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

[1]Candiolo Cancer Institute – FPO IRCCS, 10060 Candiolo, Torino, Italy

[2]Department of Oncology, University of Torino, 10060 Candiolo, Torino, Italy

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

Correspondence: livio.trusolino@ircc.it

Under drug pressure, tumours dynamically evolve multiple adaptive mechanisms that make static interrogation of genomic alterations insufficient to inform treatment decisions. Research in humans does not allow analyzing how the various tumour's regulatory circuitries are affected by therapeutic insults over time and space. Likewise, testing different precision treatments based on composite and ever-changing molecular information is hard to achieve in patients. Preclinical models are therefore needed that incorporate the biology and genetics of human cancers, navigate complex variables, and enable adequate population throughput for pinpointing randomly distributed response predictors. Patient-derived tumour xenografts (PDXs) represent dynamic entities in which cancer evolution can be monitored through serial propagation in mice. PDXs also reflect inter-patient diversity, thus enabling the nomination of response biomarkers and therapeutic targets for molecularly circumscribed tumour subgroups. Here, we discuss recent examples of the use of PDXs to tackle the continuum of precision oncology, from translational research to clinically oriented drug discovery. We elaborate on how and to what extent preclinical observations in PDXs have confirmed in some cases, and anticipated in others, findings in patients. Finally, we illustrate emerging methodological efforts that may broaden the application of PDXs by honing their predictive accuracy or by improving their versatility.

The characterization of cancer genomes has provided a catalogue of oncogenic mutations across tumours and ignited the development of therapeutic strategies tailored around individual, tumour-specific genetic aberrations with clinical actionability[1,2]. However, several issues complicate the routine implementation of therapies based on tumour genotyping. First, the response of tumours to targeted therapy may transcend their genomes[3]. For example, inhibiting the product of a single dominant oncogene could trigger compensatory signalling feedbacks, switches in cancer cell plasticity and deviations in evolutionary trajectories, all leading to the acquisition of new dependencies that substitute for those sustained by the inhibited target[4,5]. Because of this complexity, not always is tumour genomic profiling sufficient to inform therapeutic decisions and predict treatment outcome, and in many cases a *bona fide* oncogenic driver proves not to be a viable therapeutic target[6]. Second, while some genetic aberrations occur at high frequency in specific cancer types, many tumours exhibit a "long tail" distribution of rare alterations that are difficult to recognize in modestly sized patient cohorts, and are hard to qualify as driver genes rather than random passenger mutations[7].

The above considerations adduce arguments to what we believe is the essence of precision oncology: the distillation of co-dependencies and connectivities that attenuate response to targeted therapies in genetically defined contexts and the identification of combinatorial ways to tame such collateral liabilities pharmacologically. For precision oncology to manifest its full potential, platforms and data sets are needed that extend tumour molecular diversity to the extreme of reaching as much

saturation as possible. By enabling genomic profiling, biological annotation and therapeutic investigation in a multiverse of virtually unlimited patient tumour samples, PDXs 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. Here we illustrate the tapestry of functional and clinical insights that have recently emerged from PDX-based research and how studies in the field have contributed to illuminating various angles of precision oncology.

Tumour heterogeneity and evolution

In solid tumours, cancer cells are embedded in a supportive microenvironment made of stromal cells – such as fibroblasts and endothelial cells – and innate and adaptive immune cells[8]. Moreover, cancer cells are a constellation of distinct subpopulations with different phenotypic traits and genetic make-ups that are constantly moulded by time, spontaneous attrition dynamics, and therapeutic pressure[9]. Because of the variegate nature of tumour cell composition and the heterogeneous intra-tumour distribution of genetic alterations, the establishment of a PDX implies a concatenation of random events (Figure 1): (1) tumour sub-sampling for production of implantable tissue fragments introduces a certain degree of geographical bias; (2) once injected in the mouse, only a fraction of cancer cells are competent to engraft, which reduces genetic diversity due to out-competition by the fittest and most rapidly proliferating clone(s); (3) serial PDX propagation can further exacerbate PDX divergence owing to mutational evolution and phenotypic adaptation over time and space.

These peculiarities may be deemed as limitations of the methodology when pondering the accuracy of PDX models in fully phenocopying tumours of the donor patients. However, the analysis of tumour molecular deviation through repeated passages in mice has provided considerable insight into the rules that govern cancer evolutionary trajectories, clonal competition and non-genetic adaptation[10]. Likewise, the assessment of the dynamic impact of treatment on PDXs' clonal architecture and transcriptional features has advanced our understanding of the mechanisms underlying drug resistance[11].

Preservation of genetic identity

A lively debate is ongoing whether the architecture of copy number alterations (CNAs) evolves or remains stable throughout PDX propagation in mice. Ben David and colleagues used gene expression microarray data to infer CNA profiles in more than 500 PDX models across 24 cancer types[12]. This analysis revealed that approximately 60% of the models acquired at least 1 large chromosomal abnormality within a single passage, a frequency that increased to 90% when analyzing tumours from the first 4 passages[12]. As expected for a founder effect whereby model initiation imposes a strong selection pressure, genomic diversity was more evident in first- and early-passage xenografts than in xenografts at later passages.

A joint initiative of the National Cancer Institute PDXNet consortium and the EurOPDX consortium, involving a collection of more than 1,400 samples corresponding to 509 PDX models across 16 tumour types, questioned the assumption that PDXs show copy number divergence from their tumours of origin[13]. The controversy mainly stemmed from technical issues, specifically from the consideration that expression-based CNA calling has lower segmental resolution than DNA-based methods, allowing for CNA enumeration only at the scale of entire chromosomal arms. Moreover, microarray intensities of cancer cells are diluted by human stromal cells in bulk pre-implantation tumours but not in PDXs, which results in variability in the expression signals that can be erroneously interpreted as copy number changes. Copy number estimation by DNA-based measurements did not confirm mouse-induced copy number evolution and did not reveal positive selection of cancer-related genes in CNAs from PDXs compared with matched patient tumours[13]. Notably, CNA variations between original and mouse-passaged tumours were comparable to differences in multi-region tumour samples or intra-patient-samples, suggesting that PDX-associated genetic drift is more influenced by spatial heterogeneity than genetic instability.

A preliminary consensus across studies is emerging, whereby approximately 90% of the genome appears to be concordant between PDXs and original tumours. According to a reassessment of the PDXNet and EurOPDX dataset by Ben David and colleagues, a median of 10% of the genome was differentially affected by copy number variations in matched tumours and PDXs[14]. Similarly, in another collection of 536 samples across 25 cancer types, a 10% divergence between PDXs and their corresponding parental tumours was reported at the level of single-nucleotide alterations for key driver genes, along with occasional examples of PDX-associated CNA evolution that consolidated along serial passages[15]. Whether these differences, in a context of general concordance, are to be considered relevant, remains a matter of opinion.

Clinical considerations

Even in the best scenario of a high degree of molecular fidelity between PDXs and original tumours, the bottlenecks associated with model derivation and propagation, coupled with the inherent genomic instability of cancer, inevitably introduce some extent of genetic deviation. This may not affect the dominant prevalence of trunk (clonal) mutations, which are expected to be equally represented in original and propagated tumours. However, the pattern of branch (subclonal) mutations is likely to be different in passaged versus pre-implantation samples due to neutral evolution as well as selective pressure. Evidence continues to emerge that most cancers contain a minority of cells displaying innate resistance to drugs due to subclonal mutations[16], so response to therapy may be different in PDXs compared to matched original tumours.

These caveats notwithstanding, potential genomic evolution does not seem to critically affect the accuracy of therapeutic response prediction in parental tumours and derived PDXs. A retrospective

analysis of clinical outcome compared with response to the same treatment in the corresponding PDX in 92 patients with advanced solid tumours revealed a sensitivity of 96% and a specificity of 70% for the PDX drug screens, resulting in positive and negative predictive values of 85% and 91%, respectively[17]. In principle, these results bode well for the execution of prospective co-clinical trials that exploit PDXs as "avatars" for drug efficacy studies, with the aim to pass pharmacological information back to the donor patient for direct therapeutic intervention. Although initial efforts in this direction have been successfully attempted for pancreatic cancer[18], 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 to the relatively quick timescales of clinical practice.

Dynamics of genomic clones

PDX engraftment and expansion are accompanied by changes in the clonal organization of tumours. This divergence makes PDXs versatile models to trace the intra-tumour architecture of genomic diversity and to correlate clonal competition with fitness effects, which is instrumental to parsing the principles of drug resistance in heterogenous cancer cell populations (Figure 1).

Breast cancer and colorectal cancer (CRC) are the tumour settings in which more mature knowledge has been garnered. In a seminal study, genomic clonal analysis of 15 breast cancer PDXs at single-cell resolution indicated 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[19]. Over serial passaging, the spectrum of clonal expansions and declines was wider for tumours experiencing weak initial selection than for tumours characterized by massive early counterselection. Notably, parallel clonal dynamics were observed when cell populations from the same tumour were transplanted in multiple mice, with reproducible outgrowth of initially minor subclones[19]. This suggests that, in the models tested, directional clonal dynamics over time were not ascribable to stochastic processes (such as random genetic drift) but were deterministically associated with favourable mutational landscapes that conferred a predictable fitness advantage. More recently, Salehi and colleagues performed time-series fitness mapping experiments in three triple-negative breast cancer (TNBC) PDXs using single-cell CNAs as heritable genotypes to trace clonal trajectories over the course of standard-of-care chemotherapy[20]. They found that prolonged treatment with cisplatin, until development of resistance, suppressed high-fitness clones that had dominated in the absence of therapy; conversely, chemotherapy selected for phylogenetic lineages initially endowed with low fitness[20].

Engraftment-related subclonal skewing has also been documented in CRC PDXs. In four of nine tumours analyzed, dominant parental clones were less represented and minor parental subclones became dominant in PDXs compared with original tumours, with a general reduction of clonal

heterogeneity and a decreased prevalence of regionally confined subclonal mutations[21]. Kreso and colleagues monitored spontaneous and drug-induced evolutionary dynamics by combining CNA analysis and deep sequencing of mutational hotspots with lentiviral lineage tracing in 10 PDX models. After the usual clonal selection during engraftment, tumours remained genetically stable in subsequent passages[22]. However, 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, tendency to persist, or propension to decline in the face of a shared genetic ancestry[22]. In coherence with the fitness mapping conducted in breast cancer[20], treatment of mice bearing CRC PDXs with oxaliplatin preferentially eliminated persistent, high-fitness progeny and increased the proportion of previously dormant lineages[22]. However, different from breast cancer, variations in fitness landscape under drug pressure were not dictated by clonal selection of heritable genomic traits; rather, fitness was shaped by non-genetic mechanisms impacting on cell phenotypes.

Clinical considerations

The finding that cancer cell subpopulations poised to become chemorefractory showed reduced competitive ability in the absence of therapy[20,22] indicates that drug resistance has an evolutionary fitness cost, which, in principle, could be enhanced by therapeutic intervention. Intriguingly, the biological characteristics of low-fitness clones in breast cancer PDXs and of dormant lineages in CRC PDXs echo those of slow-growing persisters, which have been repeatedly identified in cell cultures after prolonged treatment with kinase inhibitors[23,24,25]. These persister cells display consistent hallmarks such as an altered chromatin organization[23], diminished apoptotic thresholds[24], and a metabolic shift to fatty acid oxidation[25]. Targeting such hallmarks – for example with histone deacetylase (HDAC) inhibitors to modulate chromatin state, BH3 mimetics to precipitate apoptosis, and blockers of fatty acid catabolism to counteract metabolic adaptation – reduced the fraction of persisters in cell-based experiments[23,24,25].

Clinical information for patients treated with analogous approaches is, at present, fragmentary. Twenty-one of 38 individuals (55%) with chemorefractory metastatic CRC treated with a combination of the HDAC inhibitor vorinostat and the antimetabolite chemotherapeutic regimen 5-fluorouracil (5-FU)/leucovorin experienced disease stabilization, and one had a partial response[26]. In contrast, the addition of the HDAC inhibitor chidamide did not improve the efficacy of cisplatin in the first-line treatment of 15 patients with advanced TNBC[27]. Phase I studies of the BCL-2/BCL-XL inhibitor navitoclax in combination with gemcitabine or carboplatin/paclitaxel in patients with solid tumours showed modest but appreciable clinical efficacy (54% and 36.8% stable disease, respectively), which was associated with relatively high toxicity[28,29]. Although clinical findings are still immature, therapies

aimed to further reduce the already limited fitness of prospectively resistant clones in heterogeneous tumours are expected to delay disease recurrence in patients.

Adaptive drug tolerance

The inherent genomic instability of tumours favours the stochastic acquisition of new mutations, some of which will afford cancer cells with a selective advantage to evade therapeutic pressure; the larger the pool of residual cells left behind by 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].

Recent evidence in PDX models has documented the importance of cellular plasticity in shaping drug response, in particular for melanoma and CRC. A common theme stemmed from PDX studies is that drug-tolerant residual cells often morph into tissue lineages that are reminiscent of those that compose normal organs or their embryonic ancestors. Single-cell RNA sequencing of *BRAF*-mutant melanoma PDXs at the zenith of maximal response to BRAF and MEK (MAPK) inhibition revealed the co-existence of distinct transcriptional states, one of which exhibited high expression of neural crest stem cell markers (melanocytes, from which melanoma arises, differentiate from neural crest progenitors during embryonic development)[30]. This transition was induced by a gene regulatory network upstream controlled by retinoic X receptor-γ and culminating into the activation of an autocrine loop in which glial cell line-derived neurotrophic factor stimulated FAK signalling[30,31] (Figure 2). Residual melanoma cells that survived MAPK blockade also displayed increased mitochondrial translation, which could be targeted by antibiotics that induce mitochondrial proteotoxic stress such as doxycyclin[32].

In the absence of EGFR signalling, actively dividing stem cells of the normal mouse intestine convert into quiescent cells that are similar to a subpopulation of slowly cycling secretory precursors committed to differentiate into Paneth cells[33-35]. Likewise, cancer cells of metastatic CRC PDXs that persisted after prolonged treatment with the clinically approved EGFR antibody cetuximab displayed signs of secretory commitment and Paneth cell-like pseudodifferentiation[36], indicating analogies between the phenotypic reprogramming that fuels quiescence in the mouse normal intestine and that occurring during manifestation of drug tolerance in human colorectal tumours (Figure 2). The lineage switch toward the secretory/Paneth cell-like fate was accompanied by a signalling shift from high EGFR pathway activity to high HER2/HER3 activity[36].

The notion that drug tolerance in CRC entails the co-option of conserved lineages or developmental pathways is supported by additional findings. In residual PDXs long exposed to standard-of-care chemotherapy (5-FU and irinotecan), clonal heterogeneity and barcode complexity were maintained[37], in line with the observation by Kreso and colleagues that CRC cells are genetically equipotent in coping with therapeutic insults[22]. Instead, lingering cells entered a reversible state that

was evocative of diapause, a period of suspended development that delays blastocyst implantation in several mammalian species[38]. Similar to diapaused blastocysts[39], residual CRC PDXs featured suppression of the mTOR pathway and upregulation of key autophagy genes[37] (Figure 2). Interestingly, a molecular adaptation resembling that of embryonic diapause has also been documented in breast and prostate cancer cells that tolerated treatment with several cytotoxic drugs[40], attesting to the generalizability of this evolutionarily conserved strategy in tumours that survive stressful conditions.

Clinical considerations

The drug-tolerant phenotypes described in residual PDXs – namely, the presence of neural crest stem cell markers in MAPK-inhibited melanomas, the Paneth cell-like transition induced by cetuximab in metastatic CRC and the diapause state experienced by CRC models exposed to chemotherapy – were verified in on-treatment biopsies from patients[30,36,37], thus confirming the reliability of PDXs in recapitulating clinical evidence. In melanoma, ongoing clinical experimentation has been inspired by the observation that FAK inhibition potentiates the anticancer effect of MAPK inactivation in residual PDXs with a neural crest stem cell phenotype[31]. In a phase Ib trial, the FAK inhibitor IN10018 is being tested together with the MEK inhibitor cobimetinib in subjects with metastatic uveal melanoma and *NRAS*-mutant metastatic melanoma (NCT04109456)[41]; similarly, a phase II trial is investigating the potential efficacy of combining the FAK inhibitor defactinib and the dual RAF/MEK inhibitor VS-6766 in patients with metastatic uveal melanoma (NCT04720417)[42]. No results are yet available for either study. Intriguingly, administration of doxycycline in a patient with metastatic melanoma under BRAF-MEK inhibitor therapy was accompanied by sudden regression of a gallbladder lesion, in line with the finding that enhanced mitochondrial translation (which is disrupted by doxycycline) counteracts the growth-inhibitory effect of MAPK blockade in melanoma PDXs[32].

As mentioned above, HER2 and HER3 signalling is adaptively upregulated in residual PDXs of metastatic CRC that persist after cetuximab treatment[36], so inhibition of HER2 and/or HER3 along with EGFR is expected to be more effective than inhibition of EGFR alone in increasing the depth of response and prolonging survival in patients. In a phase I trial in which cetuximab was administered together with the dual EGFR/HER2 inhibitor lapatinib in 6 patients with metastatic CRC, marked clinical activity was noted, with a disease control rate of 83% (33% objective response and 50% stable disease)[43]. Conversely, a phase II randomized study in 134 patients treated with 5-FU and irinotecan reported that the addition of the dual EGFR/HER3 antibody duligotuzumab provided no advantage in progression-free survival (PFS) or overall survival (OS) compared with the addition of the single-target EGFR antibody cetuximab[44]**.** In this case, results may have been biased by the potentially uneven effect of the chemotherapy backbone, which was reduced to lower dose intensity in the duligotuzumab arm owing to a higher frequency of gastrointestinal toxicity.

Anecdotal evidence suggests that inhibitors of autophagy – a functional hallmark of diapause – may be beneficial when combined with other drugs in CRC. In a phase I trial, the combination of the anti-autophagy compound hydroxychloroquine and the HDAC inhibitor vorinostat induced partial response or stable disease lasting more than two cycles in 5 of 12 CRC patients (42%)[45]. A case report of a subject with a *KRAS* mutant metastatic sigmoid adenocarcinoma documented improvement of the performance status and a reduction in the size of lung metastases soon after the concomitant administration of the MEK inhibitor binimetinib, the anti-angiogenic antibody bevacizumab and hydroxychloroquine[46]. However, in both instances the specific contribution of hydroxychloroquine within the therapeutic cocktails could not be discerned, and whether inhibitors of autophagy improve the outcome of chemotherapy in CRC patients remains to be elucidated.

Tumour composition

After tumour engraftment, the human stroma is quickly replaced by murine stroma. Therefore, the PDX transcriptome is an amalgam of human RNAs (originating from cancer cells) and mouse RNAs (originating from stromal cells). The chimeric composition of PDXs has been leveraged to deconvolute cancer cell versus stromal signals in bulk xenografts by employing bioinformatics approaches that remove mouse stromal representation, thus permitting the quantification of human cancer transcripts only (Figure 3). This strategy has led to reconsidering the biological underpinnings of a poor-prognosis CRC transcriptional subtype (CMS4) that was initially assumed to include stem-like tumours featuring traits of cancer cell epithelial-mesenchymal transition[47,48,49]. At some odds with this interpretation, species-specific expression analysis revealed that the transcript levels of CMS4 genes were mainly due to the copious presence of stromal cells rather than acquisition of a mesenchymal phenotype by cancer cells[50]. This notion does not exclude that CMS4 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 CRC patients[52] and likely contribute, at least partially, to CMS4 assignment. However, the CMS4 identity seems to be driven more by cell populations belonging to the tumour reactive stroma than by epithelial cancer cells that have undergone widespread dedifferentiation.

In a complementary perspective, dissection of the human tumour stroma through subtractive analysis of matched pre-implantation tumours, PDXs, and normal tissue samples from the same patient led to the generation of a tumour microenvironmental gene expression signature for renal cell carcinoma (RCC)[53] (Figure 3). This signature had greater discriminatory power of histologic subtypes than a signature limited to cancer cell-specific genes, underscoring the importance of microenvironmental features in defining RCC histologies. The signature also allowed to designate a highly inflamed subtype that was enriched for cells of innate and adaptive immunity, associated with clinical signs of systemic inflammation, and predicted poor survival[53]. Collectively, these results

underscore the potential of PDX-based species-specific transcriptional analyses to extract tumour classifiers with strong biological and clinical granularity.

Clinical considerations

Deciphering cancer-cell specific gene expression features that are not affected by stromal abundance may be useful to minimize the confounding variable of stromal-derived intratumoural heterogeneity in isolated biopsy samples, which may be randomly taken from the central tumour core (mainly composed of cancer cells) or the invasive front (with a higher representation of stromal cells) during routine diagnostic procedures[54]. CRIS, a new CRC classification built only on PDX human cancer-cell transcripts[55], proved to be superior to whole-tissue gene expression signatures in reducing geographical selection bias[56]. While signatures obtained from bulk tumours failed to assign samples from the same patient to the same subtype when classification scores were applied to gene expression data obtained from multiple CRC tumour regions, CRIS showed high accuracy in clustering tumour biopsies by patient-of-origin rather than region-of-origin[56]. CRIS also yielded new subtypes that only partly overlapped with transcriptional classes developed from bulk tumours and identified patients at high risk of relapse or high probability of response to EGFR inhibitors[55]. The clinical evaluation of signatures based on cancer cell-intrinsic transcripts, which are free from the confounding effects of stromal-derived intratumoural heterogeneity, is expected to deliver improved prognostic and predictive biomarkers for precision oncology decisions.

Response biomarker population studies

Response to targeted therapies occurs only in patients with genetically susceptible tumours and may be attenuated by various mechanisms of compensation[57]. By capturing inter-individual tumour diversity on a population scale, PDXs represent appropriate pharmacogenomic platforms to identify molecular determinants that enrich for potential responders. Several studies have shown that PDXs reliably phenocopy the distribution of responses observed in patients and recapitulate clinically validated correlations between drug sensitivity and biomarker positivity. PDXs have also been instrumental to prospectively discovering response predictors for new and repurposed drugs in molecularly defined tumour subsets (Figure 4).

To enable adequate coverage of representative study populations, several cancer centres have shared their PDX collections in large, distributed repositories. 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]. For some repositories, salient aspects of available PDX models (molecular characteristics, drug sensitivity, treatment history of donor patients) are described in the PDX Finder web portal (pdxfinder.org)[59] (Table 1).

Biomarker validation

PDX biobanks have been repeatedly utilized for systematic validation of response biomarkers that were initially identified through correlative analyses in patients. A pan-cancer high-throughput screen in 440 models from 16 cancer types supported 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 BYL719 in breast cancer[63] (Figure 4). Clinically established mechanisms of acquired resistance were also confirmed, namely, *BRAF* amplification and mutations in the MAPK genes *MAP2K1* (encoding MEK1) and *MAPK2* (encoding MEK2) in melanoma PDXs that had developed resistance to BRAF targeted therapy[64-66] (Figure 4)

PDX pan-cancer repositories typically include the standard spectrum of common solid cancers, but the representation of individual tumour types is inevitably fragmented into relatively small collections. Some research groups have elected to pursue biomarker validation efforts using tumour-specific PDX resources as a means to draw more powered, tissue-oriented gene-drug association maps. CRC is a paradigmatic example of such an approach. In 2011, a study in 47 metastatic CRC PDXs confirmed the long-established clinical association between *KRAS* mutations in exon 2 and *de novo* resistance to the EGFR antibody cetuximab[67]. When evaluating an additional cohort of 38 PDXs with a *KRAS* exon 2 wild-type status, the same study documented that metastatic CRC PDXs with *KRAS* mutations in exons 3 and 4 and *NRAS* mutations were also refractory to EGFR blockade[67]. This observation would obtain clinical recognition only two years later, when results from a large trial yielded solid evidence that patients with tumours exhibiting “RAS extended” mutations treated with anti-EGFR therapy had shorter PFS and OS than patients with *KRAS*/*NRAS* wild-type tumours[68] (Figure 4).

In patients with metastatic CRC negative for RAS extended mutations, higher expression of the EGFR ligands amphiregulin and epiregulin correlates with a higher probability of response to anti-EGFR therapy[69,70], likely because CRC cells with ligand-activated EGFR experience a stronger dependency on the EGFR pathway. Accordingly, a survey in 125 RAS wild-type PDX models found a substantial enrichment of cetuximab-sensitive tumours among cases with elevated levels of amphiregulin and epiregulin[71]. High levels of EGFR pathway activity transcripts (epiregulin, EGFR, and the EGFR downstream amplifier IRS2) were also detected in the CRIS-C subgroup of the CRIS CRC cancer cell-intrinsic classifier, which 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 treatment with cetuximab[36], suggesting that residual tumours that tolerate EGFR blockade are less reliant on EGFR signalling due to lower availability of EGFR agonists.

Biomarker discovery

Tumour-specific PDX collections have been successfully deployed for the nomination of novel determinants of response and resistance to clinically approved therapies. Differential gene expression analyses in a suite of 59 CRC PDXs enabled the identification of molecular profiles associated with sensitivity to some standard-of-care regimens. For example, 5-FU-responding xenografts tended to display transcriptional traits that recall those displayed by normal enterocytes and goblet cells[72], whereas 5-FU-refractory models exhibited a less differentiated phenotype characterized by high expression of the transcription factors ASCL2 and MYC, two canonical markers of WNT signalling in colonic crypt stem cells[73] (Figure 4). In the case of bevacizumab, drug sensitivity was prevalent in tumours with higher expression of genes involved in ATP synthesis-coupled mitochondrial transport, while resistance correlated with higher expression of ERF[72], a transcriptional repressor that is inactivated by the RAS-MAPK pathway[74] (Figure 4). Finally, response to EGFR blockade was more frequent in WNT-high, ASCL2/MYC-expressing tumours and was less pronounced in PDXs with elevated levels of the anti-apoptotic growth factor IGF2[72] (Figure 4). The association between poor response to cetuximab and high IGF2 expression was also reported in an independent cohort of 125 metastatic CRC PDXs and retrospectively confirmed in patients[71]. Besides CRC, transcriptomic data from PDXs have been used to estimate predictive differences between chemosensitive and chemorefractory tumours in gastric cancer. Enrichment analyses of 31 PDXs that showed different sensitivity to the combination of 5-FU and oxaliplatin put forward proficient p53 signalling and increased metabolic processes as hallmarks of responsive cases (Figure 4), and high expression of mesenchymal genes and extracellular matrix receptors as markers of resistant cases[75]. Whether all these predictive signatures can improve clinical decision making in 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 by gene candidate approaches or whole exome sequencing analyses of therapeutically annotated PDXs. Results from these investigations highlighted amplification of the *MET* and *ERBB2* genes (Figure 4) 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, which prevent antibody binding, were also identified in PDXs from patients with acquired resistance to EGFR blockade[77]. All these alterations proved to be clinically actionable, and when targeted by specific inhibitors 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 recurrent or metastatic head-and-neck squamous cell carcinoma (HNSCC) in combination with platinum and 5-FU[79]. Biomarker studies in HNSCC PDXs have provided evidence

that, similar to observations in CRC, tumours with high expression of EGFR ligands tend to be more sensitive to EGFR inhibition[80,81] (Figure 4). This association was confirmed in platinum-resistant patients who had received single-agent panitumumab, another EGFR monoclonal antibody approved for metastatic CRC[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 PDXs suggest that this response is particularly pronounced in tumours displaying unrestrained activation of the CDK4/6 and cyclin D1 cell-cycle regulatory complex due to genomic inactivation of the CDK4/6 inhibitor p16INK4A or cyclin D1 overexpression[84] (Figure 4).

Irinotecan exerts its cytotoxic activity by trapping the target enzyme topoisomerase I on DNA, leading to stalled replication forks and DNA double-strand breaks (DSBs) during replication[85]. A study in 40 PDXs has suggested that defects in the homologous recombination (HR) pathway – through which DSBs are sensed and repaired – may predict response to irinotecan in TNBC. BRCA1 and BRCA2, two key HR effectors, were mutationally inactivated or epigenetically silenced in the majority of irinotecan-sensitive TNBC PDXs[86](Figure 4). Some irinotecan-sensitive models also displayed high expression of SLFN11 (86), a putative DNA/RNA helicase that is recruited to the stressed replication forks and triggers lethal replication block in response to exogenously induced DNA damage[87,88] (Figure 4). Conversely, SLFN11-low, irinotecan-resistant tumours had an improved response when irinotecan was combined with an inhibitor of ATR[86], another component of the DNA damage response machinery on which cells under replication stress become dependent when SLFN11 protein availability is limited[88].

The multi-kinase inhibitor lenvatinib is frequently used in patients with advanced hepatocellular carcinoma (HCC), but the overall response rate is only 24%[89]. One potential mechanism of *de novo* resistance has been attributed to lenvatinib-induced feedback activation of EGFR, which appeared to be particularly pronounced in HCC cells with high EGFR expression (Figure 4). Accordingly, the combination of lenvatinib and the EGFR inhibitor gefitinib elicited marked tumour control in EGFR-high, but not in EGFR-low, HCC PDX models[90].

Altogether, population trials in PDXs 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, when testing investigational therapies, pharmacological experiments in PDXs were more stringent than those obtained in cultured cell lines. For example, while concomitant blockade of MEK and IGF1R exerted synergistic growth-inhibitory effects in a panel of cell lines from CRC, non-small cell lung cancer and pancreatic cancer, 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 de-prioritize false positive candidates that emerge from cell line-based drug screens, likely reducing attrition in drug development.

Clinical considerations

Results from PDX studies have shown that amplification of the *ERBB2* oncogene, which leads to overexpression and constitutive activation of the encoded receptor HER2, associates with poor response to anti-EGFR therapy in preclinical models of metastatic CRC[67,77]. In confirmation of this finding, several retrospective studies have documented worse outcomes (shorter PFS and OS and lower objective response rates) in metastatic CRC patients with *ERBB2*-amplified tumours who received cetuximab alone or in combination with chemotherapy relative to patients with non-amplified tumours[91-93]. PDX-based investigation has also been instrumental to define an optimal treatment regimen for cetuximab-resistant, *ERBB2*-amplified metastatic CRC. While single-agent therapy with the HER2 monoclonal antibody trastuzumab or the dual EGFR-HER2 tyrosine kinase inhibitor lapatinib failed to regress *ERBB2*-amplified PDX models, the combination of trastuzumab and lapatinib induced marked and durable tumour shrinkage[94]. The mechanism underlying this synergy is ascribable to the ability of trastuzumab to counteract compensatory activation of the HER2 signalling partner HER3, which was triggered by prolonged treatment with lapatinib alone[94]. When translated to heavily pre-treated patients with HER2-positive metastatic CRC, the combination therapy resulted in a 30% objective response rate (8/27) and a 74% disease control rate (20/27)[95]. These data compare favourably with the rates achieved with other modalities approved for the advanced-line treatment of metastatic CRC, such as the multikinase small-molecule inhibitor regorafenib[96,97] and chemotherapy with trifluridine plus tipiracil[98,99], and attest to the value of PDX-derived therapeutic results for predicting drug clinical efficacy.

Importantly, poor response in PDXs also anticipated suboptimal outcomes in patients. The pan-HER inhibitor neratinib was ineffective in metastatic CRC PDXs exhibiting *ERBB2* activating mutations[78]. Likewise, no objective responses were seen after treatment with neratinib in 12 patients with CRC harbouring mutations in *ERBB2* or *ERBB3*[100]. A survey of drug sensitivity in 32 CRC PDXs with *KRAS* mutations indicated limited efficacy of dual PI3K and MEK pathway blockade, with disease stabilization as best response[101]. In the same vein, no tumour regressions were observed in 21 patients with *KRAS* mutant metastatic CRC treated with a combination of selumetinib (a MEK inhibitor) and MK-2206 (which targets AKT, a downstream PI3K transducer)[102].

The observation that lenvatinib-refractory HCC PDXs responded to the combination of lenvatinib and gefitinib[90] spurred the design of a clinical study in patients with unresectable HCC, whose tumours had progressed on lenvatinib monotherapy. Since results in PDXs showed that tumours with elevated EGFR protein levels were particularly susceptible to the combination of lenvatinib and gefitinib, only patients with EGFR-overexpressing HCCs were enrolled in the study. Data from an interim analysis of 12 patients suggest promising clinical activity of the dual therapy, with a 33% objective response rate observed so far[90].

Overall, the prominent examples of biomarker discovery and target validation presented above illustrate the contribution of PDX-based research to more informed patient stratification and delineate successful paths to the clinic of novel or repositioned therapies for patients with hard-to-treat tumours.

Future directions

PDXs grow and evolve in severely immunocompromised mice, and the human tumour stroma is substituted by murine components over consecutive passages. Hence, PDXs are intrinsically unfit to recapitulate heterotypic interactions between cancer cells, stromal cells, 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 cross-react with receptors expressed by human cancer cells, and *vice versa*[103]; lack of a species-compatible stroma in PDXs makes it difficult to evaluate the contribution of the tumour microenvironment to therapeutic response and complicates the identification of pharmacodynamic biomarkers for drugs against microenvironmental targets, such as angiogenesis inhibitors.

The substitution of mouse stromal cells with their human counterparts is hard, if not impossible, to achieve with the current technologies. On the contrary, ongoing efforts are increasingly perfecting mouse humanization procedures for developing more holistic PDX models that contain human immune components[104,105] (Figure 5). Severely immunodeficient mice can be engrafted with various types of human leukocytes, with each approach showing drawbacks. Peripheral blood mononuclear cells (PBMCs) can be easily collected from patients who donate their tumours 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[106-108]. CD34-positive human haematopoietic stem cells give rise to various lineages of human blood cells throughout the animal's lifespan[109], but they are hardly obtainable from debilitated cancer patients. Human haematopoiesis in host mice can be supported by the introduction of mesenchymal stromal cells and by the replacement of endogenous murine cytokines with their human equivalents[110-112], which optimizes mouse humanization but also complicates procedures. Based on these considerations, it is likely that humanized PDX models will be increasingly used for selected proof-of-concept studies, for example to investigate the impact of immunotherapy on the function and localization of immune effector cells or to detect immunologically and clinically relevant tumour antigens. However, it is difficult to envision a routine deployment of such models to identify patient- and tumour-specific biomarkers of response to immunotherapy on a population scale.

PDX experimentation is notoriously expensive, labour-intensive and time-consuming. Hence, initial attempts have been undertaken that leverage the logistic advantages of scale, cost and time offered by lower model organisms – in particular, zebrafish – for phenotypic testing of drug

response[113,114] (Figure 5). In their early larval development, zebrafish do not have a competent adaptive immune system, thus representing good recipients for the xenotransplantation of human tissues. Zebrafish larvae PDXs from patients with CRC who did not relapse on adjuvant therapy with 5-FU and oxaliplatin showed a higher number of apoptotic cells after treatment with the same chemotherapeutic regimen than PDXs from patients with clinical evidence of early recurrence[115]. Moreover, in consonance with clinical observations, zebrafish PDX models bearing *KRAS* mutant tumours did not respond to cetuximab[115]. Also this system, however, has limitations. After the 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; further, only very small numbers of cancer cells can be transplanted due to the minute dimensions of zebrafish larvae, which renders tumour visualization difficult. To overcome these hurdles, an immune-deficient model of adult zebrafish has been developed that enables the long-term engraftment of larger amounts of human cancer cells, including fragments of patient tumours[116]. By utilizing this model, the combination of the PARP inhibitor olaparib and the genotoxic agent temozolomide was identified as an effective therapy for rhabdomyosarcoma[116] and is now being tested in a phase I clinical trial in adult patients with recurrent/metastatic Ewing's sarcoma or rhabdomyosarcoma following failure of prior chemotherapy (NCT01858168)[117]. The field is still in its infancy, and large-scale comparative studies are needed to define the extent to which genetic and transcriptional heterogeneity is maintained in zebrafish xenotransplants compared with original patients' tumours. However, proof-of-concept clinical prediction efforts appear to deliver encouraging results.

Humanized PDXs and zebrafish have the merit of reflecting organismal complexity, but their throughput remains low. Moreover, xenograft procedures are subject to application of the 3R's principles (reduction, refinement and replacement), which legitimately impose some control over the number of laboratory animals to be utilized for experimental purposes. To address these limitations, platforms for high-content *ex vivo* drug testing have been created using patient-derived or PDX-derived short-term explants from different cancer types (Figure 5). Results from these cultures confirmed known mechanisms of drug sensitivity and resistance (for example, sensitivity to PARP blockade in breast cancer models with somatic *BRCA1* promoter methylation or germline *BRCA1* mutations, and resistance in models with genetic loss of non-homologous end-joining genes)[118]; documented genetic and non-genetic mechanisms of acquired resistance to targeted therapies (for example, adaptive upregulation of SRC kinase activity after ALK inhibition in ALK-translocated lung tumours)[119]; and identified genetic predictors useful for drug repurposing (for example, EGFR mutations and amplification as predictors of response to the Bruton kinase inhibitor ibrutinib in glioma)[120].

Although short-term explants are expected to preserve the molecular characteristics of parental tumours, they rapidly exhaust their proliferative capacity. In recent years, three-dimensional *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 extended periods[121] (Figure 5). Similar to PDXs, tumour organoids have proven useful to explore the genetic and functional underpinnings of intratumour heterogeneity, including the hierarchy and plasticity of cancer stem cells[122], the trajectories of cancer phylogenetic evolution[123] and the patterns of signalling dynamics and transcriptional outputs at the single-cell level[124,125]. Initial evidence suggests that tumour organoids can also trigger antigen-specific expansion of tumour-reactive cytotoxic T cells and antibody-dependent tumour cytotoxicity when cultured with autologous PBMCs[126] or as cohesive units incorporating en bloc the tumour epithelium and its endogenous immune repertoire[127]. Drug sensitivity profiles in organoids have shown initial signs of consistency with patient response, with concordant results for several cytotoxic agents in gastrointestinal tumours[128,129], and have been used to guide effective therapeutic decisions in a donor patient with TNBC[130]. However, a recent prospective study in which CRC avatar organoids were used to inform experimental treatments in donor patients suggested that organoids might not be universally predictive, as strong pharmacological effects observed in organoids did not translate into durable clinical benefit in patients[131]. This discrepancy advocates 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 compound activity *in vivo*.

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

Conclusions

PDX models have proven valuable to explore many different facets of precision oncology in the preclinical space. The use of PDXs to study the clonal dynamics of tumour evolution has contributed substantial knowledge to understanding how genetic and adaptive responses to drug pressure limit therapeutic efficacy over the course of treatment. PDX collections reflecting the molecular diversity of a particular cancer type have been instrumental to nominating 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, in turn, have led to the discovery of new drug targets and the design of new therapies.

The growing appreciation that tumours are dependent not only on mutant genes but also on more elusive non-genetic factors underscores the importance of making the representation of cancer types wider, and their molecular characterization deeper, in PDX repositories. We surmise 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 map cancer liabilities and find effective ways to intercept the identified targets first in laboratory experiments, and then in patients.

KEY POINTS

- Owing to the selection bottleneck imposed by tumour engraftment and the evolutionary trajectories experienced by cancer cells along passaging, PDXs can be used to investigate tumour clonal composition and competition during spontaneous tumour progression and under drug pressure.

- Studies in PDXs at maximal response to a given therapy have provided insight into lineage-specific phenotypic adaptations, which underlie the acquisition of drug tolerance and are responsible for sustaining residual disease.

- The substitution of the human stroma by murine stroma after tumour implantation has enabled the identification of cancer cell-intrinsic and stroma-centred signatures with predictive and prognostic significance.

- Large collections of PDXs have contributed to the discovery and validation of novel response biomarkers 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 (lower organisms, *ex vivo* cultures) are being developed that complement conventional PDX models.

ACKNOWLEDGEMENTS

We thank our friends and colleagues of the Laboratory of Translational Cancer Medicine at the Candiolo Cancer Institute for comments and suggestions. Work in the authors' laboratory is supported by AIRC, Associazione Italiana per la Ricerca sul Cancro, Investigator Grant 22802; AIRC 5x1000 grant 21091 ; AIRC/CRUK/FC AECC Accelerator Award 22795; European Union's H2020 grant agreement no. 754923 COLOSSUS; and Fondazione Piemontese per la Ricerca sul Cancro-ONLUS, 5x1000 Ministero della Salute 2016. L. T. is a member of the EurOPDX consortium. All figures were initially created with Biorender.com.

COMPETING INTERESTS

L.T. has received research grants from Menarini, Merck KGaA, Merus, Pfizer, Servier and Symphogen. The remaining authors declare no competing interests.

LEGENDS TO FIGURES

Figure 1. **PDXs 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 therapeutic pressure. When tumour fragments are implanted in mice to generate PDXs, there is an initial geographical bias related to sampling, followed by a strong selection bottleneck due to successful engraftment of only a fraction of cancer cells. Serial PDX passaging also contributes to clonal evolution, which can be further exacerbated by drug treatments in recipient mice. Thus, PDXs 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.

Figure 2. **Studying phenotypic rewiring in drug-tolerant persisters using PDXs.** Research in PDXs has shown that residual tumours at maximal response to a successful therapy are made of drug-tolerant persister cells that have (re)acquired ancestral phenotypes physiologically expressed during embryogenesis or tissue regeneration. *BRAF* mutant melanoma PDXs exposed to dual BRAF/MEK blockade initially engaged a “starvation”-like transcriptional programme, which was followed by differentiation into pigment-producing cells or dedifferentiation toward either an invasive or a neural crest stem cell state[30]. Phenotypic transition to the neural crest stem cell state was driven by retinoic X receptor-γ and was accompanied by increased FAK signalling, which was caused by autocrine production of glial-derived neurotrophic factor[30,31]. Accordingly, concurrent blockade of BRAF/MEK and FAK reduced the emergence of residual cells with a neural crest stem cell state, minimized residual disease, and delayed the development of resistance[31]. In metastatic CRC, tumour remnants surviving prolonged exposure to the EGFR antibody cetuximab exhibited reduced activity of the transcriptional co-activator YAP, In turn, YAP inhibition led to increased expression of transcription factors that specify the secretory fate of intestinal progenitors (such as ATOH1) and of markers of Paneth cell terminal differentiation (such as DEFA5), together with compensatory upregulation of HER2 and HER3 (36). The Paneth-like cells that compose CRC residual tumours were reminiscent of slowly cycling secretory precursors identified in the normal mouse intestine, which are committed to differentiate into Paneth cells when EGFR signalling is low[33-35]. Conversely, the cells of treatment-naïve CRC tumours resembled actively dividing intestinal stem cells that, during post-injury intestinal regeneration, reduce their differentiation capacity towards Paneth cells and contribute to tissue reconstitution in an EGFR- and YAP-dependent manner[133]. The phenotype of CRC PDXs that tolerated treatment with 5-FU and irinotecan echoed that of diapause, an interval of

suspended development that may occur in blastocysts before implantation and is characterized by diminished mTOR signalling and upregulation of autophagy genes[37-40]. BRAFi, BRAF inhibitor; EGFRi, EGFR inhibitor; FAKi, FAK inhibitor; GDNF, glial-derived neurotrophic factor; NCSC, neural crest stem cells; RXRγ, retinoic X receptor-γ; SMC, starved-like melanoma cells.

Figure 3. **Deciphering the contribution of cancer cells versus stromal cells for tumour molecular subtyping and prognosis using PDXs.** In human tumours engrafted in mice, the human tumour stroma is rapidly substituted by murine components. Therefore, PDXs are chimeras made of human cancer cells and murine stromal cells, and gene expression profiles from bulk xenografts are a mixture of human and murine transcripts. Using subtractive bioinformatic approaches to remove mouse RNA and extract human-only (i.e., cancer cell-only) transcripts from PDX microarray data, consensus molecular subtypes identified in CRC bulk tumours were still detectable in PDX tissues with the exception of subtype 4, which was almost completely lost in PDXs. This analysis led to a reconsideration of the molecular features of subtype 4, which proved to be mostly driven by stromal abundance rather than cancer cell mesenchymal dedifferentiation as originally assumed[50]. Similarly, subtractive analyses of matched parental tumours, PDXs and normal tissues from the same patient were performed in RCC samples; by doing so, a human stromal subtype enriched for innate and adaptive immune cells was established, which correlated with systemic inflammatory manifestations and predicted poor survival[53]. CMS, consensus molecular subtype.

Figure 4. **PDXs as platforms for biomarker validation and discovery.** Population studies in PDXs have validated clinical correlations between biomarker positivity and response (green) or resistance (red) to several targeted therapies in different cancer types. New predictive determinants have also emerged from discovery studies with PDXs, and some of them have been confirmed clinically. When druggable, resistance biomarkers (for example, HER2 in cetuximab-resistant CRC and EGFR in lenvatinib-resistant HCC) have proven to be effective therapeutic targets in PDXs, setting the stage for biomarker-driven clinical trials. AREG, amphiregulin; EREG, epiregulin. FOLFOX, 5-FU, leucovorin and oxaliplatin.

Figure 5. **Emerging patient-derived cancer models.** The features of humanized mouse models, zebrafish and patient-derived cultures are visualized and benchmarked against a series of parameters. Humanized PDX models recapitulate the spectrum of human genotypic and phenotypic traits more comprehensively than any other experimental system but at the same time have important drawbacks related to HLA compatibility between donor tumours and donor immune cells, cytokine species-specificity for adequate human haematopoiesis in mice, and scalability. Zebrafish and patient-derived cell cultures are more prone to high-throughput procedures, with cultures having the

further advantage of avoiding animal experimentation. However, both models remain in their infancy in terms of biological conclusiveness and clinical generalizability of pharmacological results. We surmise that the availability of model systems characterized by increasing organismal complexity will facilitate the implementation of stepwise drug development pipelines, whereby the prioritization of hits shortlisted from initial screens in simpler models culminates in ultimate go/no-go decisions based on therapeutic outcomes in PDXs and humanized mice.

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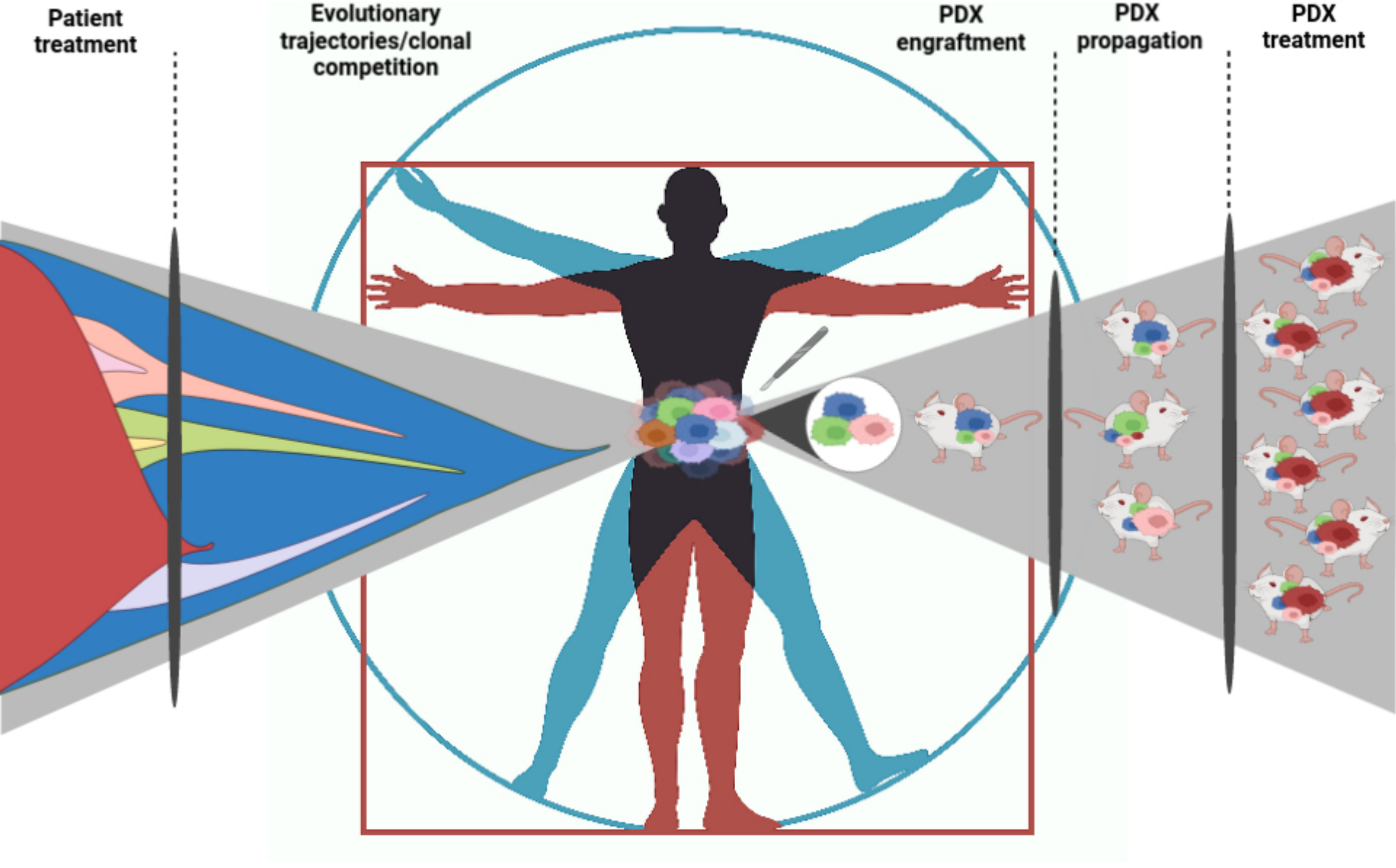
Patient treatment
Evolutionary trajectories/clonal competition
PDX engraftment
PDX propagation
PDX treatment

Fig.1

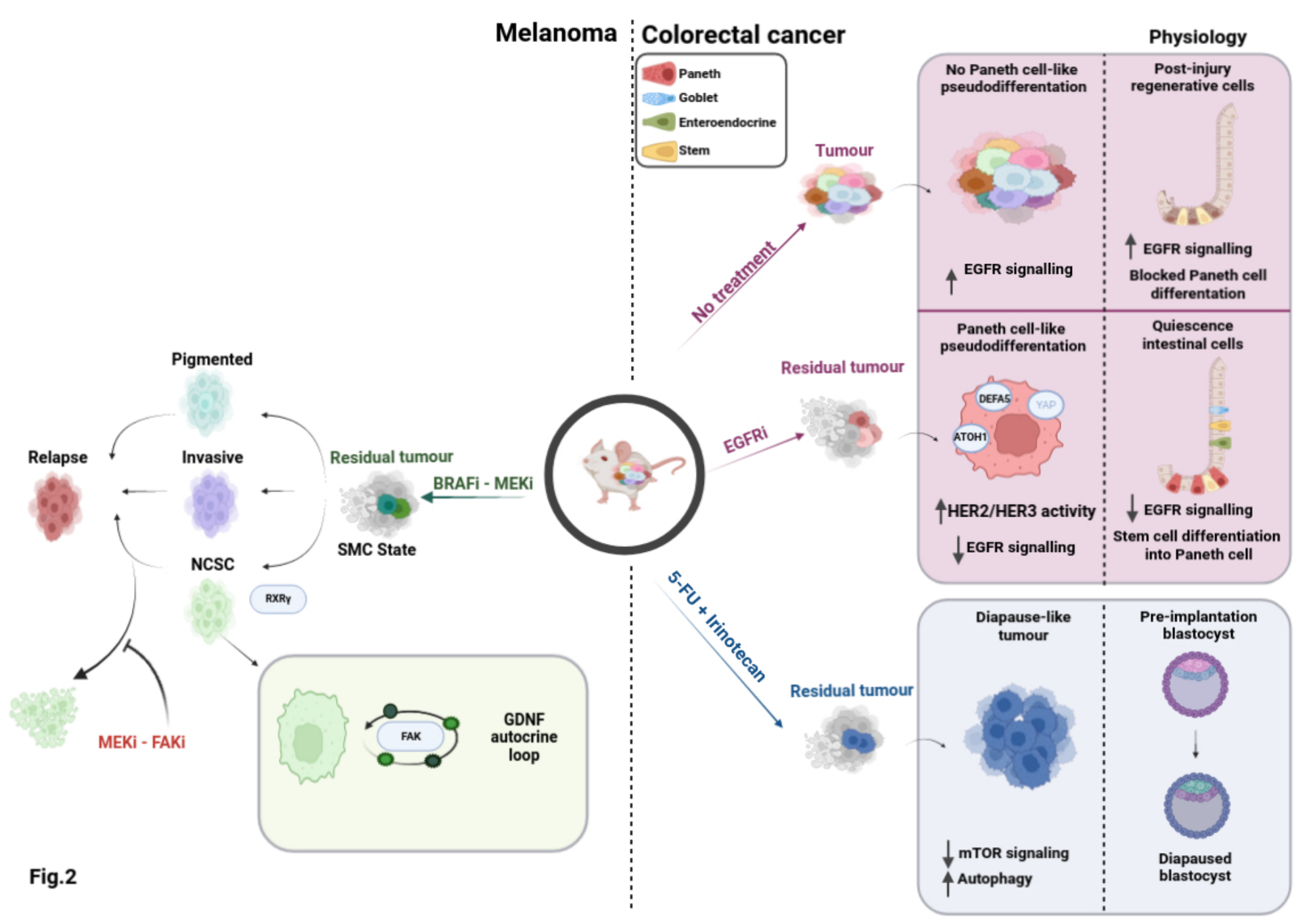
Melanoma
Colorectal cancer
Physiology
Paneth
Goblet
Enteroendocrine
Stem
Tumour
No treatment
No Paneth cell-like pseudodifferentation
EGFR signalling
Post-injury regenerative cells
EGFR signalling
Blocked Paneth cell differentation
Residual tumour
EGFRi
Paneth cell-like pseudodifferentation
DEFA5
YAP
ATOH1
HER2/HER3 activity
EGFR signalling
Quiescence intestinal cells
EGFR signalling
Stem cell differentiation into Paneth cell
5-FU + Irinotecan
Residual tumour
Diapause-like tumour
mTOR signaling
Autophagy
Pre-implantation blastocyst
Diapaused blastocyst
Pigmented
Relapse
Invasive
NCSC
RXRγ
Residual tumour
BRAFi - MEKi
SMC State
MEKi - FAKi
FAK
GDNF autocrine loop

Fig.2

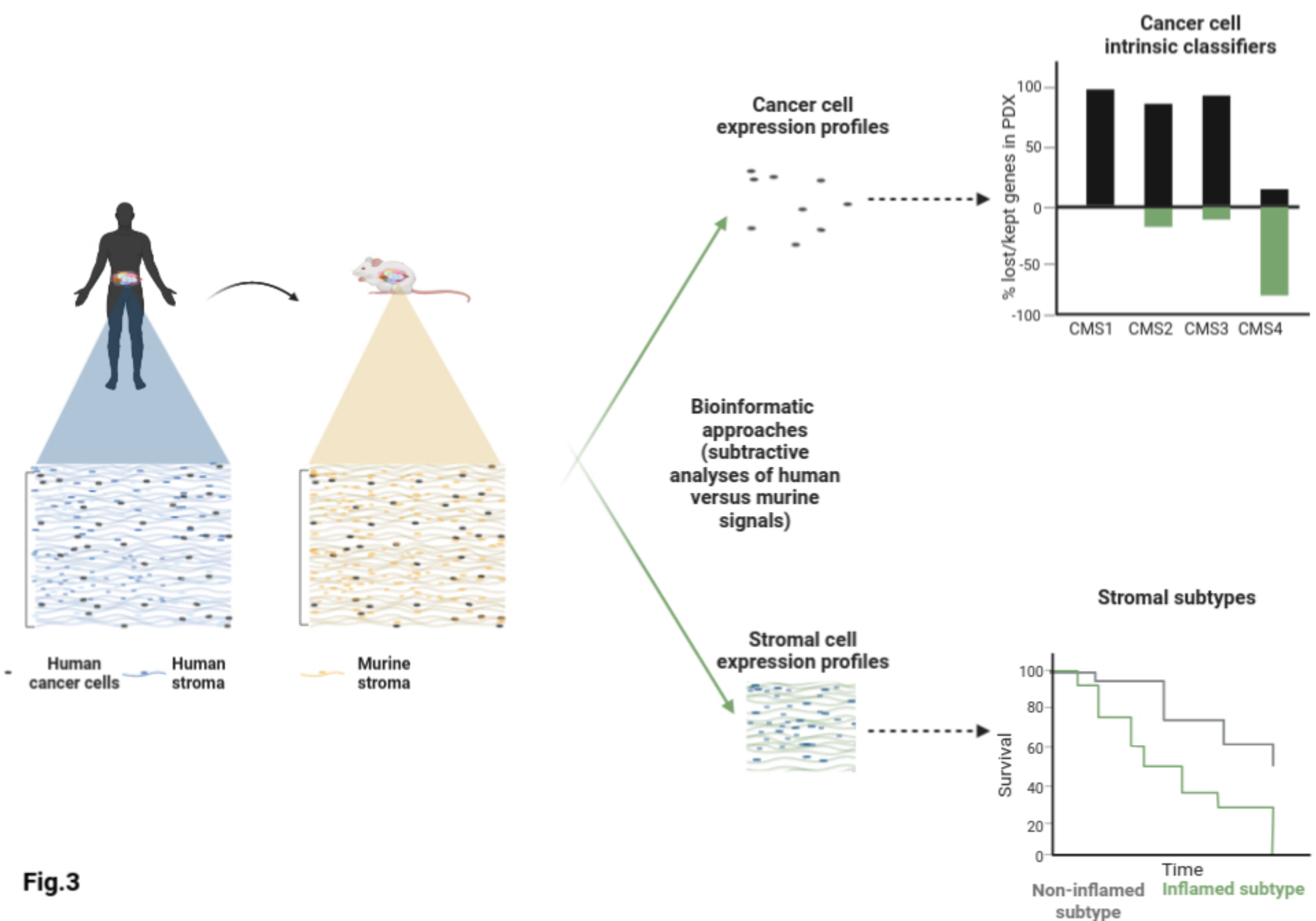
Human cancer cells
Human stroma
Murine stroma
Cancer cell expression profiles
Bioinformatic approaches (subtractive analyses of human versus murine signals)
Stromal cell expression profiles
Cancer cell intrinsic classifiers
% lost/kept genes in PDX
100
50
0
-50
-100
CMS1
CMS2
CMS3
CMS4
Stromal subtypes
Survival
100
80
60
40
20
0
Time
Non-inflamed subtype
Inflamed subtype

Fig.3

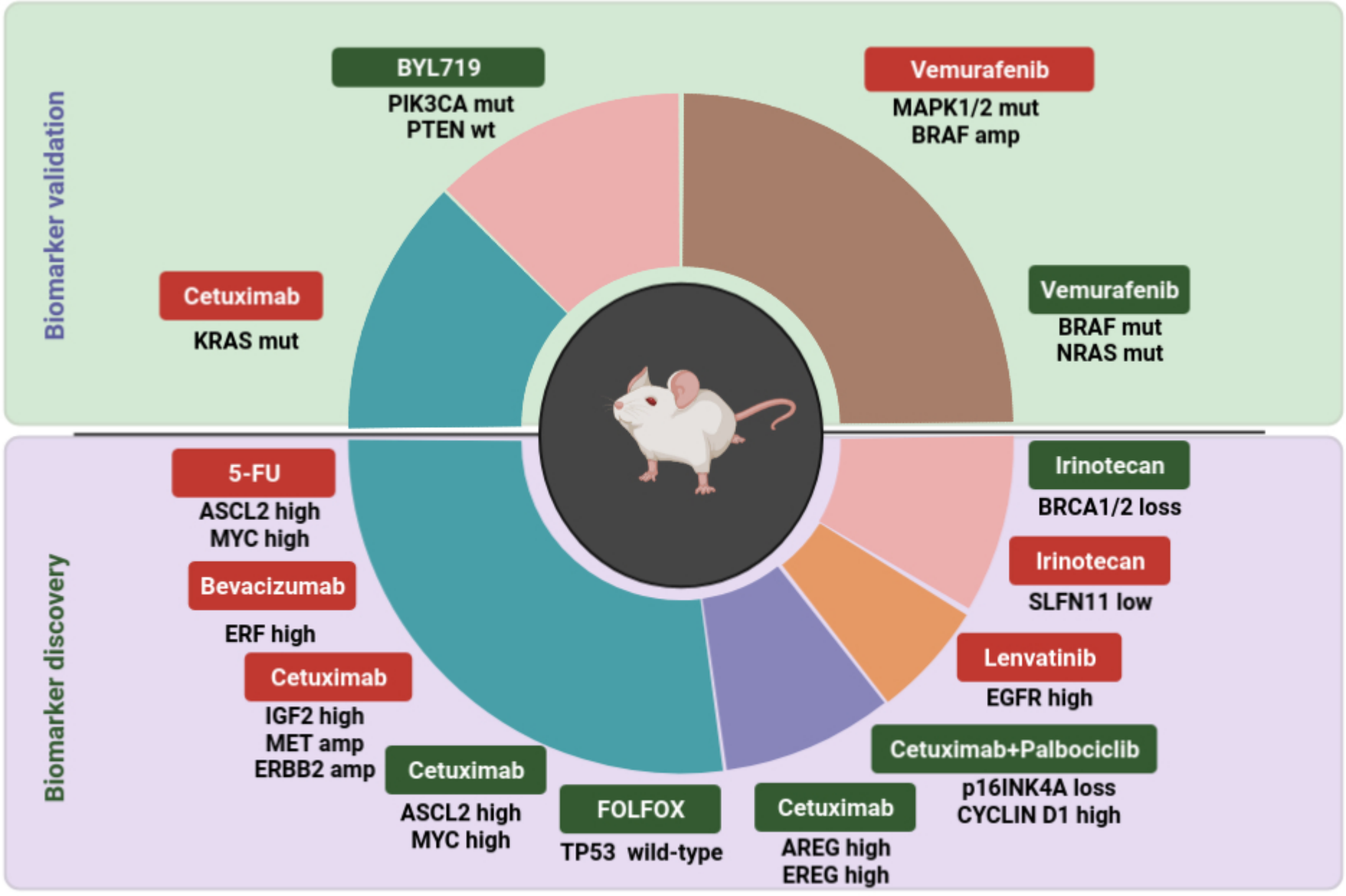
Biomarker validation
BYL719
PIK3CA mut
PTEN wt
Vemurafenib
MAPK1/2 mut
BRAF amp
Cetuximab
KRAS mut
Vemurafenib
BRAF mut
NRAS mut
Biomarker discovery
5-FU
ASCL2 high
MYC high
Bevacizumab
ERF high
Cetuximab
IGF2 high
MET amp
ERBB2 amp
Cetuximab
ASCL2 high
MYC high
FOLFOX
TP53 wild-type
Cetuximab
AREG high
EREG high
Cetuximab+Palbociclib
p16INK4A loss
CYCLIN D1 high
Lenvatinib
EGFR high
Irinotecan
SLFN11 low
Irinotecan
BRCA1/2 loss
Colorectal
Breast
Melanoma
Head&Neck
HCC

Fig.4

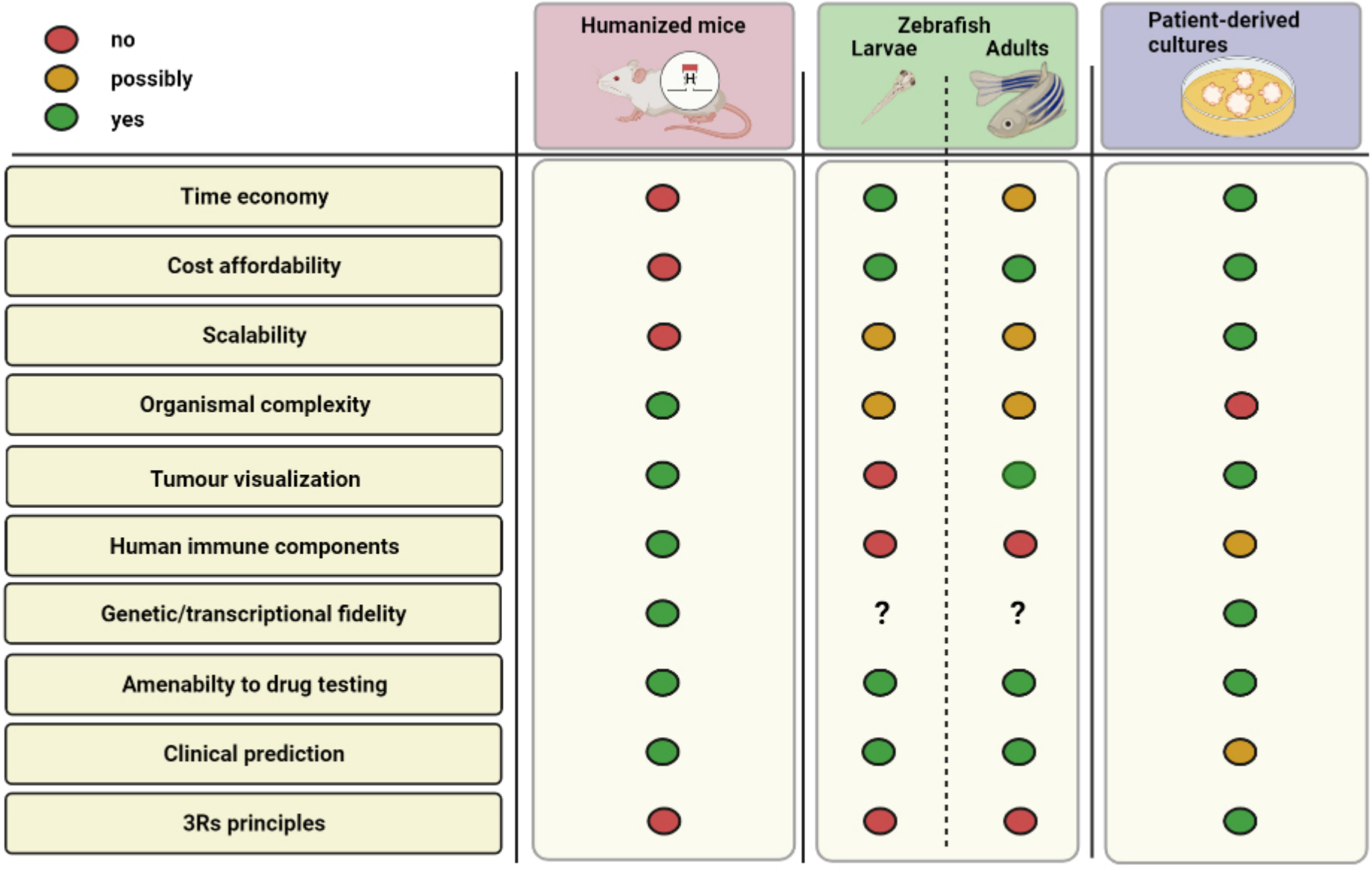
no
possibly
yes
Humanized mice
Zebrafish
Larvae
Adults
Patient-derived cultures
Time economy
Cost affordability
Scalability
Organismal complexity
Tumour visualization
Human immune components
Genetic/transcriptional fidelity
Amenabilty to drug testing
Clinical prediction
3Rs principles
?
?

Fig.5

Table 1.**Facts and figures of international PDX repositories**

Repository	**Contributing institution (number of models)[a]**	**Tumour types (number of models)[b]**	**Sites (number of models)**	**Molecular annotations (number of models)**	**Therapeutic annotations (number of models)**
EurOPDX	IRCC (715) VHIO (122) UOC (59) LIH (40) TRACE (31) Curie (16) UOM (12) UMCG (8) NKI (7)	Digestive system (842) Breast (86) Nervous system (40) Reproductive system (13) Skin (8)	Primary (413) Metastasis (584) Undefined (13)	SNVs/indels (653) CNA (505) Gene expression (266)	283
JAX PDX	JAX (426)	Digestive system (100) Thoracic cavity (77) Haematopoietic/lymphoid tissues (47) Breast (44) Connective/soft tissues (38)	Primary (229) Metastasis (145) Undefined (52)	SNVs/indels (353) CNA (300) Gene expression (379)	90
PDXnet	MD Anderson (316) Winstar-MD Anderson-Penn (256) WUSTL (119) BCM (51)	Digestive system (225) Thoracic cavity (129) Skin (119) Breast (102) Connective/soft tissues (11)	Primary (263) Metastasis (452) Undefined (27/	SNVs/indels (534) CNA (534) Gene expression (534)	0
PPTC	PPTC (261)	Haematopoietic/lymphoid tissues (90) Nervous system (76) Connective/soft tissues (62) Endocrine system (2) Digestive system (2)	Primary (164) Undefined (97)	SNVs/indels (240) CNA (252) Gene expression (252)	0

Others	DFCI-CPDM (731) CRL (540) PDMR-NCI 378 PMLB 284 SJCRH 170	Digestive system (652) Haematopoietic/lymphoid tissues (259) Thoracic cavity (184) Connective/soft tissues (157) Reproductive system (154)	Primary (763) Metastasis (435) Undefined (905)	SNVs/indels (966) CNA (529) Gene expression (468)	0

Data refer to information available in the PDX Finder web portal[59] as of May 15th, 2022. Models available at EurOPDX can also be browsed in the EurOPDX Data Portal (https://dataportal.europdx.eu/)[132]. Contributing institutions: BCM, Baylor College of Medicine, Houston, TX, USA; CRL, Charles River Laboratories, Bar Harbor, ME, USA; Curie, Institut Curie, Paris, FR; DFCI-CPDM, Dana-Farber Cancer Institute – Center for Patient-Derived Models, Boston, MA, USA; IRCC, Candiolo Cancer Institute, University of Turin, Turin, IT; JAX, The Jackson Laboratory, Farmington, CT, USA; LIH, Luxembourg Institute of Health, Esch-sur-Alzette, LU; MD Anderson, MD Anderson Cancer Center, Houston, TX, USA; NKI, Netherlands Cancer Institute, Amsterdam, NL; PDMR-NCI, Patient-Derived Models Repository – National Cancer Institute, Bethesda, MD, USA; PMLB, Princess Margaret Cancer Centre Living Biobank, Toronto, CA; PPTC, Pediatric Preclinical Tumor Consortium, Nationwide Children's Hospital, Columbus, OH, USA; SJCRH, St Jude Children's Research Hospital, Memphis, TN, USA;TRACE, TRACE-PDTX platform, Catholic University, Leuven, BE; UMCG, University Medical Center, Groningen, NL; UOC, University of Cambridge, Cambridge, UK; UOM, University of Manchester, Manchester, UK; VHIO, Vall d'Hebron Institute of Oncology, Barcelona, ES; WUSTL, Washington University, St. Louis, MO, USA. CNA, copy number alteration; SNV, single nucleotide variation. [a]For each repository, tumour types include those that form the top 5 largest collections. [b]For effective parsing of the PDX Finder database, tumours are catalogued based on anatomical location.