Trends in Cancer

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Review

# Precision neuro-oncology for children: Time to gear up!

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**Molecular profiling is central to pediatric precision oncology, yet only 15–20% of patients benefit from biology-guided treatments. This limitation underscores the urgent need for functional precision medicine, which interrogates therapeutic vulnerabilities using patient-derived models. Despite hurdles in establishing these models, such as limited biopsy material and technical screening barriers, recent advances in tumoroids, patient-derived xenografts and high-throughput platforms offer an unprecedented opportunity to bridge the translational gap. In this review, we describe the evolution of patient-derived models of pediatric brain cancers, their current strengths and weaknesses and the technological innovations redefining the field. Together, these insights provide a road map for integrating functional data into clinical decision-making, ultimately aiming to 'cure more and heal better' children with brain tumors.**

## Highlights

Pediatric brain tumors have specific clinical and molecular features, and only a small fraction of patients benefit from current precision oncology approaches.

Functional precision medicine combines molecular profiling with drug sensitivity testing of patient-derived tumor models to uncover actionable vulnerabilities.

Patient-derived models generated from limited biopsy material enable clinically relevant pharmacological high-throughput screening.

Integrating drug sensitivity data with molecular profiling facilitates drug repurposing and personalized combination therapies to ultimately improve patient outcomes.

## Bringing childhood brain cancers into the era of precision oncology

In 2022, more than 210,000 new cases of pediatric tumors and over 78,000 cancer-related deaths among children were recorded worldwide [1]. The most common types of cancer in children include leukemia, brain tumors, lymphomas and extracranial solid tumors such as neuroblastomas and Wilms' tumors. These pediatric cancers are very distinct from adult ones due to their origin and their development within a growing organism. The scarcity of these cancers and their specific biological and histopathological characteristics make the development of pediatric oncology drugs particularly challenging [2,3]. Survival rates for some cancers have significantly improved (*e.g.*, complete remission in 80–90% of cases of acute lymphoblastic leukemia). However, such progress remains more difficult to achieve for other high-risk, recurrent or refractory pediatric cancers, such as brain tumors, due to limited treatment options [4,5].

Malignant tumors of the central nervous system (CNS) kill more children than any other cancers. In over 90% of patients with high-grade glioma (HGG), relapse is inevitable, in part due to marked inter- and intra-tumoral heterogeneity contributing to therapeutic resistance [6]. Given that these rare cancers are classified into several subtypes, which are further defined by distinct genomic alterations [3], it is necessary to devise ways to prioritize drugs that are most likely to be clinically effective [3,7]. This is one of the goals of genomics-guided precision oncology, which aims to provide pediatric and adolescent patients with treatments adapted to the molecular features of their tumors. However, most pediatric cancer genomes are characterized by a low mutational burden, which means that few molecular alterations can be therapeutically targeted, explaining in part why only a small proportion of patients clinically benefit from these precision medicine programs [2,8,9]. This limitation emphasizes the urgent need for complementary strategies that go beyond transcriptional and genomic profiling. Functional precision medicine, which combines molecular profiling and drug sensitivity testing of tumor cells derived from patient samples, could help identify treatment options when standard treatments have failed [4]. It aims to optimize the selection of patient-specific drugs by integrating tumor molecular profiling and direct *ex vivo*

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functional sensitivity testing on live tumor cells from individual patients [5]. This shift has reshaped our conceptualization of cancer as a profoundly individualized disease that requires a tailored therapeutic strategy based on both molecular and functional data [5]. A better understanding of the complex mechanisms of action of drugs appears essential. Moreover, achieving this requires clinically relevant models [6] and time constraint-adapted, statistically robust, standardized methodologies. Here, we review the strategies implemented to establish the different models and the dedicated high-throughput screening (HTS) methods, as well as the challenges and opportunities awaiting us.

## The pediatric oncology challenge: 'Curing more, healing better'

Unlike adult tumors, which often have an epithelial origin, childhood cancers arise from embryonic lineages [10]. Pediatric brain tumors are the most common solid tumors in children, accounting for approximately 25% of all pediatric cancers [11,12]. Around 60% of these tumors develop in the posterior fossa, which contains the brainstem and the cerebellum. The remaining cases are distributed between the cerebral hemispheres and the spinal cord [11]. Pediatric brain cancers are mainly composed of low-grade gliomas (LGGs) and HGGs, accounting for 60% of the cases, followed by medulloblastomas (MBs) at 20%, ependymomas comprising 8–10% of cases, craniopharyngiomas accounting for 5–10% and the rare but aggressive atypical teratoid/rhabdoid tumors (ATRT) constituting 1–2% of diagnoses (Table 1) [21]. While LGGs and craniopharyngiomas show favorable outcomes, HGGs and ATRT largely remain refractory, with survival rates below 50% and 30%, respectively [12,20,22]. The classification of MBs and ependymomas has evolved from purely histological to molecularly driven risk stratification, yet high-risk and metastatic patients still face poor prognoses [13–19,21] (Table 1).

The treatment of pediatric brain tumors presents several challenges, including the difficulty of performing complete surgical resection in certain parts of the brain and the blood–brain barrier (BBB), which limits the delivery of therapeutic agents (Box 1) [25]. Additionally, the long-term effects of cranial radiotherapy, which impair neurodevelopment and endocrine functions, and cytotoxic chemotherapy, which can induce cardiomyopathy, fertility issues, and secondary cancers, are significant concerns. The cumulative incidence of chronic disease among childhood cancer survivors thus exceeds 70% within 30 years of diagnosis [2]. While newer anticancer drugs are generally more tolerable than conventional chemotherapeutics, the long-term impact of these treatments on the growth and development of pediatric patients remains a critical consideration (Box 2) [2]. Furthermore, drug development programs dedicated to pediatric oncology present a number of challenges, including the rarity of specific cancer subtypes, the limited number of targetable mutations, the difficulties in drugging nonkinase fusion oncoproteins, transcription factors, or epigenetic regulators (such as DDX31–GFI1B, MYCN, or H3F3A), and the particular considerations involved in studying therapeutic interventions in a growing organism [2].

Considering the heterogeneity of pediatric brain tumors, biopsy and molecular profiling play an important role in patient care and research, enabling target identification and enrollment in clinical trials [28]. The widespread use of sequencing approaches, associated with the rise of targeted therapies, has led to the development of numerous precision medicine programs around the world, such as Zero Childhood Cancer (ZCC) in Australia [29,30] and multiple European programs such as INFORM (INdividualized Therapy FOr Relapsed Malignancies in Childhood) and MAPPYACTS (MoleculAr Profiling for Pediatric and Young Adult Cancer Treatment Stratification) in conjunction with the AcSé-ESMART basket trial (Table 2) [31–33]. These programs aim to identify actionable molecular targets, refine diagnoses, and improve outcomes for children with high-risk and/or recurrent cancers. More than 2,000 patients

Table 1. Molecular characteristics of pediatric brain tumors[a]

| Tumor type (frequency) | Molecular alteration subtypes | Locations | Key clinical notes | 5-year overall survival | Refs |
|---|---|---|---|---|---|
| Gliomas (60%) | LGGs | Supratentorial compartment (frontal or temporal lobes) | • 40% of all CNS tumors in children<br>• Heterogeneous<br>• Poorly proliferative<br>• Glial tumors and mixed glial-neuronal tumors<br>• Grade I or II according to the World Health Organization (WHO) | 91% | [10,12,13] |
| | HGGs are divided into four subtypes:<br>1) diffuse hemispheric glioma, H3 G34-mutant<br>2) diffuse pediatric-type HGG, H3–wild type and IDH–wild type<br>3) infant-type hemispheric glioma<br>4) DMG, H3 K27-altered | All areas of the CNS with a higher prevalence in cerebral hemispheres and midline structures (pons and thalamus) | • 20% of all pediatric CNS tumors<br>• Very aggressive<br>• Diffuse infiltration<br>• Grade III or IV according to WHO | 46% | [10,12,13] |
| MBs (20%) | WNT-activated | Cerebellum | • 10–15% of MBs<br>• Respond extremely well to existing therapies<br>• Excellent prognoses | >95% | [14–17] |
| | SHH-activated and TP53–wild type | | • 30% of MBs are SHH-activated tumors<br>• Intermediate risk disease | 76% | |
| | SHH-activated and TP53-mutant | | • High-risk clinical disease<br>• Dismal prognosis | 41% | |
| | Non-WNT/non-SHH | | • 20–40% of all cases<br>• Previously known as group 3 and group 4 MBs<br>• Frequently metastatic | 30–70% for the former group 3 (depending on high or low risk classification)<br>70–80% for the former group 4 (low or high risk) | |
| Ependymomas (8–10%) | Ten subtypes based on histology, molecular information and location | Two thirds in the posterior fossa | • Heterogeneous<br>• Fusion genes and copy number abnormalities<br>• Dismal prognosis | 60–70% | [14,15,18,19] |
| Craniopharyngiomas (5–10%) | Two subtypes: adamantinomatous and papillary | Rathke pouch (suprasellar region near the optic chiasm) | • Slow-growing benign epithelial tumors<br>• Higher recurrence rates for adamantinomatous subtypes | 97% | [12,14,20] |
| ATRT (1–2%) | SMARCB1/SMARCA4 loss | Intracranial | • Very poor prognosis | 30% | [21] |

[a]DMG: diffuse midline glioma; IDH: isocitrate dehydrogenase; WNT: Wnt protein; SHH: Sonic hedgehog.

### Box 1. Strategies to overcome the BBB for drug delivery

Several strategies have been developed to bypass or temporarily disrupt the BBB, each with distinct mechanisms and clinical applications.

**Hyperosmolar therapy** uses substances such as mannitol to osmotically open the BBB, facilitating drug delivery by transiently increasing BBB permeability. Current Phase 1/2 trials are investigating mannitol use along with intra-arterial administration of chemotherapeutics in gliomas, with promising Phase 1 results demonstrating the safety of bevacizumab (Avastin, anti-VEGF) administration after osmotic BBB disruption and a reduction in tumor volume in recurrent malignant gliomas [23].

**Convection-enhanced delivery (CED)** employs a pressure gradient to infuse drugs directly into brain tissue. Ongoing trials explore the use of CED in glioblastoma or DMG to administer antibody–drug conjugates (Omburtamab, NCT01502917), chemotherapy agents (Topotecan, NCT03154996; Irinotecan, NCT03086616), or epidrugs (Panobinostat, NCT04264143); however, technical challenges such as catheter placement and drug reflux limit its efficacy [24].

**Laser-guided interstitial thermal therapy (LITT)** can disrupt the BBB by decreasing tight junction integrity and increasing permeability for up to 6 weeks. Several clinical trials using LITT are currently underway in combination with chemotherapeutic agents (doxorubicin, lomustine and temozolomide) or immunotherapies (pembrolizumab), mainly in glioblastoma. The results of this technique are limited by the use of a single interstitial catheter and the location of the tumor [24].

**Magnetic resonance-guided focused ultrasound (MRgFUS)** noninvasively disrupts the BBB using high-intensity ultrasound to perform thermoablation of a targeted area. Ongoing studies evaluate MRgFUS with chemotherapy (carboplatin, temozolomide, etoposide and doxorubicin), immunotherapy (bevacizumab and pembrolizumab), or epidrugs (panobinostat) in gliomas. Its repeatability and safety profile are advantageous, although spatial targeting remains limited by equipment constraints [24].

Each approach offers unique benefits and limitations, with ongoing research aimed at optimizing safety and efficacy in brain tumors treatment.

have been enrolled in these different studies collectively. The identification of potentially targetable molecular alterations varies between 61% and 86%, but only 10–33% of enrolled patients received a personalized treatment, mostly in monotherapy [7,30–32]. Ultimately, the clinical benefit remains limited, with response rates below 40%. In comparison, in the largest precision oncology trial in adults, NCI-MATCH (National Cancer Institute's Molecular Analysis for Therapy Choice), 38% of the more than 6,000 enrolled patients were matched to a targeted therapy, but only 18% had access to the relevant therapy [9], ultimately performing less well than pediatric patients, thus further highlighting the relevance of functional precision medicine in adult cancers as well.

### Box 2. Improving radiotherapy: Sensitization and protection

Radiation therapy remains a cornerstone of pediatric neuro-oncology, yet its efficacy is strictly limited by the vulnerability of the developing brain. To overcome this challenge, a global shift from physical precision (*e.g.,* proton therapy) to biological precision, by simultaneously employing radiosensitization and radioprotection strategies, is necessary.

**Targeted radiosensitization** aims to amplify the lethal impact of radiation specifically within the tumor mass. By targeting the DNA damage response through PARP, ATM or ATR inhibitors, it is possible to prevent malignant cells from repairing radiation-induced double-strand breaks. This selective sensitization not only increases local control but may also allow for dose de-escalation, reducing the overall burden on healthy tissues.

**Pharmacological radioprotection** aims to bypass the true bottleneck for re-irradiation at relapse, which is the cumulative toxicity to the surrounding healthy parenchyma. Emerging evidence suggests that repurposed agents can act as protective shields. By inhibiting GSK3β, lithium has shown potential in mitigating radiation-induced cognitive decline and promoting neuronal precursor survival [26]. Beta-blockers may reduce radiation-induced neuroinflammation and vascular damage, preserving the integrity of the BBB and surrounding architecture [27].

At recurrence, many patients are ineligible for re-irradiation due to the risk of radiation-induced necrosis. By priming the tumor for destruction while shielding the brain with lithium or beta-blockers, the therapeutic window may widen enough to facilitate salvage re-irradiation. Future clinical studies should evaluate these radio-modulatory strategies to ensure that survival does not come at the cost of devastating neurological integrity.

Table 2. The various pediatric precision medicine programs

| Program / Country / associated clinical trials | Key features | Actionable alterations (%) | Patients receiving targeted therapy (%) | Objective response rate (%) | Follow-up focus | Refs |
|---|---|---|---|---|---|---|
| ZCC<br>Australia | • PRISM trial<br>• 384 patients with high-risk pediatric cancer<br>• 18+ months follow-up | 67 | 29<br>mainly targeted monotherapy (in 74%) | 36<br>improved 2-year PFS | Survival, response | [29,30] |
| MAPPYACTS<br>Europe<br>NCT02613962 | • Prospective<br>• 787 patients with recurrent/refractory malignant tumors in children and young adults<br>• Algorithm-driven (five levels) | 69<br>436/787 patients | 10 | 38 | Imaging response | [31] |
| INFORM<br>Europe | • Prospective<br>• Pediatric patients with recurrent, progressive, or high-risk cancer<br>• 1,051 patients but only 519 patients completed clinical follow-up<br>• Algorithm-driven (seven levels target prioritization) | 85.9<br>• 446/519 patients<br>• 225 (43%) characterized as very high, high, or moderate priority levels<br>• Very high observed in 42 patients (8.1%) ➔ mainly neuroblastomas and HGGs | 33<br>• 147/446 patients<br>• mainly small molecule drugs and immunotherapies<br>• Median duration of treatment: 92 days | Survival outcomes improved only in patients with high-evidence targets | Survival, real-world outcomes | [30,32] |
| MOSCATO-01<br>France<br>NCT01566019 | • Cancer with recurrent/resistant tumors<br>• Intentional tumor biopsies at recurrence or progression | 60.9<br>42/75 patients | 33<br>14 patients | – | Feasibility, clinical integration | [7] |

Despite these ambitious programs, several challenges persist. First, few patients with brain tumors have been included in these clinical studies so far. Moreover, the proportion of patients receiving targeted therapies remains scant, often due to limited access to clinical trials, drug availability, insufficient evidence supporting the use of identified targets [3,8,31] and potential influence of clinician biases or uncertainty regarding the efficacy and benefit–risk ratio of precision-guided treatments [30]. For instance, in the INFORM registry, 67% of patients did not receive a targeted agent at all, underscoring the need for the creation of biomarker-driven and combination clinical trials [32], such as INFORM2, a Phase 1/2 trial evaluating the combination of Nivolumab and Entinostat in children and adolescents with refractory high-risk malignancies as well as the AcSé-ESMART [33] and MATCH studies [32]. The methods used to classify actionable alterations vary depending on the program (*e.g.*, MAPPYACTS's five-level system *vs*. INFORM's seven-level system), highlighting the importance of carefully designing clinical trials so that every patient can benefit from targeted therapy when applicable. Studies in precision pediatric oncology have demonstrated the feasibility and potential of molecular profiling to guide treatment decisions. However, the translation of genomic findings into clinical benefit remains limited, with only a small fraction of patients receiving targeted therapies. Recent trials such as INFORM have begun to integrate functional drug testing *ex vivo* with genomic-based precision medicine to provide additional therapeutic options for patients [4,31]. However, this requires the establishment of robust preclinical models [34].

## Patient-derived models: Current landscape and limitations

The preclinical toolkit for pediatric neuro-oncology has evolved from basic cellular expansion toward more sophisticated systems that recapitulate the spatiotemporal complexity of the developing brain and more accurately predict responses to treatment (Figure 1). Historically, primary 2D cultures and neurospheres served as the standard platform for HTS due to their scalability.

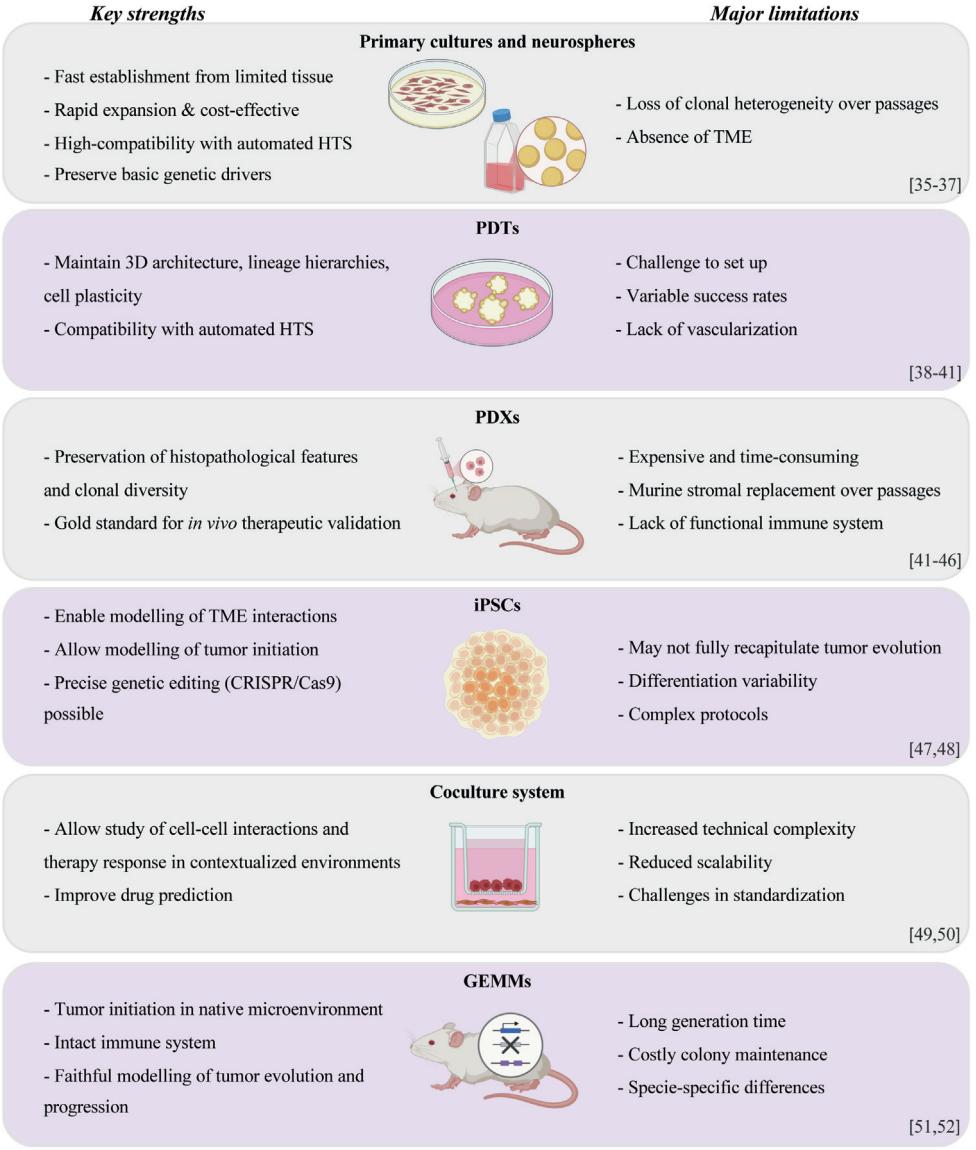


Figure 1. Overview of the diverse models in pediatric neuro-oncology. This schematic illustrates the structural components and biological interactions integrated across the modeling spectrum. The tumor microenvironment (TME) is depicted here as a dynamic neuro-oncological niche composed of the extracellular matrix, the neurovascular unit and resident brain cells (including astrocytes, microglia, and neurons), which collectively influence tumor plasticity and therapeutic resistance. While simplified platforms focus on intrinsic tumor cell properties, immunocompetent frameworks, such as syngeneic GEMMs and advanced coculture systems, are specifically designed to incorporate extrinsic signaling. These systems are indispensable for the evaluation of immunotherapies, as they provide the necessary landscape to study the recruitment, activation and exhaustion of immune effectors within a functional stroma. Ultimately, because no single system can recapitulate the full clinical reality, the strategic combination of these diverse modeling approaches is essential. Integrating these complementary platforms into a unified preclinical pipeline is the only way to bridge the gap between laboratory discovery and robust clinical translation. GEMMs: genetically engineered mouse models; HTS: high-throughput screening; iPSCs: induced-pluripotent stem cells; PDTs: patent-derived tumoroids; PDXs: patient-derived xenografts. The figure was created using BioRender [35–52].

However, these models often fail to maintain the original tumor clonal architecture [35–37]. To better preserve 3D organization and lineage hierarchies, patient-derived tumoroids (PDTs) [38–42] and patient-derived xenografts (PDXs) [42–46] have emerged as essential bridges toward biological fidelity. The field has further integrated the unique neurodevelopmental origins of these tumors, using induced-pluripotent stem cell-derived models (iPSCs) for precise genetic editing [47,48] as well as coculture systems and genetically engineered mouse models (GEMMs) that capture essential stromal and immune crosstalk [49–52]. Ultimately, since no single model captures the full clinical spectrum, preclinical strategies are moving toward integrated pipelines. Combining these complementary models is now essential to ensure robust translational success.

Limited access to tumor tissue remains a major barrier to translational research in pediatric neuro-oncology. The transition from a small biopsy to a robust experimental model requires complex amplification. The optimization of tissue dissociation and culture conditions constitutes the primary hurdles in model development. These processes require a combination of enzymatic and mechanical dissociation, followed by the use of specialized, growth factor-enriched media to maintain cellular viability and phenotype [11]. Moreover, the use of matrices that mimic the brain extracellular matrix is critical to prevent unwanted differentiation or model derivation [53,54]. To minimize the selection pressure of *in vitro* conditions, early-passage banking is mandatory. This process quickly involves scaling from initial microcultures to larger stocks and ensuring that a cryopreserved snapshot of the tumor diversity is maintained. Validation post-expansion is a multipronged process that relies on a three-pillar framework: (i) comprehensive molecular profiling to ensure the conservation of initial alterations [37,39,40,42,55]. In the context of pediatric neuro-oncology, DNA methylation profiling is essential to confirm that the model retains its original molecular subgrouping [56,57]. (ii) Histological comparison to confirm that the models maintain the expression of specific markers [39,56] and spatial organization, including mitotic activity, proliferation and differentiation status, microvascular proliferation, or perivascular pseudorosettes, which are characteristic of the tissue of origin [39,58,59]; and (iii) retrospective drug sensitivity testing as the definitive benchmark for functional validation, ensuring the model faithfully aligns with clinical data [39,42].

Despite the evolution of current patient-derived models, a significant translational gap persists, governed by an inherent trade-off between model complexity, preparation time and establishment success. The low success rate of establishment is particularly pronounced in pediatric CNS tumors due to low initial cellularity and the difficulty of replicating the brain's metabolic niche. Short-term, structurally simpler platforms, such as freshly dissociated tumor cells or early-stage 3D tumoroids, offer highly reliable success rates, making them suitable for rapid functional profiling within clinically actionable timeframes. Conversely, moving up the ladder of biological fidelity toward long-term expansion and/or complex multicellular systems significantly reduces the success rate. Furthermore, the temporal mismatch is a critical bottleneck. While aggressive tumors such as HGG or DMG can progress within weeks, establishing a robust PDX line frequently requires months, often making them retrospective tools. Crucially, as the patient's disease continues to evolve under the selective pressure of systemic therapies, the resulting PDXs may only mirror the tumor at diagnosis, failing to capture the persister clones present in the clinical setting. However, PDTs offer a more adaptive alternative. They are significantly less time-consuming and costly to establish than PDX models. They can maintain high biological fidelity and accurately recapitulate human tumor drug responses within a clinically relevant timeframe [58]. Biologically, the transition from *in vivo* to *ex vivo* imposes a fierce selective pressure. Standardized media inevitably favor specific subclones at the expense of slower-growing, chemo-resistant, stem-like populations [60,61]. Finally, the absence of a native TME, including

immune cells and vascularization, remains the Achilles' heel of current models. To overcome these constraints, the development of next-generation coculture systems is essential [62].

Addressing these inherent biological obstacles is the primary driver behind the latest innovations in screening technologies, which aim to extract high-fidelity functional data from limited patient material within clinically actionable timeframes.

## Screening technologies for patient samples: Challenges, and opportunities

Pharmacological HTS remains the cornerstone of functional profiling. By exposing patient-derived models to libraries of FDA-approved drugs and experimental compounds, immediate repositioning opportunities can be identified [55,63,64] (Box 3). To move beyond monotherapies, combinatorial drug matrices are increasingly being employed to uncover synergistic interactions, aiming to bypass the signaling pathways that frequently drive resistance [73,74]. Complementary to pharmacology, pooled CRISPR/Cas9 screens have already pinpointed critical cellular dependencies across the pediatric landscape, identifying novel targets in HGG [75] and LGG [76], as well as therapeutically targetable metabolic and signaling vulnerabilities in DMG [77] and MB [78]. Moreover, the choice of phenotypic assays critically shapes the sensitivity and specificity of these outcomes. Currently, ATP-based viability assays remain the gold standard due to their robustness and ease of automation in HTS. However, they possess inherent limitations, particularly in metabolic studies, where drug-induced changes in cellular ATP levels can occur

### Box 3. Drug repurposing: A pragmatic shortcut to pediatric precision medicine

The traditional *de novo* drug discovery pipeline is notoriously inefficient for pediatric neuro-oncology, plagued by high failure rates, prohibitive costs and a lack of commercial economic appeal for rare childhood malignancies. In this context, drug repurposing represents an intriguing strategy that identifies new indications for existing, clinically approved compounds.

#### Advantages of drug repurposing in the pediatric brain

The strategic utility of repurposed agents stems from their well-characterized clinical profiles, which substantially mitigate the safety and toxicity uncertainties typical of early-phase development. For pediatric brain tumors, this advantage is particularly pronounced when identifying drugs that exhibit high BBB crossing, a primary prerequisite for effective CNS therapies. While drug repurposing is often framed as an alternative to novel molecules, these two strategies are fundamentally complementary, particularly within combinatorial frameworks. A landmark example is the MEMMAT protocol, which synergistically integrates metronomic chemotherapy and repurposed agents with targeted therapies and intrathecal injections to bypass the BBB in recurrent MBs [65]. By using existing pharmacokinetic data, the transition from bench to such multimodal pediatric trials is accelerated, bypassing the long safety testing phases required for entirely new drugs.

#### Functional screening as a discovery engine

The integration of patient-derived models with HTS has revolutionized the identification of repurposing candidates. Large-scale chemical libraries, such as the ReFRAME or Prestwick collections, allow for the unbiased testing of thousands of approved drugs directly on patient-derived models. This approach has already yielded unanticipated hits, where drugs originally developed for metabolic (metformin [66] and statins [67]), cardiovascular (digoxin [68]), or anti-parasitic (mebendazole [69]) indications show potent selective toxicity against specific pediatric cancers. This landscape encompasses both broad-spectrum generic agents with pleiotropic effects, such as propranolol for its anti-proliferative and radio-sensitizing impact in MB [27] or fluvastatin, which was evaluated in the fluvabrex trial in patients with LGG [70]. Alongside these broad agents, targeted repurposing strategies remain a major axis. The latter is exemplified by the use of antifungal itraconazole as a Smoothened inhibitor in SHH-driven MB, demonstrating how nononcology drugs can be precisely matched to a tumor molecular landscape [71].

#### A global opportunity

Finally, drug repurposing stands as a pillar of democratized innovation. By providing cost-effective and globally available therapeutic options, it offers a unique opportunity for low- and middle-income countries to implement precision medicine, where expensive novel inhibitors remain inaccessible. Ultimately, this strategy ensures that translational progress benefits all children, offering an immediate bridge between the bench and the bedside for high-risk and recurrent diseases [72].

independently of actual cell death, potentially leading to misinterpretation of drug efficacy. To complement these metabolic readouts, newer high-throughput methods are becoming increasingly democratized, notably high-content imaging. By enabling the automated quantification of multiple parameters simultaneously, this technique provides a more multidimensional view of drug response without sacrificing the throughput necessary for large-scale discovery [34,79].

A functional precision oncology test has been developed to predict individual patient responses to radiation and temozolomide. The 3D PREDICT REGISTRY uses this test on tumor tissues isolated from patients with newly diagnosed HGG and glioblastoma. Functional results were provided within 7–10 days of tissue receipt, enabling optimization of patient management prior to therapy initiation [80]. The aim is to preferentially direct predicted nonresponders to clinical trials or toward alternative treatments potentially providing greater clinical benefit [80]. In 2023, a high-throughput *ex vivo* drug screening platform (GliExP) was able to maintain glioma stem cell populations from immediately dissociated fresh surgical tissue and perform drug screening using bespoke drug plates or current treatments, either alone or combined with radiotherapy [40]. Furthermore, others have adapted their *ex vivo* image-based drug screening platform for the functional characterization of patient glioblastoma tissues [34]. This pharmacoscopy method uses immunofluorescence staining to quantify the drug-induced specific reduction of cancer cells relative to nonmalignant TME cells to determine drug responses [34]. They identified a set of repurposable neuroactive drugs, such as the antidepressant vortioxetine, with potent anti-glioblastoma efficacy, leading to the ReVoGlio clinical trial (NCT07284628). Another study used transcriptomic-guided high-throughput drug screening in patient-derived DMG cell lines. Thanks to the molecular analyses, the authors identified vulnerabilities in the cell cycle and P53 pathways, allowing them to screen a dedicated library of 950 compounds targeting these pathways [81].

Promisingly, a prospective observational study enrolled 25 patients with relapsed or refractory solid and hematological cancers for drug testing using a library of 125 FDA-approved agents and genomic profiling. Functional screening was successfully achieved for 21 patients, and genomic profiling for 20, with a median turnaround time of 10 days and 27 days, respectively. Based on genomic profiling, only five of those 20 patients (25%) had an actionable treatment recommendation, while all 21 patients had an identified treatment option through drug testing. More interestingly, as all these patients were refractory to standard treatment, the authors demonstrated that those treated according to the results of the drug testing had a significantly longer progression-free survival (PFS) than those treated according to the physician's choice (>60 weeks for over 75% of patients *vs.* <20 weeks, respectively, $P = 0.0037$), reinforcing the interest and power of drug testing. These results underline once again the potential of functional precision medicine as a tool to guide treatment decisions in high-risk malignancies, including pediatric cancers [4]. In the same perspective, ZCC undertook *in vitro* HTS of a library of 126 anticancer drugs on 125 patient-derived samples and *in vivo* drug efficacy testing in PDX models in conjunction with comprehensive molecular profiling. The HTS results confirmed known associations between activating genomic alterations in NTRK, BRAF, and ALK and responses to matching targeted drugs, which were further validated *in vivo* in PDX models. Moreover, molecular integration coupled with HTS improved the identification of effective biomarker-driven therapeutic strategies, with, for example, a correlation of sensitivity to MEK inhibition (trametinib) potentially associated with PIK3R1 mutations and/or AKT3 gain in a subset of HGG and DMG samples [82].

One of the first limitations of HTS on patient-derived models is the scarcity of biopsy material. Standard core needle biopsies often require weeks or months of expansion as cell lines, tumoroids, or PDX models to reach the quantity needed for testing [5,9]. However, ZCC demonstrated that clinically actionable timeframes are achievable, particularly when using freshly

dissociated tumor cells, which provide the most-efficient functional readout [82]. Otherwise, miniaturization and acoustic robotic platforms allow for the testing of thousands of FDA-approved compounds using nanoliter volumes and rapid turnaround times [4]. Furthermore, there has been a significant improvement in readout systems to assess the sensitivity or resistance of patient tumor samples. From the evaluation of proliferation, survival or metabolic activity, it is now possible to integrate single-cell analysis to evaluate either bulk or subclonal phenotypic effects [9]. In another development, drug efficacy testing is now beginning to be carried out *in situ*, directly within the patient's tumor [9] with implantable microdevices, but regulatory hurdles are greater.

ZCC also demonstrated that drug response profiles appear largely independent of the expansion method used (freshly dissociated or amplified material) [82]. This allows for a multilevel screening strategy: using established cell lines for large-scale HTS to identify hits, which are subsequently validated on PDT or PDX models. This approach reduces the immediate need for large quantities of primary material while complex models are being developed. Nonetheless, standard cell lines often fail to capture the genetic heterogeneity of pediatric cancers [81]. Preserving this heterogeneity requires the use of a pharmacoscopy workflow, where samples are dissociated on the day of surgery and incubated with drugs for 48h [34] or the use of specialized conditioned media and *ex vivo* culture systems [5].

Working with viable, unfixed tissues can introduce pre-analytical variability [9]. Each step, from surgical acquisition and transport temperature to dissociation protocols, can compromise reproducibility. To overcome this, it is essential to establish standardized international protocols to ensure data consistency across laboratories [9]. Moreover, since many therapies rely on the TME, the lack of immune and stromal compartments in tumor-only cultures remains a challenge [4]. The use of mouse organotypic brain slices to better simulate the vascular and neural niches is an emerging approach in neuro-oncology [27,74,83]. Complementary to these *ex vivo* models, integrating coculture systems into HTS workflows is a priority, particularly for pediatric brain tumors where neurovascular and immune interactions critically shape therapeutic responses. In this context, the field is progressively moving beyond static monocultures toward sophisticated coculture systems integrating PDTs with stromal and immune components [79,81]. These immunocompetent platforms enable the study of immune evasion, cytokine-mediated signaling and treatment-induced microenvironmental remodeling under controlled conditions.

In parallel, microfluidic and tumor-on-a-chip technologies are rapidly expanding the experimental landscape of pediatric neuro-oncology. By recreating vascular flow, oxygen and nutrient gradients, endothelial barriers, and mechanical constraints, these systems more faithfully reproduce the physiological conditions governing immune cell trafficking and drug delivery within the brain tumor niche [82]. Beyond static spheroid coculture systems, advanced microfluidic platforms enable continuous circulation of immune cells under dynamic flow conditions, allowing the real-time investigation of immune cell migration, endothelial adhesion, extravasation, and infiltration into tumor tissues [83]. Such systems can sustain long-term cell–tissue interactions while remaining compatible with high-resolution imaging, cytokine profiling and endpoint molecular analysis [83]. This physiological fidelity is particularly relevant for evaluating advanced immunotherapies, including immune checkpoint inhibitors, CAR-T (Chimeric-Antigenic Receptor-T) cells, natural killer cell therapies, and bispecific T-cell engagers, in clinically relevant microenvironments [27,84]. Coupled with automated workflows and multiplex functional readouts, these next-generation microphysiological systems may substantially improve the predictive value of preclinical testing while remaining compatible with clinically actionable timelines and limited biopsy material.

Finally, the translation of functional data into clinical benefit requires robust data integration and international collaborations. There is a need to include multiomic integration, which combines genomic and transcriptomic signatures with functional readouts to provide a multidimensional view of drug response. This evolution is supported by advanced bioinformatics and mathematical modeling, which are essential for decoding complex data and predicting patient-specific outcomes. Transcriptomic analysis often lack integration with patient survival or prognostic data [81]. It is therefore necessary to set-up well-designed clinical trials with comprehensive data collection and high patient enrollment. Centralized bioinformatics platforms, such as PedcBioPortal, St Jude PeCan Cloud, and the PIVOT (Pediatric Preclinical *In Vivo* Testing Consortium) portal, are vital since they consolidate genomic, transcriptomic and efficacy data. Searchable catalogs (PDCM, PDX Explorer, or Childhood Cancer Data Initiative) further provide clinical, genomic, and protein annotations from PDX models. These resources enable model selection for efficacy studies by combining genomic, immunohistochemical and histopathological data [3].

## Current outlook and challenges

Given that biological signaling can change when the tumor is kept in the operating room, banking facilities or shipped to the laboratory, standardization of protocols for model establishment and rigorous quality controls will be important for conducting multicenter clinical trials [39,44,58]. Furthermore, technical efforts should focus on developing HTS-compatible models that bridge the gap between complexity and throughput. By integrating automated 3D imaging and multiparametric readouts, these platforms can move beyond simplistic viability metrics, capturing the morphological and spatial nuances of tumor response necessary for high-fidelity clinical predictions.

As molecular subtypes of pediatric brain tumors are rare and heterogeneous, it becomes essential to design large multinational trials to enroll enough patients and predict significant differences in each of these populations [2]. Innovative designs of clinical studies applicable to pediatric neuro-oncology include *N*-of-1 trials, in which a single patient acts as his or her own control; upfront window trials with a short-term evaluation of novel agents in very high-risk tumors before standard treatment; the use of Phase 0 trials with subtherapeutic microdosing to assess pharmacodynamics and target engagement to reduce the risk for children; and Phase 1/2 trials that combine Phase 1 (safety/dose) and Phase 2 (preliminary efficacy) in a single trial. These approaches can help prioritize personalization, early detection of efficacy signals and reduced exposure to ineffective treatments, while addressing the unique challenges of rare, aggressive pediatric cancers. All the examples mentioned here illustrate the need to move toward the combination of molecular profiling and HTS to improve patient access to matched therapies within clinically relevant timeframes [4,31]. By using *ex vivo* HTS and comprehensive molecular profiling at diagnosis, clinicians can identify the most effective and least toxic treatment regimens from the outset, thereby improving response rates and reducing the likelihood of adverse events [5]. This holds promise for improving long-term survival rates and the quality of life of these young patients [2]. The challenge of these future clinical trials lies in the constitution of the tumor molecular board to overcome physician hesitancy [5]. The contribution of parents and patient advocacy organizations is critical. They can provide information on facilitating access to new therapies, help ensure balance in randomized trials, facilitate research forums and raise funds [2]. Elsewhere, it is of great interest to use cerebrospinal fluid or liquid biopsy profiling to perform cell-free and circulating tumor DNA analysis, as well as emerging proteomic analyses for patient follow-up, to find early signals of failure or efficacy and enable rapid prioritization of agents [84]. As deep-learning algorithms become more prominent in helping with MRI diagnosis and interpretation, their incorporation into

assessing imaging-based endpoints can be considered, particularly in defining challenging endpoints for clinical trials in oncology, such as overall response rate and PFS (see Outstanding questions) [84].

The implementation of these pipelines integrating molecular profiling and HTS requires close and interdisciplinary collaborations between scientists, oncologists, pathologists, pharmacists, geneticists and bioinformaticians [5]. It is proposed that the optimal way to generate preclinical evidence is *via* multimodality, efficient, collaborative, preclinical investigations potentially aided by deep-learning tools [84]. This process already begins with the establishment of networks to share and exchange all the models developed during the various programs already carried out. Once established, all the models generated can be grown indefinitely and utilized for new research to develop novel therapeutic strategies, as well as to document the fidelity of the long-term models [9]. On the other hand, the creation of a data warehouse is an important axis to consider. While the NCI's PDX Network and Patient-Derived Models Repository have made the genomic data of their validated models and their associated drug responses publicly available, a specific 'data commons' for functional precision medicine would be valuable. Data present in these different warehouses could be used to predict the therapeutic responses of future patients without generating tumors for *ex vivo* HTS [9]. The systematic sharing of multiomics and phenotypic datasets will help identify the most effective therapies for these pediatric patients. In addition to collaborative efforts and sharing data and models, it is essential that all children with brain tumors can benefit from this type of clinical trial. Many patients, especially those in rural or underserved areas, face significant barriers to joining these trials [5]. That is why it is necessary to improve awareness and provide support to patients with travel assistance and/or financial incentives. Finally, it is impossible to conclude this section without mentioning the importance of patient associations, as well as parents/carers and sick children themselves, as key players in this healthcare setting.

## Concluding remarks and future perspectives

The future of pediatric neuro-oncology relies on the synergy between computational modeling and advanced bioengineering. Deep-learning approaches are poised to refine treatment scheduling by predicting the best pattern of treatment (duration, optimal dosing and timing) [85], while merging multiomics data on treatment response, genomics, epigenetics and proteomics [84]. Furthermore, this computational capacity enables a shift from single-pathway inhibition toward systems-level multidrug strategies that simultaneously target metabolism, angiogenesis, immunomodulation and neuro-tumoral crosstalk [86]. Mathematical modeling will be essential to define the optimal sequential timing of these combinations, ensuring that treatment protocols pre-emptively address tumor adaptation and resistance. Integrating deep-learning algorithms into functional precision medicine programs promises to transform data interpretation by linking pharmacological responses to molecular features, facilitating the rapid development and validation of new predictive biomarkers [84]. Simultaneously, the growing democratization of microfluidic platforms, successfully implemented in adult glioblastoma [87] and progressively adopted in MB [88], provides new opportunities to validate screening hits from limited biopsy samples. Moving forward, the hits identified through these various screening approaches could be tested in complex coculture systems, such as cells or tumoroids maintained on organotypic brain slices, thereby providing the high-fidelity frameworks necessary to model the neuro-oncological niche [27,74,83]. These next-generation, patient-centered assays will be the cornerstone for developing tailored, less toxic therapies, ensuring that precision medicine evolves from retrospective discovery to real-time therapeutic guidance (Figure 2).

### Outstanding questions

AI integration: How can deep-learning pipelines be seamlessly integrated into real-time daily clinical workflows?

Trial design: How can adaptive and basket trial designs offset high tumor heterogeneity with small sample sizes in rare malignancies?

Translational platforms: How can integrated *in vivo* models bridge the gap between retrospective validation and prospective prediction in pediatric brain tumors?

Interdisciplinary hubs: How can hospitals restructure into collaborative ecosystems that effectively bridge science, engineering, and care?

Global equity: How can international clinical trials be adapted to resource-limited settings to ensure global therapeutic equity?

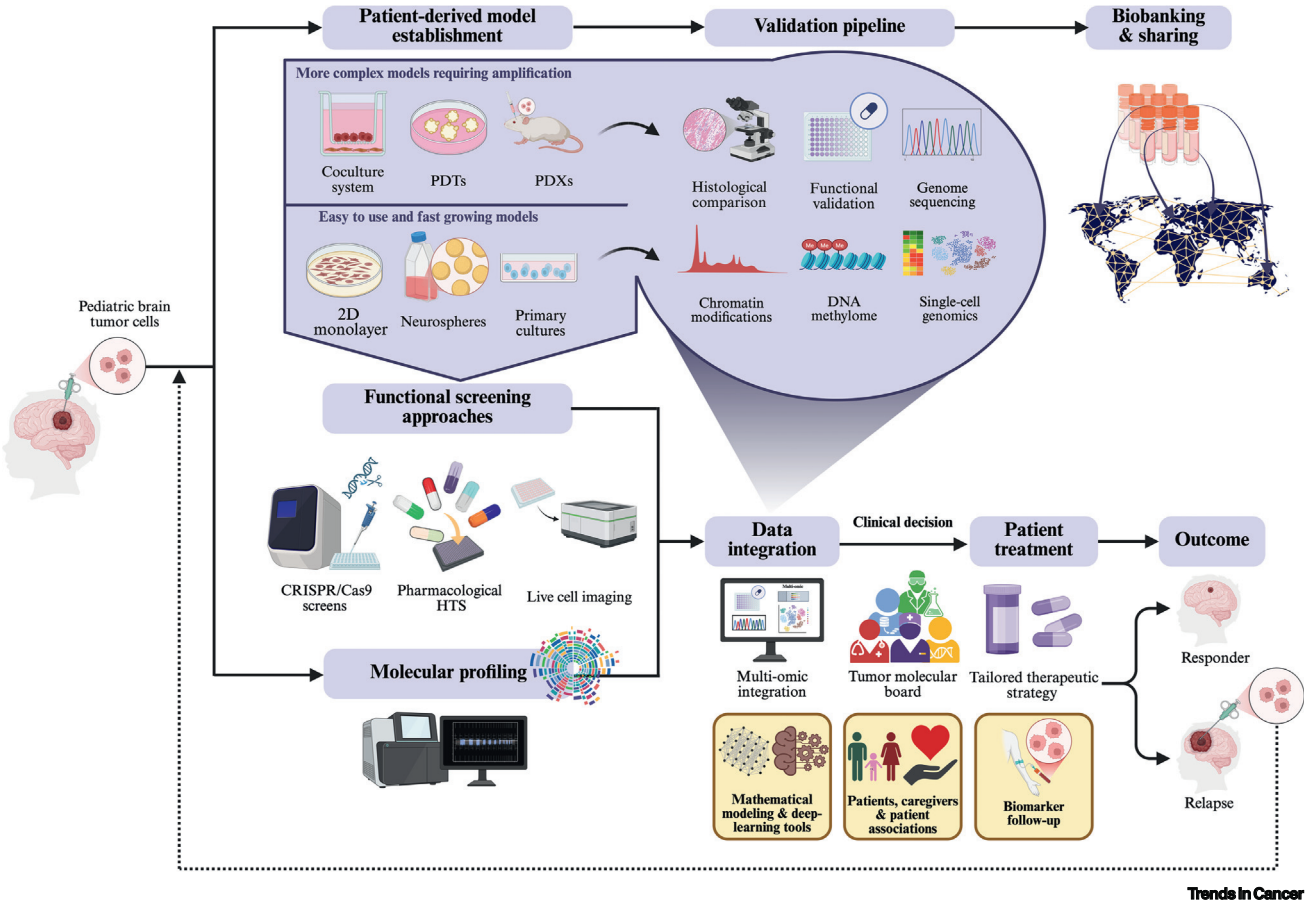


Figure 2. The future of pediatric neuro-oncology. From biopsy to *ex vivo* drug screening and molecular profiling, including model establishment, amplification and validation. Functional precision medicine combines molecular profiling with drug sensitivity testing of patient-derived tumor models. The multiomic analyses and data integration rely on deep learning and mathematical modeling to inform therapeutic decisions and ultimately improve patient outcomes. HTS: high-throughput screening; PDTs: patient-derived tumoroids; PDXs: patient-derived xenografts. The figure was created using BioRender.

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## Declaration of interests

All authors declare that they have no competing interests or other interests that might be perceived to influence the interpretations of the article.

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