Biological material collection to advance translational research and treatment of children with CNS tumours: position paper from the SIOPE Brain Tumour Group

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Paediatric CNS tumours are the most common cause of childhood cancer-related morbidity and mortality, and improvements in their diagnosis and treatment are needed. New genetic and epigenetic information about paediatric CNS tumours is transforming the field dramatically. For most paediatric CNS tumour entities, subgroups with distinct biological characteristics have been identified, and these characteristics are increasingly used to facilitate accurate diagnoses and therapeutic recommendations. Future treatments will be further tailored to specific molecular subtypes of disease, specific tumour predisposition syndromes, and other biological criteria. Successful biomaterial collection is a key requirement for the application of contemporary methodologies for the validation of candidate prognostic factors, the discovery of new biomarkers, the establishment of appropriate preclinical research models for targeted agents, a quicker clinical implementation of precision medicine, and for other therapeutic uses (eg, for immunotherapies). However, deficits in organisational structures and interdisciplinary cooperation are impeding the collection of high-quality biomaterial from CNS tumours in most centres. Practical, legal, and ethical guidelines for consent, storage, material transfer, biobanking, data sharing, and funding should be established by research consortia and local institutions to allow optimal collection of primary and subsequent tumour tissue, body fluids, and normal tissue. Procedures for the collection and storage of biomaterials and related data should be implemented according to the individual and organisational structures of the local institutions.

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Introduction

In most high-income countries, cancer is the leading, disease-related cause of death in children. CNS tumours are the most common group of solid paediatric malignancies and the most common cause of cancer-related morbidity and mortality in this age group, with an annual incidence of 4·01–5·37 per 100 000 individuals for children aged 0–15 years.[1,2] Paediatric CNS tumours comprise a group of highly heterogeneous entities, and have strikingly different clinical and biological characteristics compared with adult CNS tumours.[3]

Despite considerable advances in imaging, neurosurgery, radiotherapy, and medical treatment, survival rates for most types of paediatric CNS tumours are lower than those for childhood leukaemia and many other solid tumour types in children.[4–6] Furthermore, patients who survive CNS tumours in childhood often have impaired quality of life, including frequent and disabling endocrine and neurocognitive impairments that negatively affect their physical and mental health and also their participation in society. These deficits can result from the tumour itself but also from surgery and additional CNS-directed therapies, which are known to be particularly detrimental when applied to immature, developing brains.[7,8] Improvements in diagnosis (including shorter time to diagnosis and more accurate diagnosis and risk stratification) and treatment are urgently needed.

Biological knowledge about paediatric CNS tumours has increased in the past 5–10 years, including the identification of novel entities and subgroups with prognostic and often therapeutic implications. The improved availability of biomaterials for biological characterisation before the start of postoperative treatment is required for the benefit of individual patients and for the timely clinical validation of knowledge and further scientific progress in the field.

Importance of biological assessments

New and innovative genomic and epigenetic information is increasingly transforming the diagnostic and clinical landscape for tumours of the CNS.[9,10] For most paediatric CNS tumour types, distinct subgroups with different epidemiological, clinical, and biological characteristics have been identified, and novel subgroups continue to emerge as profiling resolution and cohort sizes increase.[11–13] Because of the high clinical relevance of genomic information, genome-wide analysis of childhood CNS tumours has become increasingly important.[12] Consequently, some of the most robustly validated new biological parameters, especially in medulloblastoma, high-grade glioma, and ependymoma, were used to define tumour entities in the revised 2016 WHO Classification of Tumors of the Central Nervous System.[3]

For example, in the previous WHO classification,[14] the diagnosis of medulloblastoma entities was based on histopathological parameters (desmoplasia, nodularity, cytological anaplasia, and large-cell appearance). According to the principles of integrated diagnosis, the

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2016 classification defines medulloblastoma entities by both histological and genetic parameters. The genetic classification includes WNT-activated, sonic hedgehog (SHH)-activated *TP53*-mutated, SHH-activated *TP53* wild type, and non-WNT and non-SHH medulloblastoma entities (including the provisional variants group 3 and group 4).[3] The biological understanding and prognostic value of other parameters in medulloblastoma continues to emerge as profiling resolution and cohort sizes increase. Novel molecular subgroups predictive of disease risk within non-WNT and non-SHH (group 3 and group 4) tumours were reported in 2017,[11] and new biomarkers defined by specific aberrations (eg, chromosome 11 loss in group 4 medulloblastomas) have been identified,[15] which now require further validation before clinical use. Studies[16,17] have also shown that biological entities of medulloblastoma are associated with different clinical, neurocognitive, and health-related quality of life outcomes, and have different frequencies of postoperative complications (eg, less frequent cerebellar mutism and motor deficits, less pronounced decline of information processing speed, and better health-related quality of life in patients who survive SHH-activated medulloblastoma compared with all other subgroups). It has been agreed internationally[18] that patients should be diagnosed according to international standards and treated, whenever possible, on the basis of a molecularly informed clinical trial. The first international clinical trials of risk-adapted therapies focusing on the assessment of clinical, pathological, and molecular biomarkers are now underway (NCT02066220, NCT01878617), designed on the basis of risk-stratification schemes defined in previous trial-based biological research studies (eg, a study by Ellison and colleagues[19]). The first insights into the biology of medulloblastoma at relapse have shown that the disease evolves clonally, and that genetic events, such as combined *TP53* mutations and *MYCN* amplification, are commonly acquired at relapse.[20,21] Assessment of distinct molecular features at relapse will thus be essential for determining treatment strategy.

In the category of paediatric high-grade gliomas, the new molecularly defined tumour entity diffuse midline glioma histone H3 K27M-mutated (grade IV) was introduced in the 2016 WHO classification, defined by the presence of K27M mutations in the histone genes *H3F3A*, *HIST1H3A*, *HIST1H3B*, or *HIST1H3C*, as well as infiltrative growth characteristics and midline location. Notably, tumours with the H3G34R/V mutation (about 10–15%) were not defined as a separate entity despite differences in age, location, outcome, and biology compared with other high-grade gliomas. Furthermore, rare paediatric high-grade gliomas such as anaplastic ganglioglioma and anaplastic pleomorphic xanthoastrocytoma carry typical (but not defining) molecular alterations but are diagnosed by histopathological criteria. For paediatric oligodendroglioma, the 1p-19q co-deletion that defines adult oligodendrogliomas (according to the 2016 WHO classification) is mostly absent. *BRAF* mutations and homozygous *CDKN2A* or *CDKN2B* deletions frequently occur in paediatric anaplastic ganglioglioma, anaplastic pleomorphic xanthoastrocytoma, and epithelioid glioblastoma. Integrated genomic, epigenomic, and transcriptomic data from across anatomical compartments of the brain are needed to define subgroups within paediatric high-grade glial tumours (malignant glioma and diffuse intrinsic pontine glioma) and to establish novel therapeutic targets.[22,23]

For supratentorial ependymoma, assessment of *RELA* fusion is required for diagnosis according to the 2016 WHO classification, whereas posterior fossa biological subgroups A and B[24] have not yet been introduced into the classification. In addition, further prognostic markers (eg, 1q gain, *CDKN2A* homozygous deletion, *TNC* expression, and *YAP1* fusion gene) have been confirmed retrospectively in multiple case series.[25] The 2017 consensus on the clinical management of intracranial ependymoma and its molecular variants states that ependymoma is a (molecularly) heterogeneous disease.[25] However, the clinical relevance of many driver epigenetic and genetic alterations, either as prognostic markers or markers predictive of therapeutic efficacy, remains to be prospectively validated.

The previous WHO classification described CNS primitive neuroectodermal tumours as an embryonal brain tumour entity.[14] Since then, it has become clear (eg, by DNA methylation profiling) that a large subset of tumours previously classified by conventional histology as CNS primitive neuroectodermal tumours can be reclassified with molecular pathological tools as other types of malignant CNS tumour and other entities.[26] Another major subset previously diagnosed as ependymoblastoma, embryonal tumour with abundant neuropil and true rosettes, or medulloepithelioma can be classified as embryonal tumours with multi-layered rosettes via *LIN28* expression analysis and 19q13.42 amplification detection.[27] Furthermore, distinct molecular entities have been described among the former group of presumed CNS primitive neuroectodermal tumours: CNS neuroblastoma with *FOXR2* activation, CNS high-grade neuroepithelial tumour with *MN1* alteration or *BCOR* alteration, and CNS Ewing sarcoma family tumour with *CIC* alteration.[26]

The majority of results from molecular profiling of atypical teratoid rhabdoid tumour tissues strongly suggest the existence of multiple molecular subgroups within this tumour type.[28] It is imperative to consolidate these early findings into a consensus molecular classification that can be applied to further tumour samples and tested against high-quality clinicopathological data to validate the prognostic nature of any molecular subgroupings.

During the past decade, it has become clear that paediatric low-grade gliomas and glio-neuronal tumours are mainly driven by altered signalling in the RAS–MAPK pathway.[29] Many pilocytic astrocytomas harbour *BRAF*

tandem duplications at chromosome 7q34, with fusion of *KIAA1549* and *BRAF* in 65% of cases, leading to loss of the regulatory N-terminal region of BRAF and the formation of fusion proteins. This *BRAF* fusion is associated with improved progression-free survival.[30] Additionally, tumorigenic *BRAF* activation occurs in ganglioglioma and pleomorphic xanthoastrocytoma, with point mutations at position 600 causing a Val600Glu substitution (*BRAF*^{V600E}). Other oncogenetic changes identified in low-grade gliomas include rearrangements of *FGFR1, MYB*, and *MYBL1*.[31] Malignant transformation, which occurs in about 2% of children with low-grade glioma, is related to homozygous deletion of *CDKN2A*.[32]

Several studies[33–36] have described the mutational landscape of CNS germ-cell tumours and highlighted the biological similarity of these tumours to their extracranial counterparts. These studies have identified mutational activation of KIT, RAS, ERK, AKT, and PI3K–MTOR pathways, representing potential targets for therapy. Given the scarcity of CNS germ-cell tumour tissue specimens available for study in North America and Europe, the collection of serum, plasma, and cerebrospinal fluid could allow non-invasive diagnosis by measuring the elevation of specific microRNAs (eg, the miR-371 to miR-373 cluster, and the miR-302 and miR-367 cluster),[37] on the basis of findings in germ cell tumour tissues.[38] Furthermore, these less invasive biospecimens could allow the identification of specific mutations through the analysis of circulating tumour DNA,[39] which could inform prognostication or the development of novel treatment strategies.

BRAF mutations are frequently present in papillary craniopharyngioma[40] and case reports show excellent responses to BRAF inhibitors in adults.[41] However, this treatment option, which potentially allows the avoidance of invasive surgery or radiotherapy and their associated complications, does not apply to adamantinomatous craniopharyngiomas in children, which frequently carry activating mutations in exon 3 of the catenin β1 gene. Despite the low availability of paediatric tissue specimens, promising biological research on adamantinomatous craniopharyngioma[42,43] has been done with the prospect of identifying targets for new therapies. This research is fundamental for children affected by adamantinomatous craniopharyngioma, but can only be continued if tumour specimens including cyst fluid are routinely sampled.

The 2007 and 2016 editions of the WHO classification discern three grades of choroid plexus tumours: classical plexus papilloma (grade I), atypical plexus papilloma (grade II), and plexus carcinoma (grade III). It has been shown that DNA methylation, single-nucleotide polymorphism, and gene expression profiles define clinically overlapping groups of choroid plexus tumours.[44]

The accelerated understanding of the biology of all paediatric brain tumour entities drove the decision for a 4th edition update of the WHO classification rather than waiting until the 5th edition. However, there is concern that the pace of change in the field creates a need to evaluate classification progress faster than is possible through standard WHO updates. Therefore, an initiative to evaluate and recommend proposed changes to future CNS tumour classifications, the Consortium to Inform Molecular and Practical Approaches to CNS Tumor Taxonomy (cIMPACT-NOW), has been initiated. The goals of cIMPACT-NOW are to facilitate input and consensus review of novel, diagnostically relevant data and to determine how such information can be practically incorporated into future CNS tumour classifications.[45]

Access to novel therapies

The European Society for Paediatric Oncology (SIOPE) has called for revisions to the EU Paediatric Medicine Regulation, aiming to increase young patients' access to innovative therapies. This call resulted in a specific report from the European Commission to the European Parliament and the Council,[46] which concluded that the concept of paediatric-use marketing authorisations has thus far failed to incentivise the development of paediatric medicines. In North America, the Research to Accelerate Cures and Equity for Children Act would require companies to apply the Pediatric Research Equity Act to any treatment with a molecular target that is relevant in adult and childhood disease. Successful biomaterial collection is a key prerequisite for preclinical research projects aiming to identify effective new drugs for children with CNS tumours, and will thus contribute to improving their access to novel therapies.

Tumour predisposition genes

In addition to entity-specific aspects, germline mutations in tumour predisposition genes in paediatric cancers are more frequent than previously thought, having been shown in 8–10% of paediatric patients with brain tumours,[47,48] and at even higher frequencies in some entities. The paediatric cancers most often associated with germline mutations include choroid plexus tumours, atypical teratoid rhabdoid tumours, medulloblastoma (eg, *TP53*-mutated SHH-activated medulloblastoma [potential Li-Fraumeni syndrome], *PTCH*-mutated or *SUFU*-mutated SHH-activated medulloblastoma [Gorlin syndrome], and *APC*-mutated WNT-activated medulloblastoma [Turcot syndrome]), high-grade glioma (mismatch repair deficiency syndromes), low-grade glioma, and ependymoma.[49] Patients with these diagnosis, as well as their families, need to be referred for genetic counselling to be informed of potential underlying predisposition syndromes.

Importance of adequately sampled and stored biomaterial

The main advantages of collecting biomaterials of adequate quality and quantity are the improved aetiopathological understanding of paediatric CNS tumours, the potential for validation and discovery of

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prognostic factors and druggable targets, the improvement of first-line and relapse treatment decisions for individual patients as well as within clinical trials and related research, the possibility to use the tissue for tumour vaccination strategies, and new insights into the biology of treatment-related acute toxicities and late effects. In addition, the frequency and clinical behaviour of tumour predisposition syndromes can only be better understood by analysing a broader series of tumour and germline material from patients with well-annotated clinical information about familial history, diagnosis, treatment, and follow-up. Biopathological characterisation is now essential for diagnosis, risk assessment, therapeutic stratification, and potential specific treatment allocation in all patients with medulloblastoma, and can form the basis of future research studies and discoveries.

Panel 1: **Advantages of adequately sampled and stored biomaterials from tumour and healthy tissue**

- Allows validation of candidate prognostic factors, and discovery of new parameters with clinical significance
- Allows storage of samples from well-documented patients treated homogeneously within prospective clinical trials (to avoid selection bias from large retrospective series and for consideration of the prognostic effect of applied treatment modalities)
- Required for validation of reported targets and identification of new druggable targets
- Forms the basis of current and future treatment decisions for individual patients at the clinical trials level, and for related research directions
- Allows the identification of relevant pathogenetic mechanisms of tumour aetiology, drivers for tumour growth, and resistance to treatments (commonly across paediatric CNS tumours, and within specific entities and subgroups) and will contribute to improving therapeutic solutions
- Increases the understanding of biological drivers for relapse, especially if paired sets of biological tissues (from initial diagnosis and relapse) are available
- Contributes to the understanding of the tumour microenvironment, other host-related factors, and immunological aspects of tumour control
- Allows the identification of appropriate diagnostic methods for liquid biopsy, and could help to identify future markers for minimal residual disease
- Facilitates identification and validation of associations of genetic polymorphisms with pharmacokinetic properties, or observed treatment-induced acute toxicities or late effects (eg, hearing deficits, cognitive impairments, or haematological toxicities)
- Will enhance knowledge about the frequency of germline mutations associated with treatment-related toxic effects or secondary malignancies, and improve recommendations for diagnostic procedures and genetic counselling
- Will be fundamental for the interpretation and comparability of clinical trial cohorts, including the evaluation of applied treatment modalities
- Will help identify surrogates for specific aspects of tumour imaging or biomarkers for tumour subgroups that can contribute to patient management
- Will allow the establishment of preclinical animal models and cell lines to test novel treatment modalities
- Will allow possible therapeutic use of the tissue (eg, for tumour vaccination strategies)
- Will ultimately speed up the urgently required scientific progress for children with CNS tumours and provide early access to new drugs in the context of appropriate clinical trials (targeting of receptors and signalling pathways, epigenetic alterations, microenvironment, and immune system)

Therefore, comprehensive neuropathological, genetic, and biological characterisation is routinely required before the start of postoperative treatment (eg, radiotherapy, or neoadjuvant or adjuvant chemotherapy) in individual patients (panel 1).

Need for tumour tissue for precision medicine

Broadly applicable methods for the genomic analysis of paediatric brain tumours, including methods for genome-wide discovery and precision medicine, have been established.[12] However, broader availability of tumour DNA and constitutional DNA is required to understand the full spectrum of frequencies and clinical implications of targeted treatments, treatment-related toxic effects, secondary malignancies, and optimal treatment and surveillance strategies for patients and their families. In this regard, it will be important to develop appropriate research models for each specific paediatric CNS tumour entity and variants to test new treatments and targeted agents. Several primary cell lines and corresponding orthotopic xenograft models have been developed for medulloblastoma[50–53] and high-grade glioma or diffuse intrinsic pontine glioma,[54–57] but representative, orthotopic, patient-derived xenograft models for other types of CNS tumours are scarce or unavailable. Thus, while collecting material for tumour characterisation, some tissue or surgical aspirate should also be collected to establish cell or organoid cultures, or animal models.[58]

In addition to the requirements of ongoing exploratory and validation research, biological data will also be needed for future diagnostic re-evaluations. Especially in long-duration clinical trials, relevant knowledge about diagnostic groups and host factors (eg, cancer predisposition or genotype variants affecting treatment efficacy) can sometimes be improved between the time of patient inclusion and data analysis. Furthermore, stored research material helps to characterise rare tumours that cannot be classified as any of the currently defined entities.

Importance of biological assessments with relevance to tumour imaging

The radiological heterogeneity of individual tumour types is increasingly apparent with advances in qualitative and quantitative analysis of conventional and advanced MRI methods.[59,60] In addition to pathological classification, emerging evidence of biological variations, particularly molecular subgroups, has stimulated interest in the field of imaging genomics or radiogenomics, which focuses on the relations between imaging phenotypes and genomics.

Correlations between *IDH* mutation status in glioma and relative cerebral blood volume have been shown.[61] Detection of 2-hydroxyglutarate on magnetic resonance spectroscopy has been proposed as a useful biomarker for gliomas with *IDH1* mutations.[62] Magnetic resonance

characteristics of paediatric medulloblastoma entities have been described on the basis of conventional imaging[63] and magnetic resonance spectroscopy.[64] Similar studies need to be done in various other paediatric brain tumours to identify imaging surrogates or biomarkers that complement their biological profile. The collection of biological material is crucial for the development of radiogenomics in paediatric neuro-oncology, and has the potential to aid decision making before surgery, guide biopsy, and allow measurement of treatment efficacy using quantitative methods.

Current situation and shortfalls

Currently, the collection of high-quality, adequately sampled and stored biomaterial is implemented successfully only in a minority of centres, mainly because of deficits in established structures, interdisciplinary cooperation, and funding. Professionally trained staff and necessary equipment are only available in some centres, and professionalisation at the central level of research consortia, including implementation of standard operating procedures at all levels involved, might also be required. Active collaboration of professionals from all involved disciplines, including neurosurgeons, neuropathologists, and paediatric oncologists, is not always established, and might be compromised because of potential conflicts of interest with other local research initiatives. In addition, the personal and infrastructural burden of successful biomaterial collection is not adequately compensated by institutional or external funding.

Proposals for improved biomaterial collection

To overcome the current limitations, strong cooperative efforts of representatives from all involved disciplines are required. The collection and storage of required biomaterial should become a routine standard for all children with CNS tumours, regardless of their inclusion in clinical trials or other research initiatives, and should become mandatory in more prospective paediatric CNS tumour trials in the future. For optimal collection in local institutions and within cooperative research groups, clear definitions of the types of biomaterials and standard operating procedures that should be used, together with thorough solutions for all associated ethical, legal, and practical issues, should be established.

Which biomaterials need to be collected and how?

To maximise the advantages of biomaterial collection and obtain a comprehensive biological understanding of tumours and host-related factors, different types of biomaterials should be collected (table).[65–68] Adequate amounts of tumour tissue, considering the safety of the patient, should be collected according to the following three criteria: (1) as unfixed, snap-frozen tissue; (2) as formalin-fixed, paraffin-embedded material; and (3) as viable, native material in transport media (or viably frozen cells) for direct tumour cell culture or xenografting in animals. Blood (as the preferred choice) or buccal swabs should be collected for germline analyses. In addition, constitutional DNA is required for comparison with the corresponding tumour tissue in genomic analyses, as tumour-specific alterations of genes, related signalling pathways, and druggable targets can only be identified and understood by comparison of tumour and germline material.

Various technical aspects of collection and storage must be carefully considered to obtain the required amounts of optimal-quality biomaterials. Successful collection requires a fundamental change in paediatric neurosurgeons' perception: they must appreciate that, apart from their primary role in performing, ideally, a gross total tumour resection without causing any additional harm to the patient, they have an equally important secondary role to do a threefold tissue-sampling procedure during surgery.

The operative procedure itself should be adapted, because much more time has to be devoted to the collection of tissue with tumour-grasping forceps from different areas of the tumour, instead of mostly using suction or ultrasonic aspirators to take out the bulk of tumour tissue. Piecemeal sampling with tumour forceps, especially in either very soft or very hard elastic tumours, can be very demanding and can prolong a surgical procedure by up to 30 min, especially if tumours are bloody. Information about the heterogeneity of tumours from MRI (diffusion-weighted sequences) or positron emission tomography could help surgeons to obtain specific tissue from various tumour areas.

Mostly self-running standard operating procedures for tissue processing should also be done by staff in the operating room, because the sampled tissue needs to be processed in parallel with tumour removal, which requires the full attention of the surgeon and the scrub nurse. The collection of material must be supervised by the neuropathologist, ensuring that proper material is taken for diagnostic procedures and biospecimen sampling. Special requirements regarding identification and sterility apply for the collection of materials that will be used for vaccine production. After the collection period, samples should be transferred by an additional person to sterile vials and immediately snap frozen and stored in –80°C freezers or liquid nitrogen (either of which should be available close to the operating room). Samples for tissue cultures need to be placed under sterile conditions into appropriate vials with media for delayed transfer to the laboratory within 24 h, or be transferred directly to the laboratory. The material allocated by the neuropathologist for diagnostic procedures will be processed by standard operating procedures. However, neurosurgeons should be aware that molecular genetic array diagnostics require additional material to extract sufficient DNA. Thus, extra time should be accounted for to achieve the required level of sampling needed.

	Processing	Purpose	Priority*	Assay
Tumour tissue				
Minimum 1 × 1·5 cm^3 or 3 × 0·5 cm^3; consider heterogeneity of tumour tissue; when stereotactic biopsy, 2 × 4 needle biopsies are recommended	FFPE tissue sampled early during dissection to obtain viable tissue (avoid crushing)	Diagnostics	1	IHC, targeted sequencing, fusion detection, or DNA methylation array
Touch imprint preparation or 1 × 0·5 cm^3	Snap frozen at –80°C after adequate drying or fixation	Diagnostics	1	FISH; DNA or RNA sequencing
1 × 0·5 cm^3	Snap frozen in N_2 or at –80°C within 20 min after resection	Research	2	DNA or RNA sequencing
1 × 0·5 cm^3	Culture medium (living cells)	Research	3	Cell culture or PDX models
1 × 0·5 cm^3	Paraformaldehyde	Research	4	Imaging
>1·5 cm^3†	Sterile, without additives, snap frozen at –80°C within 20 min after resection	Immunotherapy	1	Production of vaccine
Blood				
5 mL EDTA‡	White blood cells	Diagnostics	1	DNA
5 mL EDTA	Platelets[65]	Research	3	RNA
5 mL EDTA	Plasma[66,67]	Research	2	DNA, RNA, or extracellular vesicles§
5 mL serum	Serum[68]	Research	2	DNA, RNA, or extracellular vesicles§
Cerebrospinal fluid¶				
All available material	..	Diagnostics	1	Cytology
All other available material	..	Research	2	DNA, RNA, or extracellular vesicles§
Normal CNS tissue (obtain during placement of a ventriculoperitoneal shunt or subcutaneous intraventricular device, or during third ventriculoscopy)	FFPE or snap frozen as for tumour tissue	Research	3	IHC or DNA methylation array; FISH; DNA or RNA sequencing
Surgical aspirate (all available material)	Culture medium	Research	2	Cell culture or PDX models
Saliva or urine (1–5 mL)	..	Research	4	DNA; pharmacokinetics; extracellular vesicle analyses§

FFPE=formalin-fixed, paraffin-embedded. IHC=immunohistochemistry. FISH=fluorescence in situ hybridisation. PDX=patient-derived xenograft. EDTA=edetic acid. *1=highest priority, 2=highly important, 3=important, 4=for specific research questions. †When an operation is performed with the additional goal of obtaining tumour tissue for the preparation of dendritic cell vaccines. ‡Constitutional DNA can eventually be taken via buccal swab. §For analyses of extracellular vesicles, material should be processed within 1 h using specific procedures. ¶Cerebrospinal fluid can be collected during operation, or 14 days after operation for some treatment schedules.

Table: **Type and amount of biomaterials to be collected and proposals for collection and storage**

For standard operating procedures for tissue sampling to work well, theatre staff must be well informed and enthusiastic about undertaking the extra work that is needed. All personnel involved should understand that this tissue processing is not just for research, but will be needed for proper diagnostics, and can also affect a patient's chance of survival to the same extent as the operation and the tumour removal themselves. Understanding the importance of their role in tissue sampling collection could increase the efficiency and reliability of the personnel involved.

Sampling requires a team effort and, although sampling is done in neurosurgical theatres, the neurosurgeon's focus will primarily be on the operation itself. Thus, it is necessary to establish a tissue-processing pipeline as an interdisciplinary effort and adapt it to local conditions to include oncology, neuropathology, and theatre staff. Since patients with malignant tumours of the posterior fossa might undergo emergency surgery or weekend surgery, the standard operating procedures for tissue processing should also be organised in such a way as to be able to function at all times of the day and week.

Blood, plasma, and serum are important to elucidate the roles of circulating tumour cells, extracellular vesicles, cell-free DNA, proteins, and other key factors. Cerebrospinal fluid samples can be taken from patients with metastatic tumours, as access to macroscopic metastatic lesions is frequently limited and only possible by additional invasive procedures in these patients. Appropriate diagnostic methods for use with liquid biopsies could serve to identify new markers for minimal residual disease.[69,70] As tumour tissue from metastatic sites can otherwise only be obtained by more invasive procedures, cerebrospinal fluid could be used to detect microscopic tumour dissemination in cytospin samples, as well as to allow analysis of metastatic tumour DNA, microRNA, or proteins in the cerebrospinal fluid supernatant to enhance knowledge about metastatic tumour spread or disease progression or evolution.

Biological material should also be collected later in the disease course and after treatment. To increase biological understanding of tumour evolution and resistance mechanisms, collection of tumour tissue at the time of relapse or during autopsy is essential. Re-biopsy of

relapsed tumours should be generally recommended to ensure that the maximal amount of biological information is collected at tumour recurrence, with exceptions only if associated risks are increased in individual cases.

In addition to the specifications about the collected biomaterial outlined above, there are important considerations at the central level of research consortia or clinical trials groups, as well as at the level of local institutions. Moreover, ethical, legal, and practical aspects must be considered.

Ethical, legal, privacy, and practical aspects

Studies have shown that patients and their representatives, when given adequate information, are largely in favour of biomaterials not required for diagnostic procedures being made available for research projects.[71,72] However, important ethical, legal, privacy, and practical aspects relating to the collection, storage, shipment, and sharing of biomaterials need careful consideration (panel 2). For example, the legal definition of ownership of biomaterials and the guidelines for informed consent might vary between countries,[73] and should be considered in the context of individual patient care and international clinical trials. Because of the advantages of accurate diagnostic procedures and translational research, it is increasingly accepted that the availability of biomaterials should be a mandatory inclusion criterion for patients in clinical trials (eg, within the SIOPE-PNET5-MB trial).[74] This condition is justifiable if biomaterial is a prerequisite for the stratification of patients within a clinical trial, but also to ensure maximal scientific progress from associated biological research projects. The availability of biomaterials will facilitate future diagnostic and research evaluations of newly defined biomarkers, targets, or host factors, which could contribute to the understanding of clinical results from trials.

Biobanking

The potential advantages of centralised or decentralised (virtual) biobanking require consideration. Biomaterials can be stored centrally by academic or commercial tumour bank providers, with software systems allowing for maximal up-to-date information about the stored materials. Alternatively, the materials can be stored within local tumour banking facilities and later shipped in batches, as required, for use in further analyses (diagnostic analyses or collaborative research projects). Both centralised and decentralised storage of biomaterials allow for their use in big data analyses with bioinformatical support, and facilitate a comprehensive cataloguing of biomaterials for collaborative projects between research consortia. Regardless of storage location, proper evaluation of the samples by neuropathologists should ensure appropriate tissue representation before used in specific projects. Storage of biomaterials in aliquots allows the tissue to be used for multiple research projects.

Transparent criteria for the regulation of access to larger biomaterial series by scientists from local contributing institutions and independent researchers could positively influence the cooperation of local centres. Material transfer agreements, standard operating procedures for shipment of materials, and adequate coverage of costs could further facilitate cooperative tumour banking. It is also important to establish procedures for the coupling of tumour material data to patient data: genomic, transcriptomic, methylomic, and metabolomic data from tumour biopsies, as well as data from experiments on patient-derived cell cultures and xenografts, should ideally be stored in an international CNS tumour registry (such as the SIOPE diffuse intrinsic pontine glioma (DIPG) registry[75]) together with comprehensive anonymous clinical, radiological, and pathological data from these patients. This approach will allow for comprehensive analyses of big data. In this respect, it is worth investing in the collection of large amounts of retrospective clinical data (regarding baseline

Panel 2: **Ethical, legal, privacy, and practical aspects of storage, sharing, and shipment of biomaterials at the central level of a research consortium or clinical trials group**

- Age-appropriate information sheets for patients and their legal representatives must explain the purpose of the planned research, the recipients of the material, and the use of anonymised or pseudonymised clinical data, followed by clear forms for informed consent
- Coupling of tumour material data to patient data—including treatment and imaging findings—should be possible, allowing for comprehensive big data analyses
- Ethical approval and permissions from international, national, or local authorities should be obtained
- To provide insight into the availability of biomaterials and to identify potential for further improvements, a monitoring system for biomaterials and for associated informed consents per local hospital should be considered
- Ownership issues relating to biological tissue and clinical data, which might be different between countries, should be considered
- Advantages of centralised versus decentralised (virtual) tumour banking and procedures to check for appropriate tissue representation for interpretable biological results should be considered
- Whether the availability of biomaterial should be defined as a mandatory inclusion criterion for patients in clinical trials should be considered
- In the context of clinical trials, responsibilities of trial coordinators and local centres should be defined and adapted to applicable laws and regulations
- Adequate coverage of the local costs and shipment of biomaterials by research grants can facilitate the compliance of local institutions
- Integrated, reusable tumour box devices can facilitate shipment of frozen and unfrozen materials
- To optimise availability for both local and central research projects, respective biomaterials should be stored in appropriate aliquots
- Practical aspects of exchange and use of biomaterials should be defined by material transfer agreements between research institutions within the applicable laws and regulations
- Local researchers should be able to apply to use centrally stored material, following transparent rules for evaluation of such applications, thus allowing them to benefit from their own cooperative effort

characteristics, treatment, and survival) from multiple international groups, and to correlate these data with the analysis of genomic and epigenomic data from corresponding banked tumour samples.

National and international research consortia and clinical trials groups should consider aspects of data collection and storage of biomaterials, and discuss them early in the planning phase of collaborative projects so that specific national requirements can be implemented in a timely manner. Sustainability of data beyond individual projects and connection of data at overarching levels should also be considered. Large-scale sequencing done by the International Cancer Genome Consortium and Paediatric Cancer Genome Project[47] showed that the genetic and epigenetic repertoires of driver mutations in specific childhood malignancies differ from those of common adult-type malignancies. To bring about much-needed improvements regarding the availabilities of new drugs for children with brain tumours, paediatric platforms such as ACCELERATE have been proposed by the Cancer Drug Development Forum, Innovative Therapies for Children with Cancer, the European Network for Cancer Research in Children and Adolescents, and SIOPE.[76] These platforms aim to establish and improve paediatric drug-development programmes informed by mechanism-of-action knowledge, with aggregated databases of biological drug targets for paediatric tumours, to ultimately enable prioritisation and more rapid conduct of early-phase clinical trials for paediatric malignancies.

***Panel 3:* Aspects of standard operating procedures to be considered in local institutions**

Staff from all involved disciplines (eg, neurosurgeons, operating room staff, [neuro] pathologists, paediatric oncologists, and research nurses) should be aware of the importance of the availability of adequate biomaterials, and define the practical steps of collection, storage, and shipment of samples according to local structures. These steps include:

- Obtaining information and consent from patients and their legal representatives: preoperative oral consent in emergency cases, and written consent later; biobanking, research, or trial documents might require separate consent
- Determining amount and types of tissue, blood, and other biomaterials to be collected
- Neurosurgical considerations (frozen section, debulking, surgical aspirate, infiltration zone, healthy material, cerebrospinal fluid); freezing and fixation of maximal amounts of material; as neurosurgical interventions can be undertaken during the night or weekend, standard operating procedures should be established for the adequate storage of tissues outside of regular daytime working hours.
- Neuropathological diagnosis and reference assessments
- Sending MRI data via digital route or anonymised and coded CD-ROM
- Providing adequate short-term storage of tumour tissue and other materials such as blood, mucosa, saliva, and urine (labelling of samples, snap-freezing and storage at –80°C in operating room within 20 min)
- Transferring materials to long-term storage, or shipping samples according to standard operating procedures
- Ensuring trial-specific requirements are met (eg, touch imprint preparation for fluorescence in situ hybridisation)
- Supplying material for cell culture in specific sterile cell culture medium
- Filing documentation of collected materials per study in institution-specific lists or databases
- Confirming received materials at research institute
- Establishing procedure for prioritisation of pathology in case of sparse material

Implementation of practical solutions according to the structures of local institutions

The collection and storage of biomaterials can only be achieved successfully if all relevant steps are optimised in each local participating centre. As staff and organisational structures vary between local participating centres, a general schema might not work in all centres in the same way. Collection and storage procedures should be adapted by local institutions to suit their individual structures, ideally by a dedicated local coordinator that is supported by all the disciplines that are involved (panel 3). The practical tasks and responsibilities associated with the biomaterial tissue collection, storage and analysis should be defined and assigned to dedicated individuals, and specific education and training modules should be developed.

Conclusion

The availability of adequately sampled and stored biomaterial can confer multiple scientific and clinical advantages, such as allowing identification and validation of new and previously described prognostic factors and druggable targets. Improved sampling of biomaterial is also a major prerequisite for improving survival rates and reducing treatment-related late-effects in children with CNS tumours.

In addition to increasing knowledge about the roles of conventional treatment modalities in biologically well-defined tumour entities and subgroups, we must ensure that children are not left behind while precision oncology offers new treatment solutions for adult cancers.[77] As paediatric tumours are clinically and biologically highly distinct from adult cancers, precision medicine approaches should be redeveloped in paediatric oncological diseases that can use informative biomaterial. Ideally, data from tumour tissues and biomaterials would be coupled to corresponding anonymous patient data—such as demographic data, diagnostic features, and radiological, pathological, treatment, and outcome data—as exemplified by the SIOPE DIPG registry.

Only with widely available and informative biological material can profound improvements be achieved in reasonable time, both for individual patients and future clinical trial participants. Without proposed improvements in biomaterial collection, optimal patient care cannot be delivered at the levels of diagnostic assessments, applied treatment components, or aftercare. Likewise, the urgently required scientific progress in the field will be significantly delayed or impeded.

In summary, tumour tissue and other biomaterials should be collected from all children with CNS tumours,

and this practice will become increasingly mandatory in prospective trials of paediatric CNS tumours. Strong cooperative efforts by representatives from all involved disciplines, in local institutions and within cooperative research groups, are required to efficiently implement the collection and storage of required biomaterial.

Contributors

The manuscript was mainly written by SR and SWVG. All coauthors contributed to the content of the manuscript from the perspective of the SIOPE Brain Tumour Group they represent, as well as commenting on general aspects of this Policy Review.

Declaration of interests

CJ has received grants from Roche and Genentech. FD has received personal fees from BMS, Servier, Tesaro Oncology, and Celgene. GAS has received personal fees, non-financial support, honoraria for lectures, and travel support, and chaired advisory boards for Biomarin Inc; has participated in clinical trials for Shire Inc; and has received personal fees from Sucampo (now Malinckrodt). All other authors declare no competing interests.

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