, 21 (55%) had disease stabilization and 1 (3%) had a partial response[28]. By contrast, addition of the HDAC inhibitor chidamide to first-line cisplatin did not meet the predefined efficacy criteria in a study involving 15 patients with advanced-stage TNBC[29]. Phase I studies of navitoclax, an inhibitor of anti-apoptotic BCL-2 family proteins, in combination with gemcitabine or carboplatin–paclitaxel in patients with solid tumours showed modest but appreciable clinical efficacy (54% and 36.8% stable disease rates, respectively) and high toxicity[30,31]. Although the clinical data remain immature, therapies aimed at further reducing the already limited fitness of chemotherapy-resistant cell subpopulations in heterogeneous tumours would be expected to delay disease recurrence in patients.

Adaptive drug tolerance

Current evidence. The inherent genomic instability of tumours favours the stochastic acquisition of new mutations, some of which provide cancer cells with a selective advantage to evade therapeutic pressure; the larger the pool of residual cells not eliminated by a particular treatment, the higher the probability that a drug-resistant clone will emerge. 'Lingering' cells that withstand therapy usually do so by implementing non-genetic mechanisms of drug tolerance, which entails various modalities of phenotypic adaptation[5].

In the past few years, studies in PDX models have demonstrated the importance of cellular plasticity in shaping sensitivity to therapy, in particular in melanoma and CRC. A common theme that stems from PDX studies is that drug-tolerant residual cells often undergo phenotypic transitions into lineages that are reminiscent of those that compose non-transformed tissues or their embryonic ancestors. Single-cell RNA sequencing in PDX models of *BRAF*-mutant melanoma captured at the moment of maximal tumour shrinkage during exposure to inhibitors of the MAPK pathway effectors BRAF and MEK revealed the coexistence of distinct transcriptional states, one of which was characterized by high expression of markers for neural crest stem cells (the progenitors of melanocytes, from which melanoma arises)[32]. This transition is induced by an upstream gene-regulatory network controlled by the retinoic acid receptor RXRγ and culminates in the activation of an autocrine loop in which GDNF stimulates FAK signalling[32,33] (FIG. 2). Residual melanoma cells that survived inhibition of the MAPK pathway also had

increased mitochondrial mRNA translation that could be targeted by antibiotics that induce mitochondrial proteotoxic stress, such as doxycycline[34].

In the absence of EGFR signalling, actively dividing stem cells in the non-transformed mouse intestine convert into quiescent cells that are similar to a

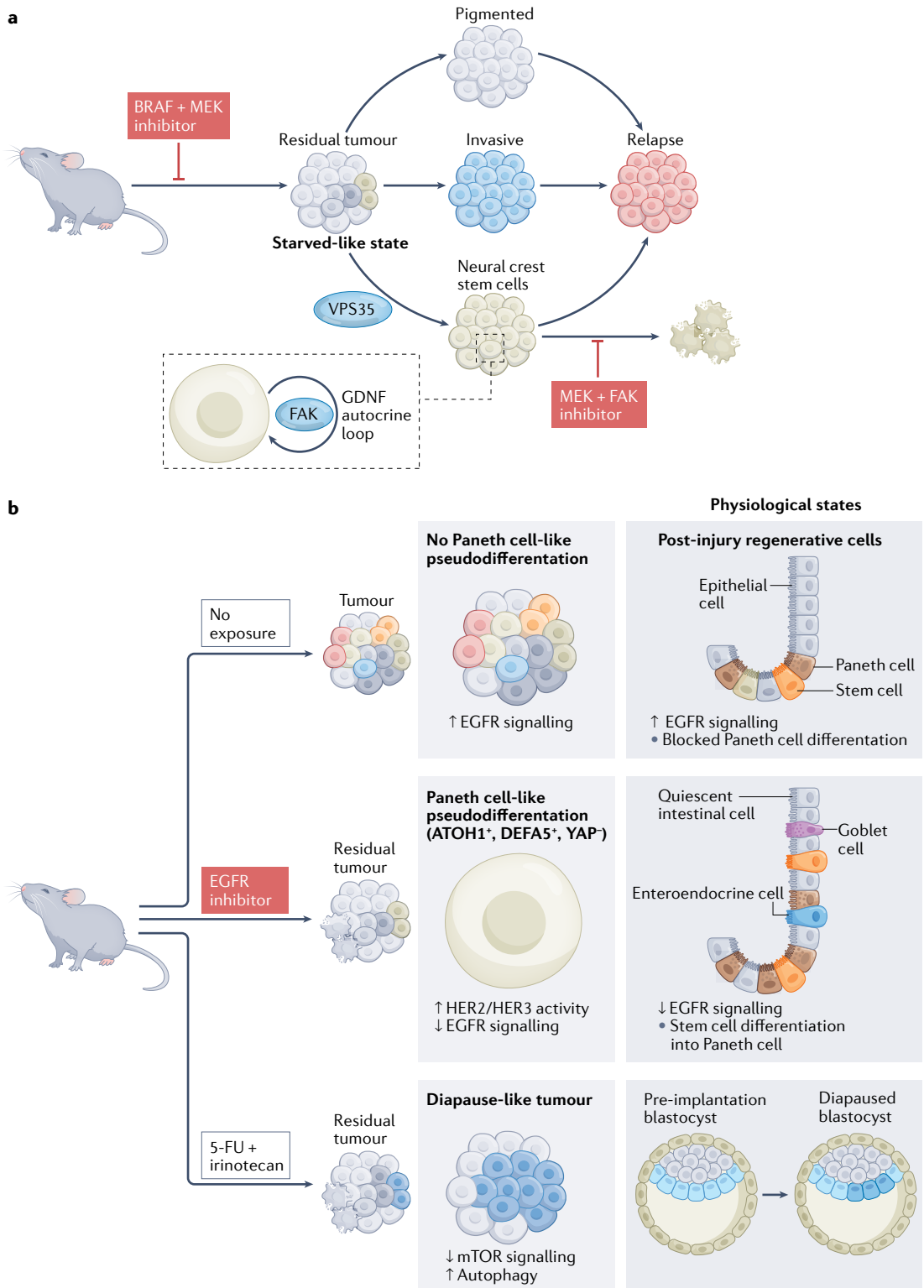

Fig. 2 | **Studying phenotypic rewiring in drug-tolerant 'persister' cells using PDX models.** Research in patient-derived xenograft (PDX) models has shown that residual tumours in mice, captured at the moment of maximal tumour shrinkage during exposure to a given therapy, contain drug-tolerant 'persister' cells that have (re-)acquired ancestral phenotypes physiologically expressed during embryogenesis or tissue regeneration. Examples are provided from *BRAF*-mutant melanoma PDX models exposed to BRAF and MEK inhibitors[32,33] (part **a**), and metastatic colorectal cancer PDX models after prolonged exposure to the anti-EGFR antibody cetuximab[38] or 5-fluorouracil (5-FU) and irinotecan[39] (part **b**).

subpopulation of slow-cycling secretory precursors committed to differentiate into Paneth cells[35–37]. Likewise, cancer cells in metastatic CRC PDXs that persist after prolonged exposure to the clinically approved anti-EGFR antibody cetuximab have signs of secretory commitment and Paneth cell-like pseudodifferentiation[38], indicating analogies between the phenotypic reprogramming that fuels quiescence in the mouse non-transformed intestine and that associated with drug tolerance in human CRC (FIG. 2). This lineage switch is accompanied by a shift in cellular signalling, from high EGFR pathway activity to high HER2 and HER3 activity[38].

Other studies have supported the notion that drug tolerance in CRC entails the co-option of conserved cell lineage and/or developmental pathways. In residual PDXs, that is, the tumour remnants that survive long-term exposure to drugs, clonal heterogeneity and complexity were maintained after exposure to standard-of-care chemotherapy (5-fluorouracil–irinotecan)[39], in line with the observation that heterogeneous CRC cells are genetically equipotent in coping with therapeutic insults[24]. Instead, cancer cells of residual PDXs entered a reversible state that was evocative of diapause, a period of suspended development that delays blastocyst implantation in several mammalian species[40]. Similar to diapaused blastocysts[41], residual CRC PDXs had suppression of an mTOR pathway signature and upregulation of key autophagy genes[39] (FIG. 2). Interestingly, a molecular adaptation resembling that of embryonic diapause has also been described in cultured breast and prostate cancer cells that tolerated long-term exposure to several cytotoxic drugs[42], attesting to the generalizability of this evolutionarily conserved strategy in tumours that survive stressful conditions.

Clinical considerations. The drug-tolerant phenotypes described in residual PDX models — namely, the presence of neural crest stem cell markers in melanomas with inhibition of the MAPK pathway, the Paneth cell-like transition induced by cetuximab in metastatic CRC, and the diapause state observed in models of CRC with long-term exposure to chemotherapy — have been verified in on-treatment biopsy samples from patients[32,38,39], thus confirming the reliability of PDX models in recapitulating tumour adaptive processes occurring in patients. In melanoma, the observation that FAK inhibition potentiates the anticancer effect of MAPK inactivation in residual PDX models with a neural crest stem cell phenotype[33] prompted an ongoing phase Ib trial testing the FAK inhibitor IN10018 together with the MEK inhibitor cobimetinib in patients with metastatic uveal melanoma and *NRAS*-mutant metastatic melanoma (NCT04109456). Similarly, a phase II trial is investigating the combination of the FAK inhibitor defactinib and the dual RAF–MEK inhibitor avutometinib in patients with metastatic uveal melanoma (NCT04720417). No results are yet available from either study. Intriguingly, administration of doxycycline in a patient with metastatic melanoma receiving BRAF–MEK inhibitors was accompanied by sudden regression of a gallbladder lesion, in line with the finding that enhanced mitochondrial mRNA translation (which is disrupted by doxycycline) counteracts the growth-inhibitory effect of MAPK blockade in PDX models of melanoma[34].

As mentioned earlier, HER2 and HER3 signalling is adaptively upregulated in residual metastatic CRC PDXs, and persists after exposure to cetuximab[38]. Therefore, inhibition of HER2 and/or HER3 along with EGFR might increase the depth of response and prolong survival durations compared to EGFR inhibition alone in patients. In a phase I trial, cetuximab was administered together with the dual EGFR–HER2 inhibitor lapatinib in six patients with metastatic CRC, with a disease control rate of 83% (with 33% and 50% of patients having an objective response or stable disease, respectively)[43]. Conversely, in a randomized phase II trial involving 134 patients with CRC, addition of the anti-EGFR–HER3 bispecific antibody duligotuzumab to 5-fluorouracil–irinotecan provided no progression-free survival (PFS) or overall survival (OS) advantage over the addition of cetuximab[44]. These results might have been biased by the potentially uneven effect of the chemotherapy backbone, which was reduced to a lower dose intensity in the duligotuzumab arm owing to a higher incidence of gastrointestinal toxicities.

Anecdotal evidence suggests that inhibitors of autophagy (a functional hallmark of diapause) might be beneficial when combined with other drugs in patients with CRC. In a phase I trial, the combination of the autophagy inhibitor hydroxychloroquine and vorinostat induced partial responses or stable disease lasting more than two cycles in 5 of 12 patients with CRC (42%)[45]. Moreover, an improvement in performance status and a reduction in the size of lung metastases was reported in a patient with *KRAS*-mutant metastatic sigmoid adenocarcinoma soon after concomitant administration of the MEK inhibitor binimetinib, the anti-angiogenic antibody bevacizumab and hydroxychloroquine[46]. In both studies, however, the specific contribution of hydroxychloroquine within the combination regimens could not be discerned. Therefore, the potential effect of autophagy inhibitors on the outcomes in patients with CRC receiving chemotherapy remains to be elucidated.

Tumour composition

Current evidence. After tumour engraftment, human stromal cells are quickly replaced by mouse stromal cells. Therefore, the PDX transcriptome is a mixture of human and mouse RNAs (originating from cancer and stromal cells, respectively). The chimeric composition of PDX models has been leveraged to deconvolute signals from cancer versus stromal cells in xenograft-derived bulk samples by using bioinformatics approaches to remove the contribution of mouse stromal cells, thus permitting the quantification of human cancer cell-specific transcripts (FIG. 3). This strategy has led to reconsideration of the biological underpinnings of the poor-prognosis CRC transcriptional subtype CMS4, which was initially assumed to comprise stem-like tumours featuring traits of cancer cell epithelial-to-mesenchymal transition[47–49]. Species-specific expression analysis revealed, however, that the presence of CMS4-related transcripts was mainly caused by abundance of stromal cells rather than

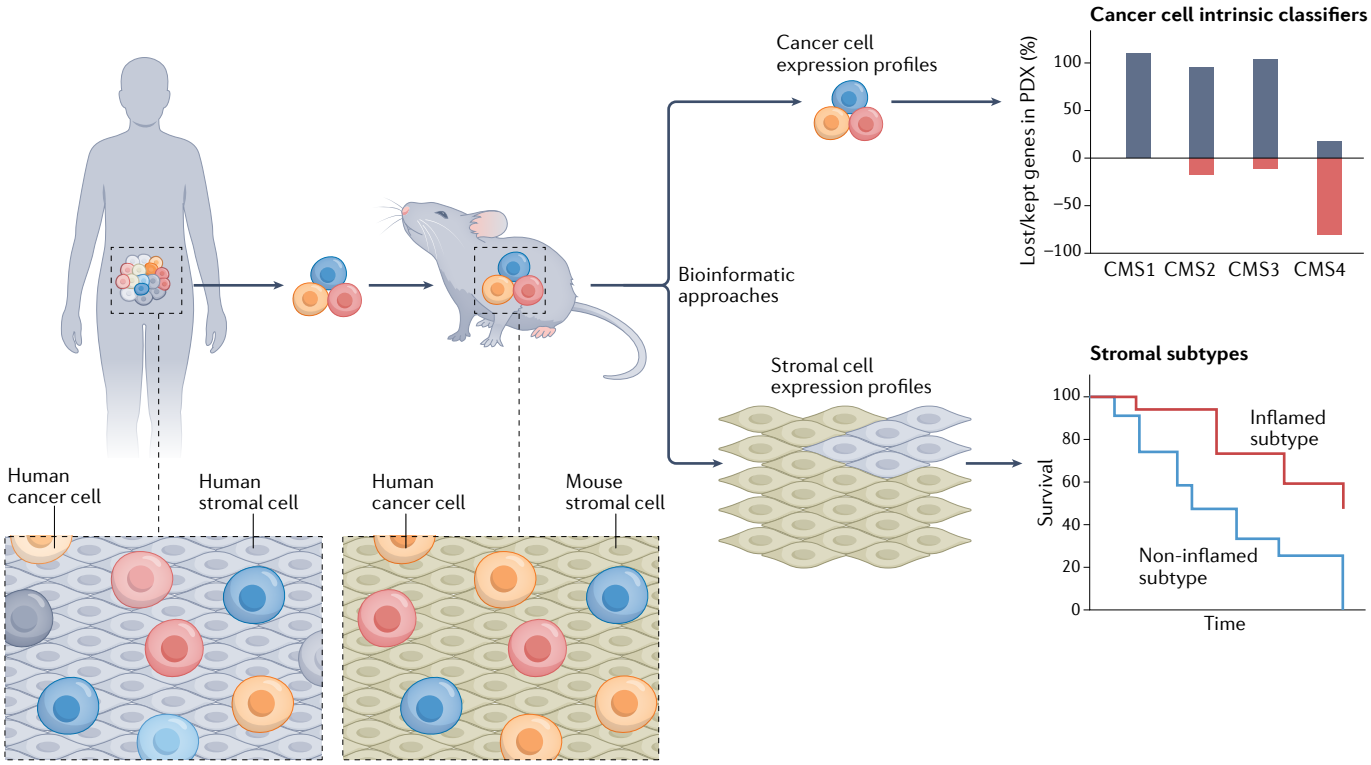

Fig. 3 | **Discriminating the contributions of cancer cells and stromal cells in PDX models.** In human tumours engrafted in mice, human stromal cells are rapidly substituted by mouse stromal cells. Therefore, patient-derived xenograft (PDX) models are chimeras comprising human cancer cells and mainly mouse stromal cells, and gene expression profiles from bulk xenografts contain a mixture of human and mouse transcripts. Subtractive bioinformatics approaches in matched tumours of origin, PDXs and normal tissues from the same patient can be used to remove mouse RNA and extract human cancer cell-specific or human stromal cell-specific transcripts from gene expression data[50,53,55]. CMS, consensus molecular subtype. Reprinted from REF.[50], Springer Nature Limited.

by acquisition of a mesenchymal phenotype by cancer cells[50]. This notion does not exclude the possibility that the CMS4 subtype also incorporates a more elusive subset of poorly differentiated cancer cells; indeed, mesenchymal markers have been found to be expressed in a fraction of CRC cell lines[51] and in tumour epithelial cells of patients with CRC[52] and probably contribute, at least partially, to the classification of tumours as CMS4. Overall, however, such a classification seems to be mainly driven by cell populations belonging to the tumour reactive stroma rather than by epithelial cancer cells that have undergone widespread dedifferentiation.

Dissection of the contribution of human stromal cells through subtractive analysis of matched pre-implantation tumours, PDXs and non-malignant tissue samples from the same patient led to the generation of a TME-related gene expression signature classification for renal cell carcinoma[53] (FIG. 3). The TME-defined signatures had greater discriminatory power for histological subtypes than signatures defined only by cancer cell genes, underscoring the importance of the TME in defining renal cell carcinoma histologies. This signature classification also resulted in the definition of a highly inflamed subtype that is enriched for innate and adaptive immune cells, associated with clinical signs of systemic inflammation, and predictive of poor survival[53]. Collectively, these results emphasize the potential of species-specific transcriptional analyses of PDXs to extract tumour classifiers with strong biological and clinical accuracy.

Clinical considerations. Deciphering cancer cell-specific gene expression features that are not affected by stromal abundance could be useful to minimize the confounding variable of stromal-derived intratumoural heterogeneity in isolated biopsy samples, which can be randomly taken from the central tumour core (mainly composed of cancer cells) or the invasive front (with a higher abundance of stromal cells) during routine diagnostic procedures[54]. CRIS, a new CRC classification built only with transcriptomic data from cancer cells in human PDXs[55], proved to be superior in reducing anatomical selection bias over whole-tissue-defined gene expression signatures[56]. While clustering bulk tumour biopsy samples by region of origin failed to consistently assign samples from the same patient to a certain subtype, CRIS accurately enabled clustering of samples by patient of origin[56]. CRIS also yielded new subtypes that only partially overlapped with transcriptional classes defined using bulk tumour data, and enabled identification of patients at high risk of relapse (with a sensitivity and specificity of 0.68 and 0.75, respectively, for 5-year distant metastasis-free survival) or with a high probability of response to cetuximab (OR 8.23)[55]. The clinical evaluation of signatures based on cancer cell-intrinsic transcripts, which are free from the confounding effects of stromal cell-derived

intratumoural heterogeneity, is expected to deliver improved prognostic and predictive biomarkers for precision oncology decisions.

Studies of predictive biomarkers

Response to targeted therapies occurs only in patients with genetically susceptible tumours and can be attenuated by various mechanisms of compensation[57]. By capturing interpatient tumour diversity on a scale compatible with that achievable in clinical trials, PDX models are appropriate pharmacogenomic platforms to identify molecular determinants that enrich for potential responders. Several studies have shown that PDX models reliably phenocopy the distribution of responses observed in patients and recapitulate clinically validated correlations between drug sensitivity and biomarker positivity. PDX models have also been instrumental in prospectively discovering predictors of response to new or repurposed drugs in molecularly defined tumour subsets (TABLE 1).

To enable adequate coverage of representative study populations, several cancer centres have shared their PDX collections in large, distributed repositories (Supplementary Table 1). These initiatives are meant not only to build ample PDX resources for the global scientific community, but also to formulate consensus guidelines for standard operating procedures and metadata harmonization[58]. Salient aspects (such as molecular characteristics, drug sensitivity and treatment history of the donor patients) of the available PDX models in some repositories are described in the PDX Finder web portal[59].

Table 1 | **PDX models as platforms for biomarker validation and discovery**

Tumour type	Therapeutic agent	Biomarkers of drug sensitivity	Biomarkers of drug resistance
Validation studies			
Melanoma	Vemurafenib	*BRAF* mut *NRAS* mut	*MAPK1/2* mut *BRAF* amp
Colorectal cancer	Cetuximab	NA	*KRAS* mut
Breast cancer	Alpelisib	*PIK3CA* mut *PTEN* wt	NA
Discovery studies			
Colorectal cancer	5-Fluorouracil	NA	*ASCL2*^{high} *MYC*^{high}
	Bevacizumab	NA	*ERF*^{high}
	Cetuximab	*ASCL2*^{high} *MYC*^{high}	*IGF2*^{high} *MET* amp *ERBB2* amp
	FOLFOX	*TP53* wt	NA
Breast cancer	Irinotecan	*BRCA1/2* loss	*SLFN11*^{low}
Head and neck cancer	Cetuximab	*AREG*^{high} *EREG*^{high}	NA
	Cetuximab–palbociclib	*CDKN2A* loss *CCND1*^{high}	NA
Hepatocellular carcinoma	Lenvatinib	NA	*EGFR*^{high}

amp, amplification; FOLFOX, 5-fluorouracil, leucovorin and oxaliplatin; mut, mutation; NA, not applicable; PDX, patient-derived xenograft; wt, wild-type.

Biomarker validation

Current evidence. PDX repositories have been repeatedly used for systematic validation of predictive biomarkers that were initially identified through correlative analyses in patients. A high-throughput screen of 440 PDX models from 16 cancer types confirmed several genotype–drug response associations already observed in the clinic[60], including *BRAF* and *NRAS* mutations as predictors of response and de novo resistance, respectively, to the BRAF inhibitor vemurafenib in melanoma[61,62], and *PIK3CA* mutations in the presence of a *PTEN* wild-type status as predictors of response to the PI3K inhibitor alpelisib in breast cancer[63] (TABLE 1). Clinically established mechanisms of acquired resistance were also confirmed, namely *BRAF* amplification and mutations in the MAPK-related genes *MAP2K1* and *MAPK2* (encoding MEK1 and MEK2, respectively) in PDXs from patients with melanoma who had developed resistance to BRAF inhibitors[64–66] (TABLE 1).

PDX pan-cancer repositories typically include a spectrum of common solid cancers, but the representation of tumour types by organ of origin is inevitably fragmented into smaller collections. Some research groups have pursued biomarker validation studies using tumour-specific PDX resources as a means to establish better-powered, tissue-oriented gene–drug association maps. CRC provides a paradigmatic example of such an approach. A study in 47 metastatic CRC PDXs published in 2011 confirmed the long-established clinical association between *KRAS* mutations in exon 2 and intrinsic resistance to cetuximab[67]. In the same study, evaluation of an additional cohort of 38 metastatic CRC PDXs with wild-type *KRAS* exon 2 status revealed that *KRAS* mutations in exons 3 and 4 and *NRAS* mutations (referred to as *RAS* extended mutations) also confer resistance to cetuximab[67].

In patients with metastatic CRC not harbouring *RAS* extended mutations, higher expression of the EGFR ligands amphiregulin and epiregulin correlates with a greater probability of response to cetuximab[68,69], probably because CRC cells with ligand-activated EGFR have a stronger dependency on the EGFR pathway. Accordingly, a survey of 125 *RAS* wild-type PDXs found a substantial enrichment of cetuximab-sensitive tumours among those with elevated levels of amphiregulin and epiregulin[70]. High levels of transcripts of EGFR pathway components (including EGFR, but also epiregulin and IRS2) were also detected in a subgroup of the CRIS CRC cancer cell-intrinsic classifier that was particularly enriched for cetuximab-responsive tumours[55]. Notably, the abundance of epiregulin and amphiregulin decreased in tumour remnants from PDXs that regressed but did not disappear after prolonged exposure to cetuximab[38], suggesting that residual tumours that tolerate EGFR blockade are less reliant on this signalling pathway owing to lower availability of EGFR agonists.

Clinical considerations. The role of *RAS* extended mutations in the resistance to cetuximab of metastatic CRC was only confirmed in a clinical study 2 years after being described in PDX models. Indeed, the results of a phase III trial yielded solid evidence that patients with tumours

exhibiting such mutations treated with the anti-EGFR antibody panitumumab had shorter PFS and OS durations than those with wild-type tumours[71] (TABLE 1).

Biomarker discovery

Current evidence. Tumour-specific PDX collections have been successfully leveraged for the identification of novel determinants of response and resistance to clinically approved therapies. Differential gene expression analyses in a panel of 59 CRC PDXs enabled the identification of molecular profiles associated with sensitivity to some standard-of-care regimens. For example, PDXs sensitive to 5-fluorouracil tend to have a transcriptional signature similar to that of non-malignant enterocytes and goblet cells[72], whereas PDXs resistant to this drug have a less-differentiated phenotype characterized by high expression of the transcripts of *ASCL2* and *MYC*, two transcription factors that are canonical markers of WNT signalling in colonic crypt stem cells[73] (TABLE 1). In the case of exposure to bevacizumab, PDXs with higher expression of genes involved in ATP synthesis-coupled mitochondrial transport had sensitivity to this drug, whereas resistance was correlated with higher expression of *ERF*[72], a transcriptional repressor that is inactivated by the RAS–MAPK pathway[74] (TABLE 1). Finally, sensitivity to EGFR inhibitors was greater in WNT^{high}, *ASCL2*, *MYC*-expressing PDXs and less pronounced in those with elevated levels of transcripts of the anti-apoptotic growth factor *IGF2* (REF.[72]) (TABLE 1). The association between poor sensitivity to cetuximab and high *IGF2* expression has also been reported in an independent cohort of 125 metastatic CRC PDXs and retrospectively confirmed in patients[70].

Transcriptomic data from PDXs have also been used to discriminate between chemosensitive and chemorefractory gastric cancers. Enrichment analyses of 31 PDXs that showed different sensitivity to 5-fluorouracil–oxaliplatin underscored proficient p53 signalling and increased metabolic processes, in particular more active mitochondrial fatty acid metabolism, as hallmarks of sensitivity to this combination (TABLE 1), and high expression of mesenchymal genes and extracellular matrix receptors as markers of resistance[75]. Whether all these predictive signatures can improve clinical decision-making in patients with CRC and gastric cancer remains to be determined.

Genetic determinants of resistance to EGFR inhibition in metastatic CRC PDXs without *RAS* extended mutations have been identified through gene candidate approaches or whole-exome sequencing analyses of therapeutically annotated PDXs. These investigations highlighted amplification of *MET* and *ERBB2* (TABLE 1) as well as mutations in *ERBB2*, *EGFR*, *FGFR1*, *PDGFRA* and *MAP2K1* as potential mechanisms of primary resistance to cetuximab[67,76–78]. Mutations in the ectodomain of EGFR that prevent antibody binding were also identified in PDXs from patients with acquired resistance to cetuximab or panitumumab[77]. All these alterations proved to be clinically actionable, and when targeted by specific inhibitors re-sensitized tumours to EGFR blockade in PDX experiments[77].

In addition to being a standard-of-care therapy for patients with metastatic CRC, cetuximab is used for the treatment of those with recurrent or metastatic head and neck squamous cell carcinoma (HNSCC) in combination with platinum and 5-fluorouracil[79]. Biomarker studies in HNSCC-derived xenografts have provided evidence that, similar to observations in CRC, high expression of EGFR ligands tends to correlate with increased sensitivity to EGFR blockade[80,81] (TABLE 1). This association was confirmed in patients with platinum-resistant HNSCC who had received single-agent panitumumab[82]. Moreover, some patients with platinum-resistant or cetuximab-resistant HNSCC respond to the combination of cetuximab and the CDK4/6 inhibitor palbociclib[83]. Findings in PDX models suggest that this response is particularly pronounced in tumours with unrestrained activation of the CDK4/6 and cyclin D1 cell cycle regulatory complex owing to genomic inactivation of the endogenous CDK4/6 inhibitor p16^{INK4A} or cyclin D1 overexpression[84] (TABLE 1).

A study in 40 PDX models suggested that defects in the homologous recombination pathway (through which double-strand DNA breaks are sensed and repaired) might predict response to the topoisomerase I inhibitor irinotecan in patients with TNBC. *BRCA1* and *BRCA2*, two key homologous recombination effectors, were mutationally inactivated or epigenetically silenced in the majority of irinotecan-sensitive TNBC PDXs[85] (TABLE 1). Some irinotecan-sensitive models also had high expression of *SLFN11* (REF.[85]), a putative DNA/RNA helicase that triggers lethal replication blockade in response to exogenously induced DNA damage[86,87] (TABLE 1). Conversely, *SLFN11*^{low}, irinotecan-resistant PDXs had an improved response to irinotecan when combined with an inhibitor of ATR[85], another component of the DNA damage-response machinery on which cells under replication stress become dependent when SLFN11 protein availability is limited[87].

Patients with advanced-stage hepatocellular carcinoma (HCC) frequently receive the multitarget TKI lenvatinib; however, this drug is associated with an overall response rate (ORR) of only 24%[88]. Lenvatinib-induced feedback activation of EGFR has been proposed as a mechanism of intrinsic resistance to this drug and in a screen of cultured HCC-derived cells was particularly pronounced in those with high levels of *EGFR* expression (TABLE 1). Accordingly, the combination of lenvatinib and the EGFR inhibitor gefitinib elicited tumour control in *EGFR*^{high}, but not in *EGFR*^{low}, HCC PDXs[89].

Altogether, these studies in PDX models have streamlined the identification of biomarkers of resistance to standard-of-care treatments and have brought to the fore alternative targets for refractory tumours. Remarkably, pharmacological studies of investigational therapies in PDXs have been more stringent than those in cultured cell lines. For example, while concomitant blockade of MEK and IGF1R had synergistic growth-inhibitory effects in a panel of CRC, non-small-cell lung cancer and pancreatic ductal adenocarcinoma cell lines, the same combination therapy was not superior to individual MEK inhibition in tumour type-matched PDXs[60]. The higher specificity of results in PDXs is expected to

deprioritize false-positive candidates that emerge from cell line-based drug screens, which could help to reduce attrition in drug development.

Clinical considerations. Results from studies in PDX models have shown that amplification of *ERBB2*, which leads to overexpression and constitutive activation of the encoded receptor HER2, associates with poor activity of anti-EGFR antibodies in preclinical models of metastatic CRC[67,77]. This finding has been confirmed in several retrospective studies documenting shorter PFS and OS, and lower ORRs with cetuximab alone or combined with chemotherapy in patients with metastatic *ERBB2*-amplified CRC relative to those with non-*ERRB2*-amplified tumours[90–92]. Studies in PDXs have also been instrumental in defining the optimal treatment regimen for cetuximab-resistant, *ERBB2*-amplified metastatic CRC. While exposure to either the anti-HER2 monoclonal antibody trastuzumab or the dual EGFR–HER2 TKI lapatinib did not have activity in *ERBB2*-amplified PDX models, the combination of the two agents induced marked and durable PDX shrinkage[93]. The mechanism underlying this synergy was ascribed to the ability of trastuzumab to counteract compensatory activation of the HER2 signalling partner HER3, which was triggered by prolonged exposure to lapatinib alone[93]. In heavily pretreated patients with HER2-positive metastatic CRC, trastuzumab–lapatinib was associated with an ORR of 30% (8 of 27 patients) and a disease control rate of 74% (20 of 27 patients)[94]. These data compare favourably with the rates achieved with other modalities approved for the advanced-line treatment of metastatic CRC, such as the multitarget TKI regorafenib[95,96] or trifluridine plus tipiracil[97,98], and attest to the value of results from PDX studies for predicting clinical drug efficacy.

Importantly, poor activity in PDX models can also anticipate suboptimal outcomes in patients. The pan-HER inhibitor neratinib was ineffective in metastatic CRC PDXs harbouring *ERBB2*-activating mutations[78]. Likewise, no objective responses were observed among 12 patients with CRC harbouring mutations in *ERBB2* or *ERBB3* who received neratinib in a basket trial[99]. A survey of drug sensitivity in 32 CRC PDXs harbouring *KRAS* mutations indicated limited efficacy of dual inhibition of PI3K (with dactolisib) and MEK (with selumetinib), with disease stabilization (defined as +35% to −50% change in size from baseline at 3 weeks) as the best outcome in 70% of the models tested[100]. Similarly, no objective responses were observed among 21 patients with *KRAS*-mutant metastatic CRC who received a combination of selumetinib and the AKT inhibitor MK-2206 (REF.[101]).

The observation that lenvatinib and gefitinib have antitumour activity in lenvatinib-refractory HCC PDXs[89] spurred the design of an early-phase trial testing this combination in patients with unresectable HCC and disease progression on lenvatinib monotherapy. Given that results in PDXs showed that tumours with elevated EGFR levels were particularly susceptible to the combination of lenvatinib and gefitinib, only patients with EGFR-overexpressing HCCs were enrolled in the trial. Data from an interim analysis of 12 patients suggest promising clinical efficacy of the dual therapy, with a 33% ORR observed at the time of this report[89]. Overall, the prominent examples of biomarker discovery and target validation discussed illustrate the contribution of studies in PDX models to enable better-informed patient stratification and delineate successful paths to the clinic of novel or repositioned therapies for patients with hard-to-treat tumours.

Upcoming experimental approaches

Humanized mouse PDX models

PDX models grow and evolve in severely immunocompromised mice, and human stromal cells are substituted by mouse components over consecutive passages. Hence, PDXs are intrinsically unfit to recapitulate heterotypic interactions between cancer, stromal and immune cells. This limitation is compounded by the fact that, in some cases, cytokines and growth factors produced by mouse stromal cells do not crossreact with receptors expressed by human cancer cells and vice versa[102]; this lack of a species-compatible TME in PDXs makes it difficult to evaluate the contribution of the TME to drug sensitivity, and complicates the identification of pharmacodynamic biomarkers for drugs targeting TME components, such as anti-angiogenic agents.

Substitution of mouse stromal cells with their human counterparts is hard, if not impossible, to achieve with the current technologies. Conversely, ongoing efforts are increasingly improving mouse humanization procedures for developing more holistic PDX models that include human immune components[103,104] (TABLE 2). Severely immunodeficient mice can be engrafted with various types of human leukocytes. However, each approach has drawbacks. Peripheral blood mononuclear cells can be easily collected from patients who donate their tumour for PDX generation, which avoids the immune reactions engendered by HLA mismatch; however, mature leukocytes from peripheral blood rapidly extinguish and cause xenogeneic graft-versus-host disease, limiting the time window of experimental testing to only a few weeks[105–107]. $CD34^+$ human haematopoietic stem cells give rise to various lineages of human blood cells throughout the lifespan of the recipient animal[108], but they are hard to obtain from frail patients with cancer. Human haematopoiesis in host mice can be supported through the introduction of mesenchymal stromal cells and the replacement of endogenous mouse cytokines with their human equivalents[109–111], which optimizes mouse humanization but also complicates procedures. Given these considerations, humanized PDX models might become increasingly used for selected proof-of-concept studies; for example, to investigate the effect of immunotherapy on the function and localization of immune effector cells or to detect immunologically and clinically relevant tumour antigens. Nevertheless, envisioning a routine deployment of such models to identify patient-specific and tumour-specific biomarkers of response to immunotherapy on a large scale is difficult.

Zebrafish PDX models

PDX experimentation is notoriously expensive, labour-intensive and time-consuming. Hence, initial attempts have been undertaken that leverage the logistic advantages

Table 2 | **Emerging preclinical patient-derived cancer models compared with conventional PDXs**

Feature	Conventional PDXs	Humanized mice	Zebrafish		Ex vivo cultures
			Larvae	Adults	
Time economy	No	No	Yes	Possibly	Yes
Cost affordability	No	No	Yes	Yes	Yes
Scalability	No	No	Possibly	Possibly	Yes
Recapitulates organism complexity	Yes	Yes	Possibly	Possibly	No
Tumour visualization	Yes	Yes	No	Yes	Yes
Presence of human immune components	No	Yes	No	No	Possibly
Genetic and transcriptional fidelity with respect to human samples	Yes	Yes	Unclear	Unclear	Yes
Amenability to drug testing	Yes	Yes	Yes	Yes	Yes
Prediction of clinical response	Yes	Possibly	Yes	Yes	Possibly
Fully compliant with the 3R Principle[a]	No	No	No	No	Yes

PDX, patient-derived xenograft. [a]3R (replacement, reduction, refinement) Principle on the protection of animals used for scientific purposes.

of scale, cost and time offered by non-mammal model organisms — in particular, zebrafish — for phenotypic testing of drug activity[112,113] (TABLE 2). In their early larval development, zebrafish do not have a competent adaptive immune system, and thus are suitable recipients for xenotransplanted human tissues. Zebrafish larvae PDXs from patients with CRC who did not have disease relapse on adjuvant therapy with 5-fluorouracil–oxaliplatin had a higher number of apoptotic cells after exposure to this chemotherapeutic regimen relative to those from patients with clinical evidence of early recurrence[114]. Moreover, in consonance with clinical observations, zebrafish PDX models harbouring *KRAS*-mutant tumours did not respond to cetuximab[114].

This system, however, also has limitations. After an initial period of functional immaturity during the larval stage, adaptive immunity rapidly ensues and leads to rejection of engrafted human cells, thus reducing the timing of tumour growth assessment to 1–2 weeks only. Furthermore, only very small numbers of cancer cells can be transplanted owing to the minute dimensions of zebrafish larvae, which renders tumour visualization problematic and makes it difficult to recapitulate the heterogeneity of the tumour of origin. To overcome these hurdles, an immune-deficient adult zebrafish model has been developed that enables the long-term (beyond 1 month) engraftment of larger amounts of human cancer cells, including fragments of patient tumours[115]. In such a model, the combination of the PARP inhibitor olaparib and the genotoxic agent temozolomide was identified as an effective therapy for rhabdomyosarcoma[115] and is now being tested in a phase I clinical trial in adults with recurrent or metastatic Ewing sarcoma or rhabdomyosarcoma who had previously received chemotherapy (NCT01858168). The field is in its early stages, and large-scale comparative studies are needed to define the extent to which genetic and transcriptional heterogeneity is maintained in zebrafish xenotransplants compared with their tumours of origin; however, proof-of-concept clinical prediction efforts seem to have delivered encouraging results.

Ex vivo cultures

Humanized mouse PDX models and zebrafish PDX models have the advantage of reflecting organismal complexity. However, their throughput remains low because these xenograft procedures are methodologically demanding and are subject to the 3R Principle (reduction, refinement and replacement), which legitimately limits the number of laboratory animals to be used for experimental purposes. To address these limitations, platforms for high-throughput ex vivo drug testing have been created using patient-derived or PDX-derived short-term (1–2 weeks) explants from different cancer types (TABLE 2). Data from these cultures have enabled: (1) confirmation of known mechanisms of drug sensitivity (for example, sensitivity to PARP inhibitors in breast cancers harbouring somatic *BRCA1* promoter methylation or germline *BRCA1* mutations) and known mechanisms of drug resistance (for example, resistance to PARP inhibitors in breast cancers with loss of non-homologous end-joining genes)[116]; (2) elucidation of genetic and non-genetic mechanisms of acquired resistance to targeted therapies, such as adaptive upregulation of SRC kinase activity after ALK inhibition in *ALK*-rearranged lung tumours[117]; and (3) identification of genetic predictors useful for drug repurposing, such as *EGFR* mutations and amplification as predictors of response to the BTK inhibitor ibrutinib in models of glioma[118].

Although short-term explants are expected to preserve the molecular characteristics of the tumour of origin, their proliferative capacity is rapidly exhausted. In the past few years, 3D in vitro organotypic cultures have been developed that overall maintain the phenotype, genetic diversity and transcriptional features of original tumour samples and can be passaged for months or even years[119] (TABLE 2). Similar to PDX models, tumour organoids have proven useful for exploring the genetic and functional underpinnings of intratumour heterogeneity, including the hierarchy and plasticity of cancer stem cells[120], the trajectories of cancer phylogenetic evolution[121] and the patterns of signalling dynamics and transcriptional outputs at the single-cell level[122,123]. Initial evidence suggests that tumour organoids can also trigger antigen-specific expansion of tumour-reactive cytotoxic T cells and antitumour antibody-dependent cellular cytotoxicity when cultured with autologous peripheral blood mononuclear cells[124] or as cohesive units incorporating the tumour epithelium and its endogenous immune repertoire en bloc[125]. Drug sensitivity profiles in organoids have shown initial signs of consistency with patient responses, with concordant results for several cytotoxic agents in gastrointestinal tumours[126,127], and have been used to guide effective therapeutic decisions in a donor patient with TNBC[128]. Nevertheless, in a prospective study using CRC avatar organoids published

in 2021, the robust pharmacological effects observed in organoids did not translate into durable clinical benefit in patients, suggesting that organoids might not be universally predictive[129]. This discrepancy underscores the need for more refined metrics and readouts to assess cell viability in organoids and suggests that, at least for some agents, ex vivo pharmacology highly differs from in vivo activity.

Future directions

Ultimately, we envision a scenario of increasing complexity in which the logistical burden inherent to PDX-based mouse experimentation will be reduced by first performing preliminary drug screens using less laborious models, such as organoids and zebrafish, followed by validation of prioritized hits in mice. This stepwise approach would be likely to facilitate the use of patient-derived models for investigating clinically actionable vulnerabilities, such as those related to cancer metabolism and epigenetic modifications, that have been traditionally studied using conventional cell lines.

Conclusions

PDX models have proven valuable in exploring many different facets of precision oncology in the preclinical space. The use of PDX models to study the clonal dynamics of tumour evolution has provided substantial knowledge on how genetic and adaptive responses to selective pressures over the course of treatment limit therapeutic efficacy. PDX repositories encompassing the molecular diversity of a particular cancer type have been instrumental in identifying biomarkers that predict sensitivity or resistance to a given therapy, thus improving clinical decision-making and rational patient stratification. The identification of molecularly enriched responder populations has also spurred clinical translation hypotheses that have, in turn, led to the discovery of new drug targets and the design of new therapies.

The increasing appreciation of the fact that tumours are dependent not only on mutant genes but also on more elusive non-genetic factors underscores the importance of increasing the representation of cancer types and deepening their molecular characterization in PDX repositories. We believe that the refinement and expansion of ex vivo and in vivo preclinical models, coupled with the increasing potential of functional perturbation approaches, will provide unprecedented opportunities to systematically identify cancer liabilities and find effective ways to target them first in preclinical experiments and then in patients.

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