

Title: Building a Pediatric CNS Tumor Liquid Biopsy Program: Overcoming Barriers to Expand Access and Collaboration

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Background: Circulating tumor DNA (ctDNA) analysis has emerged as a promising tool for the management of pediatric central nervous system (CNS) tumors. However, implementation remains concentrated to a relatively limited number of specialized centers with established clinical research infrastructure and sequencing expertise. Many pediatric neuro-oncology programs face substantial barriers to developing similar capabilities, limiting broader participation in and contribution to liquid biopsy research.

Methods: We describe the development of a pediatric CNS ctDNA program at a quaternary children's hospital with limited research infrastructure and genomic testing capabilities. Key elements include protocol development, Institutional Review Board approval, patient identification and enrollment, longitudinal blood and cerebrospinal fluid (CSF) collection workflows, specimen processing and storage procedures, and partnerships for molecular sequencing and analysis.

Results: Fifty-seven patients were enrolled over four years, resulting in collection of 417 longitudinal blood and cerebrospinal fluid samples. Standardized workflows for enrollment, specimen collection, processing, storage, and clinical annotation were successfully implemented. Strategic funding facilitated stability of a sample biorepository. Successful implementation required coordination among a multidisciplinary team spanning clinical care, research operations, laboratory sciences, and institutional administration.

Conclusions: Development of a pediatric CNS tumor liquid biopsy program is feasible at institutions without extensive pre-existing liquid biopsy infrastructure. Structural, operational and cultural barriers can be overcome by establishing standardized collection workflows, multidisciplinary collaboration, and external partnerships. Our experience provides a practical framework for other centers seeking to participate in pediatric CNS tumor liquid biopsy research.

Keywords- biorepository, ctDNA, liquid biopsy

Key points

- Expanding institutional participation in pediatric CNS tumor liquid biopsy research is essential to advance clinical translation.
- Establishing a circulating tumor DNA biorepository is feasible even at centers with limited research infrastructure.

Importance of the study:

Expansion of pediatric neuro-oncology participation in liquid biopsy research and clinical care can be accelerated through shared experiences in developing a liquid biopsy program, helping reduce barriers to implementation and broaden access to this emerging technology.

Lay summary:

Circulating tumor DNA (ctDNA) has the potential to improve monitoring and treatment of children with brain tumors. However, most ctDNA programs are limited to large research centers with specialized research infrastructure. We describe how our institution successfully developed a pediatric brain tumor liquid biopsy program despite limited existing infrastructure or experience with ctDNA analysis. Over four years, we enrolled 57 patients and collected 417 longitudinal samples. Through standardized workflows, multidisciplinary collaboration, and

external partnerships for molecular testing, we established a sustainable biorepository and research program that may serve as a model for other centers.

Introduction

Over the past decade, clinical application of liquid biopsy and circulating tumor DNA (ctDNA) analysis has rapidly gained traction, emerging as a promising tool in the management of pediatric patients with central nervous system (CNS) tumors. However, the current liquid biopsy landscape in pediatric CNS tumors reveals an inequality in scientific expertise, research impact, and clinical application across institutions. Many of the landmark studies and major advances in pediatric CNS tumor liquid biopsy have originated from a relatively small number of leading centers (1-12). These institutions possess integrated translational pipelines linking their clinical neuro-oncology teams to skilled molecular pathologists and bioinformaticians overseeing high-throughput sequencing platforms in College of American Pathologists (CAP)/Clinical Laboratory Improvement Amendments (CLIA)-certified genomic laboratories for institutions in the United State of America or in laboratories with equivalent qualifications at international institutions. They also benefit from large patient volumes, multidisciplinary research teams capable of blood and cerebrospinal fluid (CSF) collection, and reliable grant funding. In contrast, many neuro-oncology programs struggle to gain momentum in this space due to lack of access to the infrastructure required to routinely implement ctDNA analysis.

If more pediatric oncology centers began routinely collecting and banking serial blood and CSF samples from children with CNS tumors, it would have the potential to dramatically accelerate

the field by expanding the available datasets needed to refine assays, validate biomarkers across tumor types, and perform large prospective studies. Expanded participation could also enable the creation of multi-institutional liquid biopsy repositories, analogous to existing imaging and tissue consortia, facilitating rapid discovery of biomarkers for minimal residual disease, treatment response, and early relapse detection. Ultimately, this access could accelerate the progress towards the end goal of liquid biopsy improving overall survival and quality of life for children with CNS tumors.

Herein, we describe our single institution experience at a quaternary children's hospital developing a liquid biopsy program with established workflows to collect blood and CSF from patients with newly diagnosed or relapsed/refractory CNS tumors. We detail the process of protocol development, Institutional Review Board (IRB) approval, patient identification, sample collection and processing, cataloguing and long-term storage, establishment of a biorepository, and considerations for collaborative partners to facilitate molecular sequencing and bioinformatic analysis. We also assess the key components of the large multidisciplinary team required to build a liquid biopsy program as well as the financial barriers that must be overcome for program success.

At the time of project initiation, our institution lacked several infrastructural components commonly present at leading liquid biopsy centers, including an established workflow for longitudinal sample collection and storage, dedicated clinical research staff for enrollment and clinical annotations, and local capacity for ctDNA extraction and high throughput ctDNA sequencing. With many challenges to overcome, the initial focus of this project was on

establishing a feasible workflow for sample collection and biobanking. After successful implementation, the secondary focus became identifying clinically meaningful questions that could be addressed using the banked specimens and establishing collaborative partnerships to facilitate sample sequencing and analysis. The successful development of our longitudinal biorepository and collaborative analytic infrastructure may serve as a blueprint for other centers seeking to implement similar programs.

Methods

Institutional Review Board Approval

The first step in establishing our ctDNA biorepository involved obtaining Institutional Review Board (IRB) approval (**Figure 1**). To allow for flexibility in the future direction of studies utilizing these samples, our study's proposed aims were intentionally open-ended. Our stated aim was to collect, process, and bank extra plasma and/or CSF samples from pediatric neuro-oncology patients for use in ctDNA analysis as well as inclusion in future research projects and/or collaborations. Because all sample collections were obtained at the time of clinically indicated blood draws or procedures during which CSF was already being collected for clinical purposes, the IRB determined the study to involve no greater than minimal risk. Safe blood and CSF collection volumes were established based on patient age and body surface area. Specimen collection remained within these predefined limits, and clinical need was prioritized over research sample acquisition. IRB fees related to protocol review and study start-up were waived.

Funding

Institutional pilot grant funding was obtained for expenses related to study start up and initial sample collection and processing. More substantial recurring funding was secured from private philanthropic organizations as more patients were enrolled and sample accrual accelerated. These funds were also utilized to secure protected time for key clinical research staff, cover expenses related to molecular sequencing of longitudinal samples, as well as to support travel to present research findings at academic conferences.

Patient Identification and Informed Consent

Pediatric patients with newly diagnosed or treatment-refractory/recurrent CNS tumors were eligible for study inclusion. Patients were identified through multidisciplinary neuro-oncology tumor board, neurosurgery team morning report, overnight oncology sign-out, and pediatric intensive care unit consults. In the early stages of this study, priority was given to patients with higher-grade, aggressive tumors. We broadened our catchment to all brain tumor types as our workflow became more refined and efficient. When feasible, patients were approached for consent discussion prior to neurosurgical intervention or tumor resection. Patients were enrolled after completing the informed consent process with their oncologist, advanced practice provider, or an assigned clinical research coordinator affiliated with the study (**Figure 1**).

Biospecimen Sample Collection and Processing

Initial blood and CSF (where applicable) samples were collected at the time of study enrollment. Additional longitudinal samples were taken throughout the patient's treatment course and off-therapy surveillance period. Collection occurred at the time of scheduled clinical lab draws, following venous access to facilitate imaging studies, or during planned CSF collection

procedures (e.g. lumbar puncture, Ommaya reservoir tap). Plasma was collected approximately every three months for patients actively on chemotherapy and additionally at times of significant change in clinical trajectory, including at time of relapse or progression. For patients off-therapy, plasma samples were taken at intervals dictated by their surveillance imaging schedule. No specimen collections or procedures were performed solely for research purposes (**Figure 1**). Standardized protocols for sample collection and processing were established through review of literature, existing biorepository procedures and in discussion with external collaborators.

Venous blood samples at target volumes up to 20 mL, not exceeding 3mL/kg of body weight per 24 hours, were collected in EDTA tubes and processed within one to three hours of collection. Samples were centrifuged at 1500 relative centrifugal force (rcf) for 15 minutes at 4 °C. Plasma was aliquoted into 1-2mL samples. Two 0.5-1mL cell pellet aliquots were also reserved. Cerebrospinal fluid samples at target volumes of 1-10mL were collected intraoperatively or by lumbar puncture, Ommaya reservoir tap, or an external ventricular drain and placed into sterile untreated tubes. Within 15 minutes, samples were centrifuged at 3000 rcf for 15 minutes at 4 °C. The supernatant was aspirated and divided into 1-2mL aliquots.

Storage and Shipping

Processed plasma, cell pellet, and CSF samples were placed in a -80 °C freezer within 1-3 hours of collection and then transferred to the vapor phase LN2 (-196 °C) for long term storage until time of future batched analysis. To preserve patient privacy, patients and samples were deidentified by assigning an identification code separate from the patient's medical record number. Identification codes and storage of samples were tracked in SoftWear. Once a

collaborating laboratory was identified, select patient samples and at least one blood pellet were sent on dry ice along with accompanying histologic tumor slides for further processing.

Clinical Annotation

Clinical demographics such as patient age, gender, diagnosis, tumor location, and tumor molecular features were documented at the time of patient enrollment. Throughout each patient's treatment course, detailed clinical annotation including overall clinical status, presence or absence of active disease at time of sample collection based on most recent radiographic images, and concurrent therapies (e.g. chemotherapy, clinical trials, radiation therapy) was recorded at each collection timepoint. The type (e.g. plasma vs. CSF), volume, and source (e.g. IV, LP, Ommaya) of each specimen collection was also tracked.

Cost-Analysis

To provide a generalizable framework for centers seeking to establish a longitudinal ctDNA biorepository, we performed a cost-analysis to determine the most common expenses associated with this process. Acknowledging that every institution has unique infrastructure and is likely beginning the biorepository process from different starting points with variable levels of experience and available equipment, we opted to avoid citing exact monetary value of these expenses in favor of identifying more broad cost categories that can be applied to each individual center. Programmatic costs were organized into major domains including personnel, equipment, materials and supplies, service-related expenses, and institutional overhead. Within these domains, expenses were further categorized into fixed and/or variable cost structures, as well as a combination of one-time capital investments versus recurring operational expenses.

Results

Patient enrollment and sample collection:

Fifty-seven patients enrolled on the study over the first four-year period since IRB approval (**Figure 2A**). Twenty-one patients enrolled in year one, followed by an average of 12 patients per year for a total of 57 patients over the four-year period. Within the 57 patients, the average age at diagnosis was 10 years (range 1-18 years old, SD 5.5). The most common race/ethnicity was White (n=27, 47%), followed by Hispanic (n=19, 33%) and Black (n=7, 12%). A total of 92 longitudinal samples were collected in the first year followed by 121, 95, and 109 samples in years two through four, respectively (**Figure 2B**), totaling 417 samples (~7.3 samples/patient). This included 358 blood samples and 59 CSF samples. The average quantity of CSF and whole blood obtained was 5.18mL (range 0.75-20mL) and 10.1mL (range 2.5-20.75mL) respectively. The most common diagnoses of enrolled patients included medulloblastoma (n=19, 33%), diffuse midline glioma (n=9, 15%) and high-grade gliomas (n=7, 12%) (**Figure 2C**). Most patients (41/57, 71.9%) were enrolled within one week of their initial diagnosis.

Timeline of key events

A simplified chronological timeline of key events and project milestones provides a potential blueprint for other groups to follow while establishing their own ctDNA biorepository (**Figure 3**). This project was initially kickstarted by receipt of an internal \$15,000 departmental pilot grant. These early funds were designated to cover potential IRB administrative fees, clinical research coordinator effort, and startup laboratory fees for supplies related to sample collection, processing, and storage. Institutional Review Board submission, review, and acceptance took

approximately three months under the direction of a clinical research coordinator (CRC). Patient enrollment and sample collection began promptly following IRB approval. A second \$15,000 trainee support grant was secured to support further sample collection.

In anticipation of increasing financial needs as the project gained momentum, we then turned to outside philanthropic support for more substantial funding including an annual recurring endowment that would cover CRC effort, sample processing and storage, and future sequencing fees. Patient enrollment and sample collection continued over an approximately 3-year period until there were enough samples for initial batch selection for further sequencing and ctDNA analysis. Without an established ctDNA laboratory at our institution with the ability to perform ctDNA extraction, library preparation, and high-throughput sequencing, we partnered with a third-party, independent research institution to perform this analysis. By the approximate end of year five from time of initial study approval, two full batches of samples had completed the entire process from collection to sequencing and complete data analysis (**Figure 3**).

Cost-analysis

At our institution, some of the initial start-up expenses identified in our cost-analysis were able to be bypassed or subsidized by pre-existing institutional infrastructure. Nonetheless, it is critical to consider the financial burden these expenses could have on centers lacking such infrastructure. Personnel costs represented a substantial fixed annual investment. This could include effort from a physician-scientist/faculty director, clinical research coordinator, nurse coordinator, and/or laboratory technician, each contributing to regulatory oversight, patient enrollment, sample processing, and longitudinal clinical annotation (**Table 1**). Requisite personnel expenses likely

vary significantly between institutions depending on patient volume, faculty size, geographic region, extent of protected academic time for faculty, and available grant funding. Other identified equipment costs primarily incurred during program initiation include centrifuges, ultra-low temperature freezers, liquid nitrogen storage systems, and data management infrastructure. Ongoing maintenance costs can be scaled modestly with program expansion. Notably, many of these equipment infrastructure components may already exist at participating institutions, allowing for cost-sharing or partial allocation within existing laboratory environments. Other potentially substantial institutional overhead costs include fees to secure sufficient laboratory space and biosafety infrastructure (e.g. freezers, centrifuges, pipettes, and software for tracking) for sample processing. These items are often considered shared resources and were supported at the institutional level via a fixed cost structure or incorporated through variable indirect cost allocation structures.

Beyond these potentially significant fixed costs, several additional expenses follow a variable cost volume-based model. These included cost of materials and supplies required for specimen processing and storage (e.g., aliquoting supplies, reagents, cryopreservation materials), as well as service-related expenses such as specimen shipment, liquid nitrogen tank refills, and external laboratory fees for downstream ctDNA extraction, sequencing, and analysis. Additional potential variable costs included sample acquisition from external sources, pathology consultation for tissue review, and regulatory expenses related to IRB maintenance, which can vary by institutional policy (**Table 1**).

Sample Analysis

From the 417 total collected samples, 140 plasma and 27 CSF samples underwent further processing with cell-free DNA (cfDNA) extraction and subsequent sequencing. The average cfDNA yield was 16.4 ng (SD 23.9) from plasma samples and 65.7 ng (SD 155 ng) from CSF samples. Targeted next generation sequencing and shallow whole genome sequencing was performed on the samples. Analysis of the whole genome sequencing included copy number alteration and DNA fragmentomic analysis. Results from these molecular analyses will be reported separately.

Multidisciplinary Team Involvement

Establishing a collaborative workflow with effective delegation to defined personnel was crucial to the success of this project. This comprehensive team included personnel from multiple departments across the hospital resulting in a large multidisciplinary group. Although institutional structures may vary, the key components of our program included a collaborative effort from clinical providers, nursing and clinical coordination, research operations, bioinformatics and data scientist, sample collection and biorepository processing, lab and genomic analysis, institutional and administrative support, and external collaborators (**Figure 4**; as discussed further in the Discussion).

Discussion

Circulating tumor DNA analysis from blood and CSF has been shown to be both technically feasible and clinically informative for pediatric CNS tumors. Realizing the full clinical potential of liquid biopsy will require a coordinated effort to move beyond proof-of-concept studies toward large-scale validation, standardization, and clinical implementation. Central to this effort

will be expanding participation across a broader network of pediatric neuro-oncology centers committed to systematic, longitudinal biospecimen collection. Increased institutional engagement would accelerate progress toward standardizing pre-analytical protocols, refining optimal sequencing strategies, enabling multi-institutional collaborative trials, and generating the large datasets needed to establish clinical validity and utility. Without broader participation, progress will likely remain constrained to a limited number of specialized centers, slowing the translation of these promising technologies into routine clinical care.

In the last decade, several publications have highlighted the challenges of establishing and sustaining biorepositories, emphasizing the financial, operational, and social factors necessary for long-term success (13-15). While these reports have described institution-specific biobanking experiences and strategies to address common barriers, few have focused on the unique challenges associated with ctDNA biobanking in pediatric brain tumors. Furthermore, the literature has largely emphasized sample collection and storage, with limited discussion of the processes required for sample utilization. This study aims to address these gaps by describing both the establishment of a longitudinal pediatric CNS tumor ctDNA biorepository and the pathway from specimen collection to downstream molecular analysis.

The prospect of building a liquid biopsy program from scratch is certainly daunting. It is important to note that creating a comprehensive, standalone pipeline covering all aspects of the ctDNA workflow does not need to be the objective of each individual institution. Smaller pediatric oncology centers – or at least those less experienced in the liquid biopsy field – can still contribute meaningfully. One of the consistent limitations mentioned in several of the seminal

pediatric CNS liquid biopsy papers is the scarcity of longitudinally collected ctDNA samples. Therefore, any center can contribute serial samples of paired plasma and CSF with detailed clinical annotation of radiographic findings at correlating timepoints, details of treatment regimens, and long-term updates of clinical outcomes. This biological material can then be shared with collaborating centers when a research project or trial is identified that aligns with the types of available samples. These centers can also enroll patients on multi-institutional collaborative studies or consortium-based trials with centralized ctDNA extraction and sequencing capabilities.

An imbalance between growing scientific interest in ctDNA analysis for pediatric CNS tumors and the relatively small number of institutions actively leading these efforts reflects not a lack of interest, but rather the presence of various barriers that limit broader participation. These obstacles can be categorized as structural, operational/logistical, and cultural/institutional. Identifying successful ways to bypass or overcome these obstacles is critical for the successful development of new liquid biopsy programs (**Table 2**)

Structural

Barriers to establishing liquid biopsy programs manifest first at the structural level, where participation in ctDNA research is heavily influenced by access to institutional infrastructure. Many of the leading centers in this field have developed integrated translational pipelines that link clinical neuro-oncology programs with in-house molecular laboratories, bioinformatics teams, and high-throughput sequencing capabilities. In contrast, smaller or less-resourced institutions often lack access to specialized laboratory infrastructure, including CAP/CLIA-

certified sequencing platforms or the personnel required to support assay development and data analysis. Establishing and maintaining such platforms requires significant financial investment, technical expertise, and regulatory oversight, placing them out of reach for many pediatric centers. In parallel, the absence of dedicated bioinformatics support further constrains the ability to process, analyze, and interpret complex sequencing data. This creates a layer of dependency on specialized external collaborators for downstream processing, which introduces additional regulatory requirements, logistical complexity, delays in data generation, and costs. Furthermore, traditional funding mechanisms tend to favor established programs with existing infrastructure and preliminary data, reinforcing a cycle in which well-resourced centers continue to expand their capabilities while emerging programs face difficulty securing the support needed to initiate similar efforts.

In our own experience, we were able to avoid what would otherwise have been exorbitant start-up expenses by utilizing features of our existing - albeit limited - institutional research infrastructure. For example, we were able to allocate freezer space in the pathology lab for our longitudinal plasma and CSF samples and utilize already-purchased centrifuges, pipettes, and aliquot tubes for sample processing. Similarly, we identified an experienced clinical research coordinator within our oncology division with bandwidth to take on additional responsibilities and re-allocated their time to include overseeing the regulatory aspects of our ctDNA efforts (**Table 2**). Taking advantage of these existing resources reduced upfront costs during the early stages of our project. By utilizing smaller institutional grants, we were able to cover start-up expenses and establish a workflow. After demonstrating the feasibility of our liquid biopsy program, we were able to secure additional funding with philanthropic organizations to support

sequencing and bioinformatic analyses through our external collaborators. This staged funding model may be particularly relevant for emerging programs seeking to establish early momentum without reliance on large external grants.

Our cost framework highlights that the financial requirements for establishing a biorepository are not solely driven by start-up expenses and high-cost sequencing platforms, but rather by the cumulative operational demands of longitudinal biospecimen collection, processing, and annotation (**Table 1**). By prioritizing development of standardized workflows and leveraging existing institutional infrastructure and external partnerships for molecular analysis, the overall financial burden can be mitigated substantially. This model enables centers without in-house sequencing capabilities to participate meaningfully in ctDNA research through high-quality biospecimen contribution, supporting a more distributed and collaborative research ecosystem. Collectively, these findings demonstrate that while establishing a longitudinal biorepository requires coordinated investment across multiple cost domains, most expenses are scalable and can be adapted to local resources, supporting broader institutional participation beyond highly resourced centers.

Operational/Logistical

Beyond infrastructure limitations, there are substantial operational challenges associated with implementing a ctDNA program. Standardization of pre-analytical variables—including specimen collection, processing, and storage—is critical for generating reproducible and biologically meaningful results, yet these workflows are often not well-established or widely utilized, even at leading centers (16-18). In the absence of standardized workflows and dedicated

personnel, variability in sample collection, processing time, centrifugation protocols, and storage conditions can significantly impact specimen quality and downstream assay performance. This further limits the ability to combine specimens and collaborate with other institutions. The successful collection of high-quality, longitudinal ctDNA samples at clinically relevant timepoints—particularly from CSF— adds further complexity and requires precise coordination across multiple clinical environments, including operating rooms, inpatient units, outpatient clinics, and procedural suites. This necessitates clear communication across multidisciplinary teams, including oncologists, neurosurgeons, nursing staff, and clinical research coordinators (**Figure 4**). In many institutions, limited research staffing and competing clinical demands make it difficult to sustain such efforts over time. These challenges are amplified in centers where clinical staff must balance research activities with competing clinical responsibilities, limiting the reliability and reproducibility of sample acquisition. Additionally, many institutions lack integrated data management systems capable of linking biospecimens with longitudinal clinical, radiographic, and treatment data, thereby reducing the overall utility of collected samples for downstream analyses.

As an institution where clinical staff is balancing research and clinical responsibilities, we were tasked with overcoming these operational and logistical barriers. As such, we chose to initiate this project with a small group of individuals (i.e. neuro-oncology physician, biorepository manager, and CRC) to establish standardized protocols. Within this small group, protocolized workflows for approaching and consenting patients, communication between teams for sample collection, sample processing and storage, and clinical annotations were established. Early patient enrollment revealed areas for improvement within the established workflows, prompting

modifications that enhanced the efficiency and consistency of sample collection, processing, and data annotation. Following identification of a research question and establishment of a partnership with the sequencing laboratory, the team expanded to include additional personnel, each trained in defined responsibilities and protocolized workflows (**Figure 4**). Clinical providers including neuro-oncologists, neurosurgeons, and advanced practice providers were often the first members of the team to identify and interact with eligible patients. Appropriate communication between these groups ensured potential patients were identified early in the diagnosis. Radiologists were essential for initial differential diagnosis and subsequent disease status on follow-up MRIs. Pathologists played a key role in patient diagnosis and identification of viable tumor cells for sequencing purposes. Nursing and clinical coordination included the bedside nurses who assisted with blood draws, the clinical research nurse (CRN) to place orders at specimen collection timepoints, and the nurse coordinators who were the main point of contact for the family during and after treatment.

From the research team, initial efforts towards establishing the IRB required a clinical research coordinator (CRC) to ensure appropriate submission of all required documents including consent forms. Additional research team requirements included support for organizing the collection of samples, sample labeling and processing support, and study database management to keep track of clinical annotations, patient enrollment and specimen collection timepoints. Consenting patients was a combined effort between the neuro-oncology physician, CRN, and CRC. Sample collection and biorepository processing were supported by phlebotomists for blood collection as well as procedural nurses for CSF collection. As previously mentioned, the established biorepository at our institution was able to help support sample centrifugation, aliquoting, and

quality control. Samples collected before or after opening hours could be processed by the CRC and stored until the next business day.

Because our institution lacked in-house capabilities for ctDNA extraction, library preparation, sequencing, and bioinformatic analysis, we partnered with an external laboratory that has these capabilities. We utilized an existing collaboration with a laboratory in a private, non-profit research institute that provided expertise in molecular laboratory diagnostics, sequencing operations, laboratory management, and genomic analysis. Launching a relationship with a new institute involved negotiating a Research Collaboration Agreement covering operational, legal, publication, data rights, and fiscal terms. The laboratory had a preexisting pipeline for analyzing cfDNA. Additional personnel were available for analysis including bioinformaticians, data scientists, and database administrators. Lastly, institutional and administrative support was imperative to the success of the project. Department leadership, financial managers, legal specialist and information technology support all contributed to the background stability of the project (**Figure 4**).

Cultural

Less tangible, yet equally important, are the strategic and cultural barriers that may limit institutional engagement. Centers without prior experience in liquid biopsy research may perceive the field as prohibitively complex or inaccessible, particularly when compared to well-established programs producing high-impact data. This perception can lead to hesitation in committing resources toward program development, especially in environments where research infrastructure is already limited. Furthermore, the absence of clearly defined entry points for

collaboration with established sequencing centers may hinder participation, reinforcing a cycle in which only a small number of institutions contribute meaningfully to the growing body of literature. Moreover, these efforts are frequently driven by a small number of motivated individuals without dedicated protected time or institutional support, raising concerns regarding sustainability and scalability. Without broader institutional buy-in, even well-designed protocols may struggle to achieve consistent implementation.

An additional consideration is the perception that limited patient volume inherently restricts meaningful contribution to this field. While high-volume centers are well positioned to generate large datasets, this perspective overlooks the value of distributed, multi-institutional sample collection. Many smaller centers care for clinically and demographically diverse patient populations that are underrepresented in existing datasets. The primary limitation, therefore, is not simply patient volume, but rather the absence of infrastructure and workflows to systematically capture, annotate, and share these biospecimens. Expanding participation across a broader network of institutions has the potential to improve the representativeness of ctDNA datasets and enhance the generalizability of emerging biomarkers.

In this context, our experience demonstrates that these barriers, while substantial, are not insurmountable. Rather than attempting to replicate the comprehensive infrastructure of larger centers, we focused on developing a pragmatic and scalable workflow centered on systematic biospecimen collection and biobanking, leveraging existing clinical touchpoints without introducing additional procedural burden to patients. By embedding sample acquisition into routine clinical care, utilizing a flexible IRB framework with open-ended study aims, and

prioritizing longitudinal collection with detailed clinical annotation, we were able to establish a high-quality biorepository in a resource-constrained setting. This approach directly addresses one of the most consistently cited limitations in the field - the scarcity of longitudinally collected samples.

A critical component of this model is the deliberate integration of external partnerships for downstream molecular analysis. By collaborating with established laboratories or larger institutions equipped for ctDNA extraction, library preparation, and sequencing, liquid biopsy programs without internal genomic infrastructure can still contribute to and benefit from high-quality translational research. This distributed framework effectively decouples biospecimen generation from sequencing capacity, lowering the barrier to entry and enabling broader institutional participation. Importantly, such collaborations also facilitate knowledge exchange, providing opportunities for capacity building in bioinformatics and translational science over time.

Conclusions

Taken together, our workflow highlights a decentralized model of participation in ctDNA research, in which smaller centers contribute high-quality, longitudinally annotated biospecimens while leveraging collaborative networks for advanced molecular analysis. Broader adoption of this model could accelerate the development of multi-institutional biorepositories, improve assay validation across diverse patient populations, accelerate biomarker discovery, and reduce overreliance on a limited number of highly resourced centers. Ultimately, such an approach may be essential to ensure that advances in precision medicine are informed by representative patient

populations. By reframing the threshold for engagement—from comprehensive technical capability to high-quality biospecimen contribution—more institutions can play an active role in advancing the field. Ultimately, broadening participation in liquid biopsy research will be critical to realizing its full potential as a clinically actionable tool for improving outcomes in children with CNS tumors.

Future efforts of the field should focus on lowering barriers to entry through the development of standardized collection protocols, centralized processing hubs, and consortium-based regulatory frameworks that streamline collaboration across institutions. Funding mechanisms specifically designed to support early-stage infrastructure at emerging programs may further enhance participation in this space. As the field moves toward clinical implementation, ensuring broad institutional engagement will be critical to generating the robust, generalizable datasets required to establish ctDNA as a clinically actionable tool in pediatric neuro-oncology. As clinical utility increases, serial CSF acquisition may become more routinely incorporated into clinical care, highlighting the need for standardized protocols for preventing post procedure complications including post-spinal headaches. Continued improvements in the speed of technology and turnaround time for results are imperative for this real-time clinical implementation.

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B.P. Methodology, Writing – review & editing

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Ethics approval: Study was approved by IRB 21-240.

Data availability: Data generated and analyzed during the current study are not publicly available due to patient privacy considerations. De-identified data may be made available from the corresponding author upon reasonable request and with appropriate institutional approvals.

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Figure 1. Patient Enrollment and Sample Collection Workflow: The biorepository was developed through a stepwise process including IRB approval, patient enrollment, specimen collection, and biospecimen processing/storage. Blood and CSF samples were collected longitudinally from enrolled patients during routine clinical care in the operating room, oncology clinic, radiology department, and inpatient units. Samples underwent standardized processing consisting of specimen intake, centrifugation, aliquoting, and storage at -80°C for future molecular analyses. Created with BioRender.com.

Figure 2. Patient enrollment, sample collection and diagnosis distribution: **A.** Patient enrollment over the first four years of the biorepository. Bars represent the number of newly enrolled patients each year, while the line indicates the cumulative number of enrolled patients over time.

(B) Specimen collection over time. Bars represent the number of blood and/or CSF specimens collected annually, while the line demonstrates the cumulative number of banked specimens.

(C) Distribution of enrolled patients by tumor diagnosis.

Figure 3. Timeline of Key Events: Timeline illustrating key milestones in the establishment and growth of the biorepository over the first five years. Colored markers denote institutional grant funding (blue), philanthropic funding (red), and project milestones (gold).

Figure 4. Multidisciplinary Team Structure. The successful development of a pediatric ctDNA biorepository at our institution required coordinated efforts across multiple disciplines. Together, these groups form the infrastructure necessary to support longitudinal biospecimen

collection, molecular characterization, and translational liquid biopsy research in pediatric CNS tumors. Created with BioRender.com.

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Lay summary:

Circulating tumor DNA (ctDNA) has the potential to improve monitoring and treatment of children with brain tumors. However, most ctDNA programs are limited to large research centers with specialized research infrastructure. We describe how our institution successfully developed a pediatric brain tumor liquid biopsy program despite limited existing infrastructure or experience with ctDNA analysis. Over four years, we enrolled 57 patients and collected 322 longitudinal samples. Through standardized workflows, multidisciplinary collaboration, and external partnerships for molecular testing, we established a sustainable biorepository and research program that may serve as a model for other centers.

Cost Type	Description	Cost Category
Personnel		
Physician/Faculty Director	Part of Salary and Fringe- Level of effort may be cost shared by the institution via protected time	Fixed annual
Research/Lab Technician	Part of Salary and Fringe- Individual responsible for processing and tracking all samples. Regulatory responsibilities.	Fixed annual
Clinical Research Coordinator	Part of Salary and Fringe- Individual responsible for regulatory oversight and participant coordination	Fixed annual
Equipment		
Centrifuge	Acquisition and annual maintenance service contracts*	Capital Investment/Variable based on volume if using a central shared service
Freezer	Acquisition and annual maintenance service contracts*	Capital Investment/Variable based on volume if using a central shared service
Liquid nitrogen tank	Acquisition and annual maintenance service contracts*	Capital Investment/Variable based on volume if using a central shared service
Computer/Software programs	Computer for staff which must include software to track samples	Capital Investment and ongoing annual renewal costs
Materials and supplies		
Aliquoting supplies	Pipettes, tubes, etc.	Variable based on volume
Lab consumables and supplies	Chemicals, reagents, dry ice, shipping boxes, etc.	Variable based on volume
Service costs		
Sample acquisition	Obtaining samples from existing biorepositories and sites	Variable based on volume
Shipping costs	If using outside laboratory for sample sequencing or analysis	Variable based on volume
Liquid nitrogen	Monthly cost from gas provider	Variable based on volume
DNA sequencing	External providers generally provide a per sample rate	Variable based on volume
Sample analysis services	External providers generally provide a per sample rate	Variable based on volume
Other costs		
External consults (pathology, other specialties)	May be based on salary and fringe for internal staff or a consulting rate. Typically charged as a per consultation cost for reviewing tissue slides	Variable based on need
Institutional Review Board costs	Regulatory fees based on institutional fee schedule. Initiation and annual review of protocol. Some institutions may also charge a fee for amendments.	Annual and variable
Overhead costs		
Lab space	Access to designated space for sample collection and processing. Some institutions calculate an infrastructure cost based on an allocation model	Fixed, institution investment
Access to Hood	Access to a hood for sample processing. Some institutions calculate an infrastructure cost based on an allocation model	Fixed, institution investment

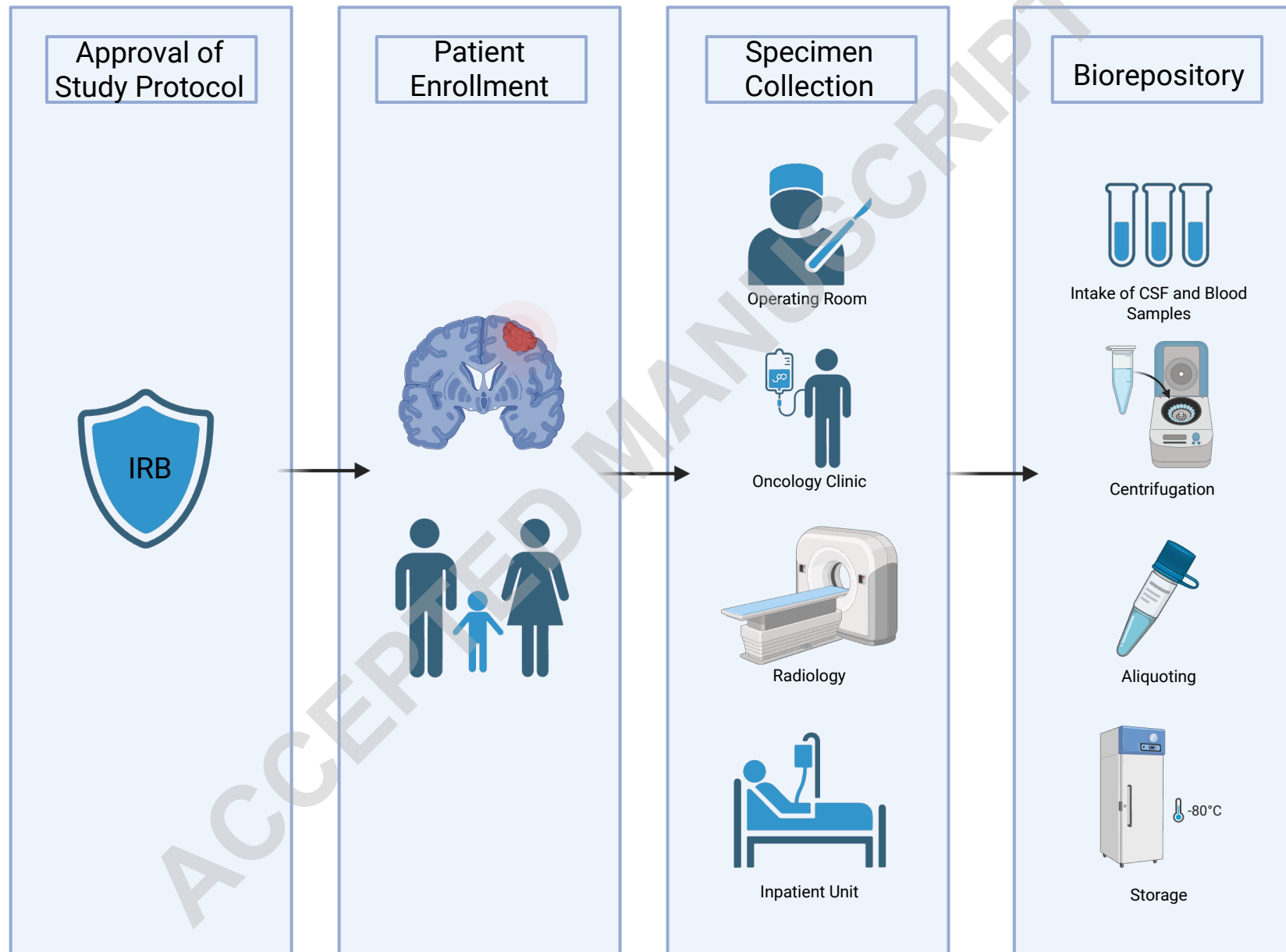
* Most institutions calculate a 5-year depreciation plan on capital acquisitions. It would be advisable to leverage any existing resource via a partial contribution to expense.

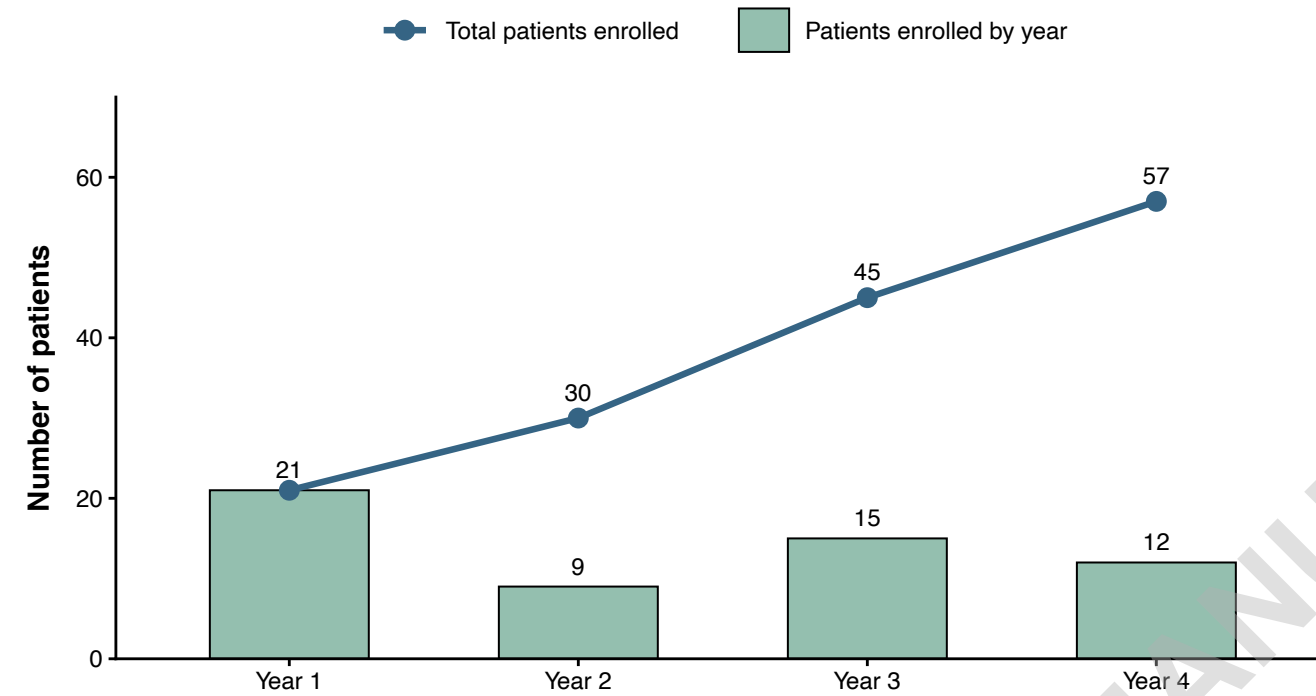
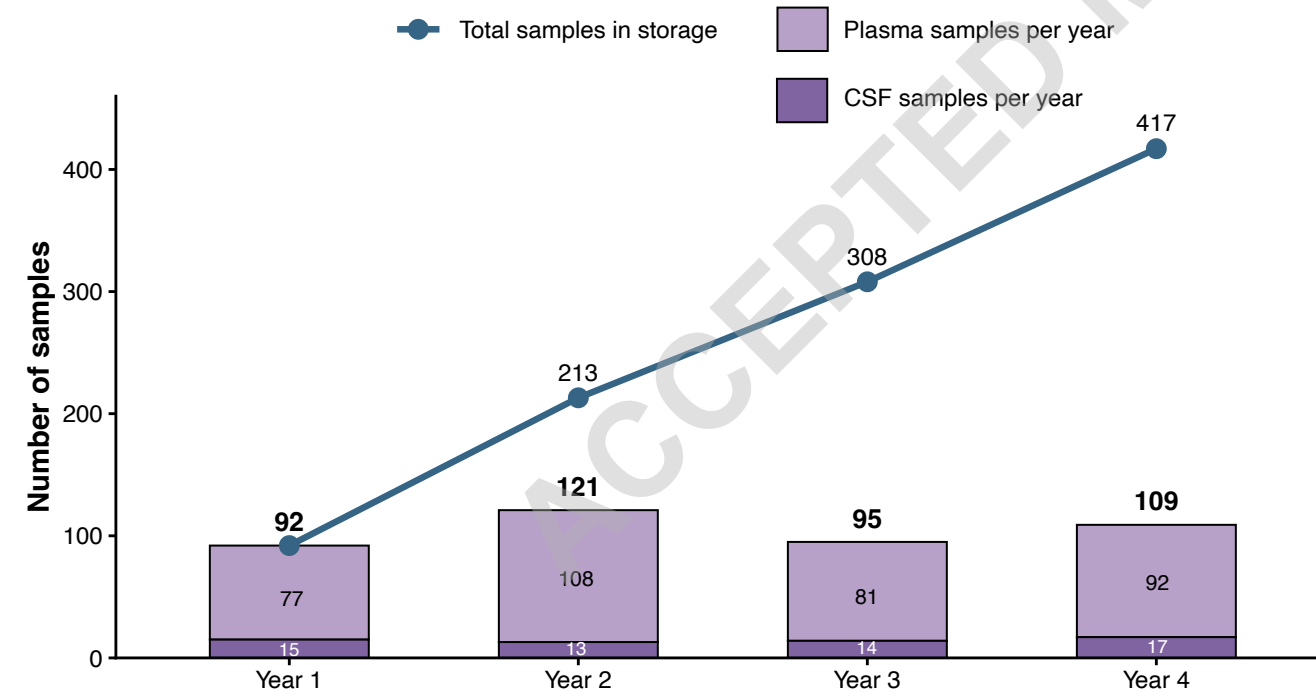
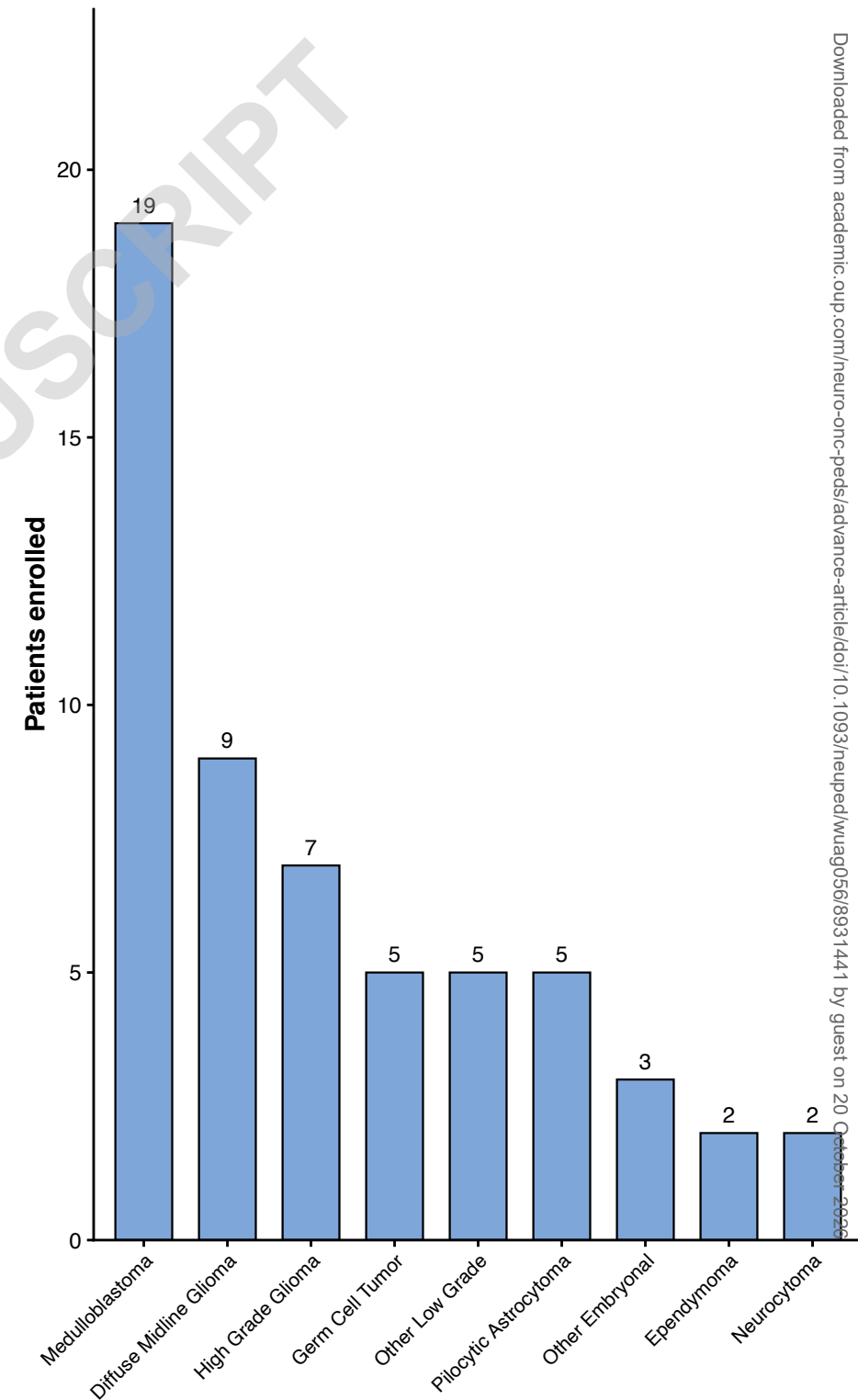
Table 1. Costs categories associated with a pediatric CNS tumor biorepository

Domain	Barrier	Impact	Practical Solution / Strategy
Structural			
Infrastructure	Lack of CAP/CLIA-certified sequencing facilities	Inability to perform ctDNA extraction, library preparation, and high-depth sequencing locally	Partner with external academic or private laboratories with established ctDNA platforms; defer sequencing while prioritizing biospecimen collection
Bioinformatics	Limited access to bioinformatics expertise	Inability to process, analyze, and interpret sequencing data	Utilize collaborator-based analysis pipelines; incorporate bioinformatics support through external partnerships; consider centralized analysis models
Biorepository Infrastructure	Limited access to sample processing and storage equipment (centrifuge, freezer)	Inability to properly preserve samples risks sample degradation and loss	Share equipment with existing institutional laboratories; purchase equipment as study grows and funds accrue. Consider collecting samples in tubes containing preservative agents and shipping to genomic laboratories for processing.
Operational/logistical			
Regulatory / IRB	Perceived complexity of protocol approval	Delays in study initiation	Design minimal-risk protocols requesting additional CSF or plasma samples collected during standard care; use broad, flexible study aims to allow future use
Research Staffing	Limited availability and bandwidth of clinical research coordinators	Difficulty sustaining longitudinal sample collection and annotation	Start with small-scale enrollment; expand staffing as funding increases
Clinical Workflow Integration	Lack of standardized processes for sample collection and processing	Variability in sample quality; reduced reproducibility	Develop simple, protocolized workflows and disperse to all parties involved with sample collection; emphasize frequent communication between teams
Financial Resources	High cost of startup including consumables, personnel and sequencing	Limits program initiation and sustainability	Start with sample collection and storage; pursue smaller internal pilot grants and philanthropic funding; delay high-cost sequencing until adequate sample accrual
Data Annotation	Inconsistent or incomplete clinical data collection	Reduces downstream research utility	Standardize clinical annotation (radiographic status, treatment timeline, outcomes) at each collection timepoint and designate personnel to task
Cultural			
Patient Volume	Relatively lower patient numbers compared to large academic centers	Reduced statistical power and slower accrual	Focus on longitudinal sampling per patient; contribute to multi-institutional consortia
Institutional Experience	Limited prior experience in liquid biopsy research	Hesitation to initiate new programs	Begin with a focused, feasible component (biobanking); build expertise incrementally through collaboration with field experts
Perception / Culture	Belief that full in-house sequencing capability is required	Discourages participation from smaller centers	Reframe contribution model: emphasize value of high-quality samples with clinical annotation
Collaboration Barriers	Lack of established partnerships with sequencing centers	Limits ability to translate samples into data	Proactively seek academic collaborations; leverage existing networks, working groups, and consortia to identify partners
Time to Impact	Delay between sample collection and downstream sequencing	Slows publication and perceived productivity	Batch samples for future sequencing; emphasize repository-building phase as success in foundational infrastructure

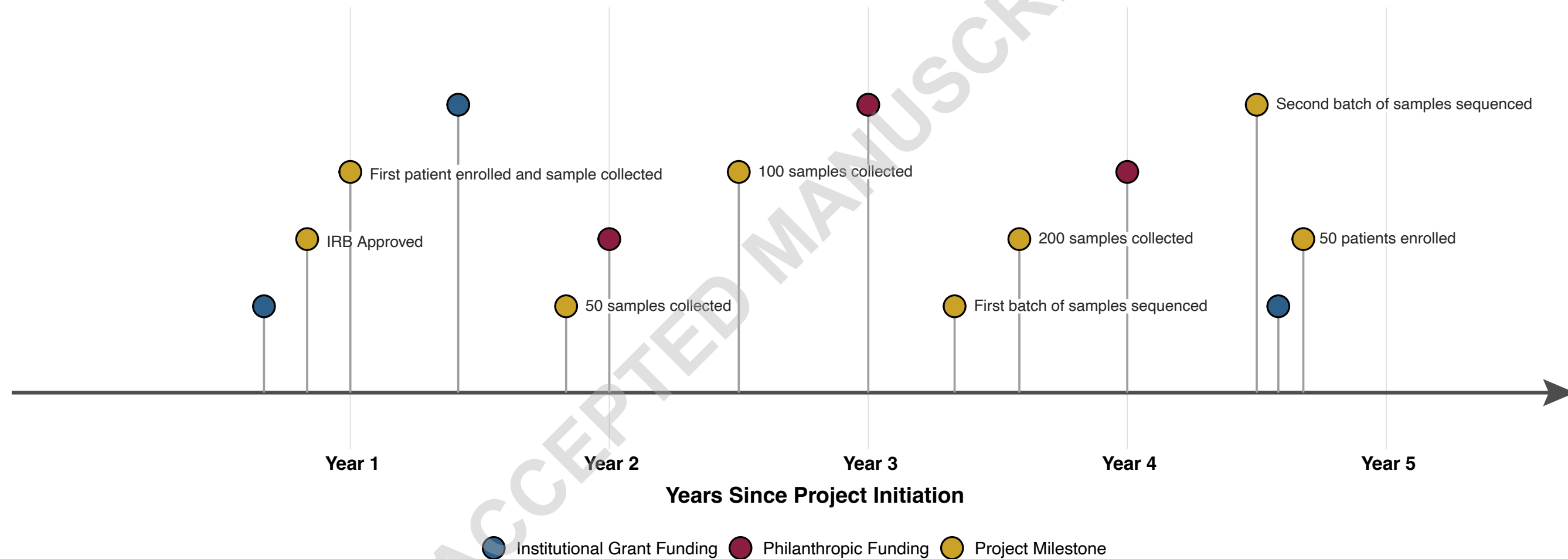
Table 2. Common barriers to developing a ctDNA biorepository with suggested solutions or strategies

Figure 1



A. Patient Enrollment Over Time**B. Sample Collection Over Time****C. Patients Enrolled by Diagnosis**

Development Timeline for Pediatric CNS Tumor ctDNA Biorepository



Multidisciplinary Team Essential for Pediatric CNS ctDNA Biorepository Program

