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***Ex vivo* drug screening: an emerging paradigm in the treatment of childhood cancer**

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Abstract

Developing and providing the right therapy for the right patient (or personalised targeted treatments) is key to reducing side-effects and improving survival in childhood cancers. Most efforts aiming to personalise childhood cancer treatment use genomic analysis of malignancies to identify potentially targetable genetic events. But it is becoming clear that not all patients will have an actionable change, and in those that do there is no additional way to determine if treatments will be effective. *Ex vivo* drug screening is a laboratory technique used to test the effects of various drugs or compounds, on biological tissues or cells that have been removed from an organism. This information is then used to predict which cancer treatments will be most effective based on the therapeutic response in the tissue or cells removed from that individual. Its utility in personalising treatments in childhood cancer is increasingly recognised. In this review we describe the different methods for *ex vivo* drug screening and the advantages and disadvantages of each technique. We also present recent evidence that *ex vivo* screening may have utility in a variety of childhood malignancies including an overview of current clinical trials appraising its use. Finally, we discuss the research questions and hurdles that must be overcome before *ex vivo* screening can be widely used in pediatric oncology.

Introduction

Approximately 85% of children diagnosed with cancer in the developed world are expected to survive [1]. Yet this headline figure hides great discrepancies in the outcomes for different types of childhood malignancies. Relatively common cancers such as acute lymphoblastic leukaemia have excellent survival rates [2] , but most children with relapsed or refractory solid malignancies have little prospect of cure [3]. In addition, even in cancers which are curable, the burden of cure is high. Traditional cytotoxic chemotherapy agents used in most treatment regimens are associated with both short and long-term toxicity, which can be severe and life changing [4]. It is likely that personalised, targeted treatments will be required before substantial improvements in outcomes and long-term toxicity can be made. However, appraising the efficacy of novel treatments is often challenging in clinical trials given the rarity of pediatric cancers and new methods to identify which children are likely to respond are therefore needed.

The majority of efforts aiming to personalise childhood cancer treatment rely on genetic sequencing of tumors and matching potential therapies to actionable mutations. However, only a small number of patients receive effective treatments based on such an approach. For example, in a recent international trial, children with relapsed/refractory malignancies had whole-exome sequencing (WES) and RNA sequencing (RNA-seq) of their tumor. Around 30% of patients were found to have an actionable mutation but this only translated to a 17% objective response rate with targeted treatments [5].

Another complementary method to identify effective treatments is *ex vivo* drug screening. This is a laboratory technique used to test the effects of various drugs or compounds, on biological tissues or cells that have been removed from an organism. In this review we outline the premise of *ex vivo* drug screening and then discuss the different methods by which it can be performed. We also give an overview of its development and current use in pediatric cancers including current clinical trials in

which it is being evaluated. Finally, we discuss the challenges to implementation of *ex vivo* screening which need to be addressed before its full potential can be fulfilled.

What is *ex vivo* drug screening?

Ex vivo drug screening is a laboratory testing platform that aims to predict which cancer treatments a patient will respond to best. At its simplest, it refers to the process of removing tumor cells or tissue from a patient, screening these cells for their response to a specific panel of drugs, and then interpreting the results to guide the treatment of an individual patient (Figure 1). The results can be used to confirm drug sensitivity patterns predicted from molecular genetics [6] and/or inform treatment options when standard therapies have been exhausted [7]. It is not a new concept, having first been described nearly 70 years ago [8] but efforts to integrate it into clinical oncology practice have generally been limited and unsuccessful due to variability in responses and lack of reproducibility [9]. However, improved methodologies, readouts, and classes of drugs that can be tested have led to recent encouraging results [7] particularly when combined with targeted therapies guided by genomics [10].

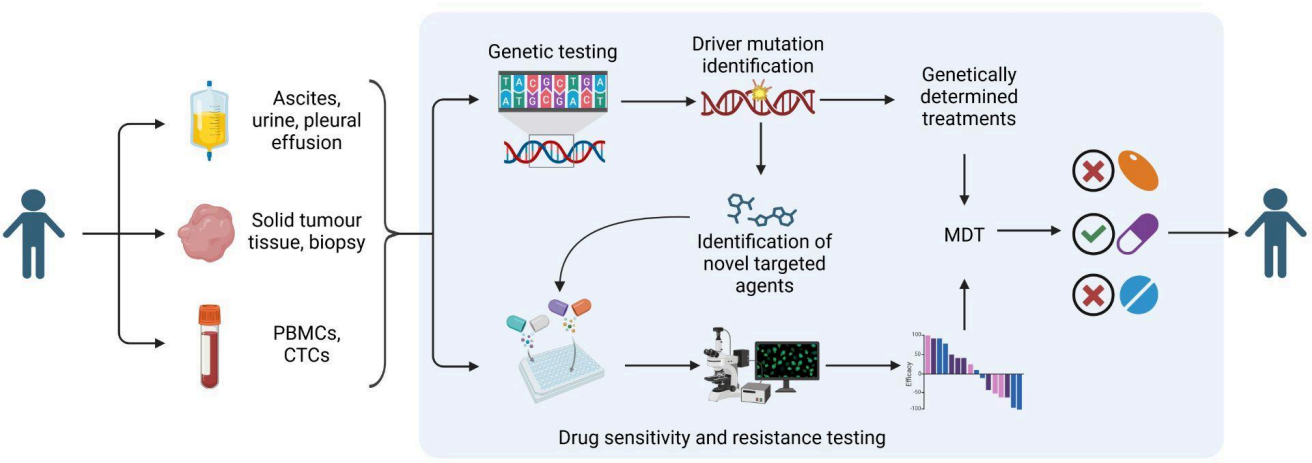

Figure 1. An overview of *ex vivo* drug testing and how it can be integrated into clinical practice. The patient's tumor is sampled at diagnosis and/or relapse. Tumor cells are then processed and analysed on an *ex vivo* drug sensitivity screening platform where multiple potential therapies can be tested. The results are then discussed at a molecular tumor board, often in conjunction with results from genomic profiling to inform treatment decisions.

There are several *ex vivo* drug screening techniques available which can be categorised according to their biological complexity. The methods (summarised in Figure 2) are not mutually exclusive and can be complementary, but we will consider each in turn.

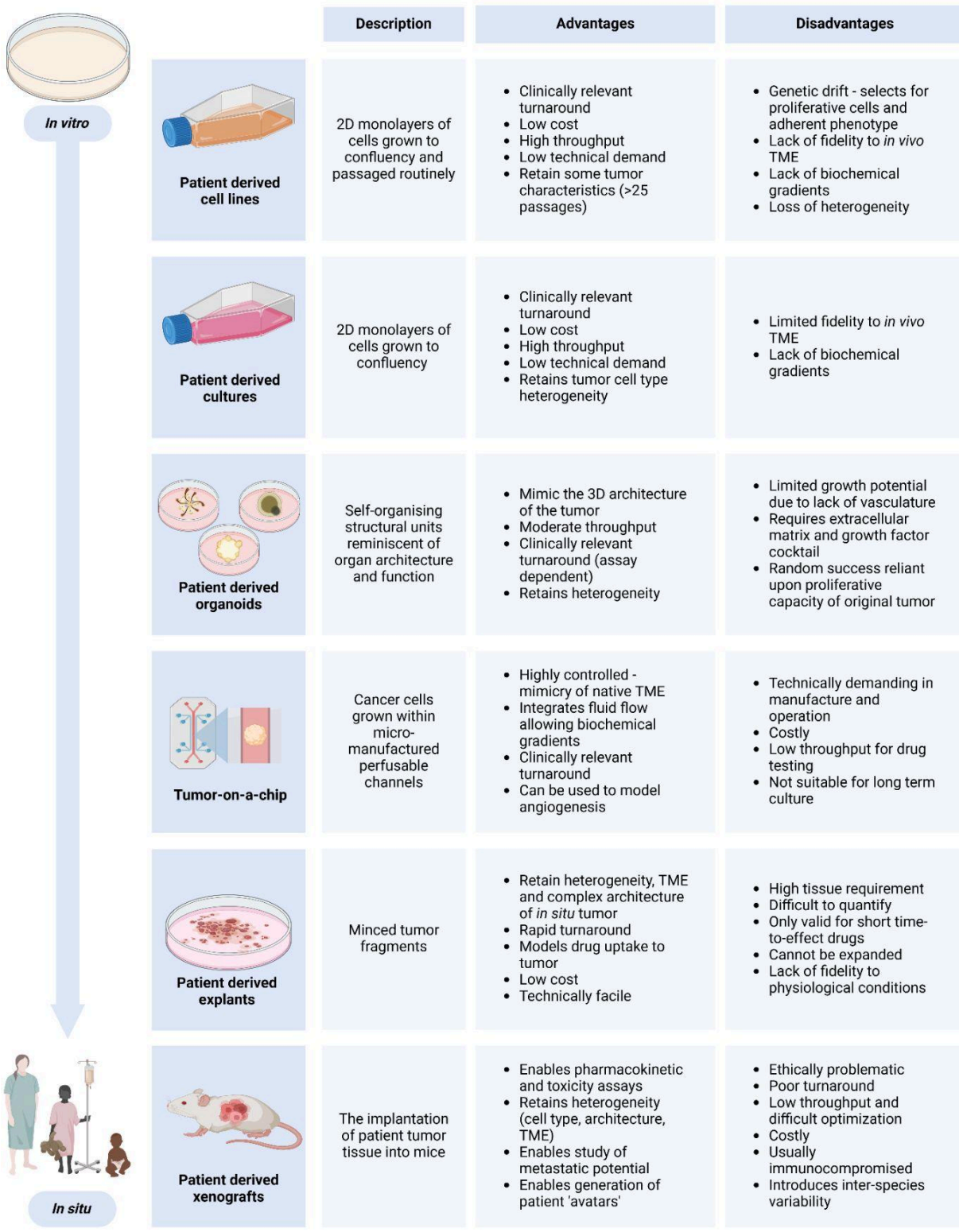

Figure 2. The main methods of *ex vivo* drug screening together with their advantages and disadvantages, and a scale of fidelity to the original patient and tumor. TME – tumor microenvironment.

Patient derived cell lines (PDCL)

For decades, basic and preclinical cancer research has been based on the use of cell lines, originally derived from patients, but adapted to grow indefinitely in artificial culture conditions. Whilst revolutionising cancer research, established cell lines differ from patient tumors in several ways. They have low cell heterogeneity and lack immune and stromal components [11]. There are also multiple culture adaptation artefacts, including rapid genetic diversification occurring as a result of positive clonal selection highly sensitive to culture conditions [12]. This is thought to contribute to the low interlaboratory reproducibility of cell-based pharmacogenomics screening and high drug attrition rates in oncology [13].

The development of primary patient-derived cell lines (PDCLs) can overcome some limitations with a more faithful reproduction of the microenvironment of the disease and individual tumor genetics. PDCLs are *ex vivo* populations directly derived from tissue samples, usually core biopsies, fine needle aspirates, pleural effusions or resections [14]. The excision of malignant and healthy tissue helps to preserve cell phenotypes and the heterogeneity of cancer subpopulations, both of which help to mimic the tumor microenvironment. They can also preserve cancer cells with stem cell phenotypes known to play an important role in the development of drug resistance [15].

There are several methods to develop PDCLs which have been comprehensively reviewed elsewhere [16]. There are an increasing number of reports of PDCLs being generated from adult patients with malignancies with poor outcomes which have the potential to inform future therapy. Kim *et al*. (2019) showed it was possible to generate 23 PDCLs from 96 malignant effusions of 77 patients with advanced lung adenocarcinoma. *In vitro* response to targeted therapies including tyrosine kinase inhibitors reflected patient treatment responses. It was also possible to sequence a proportion of the PDCLs and identify targetable mutations such as *BRAF K601E*. The cells harbouring this driver mutation showed *in vitro* sensitivity to dabrafenib and trametinib suggesting this could be a potentially

novel treatment option for the patient [17]. Brodin *et al*. demonstrated that soft tissue sarcoma cultures could be established from patient biopsies with a success rate of nearly 60%. Genomic analysis and drug sensitivity testing helped to identify the cSrc inhibitor dasatinib as an active drug in sarcomas. It was also shown that *ex vivo* drug sensitivity correlated with patient response for a variety of conventional and targeted treatment options in relapsed and refractory disease [18].

Despite these encouraging reports, PDCLs have several disadvantages. Culturing cells *in vitro*, can result in genetic drift and divergence from their parental tumors [19]. The successful generation of PDCLs is also variable in the literature but is typically not higher than 50% [14]. Perhaps unsurprisingly the biggest predictor of failure is a failure to visualise cancer cells on microscopy after tissue dissociation, highlighting the importance of obtaining representative biopsies and appropriate dissociation techniques [14].

Patient derived cultures

An alternative approach to using PDCLs is the study of drug action directly in patient derived cell cultures (PDCs). This involves the generation of a cellular suspension which can be derived from dissociation of a solid tumor, or extraction of cellular components from liquid malignant fluid such as ascites or a pleural effusion. In contrast to PDCLs, the cellular suspension is not grown or passaged prior to drug screening. This technique therefore has the advantage of being able to screen a large number of drugs in a relatively short time (3-5 days). It is clear that one of the challenges in using PDCs is developing a robust clinical pipeline to rapidly transfer patient samples for processing and analysis. Recently Gagg *et al.*, described their experience of using PDCs from adult patients with glioblastoma to accurately identify *MGMT* status through response to temozolomide and screen 30 potential therapies, which could act as a model for other solid tumors in adults and children [20].

There are a number of case reports in adults with rare cancers supporting the use of this technique to identify potentially effective treatment options [21,22]. Limitations of PDCs include loss of spatial

tissue microenvironment and architecture which can affect the response of certain categories of drugs such as immunotherapies. PDCs are also only viable for a short period of time meaning drugs which exert their maximum effects after several cell divisions may not be readily identified.

Patient organoids

Organoids are 3D *in vitro* cultures of human cells, which in the case of malignancy are typically derived from biopsy or surgical resections. Their generation involves the isolation of primary tumor tissue, dissociation into single cells or microaggregates, followed by expansion of the cells as 3D structures [23]. This often requires highly optimised and tissue specific culture environments or matrix support such as Matrigel [23]. Their main advantage over 2D models such as PDCLs and PDCs is that they are thought to more closely resemble the patient's tumor with regards to tumor heterogeneity and genetics. It is also possible to incorporate heterologous, stroma-derived cells, such as cancer-associated fibroblasts, immune cells and endothelial cells into organoid cultures, potentially from the same patient. Organoids are therefore thought to be a more representative model of tumors than 2D models. For example, a study comparing DNA copy numbers in breast tumor organoids and 2D cell lines to patient tumors found greater concordance between tumor samples and organoids than with 2D cell lines [24].

There is increasing evidence that drug responses measured *ex vivo* in organoids correlates to clinical outcomes in patients. In a seminal study, Vlachogiannis *et al*., (2018) showed patient derived organoids from heavily pretreated colorectal and gastroesophageal cancer patients could predict treatment response in clinical trials [25]. Organoids derived from an adult patient with glioblastoma multiforme were also able to predict response to everolimus [26].

However, there are a number of limitations with organoids. Firstly, the derivation of organoids is not possible for all malignancies. For example, non-small cell lung cancer has a low establishment rate due to overgrowth of tumor organoids by normal airway cells [27]. Similar technical issues have been

observed for liver [28], prostate [29] and endometrial cancers [30]. Some of these issues can be overcome by use of growth factors or addition of drugs to achieve pure cancer cell populations but the effect of these on tumor representation, including recapitulation of the tumor microenvironment, and the ability to predict treatment response is uncertain [23]. Another problem is the length of time it takes to generate organoids. In the majority of cases, it is weeks or even months limiting the clinical value to patients who often have a limited lifespan.

Assembloids is a new term to describe systems analogous to organoids but with more complexity incorporating additional elements such as immune cells and capillaries. They have been defined as self-organising cell systems arising from combinations of different organoids or cell types and have been developed to overcome some perceived limitations of organoids [31]. An assembloid model of bladder urothelial carcinoma has been developed and there are reports of models being used to predict drug response such as in malignant melanoma [32] and lung cancer [33]. But their use in *ex vivo* drug screening is still in its infancy and their future role in the field remains unclear.

Tumor-on-chip

Another model for *ex vivo* drug screening is tumor-on-chip. This technology aims to emulate the physiological environment and functionality of human organs or tumors on a chip for disease modelling and drug testing. The chip takes the form of a microfluidic device with networks of tiny microchannels for guiding and manipulating minute volumes of solution. The organ or tumor refers to miniature tissues grown and residing in the microfluidic chips, which can mimic one or more tissue-specific functions. It is a rapidly expanding field and has recently been comprehensively reviewed [34]. As with other models the aim is to recreate cell connections and tumor micro-environment more accurately.

The translational potential of these systems has been illustrated for a number of different cancers but there is little experience with pediatric malignancies. Yi *et al*., developed a glioblastoma-on-a-chip

using patient-derived tumor cells, decellularized extracellular matrix from brain tissue, and vascular endothelial cells [35]. They reported that resistance to treatment with temozolomide observed in patients could be replicated in their system, and trialled a number of other targeted treatments. Other studies have been performed for diffuse large B cell lymphoma [36] and osteosarcoma [37]. However, experience in using these systems to directly influence the treatment of patients is limited.

Patient derived explants (PDEs)

Patient derived explants (PDEs) are formed by dissecting fresh, surgically resected tumor tissue and placing these under defined culture conditions prior to drug response testing. They have several advantages compared to other models including being more economical and significantly they are also thought to maintain the phenotype and microenvironment of the individual tumor. This can make them particularly useful for testing immunotherapy as these agents require an intact human tumor micro-environment to be effective [38].

They have been widely used for evaluation of tumor response to treatments in a variety of different malignancies in adults but there is limited experience in pediatric tumors. For example, a group treated glioblastoma PDEs with temozolomide and found that individual tumors displayed different susceptibility to the treatment, consistent with clinical observations [39]. In common with other *ex vivo* screening techniques, there is interest in combining PDEs with other information such as genomics. Some groups have then used novel learning algorithms to predict the therapeutic efficacy of targeted and cytotoxic drugs. Such an approach achieved 100% sensitivity in predicting response in a cohort of head and neck and non-small lung cancer patients [40].

There are a number of limitations with PDEs. Perhaps the most significant is their short-term viability with most lasting a maximum of 72 hours [41]. Another disadvantage is the need for relatively large amounts of tissue which will usually have to be from surgical resections rather than biopsies.

Patient derived xenografts

Patient derived xenografts (PDX) involve transplantation of human tumor tissue into animals that have been modified to accept the graft [42]. Traditional PDX models are generated by implantation or injection of human malignant cells into the flank, peritoneum, or tail vein of mice which are immunodeficient. Whilst invaluable in pre-clinical research there is recent interest in using PDX in "real-time" personalised medicine platforms to better predict the likely effectiveness of treatments. Such PDX models are known as "avatars" or "mirror models" and are usually mice but there are other models based on zebrafish [43] or *Drosophilia melanogaster* genetically modified to reflect the patient's tumor [44].

There is increasing experience with using PDX models to inform treatment options after studies demonstrated that PDXs accurately replicate patient outcomes and can predict response to different treatments [45]. For example, in a pilot study, PDXs were developed for 14 patients with refractory solid tumors and screened with more than 200 treatment regimens. An effective treatment was identified for 12 patients with objective response rates of 88% [46]. PDXs have now been successfully established for a variety of pediatric solid malignancies including Wilms tumors, rhabdomyosarcomas, neuroblastomas and high-grade sarcomas [47]. The recent MAPPYACTS trial which aimed to identify targetable genetic alterations in pediatric patients with relapsed and refractory solid tumors also had an ancillary arm which has led to the development of a PDX bank for pediatric tumors. Over the four years of the study, nine participating research laboratories established 131 xenografts from a range of pediatric malignancies [5]. They represent a valuable tool for future research studies, and are accessible for pre-clinical research via the Medicines Initiative (IMI2) ITCC-P4 project (https://www.itccp4.eu).

Despite their potential, there are significant challenges to introducing *ex vivo* screening using PDXs into routine clinical practice. There is considerable cost involved in their generation [48] whilst

engraftment rates vary widely affected by tumor type and transplantation site [49]. Concerns have been raised about whether PDXs accurately reflect the tumor micro-environment affecting their ability to predict treatment responses accurately [48]. If immunodeficient PDXs are used, this makes testing immunotherapy very challenging, if not impossible. Furthermore, the number of animals sacrificed to predict therapeutic response for a single patient may be difficult to justify ethically for patients and funders [50]. Lastly, it can take significant amounts of time to generate PDX models [51] limiting their use for patients with a poor life expectancy,

***Ex vivo* drug screening in pediatric cancers**

In many ways pediatric cancers are ideally placed to benefit from the potential of *ex vivo* drug screening. Clinical trials are difficult because of the rarity of the diseases, regulatory requirements and limited commercial interest. Genetic sequencing of tumors has become increasingly common, but the incidence of targetable molecular findings is variable, in most studies being around 30-50% [5,52]. *Ex vivo* screening may therefore serve two purposes – to validate targetable genetic mutations whilst also highlighting novel treatments for patients, particularly in the relapsed and/or refractory setting.

Table 2 gives an overview of published experience using *ex vivo* screening in pediatric cancers which is then discussed separately for haematological malignancies and solid tumors. We then detail current clinical trials in which it is being appraised. Lastly, we outline some of the challenges of implementing routine *ex vivo* drug screening.

Malignancy	Study details	*Ex vivo* method used	Reference
Haematological			
Relapsed/refractory ALL	12 patients with relapsed disease and 5 with refractory disease included. Samples screened against 60 drugs using PDX/patient derived culture platform. Patient with refractory T-ALL treated with dasatinib on the basis of screening and achieved a 5-month remission	1. Patient derived cultures 2. PDX	[53]
Pediatric lymphoma/leukaemia	Retrospective case series. 14 patients with leukaemia/lymphoma at diagnosis/relapse underwent functional drug screening +/- genomic profiling. Four AML patients had treatment informed by functional drug screen.	1. Patient derived cultures	[54]
AML	31 pediatric AML patients screened at diagnosis using an automated flow cytometry platform assessing 78 drugs/doses. Drug screen results correlated with MRD, relapse-free survival and suggested novel treatment options.	1. Patient derived cultures	[55]
Relapsed mixed-phenotype acute leukaemia	Single case report. Blasts from 12 year old with relapsed mixed phenotype acute leukaemia screened against panel of drugs. High sensitivity to venetoclax and intermediate sensitivity to azacitidine identified. Venetoclax/azacitidine combination treatment commenced leading to transient remission for several months.	1. Patient derived cultures	[56]
ALL	Samples from 805 children with newly diagnosed ALL tested against 18 therapeutic agents. Drug sensitivity found to correlate with MRD and potential efficacy of dasatinib in T-ALL highlighted.	1. Patient derived cultures	[57]
B-ALL	Samples from 34 patients with B-ALL and a poor prognosis screened against 174 compounds. Nine potentially effective treatments identified.	1. PDX derived samples cultured	[58]
Solid tumors			
Ependymoma	Limited study examining *ex vivo* screening in two patients (one 21 year old, one 5 month old) with grade III ependymoma. Results not used to inform treatment but felt to reflect clinical behaviour of disease.	1. Patient derived cultures including cancer-like stem cells. 2. PDX model in adult patient	[59]
Relapsed rhabdomyosarcoma	Single case report of a 7 year old child with relapsed rhabdomyosarcoma who had progressed through multiple treatments. *Ex vivo* drug screening against a panel of 103 drugs undertaken and combined with genomic screening. Vincristine/irinotecan/temozolomide	1. Patient derived cultures	[60]

	identified as potentially efficacious and resulted in clinical response.		
Relapsed/refractory solid tumors	Subset of INFORM (INdividualized Therapy FOr Relapsed Malignancies in Childhood) study. In 65 successfully screened samples, 72% had at least one drug hit and 81% of cases had a drug hit when no targetable mutation had been identified. Findings correlated with clinical response in proportion of cases	1.Patient derived organoid cultures	[61]
High risk solid tumors (defined as expected survival rate < 30%)	56 high-risk solid tumor pediatric cancer patients enrolled. Patients had genomic analysis combined with drug screening in both patient derived culture and PDX models. Results demonstrated drug screening feasible in 52% of patients and could have led to novel treatment options in 10% of patients	1. Patient derived cultures 2. PDX models	[62]
High risk solid tumors (defined as expected survival rate < 30%)	125 patient-derived samples screened against a library of 126 anticancer drugs. Drug screening confirmed known associations between activating genomic alterations in *NTRK*, *BRAF*, and *ALK* and responses to matching targeted drugs. Also identified biomarkers of sensitivity to WEE1 and MEK inhibition. Clinical responses in patients correlated with those in PDX models.	1. Patient derived cultures 2. PDX models	[63]
Relapsed/refractory solid and hematological malignancies	21 out of 25 patients with relapsed/refractory pediatric cancer had samples successfully screened against up to 125 drugs with all patients subsequently having treatment options identified. 5/6 patients who received treatments recommended by drug screening/genomic analysis had a 1.3-fold improvement in progression-free survival compared to previous therapy.	1. Patient derived cultures	[10]

Table 1. Publications describing the use of *ex vivo* drug screening in pediatric malignancies.

T-ALL - T-cell acute lymphoblastic leukaemia; PDX - patient derived xenograft; AML - acute myeloid leukaemia; MRD - measured residual disease; B-ALL - B-cell acute lymphoblastic leukaemia. PDX - patient derived xenograft.

Haematological malignancies

Ex vivo screening programmes in haematological malignancies are more established, primarily due to the accessibility of samples from large national and international trials and the need for less complex sample processing. The first studies from adult patients were published more than a decade ago and it has been shown *ex vivo* drug screening in adult patients with aggressive hematological malignancies including AML and ALL [7,64–69] can inform novel treatment strategies resulting in clinical responses.

In pediatrics, experience is more limited, but is increasing. A proof-of-concept study in drug resistant ALL screened 12 patients with relapsed disease and five with refractory disease against a panel of 60 clinical drugs and pre-clinical compounds in co-cultures of bone marrow stromal cells and PDX models [53]. Individual patterns of marked drug resistance and responses to new agents of immediate clinical relevance were detected including the BCL2-inhibitor, venetoclax in B-cell precursor ALL. Unexpected sensitivity to the tyrosine kinase inhibitor, dasatinib was detected in two T-cell ALL (T-ALL) cohorts. Based on drug profiling information, a patient with refractory T-ALL was treated with dasatinib and achieved a 5-month remission. Further studies in pediatric ALL have demonstrated results from *ex vivo* drug screening correlate with MRD whilst also identifying potentially novel treatments including again dasatinib in T-ALL [57,58].

It has also been shown *ex vivo* drug sensitivity and clinical response in pediatric acute myeloid leukaemia (AML) patients correlates with minimal residual disease percentage and one year relapse-free survival [55]. Further support for a possible role of *ex vivo* drug screening in AML came from a retrospective review of patients with haematological malignancies who underwent genomic profiling and *ex vivo* drug screening of cancer cells. All four patients with refractory AML who had *ex vivo* drug screening had their treatment altered [54]. Finally a recent case report in a 12 year old patient with relapsed mixed phenotype acute leukaemia described using *ex vivo* drug screening to

identify the novel treatment combination venetoclax/azacitidine which led to a transient clinical response for several months [56].

Ex vivo drug screening in solid tumors

Recent reports are encouraging in supporting a role for *ex vivo* screening to personalise therapy for solid childhood cancers. One of the most significant was part of the TARGET pilot study of the Australian ZERO precision Childhood Cancer Programme. Fifty-six children with high-risk cancer, with an expected survival of <30%, were consecutively enrolled. There was an equal distribution of patients at diagnosis and relapse/refractory disease, which included 48% with central nervous system (CNS) tumors, 38% non-CNS solid tumors, and 14% hematologic malignancies. Patients underwent genomic analysis and *ex vivo* testing was attempted using high-throughput drug screening on tumor derived cells and through the generation of PDX murine models. The authors reported that across the whole testing platform, treatment options were identified for 70% of patients, whilst drug screening and/or PDX model generation was possible for 52% of patients. Only the molecular therapeutic recommendations were provided to treating oncologists, so the *ex vivo* results did not have a direct clinical impact. However, there was a strong correlation between *ex vivo* drug screening and the clinical responses in patients. It was found 4/14 molecular, 4/5 high throughput screening and 4/8 PDX predictions correctly forecast a response in the patient receiving that specific drug. 10% of patients had a therapy identified through drug screening that would not have been found through genomic analysis alone [62]. Further work based on patients enrolled in the same study showed drug screening confirmed known associations between activating genomic alterations in *NTRK*, *BRAF*, and *ALK* and responses to matching targeted drugs. In addition, effective combinations could be predicted by correlating sensitivity profiles between drugs [63]. The authors concluded *ex vivo* drug screening was a powerful addition to programmes aiming to personalise medicine in pediatric cancers.

Two similar studies have recently been published highlighting the possible value of *ex vivo* drug screening in childhood cancer with a poor prognosis. The international precision oncology program

INFORM enrolled children with relapsed/refractory cancer for comprehensive molecular analysis and recorded outcomes after matched targeted treatment. Whilst most patient samples were analysed solely with genetic analysis [61], drug sensitivity profiling was introduced for a subset where fresh tissue was available. The platform used was based on *ex vivo* multicellular fresh tumor tissue spheroid cultures in 384-well plates and 75-78 drugs were screened. Amongst 65 successfully screened samples, 72% had at least one drug hit and 81% of cases had a drug hit when no targetable mutation had been identified. The authors described three cases with striking parallels between drug screening and the clinical course and showed that in 9/14 cases with a tumor-driving mutation at least one predicted drug sensitivity hit was found.

A further report in a different cohort had similar encouraging findings. This was primarily a feasibility study to examine whether *ex vivo* drug screening and genomic analysis was possible in a timely manner in children with relapsed/refractory pediatric cancers [10]. The study showed it was possible to generate meaningful results within 9-10 days for both solid and haematological malignancies using drug screening compared to around 26 days when using genomic analysis. Whilst treatments were not given as part of the study, clinicians had access to the drug screen and genomic data via a tumor board. 21/25 patients enrolled in the study successfully had drug screening with treatment options identified for all. In contrast, only 5/20 patients who had genomic analysis had targetable mutations. Subsequently, 6 patients had treatments guided by the drug screen with a 1.3-fold improvement in progression-free survival compared to previous therapy. The authors published a case report from the same study describing in more detail a 7 year old with rhabdomyosarcoma who had relapse treatment successfully informed by drug screening [60].

Clinical trials utilising *ex vivo* screening in pediatric cancers

The potential of *ex vivo* drug screening in pediatric cancers is illustrated by the number of clinical trials which are now investigating *ex vivo* screening and are currently recruiting (summarised in Table 1). Some have already published early results suggesting a possible clinical impact [60,62]. All are

combining genomic sequencing of tumors +/- the germline with *ex vivo* screening results in order to either direct patient therapy or correlate results with patient outcomes. This illustrates the likely clinical impact of *ex vivo* drug screening in that it will complement rather than replace genetic profiling of tumors.

Most trials are utilising drug screens of 2-D cultures of tumor cells with one examining organoids derived from tumor cells and another using PDX models. The largest trial by far is in Australia and is aiming to capture every child in the country being treated for cancer (NCT05504772). They are planning to recruit 3500 children over 10 years. The study is analogous to trials being conducted in Europe and the US, which are examining the impact of clinical molecular profiling on treatments but is also incorporating *ex vivo* drug screening to inform treatments. The trial may provide a platform for how *ex vivo* drug screening can be incorporated into future treatment regimens for childhood cancer.

Challenges of *ex vivo* drug screening

Despite its potential and an increasing number of clinical trials in which it is being appraised, there remain challenges before *ex vivo* drug screening can enter routine clinical practice as discussed below.

Tissue acquisition

By definition, *ex vivo* drug screening requires viable tissue. With the advent of routine genome sequencing, tissue from a biopsy or surgical resection is frequently taken fresh, but is then either rapidly fixed or frozen for further analysis. Almost all *ex vivo* methods require the utilisation of fresh tissue and must therefore be performed prospectively rather than using previously stored samples. A clinical pipeline must also be established where tissue can be reliably transferred from the operating room to the *ex vivo* screening facility.

There are also increasing demands on tissue obtained at diagnosis and relapse. Most importantly, tissue is required for the diagnostic process but can also be needed for whole genome sequencing/genomic analysis and other research studies. Biopsy samples can be small, especially in conditions such as a diffuse midline glioma [70] and *ex vivo* drug screening will need to compete with these other demands for tissue. Whilst there is the potential to obtain tumor samples through other means such as by sampling effusions, ascites, cerebrospinal fluid and peripheral blood this is more challenging in solid tumors due to low cell numbers in the samples [71].

A further issue regarding tissue acquisition is intra-tumor heterogeneity and whether a biopsy from a single primary lesion represents the full spectrum of disease [72]. It has been shown in multiple different cancer types, including recently neuroblastoma [73], that genetic mutations can vary spatially at diagnosis and evolve under therapy. This raises the prospect of patients requiring multiple and repeated biopsies for *ex vivo* drug screening at different timepoints of the illness, especially if they become resistant to therapy.

Calibration and quality assurance

Information obtained from *ex vivo* drug screening is usually parametric with a value obtained (eg. 0-100) based on percentage of dead cells or percentage loss of viability. In contrast to genomic data, this then requires interpreting to decide which value for each drug predicts clinical response. When obtaining values from different drugs and/or doses these also require ranking to predict efficacy [71]. Ideally, this would be done by comparing *ex vivo* drug screening results to clinical outcomes but this requires a clinical trial which is time consuming and expensive. There are other pre-clinical strategies to calibrate *ex vivo* drug testing. For example, one strategy involves comparing drug responses in 2D or organoid models with responses in matched *in vivo* models to decide if *in vitro* drug responses are reliable [74]. Another strategy involves comparing *ex vivo* drug testing on cancer cells and healthy

cells. Regardless, careful optimisation is required to ensure results are reliable and reproducible before any *ex vivo* drug testing platform can be used clinically.

Time to obtain results

The clinical utility of *ex vivo* drug screening is heavily dependent on the speed of results. It is anticipated the majority of patients in which it has utility will have a limited lifespan and in order to benefit, results must therefore be reported in a timely manner. Whilst some techniques such as those based on patient-derived cultures can report results within several days, others which are reliant on either *ex vivo* expansion of tumor samples or PDX models can take weeks or even months to report findings. A possible strategy to solve this issue could be to perform *ex vivo* drug screening at diagnosis, especially if the prognosis is poor, and then use the results at relapse or progression after standard-of care treatment [75]. However, as already discussed it is known that genetic changes frequently occur during progression and relapse [76] which may limit the applicability of any results obtained at diagnosis.

Other limitations

There are additional limitations associated with *ex vivo* drug screening. It is almost never possible to perform in 100% of patient samples and depending on the model chosen and the type of cancer, this may be much lower. For example, in the TARGET pilot study in Australia it was only feasible in 52% of patients [62]. Finally, the cost of *ex vivo* drug screening has to be considered and its use should eventually be appraised using standard economic metrics to determine its value, especially if employed in a publicly funded healthcare system.

Conclusion

The need for personalised, targeted therapies in childhood cancer in order to significantly improve survival and reduce the toxicity of current treatments is widely accepted. There are an increasing number of methods for *ex vivo* drug screening and the challenge in the future will be to determine which, likely in combination, are most appropriate for clinical use. It also remains uncertain how *ex vivo* drug screening complements genetic profiling. Further, there are logistical, technical and economical challenges before drug screening can be widely adopted. However, there is no doubt *ex vivo* drug screening has the potential to play a significant role in personalising childhood cancer treatment and it is likely to become increasingly familiar to pediatric hemato-oncologists in the coming years.

Study name	Cancer type	Study Identifier	No. of patients	Screening methods	Study type	Primary outcome and current status at time of publication
Individualized Treatment Plan in Children and Young Adults With Relapsed Medulloblastoma	Relapsed medulloblastoma	NCT05057702	10	1. Drug screen of tumor cells 2. Whole exome and RNA sequencing of tumor	Pilot	Median time from tissue collection to issued treatment plan from the specialized tumor board. Currently recruiting.
A Study to Predict Response to Virotherapy and Immunotherapy Using an *Ex-Vivo* Three-Dimensional Patient-Derived Organoid Model of Pediatric Urological Cancers	Patients with primary/relapsed pediatric urological cancers having resection/biopsy	19-002795 (Mayo Clinic)	Not specified	1. Drug screen of 2-D culture of tumor cells and organoids 2. Sequencing of tumor	Observational	To find markers of tumor response based on drug screen/mutation profiles. Currently recruiting.
Driving Therapeutic Progress of Childhood Leukemia Through Advanced Translational Research With Immediate and Long-term Impact	pediatric leukaemia	NCT04478006	150	1. Whole exome sequencing 2. RNA sequencing 3. I*n vitro* high throughput drug screening	Observational	Inform future patient stratification for identification of patients who may benefit from targeted treatments.
Precision Medicine for Every Child With Cancer (ZERO2)	All pediatric cancers	NCT05504772	3500	1. Whole genome sequencing 2. RNA sequencing 3. DNA methylation profiling 4. Targeted panel sequencing 5. *In* vitro high throughput drug screening 6. In vivo drug testing using PDX models 7. Liquid biopsy	Observational	Clinical utility of recommended personalised therapy in both high-risk and non-high-risk groups.

Table 2. Current clinical trials employing *ex vivo* screening techniques in pediatric cancers (from clinicaltrials.gov). PDX - patient derived xenograft.

References

1. Ward ZJ, Yeh JM, Bhakta N, Frazier AL, Girardi F, Atun R. Global childhood cancer survival estimates and priority-setting: a simulation-based analysis. Lancet Oncol. 2019;20: 972–983.

2. Carobolante F, Chiaretti S, Skert C, Bassan R. Practical guidance for the management of acute lymphoblastic leukemia in the adolescent and young adult population. Ther Adv Hematol. 2020;11: 2040620720903531.

3. Cho HW, Lee JW, Ma Y, Yoo KH, Sung KW, Koo HH. Treatment outcomes in children and adolescents with relapsed or progressed solid tumors: A 20-year, single-center study. J Korean Med Sci. 2018;33: e260.

4. Chang WH, Katsoulis M, Tan YY, Mueller SH, Green K, Lai AG. Late effects of cancer in children, teenagers and young adults: Population-based study on the burden of 183 conditions, in-patient and critical care admissions and years of life lost. Lancet Reg Health Eur. 2022;12: 100248.

5. Berlanga P, Pierron G, Lacroix L, Chicard M, Adam de Beaumais T, Marchais A, et al. The European MAPPYACTS trial: Precision medicine program in pediatric and adolescent patients with recurrent malignancies. Cancer Discov. 2022;12: 1266–1281.

6. Lehtomaki KI, Lahtinen LI, Rintanen N, Kuopio T, Kholova I, Makela R, et al. Clonal evolution of MEK/MAPK pathway activating mutations in a metastatic colorectal cancer case. Anticancer Res. 2019;39: 5867–5877.

7. Snijder B, Vladimer GI, Krall N, Miura K, Schmolke A-S, Kornauth C, et al. Image-based ex-vivo drug screening for patients with aggressive haematological malignancies: interim results from a single-arm, open-label, pilot study. Lancet Haematol. 2017;4: e595–e606.

8. Wright JC, Cobb JP, Gumport SL, Golomb FM, Safadi D. Investigation of the relation between clinical and tissue-culture response to chemotherapeutic agents on human cancer. N Engl J Med. 1957;257: 1207–1211.

9. Hamburger AW. Use of in vitro tests in predictive cancer chemotherapy. J Natl Cancer Inst. 1981;66: 981–988.

10. Acanda De La Rocha AM, Berlow NE, Fader M, Coats ER, Saghira C, Espinal PS, et al. Feasibility of functional precision medicine for guiding treatment of relapsed or refractory pediatric cancers. Nat Med. 2024;30: 990–1000.

11. Wilding JL, Bodmer WF. Cancer cell lines for drug discovery and development. Cancer Res. 2014;74: 2377–2384.

12. Ben-David U, Siranosian B, Ha G, Tang H, Oren Y, Hinohara K, et al. Genetic and transcriptional evolution alters cancer cell line drug response. Nature. 2018;560: 325–330.

13. Haibe-Kains B, El-Hachem N, Birkbak NJ, Jin AC, Beck AH, Aerts HJWL, et al. Inconsistency in large pharmacogenomic studies. Nature. 2013;504: 389–393.

14. Kodack DP, Farago AF, Dastur A, Held MA, Dardaei L, Friboulet L, et al. Primary patient-derived cancer cells and their potential for personalized cancer patient care. Cell Rep. 2017;21: 3298–3309.

15. Shibue T, Weinberg RA. EMT, CSCs, and drug resistance: the mechanistic link and clinical implications. Nat Rev Clin Oncol. 2017;14: 611–629.

16. Richter M, Piwocka O, Musielak M, Piotrowski I, Suchorska WM, Trzeciak T. From donor to the lab: A fascinating journey of primary cell lines. Front Cell Dev Biol. 2021;9: 711381.

17. Kim S-Y, Lee JY, Kim DH, Joo H-S, Yun MR, Jung D, et al. Patient-derived cells to guide targeted therapy for advanced lung adenocarcinoma. Sci Rep. 2019;9: 19909.

18. Brodin BA, Wennerberg K, Lidbrink E, Brosjö O, Potdar S, Wilson JN, et al. Drug sensitivity testing on patient-derived sarcoma cells predicts patient response to treatment and identifies c-Sarc inhibitors as active drugs for translocation sarcomas. Br J Cancer. 2019;120: 435–443.

19. Mouriaux F, Zaniolo K, Bergeron M-A, Weidmann C, De La Fouchardière A, Fournier F, et al. Effects of long-term serial passaging on the characteristics and properties of cell lines derived from uveal melanoma primary tumors. Invest Ophthalmol Vis Sci. 2016;57: 5288–5301.

20. Gagg H, Williams ST, Conroy S, Myers KN, McGarrity-Cottrell C, Jones C, et al. Ex-vivo drug screening of surgically resected glioma stem cells to replace murine avatars and provide personalise cancer therapy for glioblastoma patients. F1000Res. 2023;12: 954.

21. Rantala JK, Arjonen A, Hakkarainen H, Kononen J, Rantala JK, Hollmén M, et al. Ex vivo modelling of therapy efficacy for rare Krukenberg tumors – A report of two cases. Clin Oncol Res. 2020; 1–8.

22. Mäkelä R, Arjonen A, Suryo Rahmanto A, Härmä V, Lehtiö J, Kuopio T, et al. Ex vivo assessment of targeted therapies in a rare metastatic epithelial-myoepithelial carcinoma. Neoplasia. 2020;22: 390–398.

23. Clevers H, Tuveson DA. Organoid models for cancer research. Annu Rev Cancer Biol. 2019;3: 223–234.

24. Duarte AA, Gogola E, Sachs N, Barazas M, Annunziato S, R de Ruiter J, et al. BRCA-deficient mouse mammary tumor organoids to study cancer-drug resistance. Nat Methods. 2018;15: 134–140.

25. Vlachogiannis G, Hedayat S, Vatsiou A, Jamin Y, Fernández-Mateos J, Khan K, et al. Patient-derived organoids model treatment response of metastatic gastrointestinal cancers. Science. 2018;359: 920–926.

26. Loong HH-F, Wong AM, Chan DT-M, Cheung MS-H, Chow C, Ding X, et al. Patient-derived tumor organoid predicts drugs response in glioblastoma: A step forward in personalized cancer therapy? J Clin Neurosci. 2020;78: 400–402.

27. Dijkstra KK, Monkhorst K, Schipper LJ, Hartemink KJ, Smit EF, Kaing S, et al. Challenges in establishing pure lung cancer organoids limit their utility for personalized medicine. Cell Rep. 2020;31: 107588.

28. Broutier L, Mastrogiovanni G, Verstegen MM, Francies HE, Gavarró LM, Bradshaw CR, et al. Human primary liver cancer-derived organoid cultures for disease modeling and drug screening. Nat Med. 2017;23: 1424–1435.

29. Gao D, Vela I, Sboner A, Iaquinta PJ, Karthaus WR, Gopalan A, et al. Organoid cultures derived from patients with advanced prostate cancer. Cell. 2014;159: 176–187.

30. Boretto M, Maenhoudt N, Luo X, Hennes A, Boeckx B, Bui B, et al. Patient-derived organoids from endometrial disease capture clinical heterogeneity and are amenable to drug screening. Nat Cell Biol. 2019;21: 1041–1051.

31. Pașca SP, Arlotta P, Bateup HS, Camp JG, Cappello S, Gage FH, et al. A nomenclature consensus for nervous system organoids and assembloids. Nature. 2022;609: 907–910.

32. Rodrigues DB, Moreira HR, Jarnalo M, Horta R, Marques AP, Reis RL, et al. Generation of 3D melanoma models using an assembloid-based approach. Acta Biomater. 2024;178: 93–110.

33. Zhang Y, Hu Q, Pei Y, Luo H, Wang Z, Xu X, et al. A patient-specific lung cancer assembloid model with heterogeneous tumor microenvironments. Nat Commun. 2024;15: 3382.

34. Leung CM, de Haan P, Ronaldson-Bouchard K, Kim G-A, Ko J, Rho HS, et al. A guide to the organ-on-a-chip. Nat Rev Methods Primers. 2022;2: 1–29.

35. Yi H-G, Jeong YH, Kim Y, Choi Y-J, Moon HE, Park SH, et al. A bioprinted human-glioblastoma-on-a-chip for the identification of patient-specific responses to chemoradiotherapy. Nat Biomed Eng. 2019;3: 509–519.

36. Sabhachandani P, Sarkar S, Mckenney S, Ravi D, Evens AM, Konry T. Microfluidic assembly of hydrogel-based immunogenic tumor spheroids for evaluation of anticancer therapies and biomarker release. J Control Release. 2019;295: 21–30.

37. Mitxelena-Iribarren O, Hisey CL, Errazquin-Irigoyen M, González-Fernández Y, Imbuluzqueta E, Mujika M, et al. Effectiveness of nanoencapsulated methotrexate against osteosarcoma cells: in vitro cytotoxicity under dynamic conditions. Biomed Microdevices. 2017;19: 35.

38. Powley IR, Patel M, Miles G, Pringle H, Howells L, Thomas A, et al. Patient-derived explants (PDEs) as a powerful preclinical platform for anti-cancer drug and biomarker discovery. Br J Cancer. 2020;122: 735–744.

39. Merz F, Gaunitz F, Dehghani F, Renner C, Meixensberger J, Gutenberg A, et al. Organotypic slice cultures of human glioblastoma reveal different susceptibilities to treatments. Neuro Oncol. 2013;15: 670–681.

40. Majumder B, Baraneedharan U, Thiyagarajan S, Radhakrishnan P, Narasimhan H, Dhandapani M, et al. Predicting clinical response to anticancer drugs using an ex vivo platform that captures tumour heterogeneity. Nat Commun. 2015;6: 6169.

41. Schäfer WR, Fischer L, Roth K, Jüllig AK, Stuckenschneider JE, Schwartz P, et al. Critical evaluation of human endometrial explants as an ex vivo model system: a molecular approach. Mol Hum Reprod. 2011;17: 255–265.

42. Tentler JJ, Tan AC, Weekes CD, Jimeno A, Leong S, Pitts TM, et al. Patient-derived tumour xenografts as models for oncology drug development. Nat Rev Clin Oncol. 2012;9: 338–350.

43. Fazio M, Ablain J, Chuan Y, Langenau DM, Zon LI. Zebrafish patient avatars in cancer biology and precision cancer therapy. Nat Rev Cancer. 2020;20: 263–273.

44. Bangi E, Ang C, Smibert P, Uzilov AV, Teague AG, Antipin Y, et al. A personalized platform identifies trametinib plus zoledronate for a patient with KRAS-mutant metastatic colorectal cancer. Sci Adv. 2019;5: eaav6528.

45. Izumchenko E, Paz K, Ciznadija D, Sloma I, Katz A, Vasquez-Dunddel D, et al. Patient-derived xenografts effectively capture responses to oncology therapy in a heterogeneous cohort of patients with solid tumors. Ann Oncol. 2017;28: 2595–2605.

46. Hidalgo M, Bruckheimer E, Rajeshkumar NV, Garrido-Laguna I, De Oliveira E, Rubio-Viqueira B, et al. A pilot clinical study of treatment guided by personalized tumorgrafts in patients with advanced cancer. Mol Cancer Ther. 2011;10: 1311–1316.

47. Stewart E, Federico SM, Chen X, Shelat AA, Bradley C, Gordon B, et al. Orthotopic patient-derived xenografts of paediatric solid tumours. Nature. 2017;549: 96–100.

48. Malaney P, Nicosia SV, Davé V. One mouse, one patient paradigm: New avatars of personalized cancer therapy. Cancer Lett. 2014;344: 1–12.

49. Garralda E, Paz K, López-Casas PP, Jones S, Katz A, Kann LM, et al. Integrated next-generation sequencing and avatar mouse models for personalized cancer treatment. Clin Cancer Res. 2014;20: 2476–2484.

50. Williams ST, Wells G, Conroy S, Gagg H, Allen R, Rominiyi O, et al. Precision oncology using ex vivo technology: a step towards individualised cancer care? Expert Rev Mol Med. 2022;24: e39.

51. Garrido-Laguna I, Uson M, Rajeshkumar NV. Tumor engraftment in nude mice and enrichment in stroma-related gene pathways predict poor survival and resistance to gemcitabine in patients with pancreatic cancer. Clinical Cancer Research. 2011;17. doi:10.1158/1078-0432.CCR-11-0341

52. Mody RJ, Wu Y-M, Lonigro RJ, Cao X, Roychowdhury S, Vats P, et al. Integrative clinical sequencing in the management of refractory or relapsed cancer in youth. JAMA. 2015;314: 913–925.

53. Frismantas V, Dobay MP, Rinaldi A, Tchinda J, Dunn SH, Kunz J, et al. Ex vivo drug response profiling detects recurrent sensitivity patterns in drug-resistant acute lymphoblastic leukemia. Blood. 2017;129: e26–e37.

54. Eaton A, Wong V, Schiff D, Anderson E, Ding H, Capparelli EV, et al. Tumor genomic profiling and ex vivo drug sensitivity testing for pediatric leukemia and lymphoma patients. J Pediatr Pharmacol Ther. 2022;27: 123–131.

55. Strachan DC, Gu CJ, Kita R, Anderson EK, Richardson MA, Yam G, et al. Ex vivo drug sensitivity correlates with clinical response and supports personalized therapy in pediatric AML. Cancers (Basel). 2022;14: 6240.

56. Gonzales F, Guilmatre A, Barthélémy A, Lapillonne H, Pottier N, Leverger G, et al. Ex vivo drug sensitivity profiling-guided treatment of a relapsed pediatric mixed-phenotype acute leukemia with venetoclax and azacitidine. Pediatr Blood Cancer. 2022;69: e29678.

57. Lee SHR, Yang W, Gocho Y, John A, Rowland L, Smart B, et al. Pharmacotypes across the genomic landscape of pediatric acute lymphoblastic leukemia and impact on treatment response. Nat Med. 2023;29: 170–179.

58. Oikonomou A, Valsecchi L, Quadri M, Watrin T, Scharov K, Procopio S, et al. High-throughput screening as a drug repurposing strategy for poor outcome subgroups of pediatric B-cell precursor Acute Lymphoblastic Leukemia. Biochem Pharmacol. 2023;217: 115809.

59. Mathis SE, Alberico A, Nande R, Neto W, Lawrence L, McCallister DR, et al. Chemo-predictive assay for targeting cancer stem-like cells in patients affected by brain tumors. PLoS One. 2014;9: e105710.

60. De La Rocha A, Fader AM, Coats M, Espinal ER, Berrios PS, Saghira V. Clinical Utility of Functional Precision Medicine in the Management of Recurrent/Relapsed Childhood Rhabdomyosarcoma. JCO Precis Oncol. 2021;5.

61. van Tilburg CM, Pfaff E, Pajtler KW, Langenberg KPS, Fiesel P, Jones BC, et al. The pediatric precision oncology INFORM registry: Clinical outcome and benefit for patients with very high-evidence targets. Cancer Discov. 2021;11: 2764–2779.

62. Lau LMS, Mayoh C, Xie J, Barahona P, MacKenzie KL, Wong M, et al. In vitro and in vivo drug screens of tumor cells identify novel therapies for high-risk child cancer. EMBO Mol Med. 2022;14: e14608.

63. Mayoh C, Mao J, Xie J, Tax G, Chow S-O, Cadiz R, et al. High-throughput drug screening of primary tumor cells identifies therapeutic strategies for treating children with high-risk cancer. Cancer Res. 2023;83: 2716–2732.

64. Pemovska T, Kontro M, Yadav B, Edgren H, Eldfors S, Szwajda A, et al. Individualized systems medicine strategy to tailor treatments for patients with chemorefractory acute myeloid leukemia. Cancer Discov. 2013;3: 1416–1429.

65. Swords RT, Azzam D, Al-Ali H, Lohse I, Volmar C-H, Watts JM, et al. Ex-vivo sensitivity profiling to guide clinical decision making in acute myeloid leukemia: A pilot study. Leuk Res. 2018;64: 34–41.

66. Pemovska T, Johnson E, Kontro M, Repasky GA, Chen J, Wells P, et al. Axitinib effectively inhibits BCR-ABL1(T315I) with a distinct binding conformation. Nature. 2015;519: 102–105.

67. Malani D, Kumar A, Brück O, Kontro M, Yadav B, Hellesøy M, et al. Implementing a functional precision medicine tumor board for acute myeloid leukemia. Cancer Discov. 2022;12: 388–401.

68. Kornauth C, Pemovska T, Vladimer GI, Bayer G, Bergmann M, Eder S, et al. Functional precision medicine provides clinical benefit in advanced aggressive hematologic cancers and identifies exceptional responders. Cancer Discov. 2022;12: 372–387.

69. Rowland L, Smart B, Brown A, Dettorre GM, Gocho Y, Hunt J, et al. Ex vivo drug sensitivity imaging-based platform for primary acute lymphoblastic leukemia cells. Bio Protoc. 2023;13: e4731.

70. Fu AY, Kavia J, Yadava Y, Srinivasan A, Hargwood P, Mazzola CA, et al. Biopsy of diffuse midline glioma is safe and impacts targeted therapy: a systematic review and meta-analysis. Childs Nerv Syst. 2024;40: 625–634.

71. Letai A, Bhola P, Welm AL. Functional precision oncology: Testing tumors with drugs to identify vulnerabilities and novel combinations. Cancer Cell. 2022;40: 26–35.

72. Karlsson J, Valind A, Holmquist Mengelbier L, Bredin S, Cornmark L, Jansson C, et al. Four evolutionary trajectories underlie genetic intratumoral variation in childhood cancer. Nat Genet. 2018;50: 944–950.

73. Schmelz K, Toedling J, Huska M, Cwikla MC, Kruetzfeldt L-M, Proba J, et al. Spatial and temporal intratumour heterogeneity has potential consequences for single biopsy-based neuroblastoma treatment decisions. Nat Commun. 2021;12: 6804.

74. Bhola PD, Ahmed E, Guerriero JL, Sicinska E, Su E, Lavrova E, et al. High-throughput dynamic BH3 profiling may quickly and accurately predict effective therapies in solid tumors. Sci Signal. 2020;13: eaay1451.

75. Guillen KP, Fujita M, Butterfield AJ, Scherer SD, Bailey MH, Chu Z, et al. A human breast cancer-derived xenograft and organoid platform for drug discovery and precision oncology. Nat Cancer. 2022;3: 232–250.

76. Rosswog C, Fassunke J, Ernst A, Schömig-Markiefka B, Merkelbach-Bruse S, Bartenhagen C, et al. Genomic ALK alterations in primary and relapsed neuroblastoma. Br J Cancer. 2023;128: 1559–1571.