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Future treatments will be further tailored to specific molecular subtypes of disease, specific tumor predisposition syndromes, and other biological criteria. Currently, deficits in structures and interdisciplinary cooperation are impeding the collection of high-quality biomaterial in most centers. However, successful material collection is a key prerequisite for the application of contemporary methodologies for validation of candidate prognostic factors, the discovery of new biomarkers, the establishment of appropriate pre-clinical research models for targeted agents, the faster clinical implementation of precision medicine, and for other therapeutic use of the tissue, e.g. for immunotherapies. Practical, legal and ethical aspects of consent, storage, material transfer, biobanking, data sharing, and funding must be established by research consortia and local institutions for optimal collection of primary and subsequent tumor tissue, body fluids, and normal tissue. These requirements must be adapted to the individual personal and organizational structures of the local institutions.

Policy Review

Biological material collection to advance translational research and treatment of children with CNS tumors: A position paper and practical considerations from the SIOP-Europe Brain Tumor Group

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Abstract

Pediatric central nervous system (CNS) tumors are the most common cause of childhood cancer-related morbidity and mortality. Improvements in diagnosis and treatment are mandatory. New (epi)genetic information is transforming the field dramatically. For most pediatric CNS tumor entities, subgroups with distinct biological characteristics are identified and increasingly used for accurate diagnoses and therapeutic recommendations.

Future treatments will be further tailored to specific molecular subtypes of disease, specific tumor predisposition syndromes, and other biological criteria. Currently, deficits in structures and interdisciplinary cooperation are impeding the collection of high-quality biomaterial in most centers. However, successful material collection is a key prerequisite for the application of contemporary methodologies for validation of candidate prognostic factors, the discovery of new biomarkers, the establishment of appropriate pre-clinical research models for targeted agents, the faster clinical implementation of precision medicine, and for other therapeutic use of the tissue, e.g. for immunotherapies. Practical, legal and ethical aspects of consent, storage, material transfer, biobanking, data sharing, and funding must be established by research consortia and local institutions for optimal collection of primary and subsequent tumor tissue, body fluids, and normal tissue. These requirements must be adapted to the individual personal and organizational structures of the local institutions.

Introduction

In developed countries, cancer is the leading disease-related cause of death in childhood. Pediatric central nervous system (CNS) tumors are the most common group of solid pediatric malignancies and the most common cause of cancer-related morbidity and mortality in this age group. Incidence rates between 5.37 and 4.01 per 100.000/year have been reported for children between 0 and 15 years [1,2]. Pediatric CNS tumors comprise a group of highly heterogeneous entities with strikingly different clinical and biological characteristics compared to adult CNS tumors [3]. In spite of significant advances in imaging, neurosurgery, radiotherapy, and medical treatment, survival rates for most pediatric CNS tumor patients are lagging behind the success rates of childhood leukemia and many other solid tumor types [4-6]. In addition, survivors of childhood CNS tumors most often suffer impaired quality of life, including frequent and disabling endocrine and neuro-cognitive impairments which not only negatively impact their physical and mental health but also their participation in society. These deficits are related to the tumor itself, as well as to surgery and additional CNS-directed therapies, that are known to be particularly detrimental when applied on an immature developing brain [7,8]. Improvements in diagnosis, including shorter time to diagnosis, more accurate of diagnosis and risk stratification, and better treatment are urgently needed. The biological knowledge about pediatric CNS tumors has been increasing dramatically in the past 5 to 10 years, including newly identified subgroups with prognostic and often therapeutic implications. The improved availability of biomaterials for biological characterization before the start of postoperative treatment is a prerequisite for benefits of individual patients, as well as for the timely clinical validation of current knowledge and further scientific progress in the field.

The importance of biological assessments in pediatric CNS tumors

Tumor classification and clinical relevance

New innovative genomic and epigenetic information is increasingly transforming the diagnostic and clinical landscape [9,10]. For the large majority of pediatric CNS tumor 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]. Due to the high clinical relevance, the genome-wide analysis of childhood CNS tumors has become increasingly important [12].

Consequently, some of the most robustly validated new biological parameters, especially in medulloblastoma, high grade glioma and ependymoma, have been included into the 2016 version of the WHO Classification of Tumors of the Central Nervous System [3].

For example, in the previous WHO-classification [14], the diagnosis of medulloblastoma and its subtypes was defined by histopathological parameters (desmoplasia, anaplasia, or large-cell components). In addition to histopathological features the 2016 WHO version defines medulloblastoma subgroups by molecular characteristics i.e. WNT-, sonic hedgehog (SHH)-, TP53mutated/SHH-, 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 Group 3 and Group 4 tumors, have recently been reported [11], alongside the discovery of biomarkers defined by specific aberrations (e.g. chromosome 11 loss in Group 4 medulloblastoma [15]), which now require further validation prior to clinical use. Recent analyses of neurocognitive outcomes per biological subgroups in medulloblastoma showed that biological subgroups have an association with clinical, neurocognitive and health-related quality of life outcomes, with different rates of post-operative complications (less cerebellar mutism and motor deficits, less pronounced information processing speed decline, and better health-related quality of life in surviving SHH patients) [16,17]. It has been agreed internationally that whenever possible, patients should be treated on a molecularly informed clinical trial. The first international clinical trials of risk-adapted therapies focused on the assessment of clinical, pathological and molecular biomarkers are now underway (NCT02066220, NCT01878617), based on risk stratification schemes defined in previous trial-based biological research studies, e.g. from Ellison *et al.* [18]. Finally, first insights are emerging into the biology of medulloblastoma at relapse; these have shown that the disease evolves clonally and that genetic events, such as combined TP53 mutations and MYC/N amplification, are commonly acquired at relapse [19,20]. Assessment of distinct molecular features at relapse will thus be essential for determining the treatment strategy.

In pediatric high-grade gliomas, the molecularly defined new tumor entity, diffuse midline glioma (DMG IV) has been introduced, which is exclusively defined by demonstration of K27M mutations in the H3F3A (histone 3.3) or HIST1H3A/B/C (histone 3.1) genes. This tumor entity and its WHO grade are defined by demonstration of specific histone mutations besides infiltrative growth characteristics and midline location, rather than by the usual histological criteria of malignant tumor growth. Of note, H3G34R/V tumors (about 10 to 15%) were not mentioned separately despite clear age, location, outcome and biological differences. Furthermore, rare pediatric high grade gliomas (HGG) such as WHO grade III anaplastic oligodendroglioma (AO III), WHO grade III anaplastic ganglioglioma (AGG III), and WHO grade III anaplastic pleomorphic xanthoastrocytoma (APXA III) are also mostly

defined by specific molecular findings or their absence. For pediatric AO III, the usual 1p 19 q co-deletion which defines most adult AO III according to the new WHO brain tumor classification, is mostly absent in their pediatric counterparts. BRAF mutations (as well as homozygous CDKN2A/B deletions) may characterize pediatric AGG III and APXA III. The integrated genomic, epigenomic and transcriptomic data across anatomical compartments of the brain is needed to define subgroups within pediatric high-grade glial tumors (malignant glioma and diffuse intrinsic pontine glioma) as well as novel therapeutic targets [21,22].

For ependymoma, the assessment of RELA-fusion is required for diagnoses according to the 2016 classification, while posterior fossa biological subgroups A and B (PFA and PFB [23]) have not yet been introduced into the WHO classification. In addition, further prognostic markers have been confirmed retrospectively in multiple case series (1q gain, CDKN2A homozygous deletion, TNC expression, Yap1-fusion gene) [24]. The current consensus on the clinical management of intracranial ependymoma and its molecular variants has recently been published, and states that ependymoma is a (molecularly) heterogenous disease [25]. However, the clinical relevance of many driver epigenetic and genetic alterations, either as prognostic markers or as markers predictive of therapeutic efficacy, remains to be prospectively validated.

CNS primitive neuroectodermal tumors (CNS-PNET) were classified as one entity of embryonal brain tumors in the previous WHO-classification [14]. In the meantime, it has become clear e.g. by DNA methylation profiling that a major part of tumors previously classified as CNS-PNETs, can be reclassified as other malignant CNS tumors such as high grade glioma, pleomorphic xanthoastrocytoma, atypical teratoid/rhabdoid tumor (AT/RT), ependymoma etc. [26]. Another major subset can be classified as embryonal tumors with multilayered rosettes (ETMR) by LIN28 expression analysis and 19q13.42 amplification detection [27]. New molecular entities have been described among the former group of presumed CNS-PNETs: CNS-neuroblastoma with FOXR2 activation (CNS NB-FOXR2), CNS high-grade neuroepithelial tumor with MN1 alteration (CNS HGNET-MN-1) or with BCOR alteration (CNS-HGNET-BCOR), and CNS Ewing sarcoma family tumor with CIC alteration (CNS EFT-CIC) [26].

The majority of molecular profiling performed on atypical teratoid/rhabdoid tumors tissue to date points strongly towards the existence of multiple molecular subgroups within the disease [28]. It is imperative to consolidate these early findings into a consensus molecular classification which may be applied to further tumor samples and tested against high-quality clinico-pathological data to validate the prognostic nature of any molecular sub-groupings.

During the past decade the molecular background of pediatric low grade gliomas (LGG) has become clear, showing that LGG and glial-neuronal tumors are mainly driven by altered signaling in the RAS-

MAPK pathway [29]. In particular, pilocytic astrocytoma shows mainly BRAF tandem duplications at chromosome 7q34 mainly with fusion of KIAA549 and BRAF in 65% leading to loss of the regulatory N'-terminal region of BRAF and the formation of fusion proteins. This BRAF fusion correlates with improved progression-free survival (PFS) [30]. Besides the fusion as biologic background tumorigenic BRAF activation occurs in ganglioglioma and pleomorphic xanthoastrocytoma with point mutations at position 600 with glutamate substitution for valine, BRAFV600E. Other oncogenetic changes in LGG are found in rearrangements of FGFR1, MYB and MYBL1 associated with morphologies of DNET and angiocentric glioma respectively [31]. The malignant transformation found in about 2% of children with LGG has been found to be related to additional homozygous deletion of CDKN2A [32].

The mutational landscape of CNS germ cell tumors (GCT) is becoming described [33-36] and highlighted the biological similarity of these tumors to their extracranial counterparts. These studies have identified mutational activation of KIT/RAS/ERK and AKT as well as the PI3K/MTOR pathways, representing potential targets for therapy. Given the limited CNS GCT tissue specimens available to study in North America and Europe, collection of serum/plasma and CSF may in future allow non-invasive diagnosis using the elevation of specific microRNAs (miR-371~373 and miR-302/367) [37], based on findings in GCT tissues [38]. In addition, these less-invasive biospecimens will allow the identification of specific mutations through circulating tumor DNA (ctDNA) analysis [39], which may inform prognosis and/or novel treatment strategies.

Recent research revealed the frequent presence of a BRAF mutation in papillary craniopharyngioma CP) [40] and first case reports document excellent response rates to BRAF inhibitors in adults [41]. However, this treatment option – potentially avoiding invasive surgery or radiation and associated complications – does not apply to adamantinomatous CP (aCP) in children. Despite limited availability of pediatric tissue specimen, promising biological research relating to aCP [42,43] has been published with the prospect of identifying targets for new therapies. This research is of fundamental importance to children affected by aCP but can only be continued if tumor specimen including cyst fluid are routinely sampled.

In its 2007 and 2016 editions, the WHO classification discerns three grades of choroid plexus tumors (CPT): classical plexus papilloma grade I (CPP), atypical plexus papilloma grade III (APP) and plexus carcinoma grade III (CPC). Recently several groups have shown that DNA methylation, SNP-profiling, and gene expression defines clinically overlapping groups of CPTs [44].

The accelerated understanding drove the decision for an earlier, 4th edition update of the CNS WHO rather than waiting longer for a 5th edition. However, there is still 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

tumor classifications has been announced: cIMPACT-NOW, the Consortium to Inform Molecular and Practical Approaches to CNS Tumor Taxonomy. The goal of cIMPACT-NOW is to facilitate input and consensus review of novel diagnostically relevant data and determine how such information can be practically incorporated into future CNS tumor classifications [45].

Access to novel therapies

Importantly, the SIOPe has called for revisions to the EU Pediatric 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 describing "The state of pediatric medicines in the EU - 10 years of the EU Pediatric Regulation", which concluded that the Pediatric Use Marketing Authorizations (PUMA) concept has so far failed to incentivize the development of pediatric medicines [46]. In North America, the RACE (Research to Accelerate Cures and Equity) for Children Act would require companies to apply PREA (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 tumors, and therefore will contribute to their improved access to novel therapies.

Tumor predisposition genes

In addition to the entity-specific aspects, germline mutations in tumor predisposition genes in pediatric cancer are more frequent than previously thought. They have been shown in 8.6 % of pediatric brain tumor patients, and some of the pediatric cancers most often associated with germline mutations are CPT, atypical teratoid/rhabdoid tumors, medulloblastoma (TP53-mutated SHH MB – potential Li-Fraumeni syndrome, PTCH or SUFU-mutated SHH MB – Gorlin syndrome, APC-mutated WNT MB – Turcot syndrome), HGG, LGG and ependymoma [47]. Patients and families need to be referred for genetic counseling, to be informed of potential underlying predisposition syndromes.

The importance of adequately sampled and stored biomaterial

The main advantages of the collection of biomaterials in adequate quality and quantity are the improved etiopathological understanding of pediatric CNS tumors, the validation and discovery of

prognostic factors and drugable 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 tumor vaccination strategies, and new insights in biology of acute toxicities and late-effects. In addition, frequencies of tumor predisposition syndromes and their clinical behavior can only be better understood by analyzing broader series of tumor- and germline material from patients with well-annotated clinical information about familiar history, diagnosis, treatment and follow-up. In consequence, bio-pathological characterization is now essential for diagnosis, risk assessment, therapeutic stratification and potentially specific treatment allocation in all patients with medulloblastoma, as well as underpinning future research studies and discoveries. Therefore, comprehensive bio-pathological characterization is routinely required before the start of postoperative treatment (e.g. radiotherapy, neoadjuvant or adjuvant chemotherapy) in individual patients. The most relevant arguments are listed in table 1.

The need for tumor tissue for precision medicine

Broadly applicable methods for the genomic analysis of childhood brain tumors including methods for genome-wide discovery and precision medicine have been established [12]. However, broader availability of tumor- and constitutional DNA is required to understand the full spectrum of frequencies and the important clinical implications regarding targeted treatments, treatment-related toxicities, secondary malignancies, and the optimal treatment and surveillance strategies for those patients and families. In this regard, it will be important to develop appropriate research models for each specific pediatric CNS tumor type and subtype to test new treatments and targeted agents. Currently, a number of primary cell lines and corresponding orthotopic xenograft models have been developed for medulloblastoma [48-51], and HGG/DIPG [52-55], but good orthotopic patient-derived xenograft (PDX) models for all other types of CNS tumors are scarce or lacking. Thus, while collecting material for tumor characterization, some tissue or CUSA (Cavitron Ultrasonic Surgical Aspirator) material should also be collected for the establishment of cell cultures, animal models or organoids [56].

Beyond current exploratory and validation research activities, biology data will also be needed for future diagnostic re-evaluations. Especially in long duration clinical trials, relevant improvement of knowledge on diagnostic groups as well as relevant host factors (cancer predisposition, genotype variants in treatment efficacy) can emerge between time of patient inclusion and trial data analysis. Also, stored research material helps to characterize rare tumors that do not fall into any of the currently appreciated entities.

The importance of biological assessments with relevance to tumor imaging

The radiological heterogeneity of individual tumor types is increasingly apparent with advances in qualitative and quantitative analysis of both conventional and advanced MR imaging methods [57,58]. In addition to pathological classification, the emerging evidence of the biological variations, particularly molecular subgroups has stimulated interest in the field of imaging genomics or radiogenomics that focuses on the relationship between imaging phenotypes and genomics.

Recent studies have identified correlations between IDH mutation status in gliomas and relative cerebral blood volume (rCBV) [59]. Detection of 2-hydroxyglutarate (2-HG) on MR spectroscopy has been proposed as a useful biomarker for Gliomas with IDH 1 mutation [60]. In the pediatric population MR characteristics of medulloblastoma subtypes have been described based on conventional imaging [61] and MR spectroscopy [62]. Similar studies need to be carried out in various pediatric brain tumors to identify imaging surrogates or biomarkers that complement their biological profile. The collection of biological material is central to the development of radio genomics in pediatric neurooncology. It has the potential to aid decision making prior to surgery, guiding biopsy and measure efficacy of treatment using quantitative methods.

The current situation and shortfalls

Currently, the collection of high-quality, adequately sampled and stored biomaterial is implemented successfully only in a minority of centers. This is mainly due to deficits in established structures, interdisciplinary cooperation, and funding. Professionally trained staff and required equipment is only available in some centers, and may also need to be professionalized at the central level of research consortia. Active collaboration of all involved disciplines, including neurosurgeons, neuropathologists, and pediatric oncologists, is not always established, and might be compromised due to potential conflicts of interest with other local research initiatives. In addition, the required personal and infrastructural burden for successful biomaterial collection is not adequately compensated by additional 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 must become a routine

standard for all children with CNS tumors, regardless of their inclusion in clinical trials or other research initiatives. Moreover, it must become increasingly mandatory in future prospective pediatric CNS tumor trials. For their optimal collection in local institutions and within cooperative research groups, clear definitions of the types of biomaterials with standard operating procedures (SOP) must be implemented, together with thorough solutions for all associated ethical, legal and practical aspects.

Which biomaterials need to be collected and how?

To maximize the above mentioned advantages and to obtain a comprehensive biological understanding of tumor and host-related factors, different types of biomaterials need to be collected. Adequate amounts of tumor tissue, taking into account safety of the patient, should be collected threefold: 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 tumor cell culture or direct xenografting in animals. Blood as preferred choice or buccal swabs should be collected for germline analyses. In addition, constitutional DNA is required for comparison with genomic analyses from the corresponding tumor tissue, as tumor-specific alterations of genes, related signaling pathways, and drugable targets can only be identified and understood by comparison of tumor and germline material. A list of types of biomaterials to be collected and technical aspects of collection and storage is given in Table 2.

Various technical aspects of collection and storage of biomaterials need to be carefully considered to obtain useful amounts of the required materials of optimal quality. This requires first of all a fundamental change in the pediatric neurosurgeons perception, that they play two equally important and pivotal roles in the process of treatment. Neurosurgeons need to appreciate, that apart from their primary role with regard to performing ideally a gross total tumor resection without causing any additional harm to the patients, there is an equally important secondary role by performing a threefold tissue sampling during surgery.

The operative procedure *per se* should be adapted, because much more time needs to be devoted to collect tissue with tumor grasping forceps from different areas of the tumor, instead of mostly using suction or ultrasonic aspirators to take out the bulk of tumor tissue. Piecemeal sampling with the tumor forceps is, especially in either very soft or very hard-elastic tumors, very time- and patience consuming and can prolong a surgical procedure up to 30 minutes, especially if tumors are very bloody. Information about the heterogeneity of tumors from MRI (diffusion-weighted sequences) or positron-emission-tomography may be used to specifically obtain tissue from various tumor areas.

In addition, a more or less self-running tissue processing SOP needs to be enacted among the theater staff, because the sampled tissue needs to be processed in parallel to the tumor removal, which takes full attention of the surgeon and the scrub nurse. The pathologist might be involved at that time to determine the samples collected being tumor tissue. After the end of the collection period, samples need to be transferred by a third person from the staff to sterile vials and immediately snap frozen in -80ºC freezers or liquid nitrogen, either option needs to be available close to the operating rooms. The samples for tissue cultures need to go into appropriate vials with media for delayed transfer to the lab within 24h or have to be transferred directly to the lab. Only the usual pathology for FFPE material can stay and be processed „as always“. However, neurosurgeons need to know that molecular genetic array diagnostics need additional material to extract enough DNA. Thus, generous sampling is essential and the time needs to be invested.

To make the tissue sampling SOP work well, theater staff needs to be informed and made enthusiastic for this additional work they have to cope with. There needs to be a thorough understanding in all personnel involved, that the tissue processing they are performing is not “just for research”, but has an enormous impact on the patients chances of survival, equal to the impact of operation and tumor removal itself. Understanding the importance of their role in this process will make them efficient and reliable members of the process.

Finally the sampling effort is a team effort and, despite the fact that it is done in the neurosurgical theater, the neurosurgeon’s focus will properly be on the operation itself. Thus the tissue processing pipeline needs to be established as an interdisciplinary effort and adapted to the local conditions including oncology, pathology and theater staff. Since especially malignant tumors of the posterior fossa might undergo emergency surgery or weekend surgery, the SOP for tissue processing needs to be organized in a way that it will function 24/7.

Blood, plasma and serum are important to elucidate the role of circulating tumor cells, extracellular vesicles, cell-free DNA, proteins and other key parameters. Cerebrospinal fluid samples can be used in metastatic tumors, as the access to macroscopic metastatic lesions is frequently limited and only possible by additional invasive procedures. Appropriate diagnostic methods for liquid biopsy may serve to identify future markers for minimal residual disease [63,64]. As tumor tissue from metastatic sites can otherwise only be obtained by more invasive procedures, cerebrospinal fluid (CSF) may not only be used to detect microscopic tumor dissemination in cytospin samples, but may also serve to analyze metastatic tumor DNA, microRNA (miRNA) or proteins in the CSF-supernatant, to enhance knowledge about metastatic tumor spread or disease progression/evolution.

Importantly, biologic material should also be collected later-on during the disease course and after treatment. To speed up the biological understanding of tumor evolution and the appearance of

resistance mechanisms, it is of paramount importance to collect tumor tissue at the time of relapse, or through autopsy. To ensure maximal biological information at tumor recurrence, re-biopsy of relapsed tumors should be generally recommended, 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 trial groups, as well as at the level of 'local' institutions (figure 1). Moreover, ethical, legal and practical aspects must be considered.

Ethical, legal, privacy, and practical aspects at the central level of a research consortium or clinical trials group

Studies have shown clear support from patients and their representatives, who, once in receipt of adequate information, are largely in favor that biomaterials not required for diagnostic procedures are made available for research projects [65,66]. However, important ethical, legal, privacy and practical aspects need to be considered in the process of collection, storage, shipment, and sharing of biomaterials. For example, the legal definitions for ownership of biomaterials, and guidelines for informed consent may vary between countries [67], and need to be considered for individual patient care and in the conduct of international clinical trials. Due to the advantages of accurate diagnostic procedures and translational research, it is increasingly accepted that the availability of biomaterials is defined as a mandatory inclusion criterion for patients within clinical trials (e.g. within the SIOPE-PNET5-MB trial [68]). This may not only be justified if biomaterial is a prerequisite for 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 may impact on the understanding of the clinical results of the trials. The main ethical, legal, privacy, and practical aspects of storage, sharing, and shipment of biomaterials are listed in Table 3.

Biobanking

In addition, advantages of central or decentralized (virtual) biobanking need to be considered. Biomaterials can be stored centrally by academic or commercial tumor bank providers, with software systems allowing for maximal transparency about the stored materials. Alternatively, they can be stored within the respective local tumor banking facilities, and may be shipped according to the requirements of further analyses (diagnostic analyses or collaborative research projects) in batches

at later time points. Both central and decentral storage of biomaterials will also allow its use for big data analyses with bioinformatical support, and facilitate a comprehensive cataloguing of biomaterials for collaborative projects between research consortia. In any case, SOPs to control tumor samples for appropriate tissue representation must also be implemented. Storage of biomaterials in aliquots allows the tissue to be used for multiple research projects. Transparent criteria for the access of scientists from local contributing institutions and for independent researchers to the larger biomaterial series may positively impact the cooperation of local centers. Material transfer agreements, SOPs for shipment of materials, and adequate coverage of costs may further facilitate cooperative tumor-banking. In addition, it is important to define coupling of tumor material data to patient data: genomic, transcriptomic, methylomic, metabolomics data from tumor biopsies, as well as data from experiments on patient-derived cell cultures and xenografts, should ideally be stored in an international CNS tumor registry such as the recently established SIOPE DIPG Registry [69], together with comprehensive anonymous clinical, radiological and pathology data of these patients. This will allow for comprehensive Big Data analyses. In this respect, it is of high value to invest in gathering large numbers of retrospective clinical data (baseline characteristics, treatment and survival data) from multiple international groups, and to correlate these with analysis of (epi)genomic data from corresponding banked tumor samples.

National and international research consortia and/or clinical trial groups must consider these aspects and discuss these early in the planning phase of collaborative projects, so that specific national requirements can be implemented in timely manner. Sustainability of data beyond projects and connection of data at overarching levels should be envisioned. Recent large-scale sequencing by International Cancer Genome Consortium and Paediatric Cancer Genome Project has further shown that the genetic and epigenetic repertoire of driver mutations in specific childhood malignancies differs from more common adult-type malignancies. To bring about much needed change, pediatric 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 the SIOPe [70]. These platforms aim to develop mechanism-of-action informed paediatric drug development programmes with aggregated databases of paediatric biological tumour drug targets, to ultimately enable prioritisation and conduct of early phase clinical paediatric trials more rapidly.

Local institutions need to implement practical solutions according to their structures

It has become evident that the collection and storage of biomaterials can only be achieved successfully, if all relevant steps are solved in each local participating center. As personal and organizational structures are highly different between local participating centers, a general schema may not work in all centers in the same way. The above mentioned aspects rather need to be adapted individually by the local institutions to their structures, ideally by a dedicated local coordinator supported by all other involved disciplines (see table 4). Ultimately, the practical tasks and responsibilities need to be defined and assigned to responsible individuals. Specific education and training modules should be developed.

Conclusion

The availability of adequately sampled and stored biomaterial will confer multiple advantages of highest scientific and clinical relevance, such as validation of described and identification of new prognostic factors and drugable targets. This paper aims to stress on the need of biomaterial sampling, and includes also highly relevant practical, ethical, and privacy aspects. Improved sampling of biomaterial is a major prerequisite for the improvement of survival rates for children with CNS tumors, and to reduce treatment-related late-effects.

In addition to increasing knowledge about the roles of conventional treatment modalities in biologically well-defined entities and subgroups, it must be ensured that children are not left behind while precision oncology offers new treatment solutions for adult cancers [71]. As pediatric tumors are clinically and biologically highly distinct from adult cancers, these approaches must be redeveloped in oncologic diseases, with informative biomaterial. Ideally, data from tumor tissues and biomaterials would be coupled to corresponding anonymous patient data, such as demographics, diagnostic features, radiology and pathology treatment and outcome data, as exemplified by the recently established SIOPE DIPG Registry.

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

In summary, tumor tissue and other biomaterials need to be collected from all children with CNS tumors, and will become increasingly mandatory in prospective pediatric CNS tumor trials. Strong cooperative efforts of 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.

Search strategy and selection criteria

In this review, chairs and representatives of the SIOPe Brain Tumor Group (BTG) (https://www.siope.eu/european-research-and-standards/clinical-research-council/siopecrc/european-clinical-study-groups/siope-brain-tumour-group/) have summarized their views how to efficiently improve biomaterial collection for children with CNS tumours, and why this is urgently required. This has been based on the profound experiences in the conduct of national and international multicenter clinical trials and collaborative research projects. Cited literature for this policy review has been primarily selected by relevance and actuality rather than being object of systematic literature review. SIOPe BTG is a European multidisciplinary association of healthcare professionals which leads in research, treatment (among which international clinical trials) and care of children and young people with tumors of the CNS. It is a subgroup of the European branch (SIOPe) (http://www.siope.eu/) of the International Society of Paediatric Oncology (SIOP) (http://siop-online.org/).

Contributors

The manuscript was mainly written by SR and SWVG. All coauthors contributed to the content of the manuscript from the perspective of the BTG working group they represent, as well as for general aspects of this policy review.

Declaration of interest

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References

1. Ostrom QT, Gittleman H, Fulop J, et al. CBTRUS Statistical Report: Primary Brain and Central Nervous System Tumors Diagnosed in the United States in 2008-2012. *Neuro Oncol* 2015; **17 Suppl 4**: iv1-iv62.

2. Kaatsch P, Spix C, Grabow D, Becker C. Annual Report 2016. 2016. http://www.kinderkrebsregister.de/typo3temp/secure_downloads/22605/0/17aa97a18ea4a834424f1eb1a46e6ada9829b582/jb2016_s.pdf2017).

3. Louis DN, Perry A, Reifenberger G, et al. The 2016 World Health Organization Classification of Tumors of the Central Nervous System: a summary. *Acta Neuropathol* 2016; **131**(6): 803-20.

4. Packer RJ. Childhood brain tumors: accomplishments and ongoing challenges. *J Child Neurol* 2008; **23**(10): 1122-7.

5. Pui CH, Gajjar AJ, Kane JR, Qaddoumi IA, Pappo AS. Challenging issues in pediatric oncology. *Nat Rev Clin Oncol* 2011; **8**(9): 540-9.

6. Mertens AC, Yong J, Dietz AC, et al. Conditional survival in pediatric malignancies: analysis of data from the Childhood Cancer Survivor Study and the Surveillance, Epidemiology, and End Results Program. *Cancer* 2015; **121**(7): 1108-17.

7. Armstrong GT, Liu Q, Yasui Y, et al. Long-term outcomes among adult survivors of childhood central nervous system malignancies in the Childhood Cancer Survivor Study. *J Natl Cancer Inst* 2009; **101**(13): 946-58.

8. Bhatia S, Armenian SH, Armstrong GT, et al. Collaborative Research in Childhood Cancer Survivorship: The Current Landscape. *J Clin Oncol* 2015; **33**(27): 3055-64.

9. Gajjar A, Bowers DC, Karajannis MA, Leary S, Witt H, Gottardo NG. Pediatric Brain Tumors: Innovative Genomic Information Is Transforming the Diagnostic and Clinical Landscape. *J Clin Oncol* 2015; **33**(27): 2986-98.

10. Northcott PA, Pfister SM, Jones DT. Next-generation (epi)genetic drivers of childhood brain tumours and the outlook for targeted therapies. *Lancet Oncol* 2015; **16**(6): e293-302.

11. Schwalbe EC, Lindsey JC, Nakjang S, et al. Novel molecular subgroups for clinical classification and outcome prediction in childhood medulloblastoma: a cohort study. *Lancet Oncol* 2017; **18**(7): 958-71.

12. Mack SC, Northcott PA. Genomic Analysis of Childhood Brain Tumors: Methods for Genome-Wide Discovery and Precision Medicine Become Mainstream. *J Clin Oncol* 2017; **35**(21): 2346-54.

13. Liu KW, Pajtler KW, Worst BC, Pfister SM, Wechsler-Reya RJ. Molecular mechanisms and therapeutic targets in pediatric brain tumors. *Sci Signal* 2017; **10**(470).

14. Louis DN, Ohgaki H, Wiestler OD, et al. The 2007 WHO classification of tumours of the central nervous system. *Acta Neuropathol* 2007; **114**(2): 97-109.

15. Thompson EM, Hielscher T, Bouffet E, et al. Prognostic value of medulloblastoma extent of resection after accounting for molecular subgroup: a retrospective integrated clinical and molecular analysis. *Lancet Oncol* 2016; **17**(4): 484-95.

16. Moxon-Emre I, Taylor MD, Bouffet E, et al. Intellectual Outcome in Molecular Subgroups of Medulloblastoma. *J Clin Oncol* 2016; **34**(34): 4161-70.

17. Bull KS, Kennedy CR, Bailey S, Ellison DW, Clifford SC. Improved health-related quality of life outcomes associated with SHH subgroup medulloblastoma in SIOP-UKCCSG PNET3 trial survivors. *Acta Neuropathol* 2014; **128**(1): 151-3.

18. Ellison DW, Kocak M, Dalton J, et al. Definition of disease-risk stratification groups in childhood medulloblastoma using combined clinical, pathologic, and molecular variables. *J Clin Oncol* 2011; **29**(11): 1400-7.

19. Hill RM, Kuijper S, Lindsey JC, et al. Combined MYC and P53 defects emerge at medulloblastoma relapse and define rapidly progressive, therapeutically targetable disease. *Cancer Cell* 2015; **27**(1): 72-84.

20. Morrissy AS, Garzia L, Shih DJ, et al. Divergent clonal selection dominates medulloblastoma at recurrence. *Nature* 2016; **529**(7586): 351-7.

21. Jones C, Karajannis MA, Jones DTW, et al. Pediatric high-grade glioma: biologically and clinically in need of new thinking. *Neuro Oncol* 2017; **19**(2): 153-61.

22. Mackay A, Burford A, Carvalho D, et al. Integrated Molecular Meta-Analysis of 1,000 Pediatric High-Grade and Diffuse Intrinsic Pontine Glioma. *Cancer Cell* 2017; **32**(4): 520-37 e5.

23. Pajtler KW, Witt H, Sill M, et al. Molecular Classification of Ependymal Tumors across All CNS Compartments, Histopathological Grades, and Age Groups. *Cancer Cell* 2015; **27**(5): 728-43.

24. Ramaswamy V, Hielscher T, Mack SC, et al. Therapeutic Impact of Cytoreductive Surgery and Irradiation of Posterior Fossa Ependymoma in the Molecular Era: A Retrospective Multicohort Analysis. *J Clin Oncol* 2016; **34**(21): 2468-77.

25. Pajtler KW, Mack SC, Ramaswamy V, et al. The current consensus on the clinical management of intracranial ependymoma and its distinct molecular variants. *Acta Neuropathol* 2017; **133**(1): 5-12.

26. Sturm D, Orr BA, Toprak UH, et al. New Brain Tumor Entities Emerge from Molecular Classification of CNS-PNETs. *Cell* 2016; **164**(5): 1060-72.

27. Picard D, Miller S, Hawkins CE, et al. Markers of survival and metastatic potential in childhood CNS primitive neuro-ectodermal brain tumours: an integrative genomic analysis. *Lancet Oncol* 2012; **13**(8): 838-48.

28. Johann PD, Erkek S, Zapatka M, et al. Atypical Teratoid/Rhabdoid Tumors Are Comprised of Three Epigenetic Subgroups with Distinct Enhancer Landscapes. *Cancer Cell* 2016; **29**(3): 379-93.

29. Packer RJ, Pfister S, Bouffet E, et al. Pediatric low-grade gliomas: implications of the biologic era. *Neuro Oncol* 2017; **19**(6): 750-61.

30. Hawkins C, Walker E, Mohamed N, et al. BRAF-KIAA1549 fusion predicts better clinical outcome in pediatric low-grade astrocytoma. *Clin Cancer Res* 2011; **17**(14): 4790-8.

31. Qaddoumi I, Orisme W, Wen J, et al. Genetic alterations in uncommon low-grade neuroepithelial tumors: BRAF, FGFR1, and MYB mutations occur at high frequency and align with morphology. *Acta Neuropathol* 2016; **131**(6): 833-45.

32. Mistry M, Zhukova N, Merico D, et al. BRAF mutation and CDKN2A deletion define a clinically distinct subgroup of childhood secondary high-grade glioma. *J Clin Oncol* 2015; **33**(9): 1015-22.

33. Wang L, Yamaguchi S, Burstein MD, et al. Novel somatic and germline mutations in intracranial germ cell tumours. *Nature* 2014; **511**(7508): 241-5.

34. Fukushima S, Otsuka A, Suzuki T, et al. Mutually exclusive mutations of KIT and RAS are associated with KIT mRNA expression and chromosomal instability in primary intracranial pure germinomas. *Acta Neuropathol* 2014; **127**(6): 911-25.

35. Schulte SL, Waha A, Steiger B, et al. CNS germinomas are characterized by global demethylation, chromosomal instability and mutational activation of the Kit-, Ras/Raf/Erk- and Akt-pathways. *Oncotarget* 2016; **7**(34): 55026-42.

36. Ichimura K, Fukushima S, Totoki Y, et al. Recurrent neomorphic mutations of MTOR in central nervous system and testicular germ cell tumors may be targeted for therapy. *Acta Neuropathol* 2016; **131**(6): 889-901.

37. Murray MJ, Bell E, Raby KL, et al. A pipeline to quantify serum and cerebrospinal fluid microRNAs for diagnosis and detection of relapse in paediatric malignant germ-cell tumours. *Br J Cancer* 2016; **114**(2): 151-62.

38. Palmer RD, Murray MJ, Saini HK, et al. Malignant germ cell tumors display common microRNA profiles resulting in global changes in expression of messenger RNA targets. *Cancer Res* 2010; **70**(7): 2911-23.

39. De Mattos-Arruda L, Mayor R, Ng CK, et al. Cerebrospinal fluid-derived circulating tumour DNA better represents the genomic alterations of brain tumours than plasma. *Nat Commun* 2015; **6**: 8839.

40. Brastianos PK, Taylor-Weiner A, Manley PE, et al. Exome sequencing identifies BRAF mutations in papillary craniopharyngiomas. *Nat Genet* 2014; **46**(2): 161-5.

41. Brastianos PK, Shankar GM, Gill CM, et al. Dramatic Response of BRAF V600E Mutant Papillary Craniopharyngioma to Targeted Therapy. *J Natl Cancer Inst* 2016; **108**(2).

42. Hofmann BM, Hoelsken A, Fahlbusch R, Blumcke I, Buslei R. Hormone receptor expression in craniopharyngiomas: a clinicopathological correlation. *Neurosurgery* 2010; **67**(3): 617-25; discussion 25.

43. Martinez-Barbera JP. 60 years of neuroendocrinology: Biology of human craniopharyngioma: lessons from mouse models. *J Endocrinol* 2015; **226**(2): T161-72.

44. Thomas C, Sill M, Ruland V, et al. Methylation profiling of choroid plexus tumors reveals 3 clinically distinct subgroups. *Neuro Oncol* 2016; **18**(6): 790-6.

45. Louis DN, Aldape K, Brat DJ, et al. Announcing cIMPACT-NOW: the Consortium to Inform Molecular and Practical Approaches to CNS Tumor Taxonomy. *Acta Neuropathol* 2017; **133**(1): 1-3.

46. Report from the commission to the European Parliament and the Council. State of Paediatric Medicines in the EU - 10 years of the EU Paediatric Regulation. 2017.

47. Zhang J, Walsh MF, Wu G, et al. Germline Mutations in Predisposition Genes in Pediatric Cancer. *N Engl J Med* 2015; **373**(24): 2336-46.

48. Xu J, Erdreich-Epstein A, Gonzalez-Gomez I, et al. Novel cell lines established from pediatric brain tumors. *J Neurooncol* 2012; **107**(2): 269-80.

49. Dietl S, Schwinn S, Dietl S, et al. MB3W1 is an orthotopic xenograft model for anaplastic medulloblastoma displaying cancer stem cell- and Group 3-properties. *BMC Cancer* 2016; **16**: 115.

50. Girard E, Ditzler S, Lee D, et al. Efficacy of cabazitaxel in mouse models of pediatric brain tumors. *Neuro Oncol* 2015; **17**(1): 107-15.

51. Sanden E, Eberstal S, Visse E, Siesjo P, Darabi A. A standardized and reproducible protocol for serum-free monolayer culturing of primary paediatric brain tumours to be utilized for therapeutic assays. *Sci Rep* 2015; **5**: 12218.

52. Liu Z, Zhao X, Mao H, et al. Intravenous injection of oncolytic picornavirus SVV-001 prolongs animal survival in a panel of primary tumor-based orthotopic xenograft mouse models of pediatric glioma. *Neuro Oncol* 2013; **15**(9): 1173-85.

53. Zhao X, Zhao YJ, Lin Q, et al. Cytogenetic landscape of paired neurospheres and traditional monolayer cultures in pediatric malignant brain tumors. *Neuro Oncol* 2015; **17**(7): 965-77.

54. Hashizume R, Smirnov I, Liu S, et al. Characterization of a diffuse intrinsic pontine glioma cell line: implications for future investigations and treatment. *J Neurooncol* 2012; **110**(3): 305-13.

55. Jansen MH, Lagerweij T, Sewing AC, et al. Bevacizumab Targeting Diffuse Intrinsic Pontine Glioma: Results of 89Zr-Bevacizumab PET Imaging in Brain Tumor Models. *Mol Cancer Ther* 2016; **15**(9): 2166-74.

56. Drost J, Clevers H. Translational applications of adult stem cell-derived organoids. *Development* 2017; **144**(6): 968-75.

57. Peet AC. Magnetic resonance spectroscopy and beyond for pediatric brain tumors. *CNS Oncol* 2014; **3**(3): 195-7.

58. Zarinabad N, Wilson M, Gill SK, Manias KA, Davies NP, Peet AC. Multiclass imbalance learning: Improving classification of pediatric brain tumors from magnetic resonance spectroscopy. *Magn Reson Med* 2017; **77**(6): 2114-24.

59. Kickingereder P, Sahm F, Radbruch A, et al. IDH mutation status is associated with a distinct hypoxia/angiogenesis transcriptome signature which is non-invasively predictable with rCBV imaging in human glioma. *Sci Rep* 2015; **5**: 16238.

60. Andronesi OC, Rapalino O, Gerstner E, et al. Detection of oncogenic IDH1 mutations using magnetic resonance spectroscopy of 2-hydroxyglutarate. *J Clin Invest* 2013; **123**(9): 3659-63.

61. Perreault S, Ramaswamy V, Achrol AS, et al. MRI surrogates for molecular subgroups of medulloblastoma. *AJNR Am J Neuroradiol* 2014; **35**(7): 1263-9.

62. Bluml S, Margol AS, Sposto R, et al. Molecular subgroups of medulloblastoma identification using noninvasive magnetic resonance spectroscopy. *Neuro Oncol* 2016; **18**(1): 126-31.

63. Vu-Han TL, Fruhwald MC, Hasselblatt M, et al. Identifying molecular markers for the sensitive detection of residual atypical teratoid rhabdoid tumor cells. *Cancer Genet* 2014; **207**(9): 390-7.

64. Chakravadhanula M, Tembe W, Legendre C, et al. Detection of an atypical teratoid rhabdoid brain tumor gene deletion in circulating blood using next-generation sequencing. *J Child Neurol* 2014; **29**(9): NP81-5.

65. Mitchell D, Geissler J, Parry-Jones A, et al. Biobanking from the patient perspective. *Research involvement and engagement* 2015; **1**: 1-17.

66. Page SA, Manhas KP, Muruve DA. A survey of patient perspectives on the research use of health information and biospecimens. *BMC Med Ethics* 2016; **17**(1): 48.

67. Kaye J, Briceno Moraia L, Curren L, et al. Consent for Biobanking: The Legal Frameworks of Countries in the BioSHaRE-EU Project. *Biopreserv Biobank* 2016; **14**(3): 195-200.

68. Pizer BL, Clifford SC. The potential impact of tumour biology on improved clinical practice for medulloblastoma: progress towards biologically driven clinical trials. *Br J Neurosurg* 2009; **23**(4): 364-75.

69. Veldhuijzen van Zanten SE, Baugh J, Chaney B, et al. Development of the SIOPE DIPG network, registry and imaging repository: a collaborative effort to optimize research into a rare and lethal disease. *J Neurooncol* 2017; **132**(2): 255-66.

70. Pearson AD, Herold R, Rousseau R, et al. Implementation of mechanism of action biology-driven early drug development for children with cancer. *Eur J Cancer* 2016; **62**: 124-31.

71. The Lancet O. Making paediatric precision oncology a reality. *Lancet Oncol* 2016; **17**(10): 1335.

72. Best MG, Sol N, Kooi I, et al. RNA-Seq of Tumor-Educated Platelets Enables Blood-Based Pan-Cancer, Multiclass, and Molecular Pathway Cancer Diagnostics. *Cancer Cell* 2015; **28**(5): 666-76.

73. Jeurissen S, Vergauwen G, Van Deun J, et al. The isolation of morphologically intact and biologically active extracellular vesicles from the secretome of cancer-associated adipose tissue. *Cell Adh Migr* 2017; **11**(2): 196-204.

74. Consortium E-T, Van Deun J, Mestdagh P, et al. EV-TRACK: transparent reporting and centralizing knowledge in extracellular vesicle research. *Nat Methods* 2017; **14**(3): 228-32.

75. Gautam A, Kumar R, Dimitrov G, Hoke A, Hammamieh R, Jett M. Identification of extracellular miRNA in archived serum samples by next-generation sequencing from RNA extracted using multiple methods. *Mol Biol Rep* 2016; **43**(10): 1165-78.

Policy Review

Biological material collection to advance translational research and treatment of children with CNS tumors: A position paper and practical considerations from the SIOP-Europe Brain Tumor Group

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Abstract

Pediatric central nervous system (CNS) tumors are the most common cause of childhood cancer-related morbidity and mortality. Improvements in diagnosis and treatment are mandatory. New (epi)genetic information is transforming the field dramatically. For most pediatric CNS tumor entities, subgroups with distinct biological characteristics are identified and increasingly used for accurate diagnoses and therapeutic recommendations.

Future treatments will be further tailored to specific molecular subtypes of disease, specific tumor predisposition syndromes, and other biological criteria. Currently, deficits in structures and interdisciplinary cooperation are impeding the collection of high-quality biomaterial in most centers. However, successful material collection is a key prerequisite for the application of contemporary methodologies for validation of candidate prognostic factors, the discovery of new biomarkers, the establishment of appropriate pre-clinical research models for targeted agents, the faster clinical implementation of precision medicine, and for other therapeutic use of the tissue, e.g. for immunotherapies. Practical, legal and ethical aspects of consent, storage, material transfer, biobanking, data sharing, and funding must be established by research consortia and local institutions for optimal collection of primary and subsequent tumor tissue, body fluids, and normal tissue. These requirements must be adapted to the individual personal and organizational structures of the local institutions.

Introduction

In developed countries, cancer is the leading disease-related cause of death in childhood. Pediatric central nervous system (CNS) tumors are the most common group of solid pediatric malignancies and the most common cause of cancer-related morbidity and mortality in this age group. Incidence rates between 5.37 and 4.01 per 100.000/year have been reported for children between 0 and 15 years [1,2]. Pediatric CNS tumors comprise a group of highly heterogeneous entities with strikingly different clinical and biological characteristics compared to adult CNS tumors [3]. In spite of significant advances in imaging, neurosurgery, radiotherapy, and medical treatment, survival rates for most pediatric CNS tumor patients are lagging behind the success rates of childhood leukemia and many other solid tumor types [4-6]. In addition, survivors of childhood CNS tumors most often suffer impaired quality of life, including frequent and disabling endocrine and neuro-cognitive impairments which not only negatively impact their physical and mental health but also their participation in society. These deficits are related to the tumor itself, as well as to surgery and additional CNS-directed therapies, that are known to be particularly detrimental when applied on an immature developing brain [7,8]. Improvements in diagnosis, including shorter time to diagnosis, more accurate of diagnosis and risk stratification, and better treatment are urgently needed. The biological knowledge about pediatric CNS tumors has been increasing dramatically in the past 5 to 10 years, including newly identified subgroups with prognostic and often therapeutic implications. The improved availability of biomaterials for biological characterization before the start of postoperative treatment is a prerequisite for benefits of individual patients, as well as for the timely clinical validation of current knowledge and further scientific progress in the field.

The importance of biological assessments in pediatric CNS tumors

Tumor ~~C~~classification and clinical relevance

New innovative genomic and epigenetic information is increasingly transforming the diagnostic and clinical landscape [9,10]. For the large majority of pediatric CNS tumor 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]. Due to the high clinical relevance, the genome-wide analysis of childhood CNS tumors has become increasingly important [12].

Consequently, some of the most robustly validated new biological parameters, especially in medulloblastoma, high grade glioma and ependymoma, have been included into the 2016 version of the WHO Classification of Tumors of the Central Nervous System [3].

For example, in the previous WHO-classification [14], the diagnosis of medulloblastoma and its subtypes was defined by histopathological parameters (desmoplasia, anaplasia, or large-cell components). In addition to histopathological features the 2016 WHO version defines medulloblastoma subgroups by molecular characteristics i.e. WNT-, sonic hedgehog (SHH)-, TP53mutated/SHH-, 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 Group 3 and Group 4 tumors, have recently been reported [11], alongside the discovery of biomarkers defined by specific aberrations (e.g. chromosome 11 loss in Group 4 medulloblastoma [15]), which now require further validation prior to clinical use. Recent analyses of neurocognitive outcomes per biological subgroups in medulloblastoma showed that biological subgroups have an association with clinical, neurocognitive and health-related quality of life outcomes, with different rates of post-operative complications (less cerebellar mutism and motor deficits, less pronounced information processing speed decline, and better health-related quality of life in surviving SHH patients) [16,17]. It has been agreed internationally that whenever possible, patients should be treated on a molecularly informed clinical trial. The first international clinical trials of risk-adapted therapies focused on the assessment of clinical, pathological and molecular biomarkers are now underway (NCT02066220, NCT01878617), based on risk stratification schemes defined in previous trial-based biological research studies, e.g. from Ellison *et al.* [18]. Finally, first insights are emerging into the biology of medulloblastoma at relapse; these have shown that the disease evolves clonally and that genetic events, such as combined TP53 mutations and MYC/N amplification, are commonly acquired at relapse [19,20]. Assessment of distinct molecular features at relapse will thus be essential for determining the treatment strategy.

In pediatric high-grade gliomas, the molecularly defined new tumor entity, diffuse midline glioma (DMG IV) has been introduced, which is exclusively defined by demonstration of K27M mutations in the H3F3A (histone 3.3) or HIST1H3A/B/C (histone 3.1) genes. This tumor entity and its WHO grade are defined by demonstration of specific histone mutations besides infiltrative growth characteristics and midline location, rather than by the usual histological criteria of malignant tumor growth. Of note, H3G34R/V tumors (about 10 to 15%) were not mentioned separately despite clear age, location, outcome and biological differences. Furthermore, rare pediatric high grade gliomas (HGG) such as WHO grade III anaplastic oligodendroglioma (AO III), WHO grade III anaplastic ganglioglioma (AGG III), and WHO grade III anaplastic pleomorphic xanthoastrocytoma (APXA III) are also mostly

defined by specific molecular findings or their absence. For pediatric AO III, the usual 1p 19 q co-deletion which defines most adult AO III according to the new WHO brain tumor classification, is mostly absent in their pediatric counterparts. BRAF mutations (as well as homozygous CDKN2A/B deletions) may characterize pediatric AGG III and APXA III. The integrated genomic, epigenomic and transcriptomic data across anatomical compartments of the brain is needed to define subgroups within pediatric high-grade glial tumors (malignant glioma and diffuse intrinsic pontine glioma) as well as novel therapeutic targets [21,22].

For ependymoma, the assessment of RELA-fusion is required for diagnoses according to the 2016 classification, while posterior fossa biological subgroups A and B (PFA and PFB [23]) have not yet been introduced into the WHO classification. In addition, further prognostic markers have been confirmed retrospectively in multiple case series (1q gain, CDKN2A homozygous deletion, TNC expression, Yap1-fusion gene) [24]. The current consensus on the clinical management of intracranial ependymoma and its molecular variants has recently been published, and states that ependymoma is a (molecularly) heterogenous disease [25]. However, the clinical relevance of many driver epigenetic and genetic alterations, either as prognostic markers or as markers predictive of therapeutic efficacy, remains to be prospectively validated.

CNS primitive neuroectodermal tumors (CNS-PNET) were classified as one entity of embryonal brain tumors in the previous WHO-classification [14]. In the meantime, it has become clear e.g. by DNA methylation profiling that a major part of tumors previously classified as CNS-PNETs, can be reclassified as other malignant CNS tumors such as high grade glioma, pleomorphic xanthoastrocytoma, atypical teratoid/rhabdoid tumor (AT/RT), ependymoma etc. [26]. Another major subset can be classified as embryonal tumors with multilayered rosettes (ETMR) by LIN28 expression analysis and 19q13.42 amplification detection [27]. New molecular entities have been described among the former group of presumed CNS-PNETs: CNS-neuroblastoma with FOXR2 activation (CNS NB-FOXR2), CNS high-grade neuroepithelial tumor with MN1 alteration (CNS HGNET-MN-1) or with BCOR alteration (CNS-HGNET-BCOR), and CNS Ewing sarcoma family tumor with CIC alteration (CNS EFT-CIC) [26].

The majority of molecular profiling performed on atypical teratoid/rhabdoid tumors tissue to date points strongly towards the existence of multiple molecular subgroups within the disease [28]. It is imperative to consolidate these early findings into a consensus molecular classification which may be applied to further tumor samples and tested against high-quality clinico-pathological data to validate the prognostic nature of any molecular sub-groupings.

During the past decade the molecular background of pediatric low grade gliomas (LGG) has become clear, showing that LGG and glial-neuronal tumors are mainly driven by altered signaling in the RAS-

MAPK pathway [29]. In particular, pilocytic astrocytoma shows mainly BRAF tandem duplications at chromosome 7q34 mainly with fusion of KIAA549 and BRAF in 65% leading to loss of the regulatory N'-terminal region of BRAF and the formation of fusion proteins. This BRAF fusion correlates with improved progression-free survival (PFS) [30]. Besides the fusion as biologic background tumorigenic BRAF activation occurs in ganglioglioma and pleomorphic xanthoastrocytoma with point mutations at position 600 with glutamate substitution for valine, BRAFV600E. Other oncogenetic changes in LGG are found in rearrangements of FGFR1, MYB and MYBL1 associated with morphologies of DNET and angiocentric glioma respectively [31]. The malignant transformation found in about 2% of children with LGG has been found to be related to additional homozygous deletion of CDKN2A [32].

The mutational landscape of CNS germ cell tumors (GCT) is becoming described [33-36] and highlighted the biological similarity of these tumors to their extracranial counterparts. These studies have identified mutational activation of KIT/RAS/ERK and AKT as well as the PI3K/MTOR pathways, representing potential targets for therapy. Given the limited CNS GCT tissue specimens available to study in North America and Europe, collection of serum/plasma and CSF may in future allow non-invasive diagnosis using the elevation of specific microRNAs (miR-371~373 and miR-302/367) [37], based on findings in GCT tissues [38]. In addition, these less-invasive biospecimens will allow the identification of specific mutations through circulating tumor DNA (ctDNA) analysis [39], which may inform prognosis and/or novel treatment strategies.

Recent research revealed the frequent presence of a BRAF mutation in papillary craniopharyngioma CP) [40] and first case reports document excellent response rates to BRAF inhibitors in adults [41]. However, this treatment option – potentially avoiding invasive surgery or radiation and associated complications – does not apply to adamantinomatous CP (aCP) in children. Despite limited availability of pediatric tissue specimen, promising biological research relating to aCP [42,43] has been published with the prospect of identifying targets for new therapies. This research is of fundamental importance to children affected by aCP but can only be continued if tumor specimen including cyst fluid are routinely sampled.

In its 2007 and 2016 editions, the WHO classification discerns three grades of choroid plexus tumors (CPT): classical plexus papilloma grade I (CPP), atypical plexus papilloma grade III (APP) and plexus carcinoma grade III (CPC). Recently several groups have shown that DNA methylation, SNP-profiling, and gene expression defines clinically overlapping groups of CPTs [44].

The accelerated understanding drove the decision for an earlier, 4th edition update of the CNS WHO rather than waiting longer for a 5th edition. However, there is still 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

tumor classifications has been announced: cIMPACT-NOW, the Consortium to Inform Molecular and Practical Approaches to CNS Tumor Taxonomy. The goal of cIMPACT-NOW is to facilitate input and consensus review of novel diagnostically relevant data and determine how such information can be practically incorporated into future CNS tumor classifications [45].

Access to novel therapies

Importantly, the SIOPe has called for revisions to the EU Pediatric 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 describing "The state of pediatric medicines in the EU - 10 years of the EU Pediatric Regulation", which concluded that the Pediatric Use Marketing Authorizations (PUMA) concept has so far failed to incentivize the development of pediatric medicines [46]. In North America, the RACE (Research to Accelerate Cures and Equity) for Children Act would require companies to apply PREA (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 tumors, and therefore will contribute to their improved access to novel therapies.

Tumor predisposition genes

In addition to the entity-specific aspects, germline mutations in tumor predisposition genes in pediatric cancer are more frequent than previously thought. They have been shown in 8.6 % of pediatric brain tumor patients, and some of the pediatric cancers most often associated with germline mutations are CPT, atypical teratoid/rhabdoid tumors, medulloblastoma (TP53-mutated SHH MB – potential Li-Fraumeni syndrome, PTCH or SUFU-mutated SHH MB – Gorlin syndrome, APC-mutated WNT MB – Turcot syndrome), HGG, LGG and ependymoma [47]. Patients and families need to be referred for genetic counseling, to be informed of potential underlying predisposition syndromes.

~~**The need for tumor tissue for precision medicine**~~

~~Broadly applicable methods for the genomic analysis of childhood brain tumors including methods for genome wide discovery and precision medicine have been established [12]. However, broader availability of tumor- and constitutional DNA is required to understand the full spectrum of frequencies and the important clinical implications regarding targeted treatments, treatment related~~

toxicities, secondary malignancies, and the optimal treatment and surveillance strategies for those patients and families. In this regard, it will be important to develop appropriate research models for each specific pediatric CNS tumor type and subtype to test new treatments and targeted agents. Currently, a number of primary cell lines and corresponding orthotopic xenograft models have been developed for medulloblastoma [47-50], and HGG/DIPG [51-54], but good orthotopic patient derived xenograft (PDX) models for all other types of CNS tumors are scarce or lacking. Thus, while collecting material for tumor characterization, some tissue or CUSA (Cavitron Ultrasonic Surgical Aspirator) material should also be collected for the establishment of cell cultures, animal models or organoids [55].

Beyond current exploratory and validation research activities, biology data will also be needed for future diagnostic re-evaluations. Especially in long duration clinical trials, relevant improvement of knowledge on diagnostic groups as well as relevant host factors (cancer predisposition, genotype variants in treatment efficacy) can emerge between time of patient inclusion and trial data analysis. Also, stored research material helps to characterize rare tumors that do not fall into any of the currently appreciated entities.

The importance of biological assessments with relevance to tumor imaging

The radiological heterogeneity of individual tumor types is increasingly apparent with advances in qualitative and quantitative analysis of both conventional and advanced MR imaging methods [56,57]. In addition to pathological classification, the emerging evidence of the biological variations, particularly molecular subgroups has stimulated interest in the field of imaging genomics or radiogenomics that focuses on the relationship between imaging phenotypes and genomics.

Recent studies have identified correlations between IDH mutation status in gliomas and relative cerebral blood volume (rCBV) [58]. Detection of 2-hydroxyglutarate (2-HG) on MR spectroscopy has been proposed as a useful biomarker for Gliomas with IDH 1 mutation [59]. In the pediatric population MR characteristics of medulloblastoma subtypes have been described based on conventional imaging [60] and MR spectroscopy [61]. Similar studies need to be carried out in various pediatric brain tumors to identify imaging surrogates or biomarkers that complement their biological profile. The collection of biological material is central to the development of radio genomics in pediatric neurooncology. It has the potential to aid decision making prior to surgery, guiding biopsy and measure efficacy of treatment using quantitative methods.

The importance of adequately sampled and stored biomaterial

The main advantages of the collection of biomaterials in adequate quality and quantity are the improved etiopathological understanding of pediatric CNS tumors, the validation and discovery of prognostic factors and drugable 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 tumor vaccination strategies, and new insights in biology of acute toxicities and late-effects. In addition, frequencies of tumor predisposition syndromes and their clinical behavior can only be better understood by analyzing broader series of tumor- and germline material from patients with well-annotated clinical information about familiar history, diagnosis, treatment and follow-up. In consequence, bio-pathological characterization is now essential for diagnosis, risk assessment, therapeutic stratification and potentially specific treatment allocation in all patients with medulloblastoma, as well as underpinning future research studies and discoveries. Therefore, comprehensive bio-pathological characterization is routinely required before the start of postoperative treatment (e.g. radiotherapy, neoadjuvant or adjuvant chemotherapy) in individual patients. The most relevant arguments are listed in table 1.

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The current situation and shortfalls

Currently, the collection of high-quality, adequately sampled and stored biomaterial is implemented successfully only in a minority of centers. This is mainly due to deficits in established structures, interdisciplinary cooperation, and funding. Professionally trained staff and required equipment is only available in some centers, and may also need to be professionalized at the central level of research consortia. Active collaboration of all involved disciplines, including neurosurgeons, neuropathologists, and pediatric oncologists, is not always established, and might be compromised due to potential conflicts of interest with other local research initiatives. In addition, the required personal and infrastructural burden for successful biomaterial collection is not adequately compensated by additional 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 must become a routine standard for all children with CNS tumors, regardless of their inclusion in clinical trials or other research initiatives. Moreover, it must become increasingly mandatory in future prospective pediatric CNS tumor trials. For their optimal collection in local institutions and within cooperative research groups, clear definitions of the types of biomaterials with standard operating procedures (SOP) must be implemented, together with thorough solutions for all associated ethical, legal and practical aspects.

Which biomaterials need to be collected and how?

To maximize the above mentioned advantages and to obtain a comprehensive biological understanding of tumor and host-related factors, different types of biomaterials need to be collected. Adequate amounts of tumor tissue, taking into account safety of the patient, should be collected threefold: 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 tumor cell culture or direct xenografting in animals. Blood as preferred choice or buccal swabs should be collected for germline analyses. In addition, constitutional DNA is required for comparison with genomic analyses from the corresponding tumor tissue, as tumor-specific alterations of genes, related signaling pathways, and drugable targets can only be identified and understood by comparison of tumor and germline material. A list of types of biomaterials to be collected and technical aspects of collection and storage is given in Table 2.

Various technical aspects of collection and storage of biomaterials need to be carefully considered to obtain useful amounts of the required materials of optimal quality. This requires first of all a fundamental change in the pediatric neurosurgeons perception, that they play two equally important and pivotal roles in the process of treatment. Neurosurgeons need to appreciate, that apart from their primary role with regard to performing ideally a gross total tumor resection without causing any additional harm to the patients, there is an equally important secondary role by performing a threefold tissue sampling during surgery.

The operative procedure *per se* should be adapted, because much more time needs to be devoted to collect tissue with tumor grasping forceps from different areas of the tumor, instead of mostly using suction or ultrasonic aspirators to take out the bulk of tumor tissue. Piecemeal sampling with the tumor forceps is, especially in either very soft or very hard-elastic tumors, very time- and patience consuming and can prolong a surgical procedure up to 30 minutes, especially if tumors are very

bloody. Information about the heterogeneity of tumors from MRI (diffusion-weighted sequences) or positron-emission-tomography may be used to specifically obtain tissue from various tumor areas.

In addition, a more or less self-running tissue processing SOP needs to be enacted among the theater staff, because the sampled tissue needs to be processed in parallel to the tumor removal, which takes full attention of the surgeon and the scrub nurse. The pathologist might be involved at that time to determine the samples collected being tumor tissue. After the end of the collection period, samples need to be transferred by a third person from the staff to sterile vials and immediately snap frozen in -80ºC freezers or liquid nitrogen, either option needs to be available close to the operating rooms. The samples for tissue cultures need to go into appropriate vials with media for delayed transfer to the lab within 24h or have to be transferred directly to the lab. Only the usual pathology for FFPE material can stay and be processed „as always“. However, neurosurgeons need to know that molecular genetic array diagnostics need additional material to extract enough DNA. Thus, generous sampling is essential and the time needs to be invested.

To make the tissue sampling SOP work well, theater staff needs to be informed and made enthusiastic for this additional work they have to cope with. There needs to be a thorough understanding in all personnel involved, that the tissue processing they are performing is not “just for research”, but has an enormous impact on the patients chances of survival, equal to the impact of operation and tumor removal itself. Understanding the importance of their role in this process will make them efficient and reliable members of the process.

Finally the sampling effort is a team effort and, despite the fact that it is done in the neurosurgical theater, the neurosurgeon’s focus will properly be on the operation itself. Thus the tissue processing pipeline needs to be established as an interdisciplinary effort and adapted to the local conditions including oncology, pathology and theater staff. Since especially malignant tumors of the posterior fossa might undergo emergency surgery or weekend surgery, the SOP for tissue processing needs to be organized in a way that it will function 24/7.

Blood, plasma and serum are important to elucidate the role of circulating tumor cells, extracellular vesicles, cell-free DNA, proteins and other key parameters. Cerebrospinal fluid samples can be used in metastatic tumors, as the access to macroscopic metastatic lesions is frequently limited and only possible by additional invasive procedures. Appropriate diagnostic methods for liquid biopsy may serve to identify future markers for minimal residual disease [63,64]. As tumor tissue from metastatic sites can otherwise only be obtained by more invasive procedures, cerebrospinal fluid (CSF) may not only be used to detect microscopic tumor dissemination in cytospin samples, but may also serve to analyze metastatic tumor DNA, microRNA (miRNA) or proteins in the CSF-supernatant, to enhance knowledge about metastatic tumor spread or disease progression/evolution.

Importantly, biologic material should also be collected later-on during the disease course and after treatment. To speed up the biological understanding of tumor evolution and the appearance of resistance mechanisms, it is of paramount importance to collect tumor tissue at the time of relapse, or through autopsy. To ensure maximal biological information at tumor recurrence, re-biopsy of relapsed tumors should be generally recommended, 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 trial groups, as well as at the level of 'local' institutions (figure 1). Moreover, ethical, legal and practical aspects must be considered.

Ethical, legal, privacy, and practical aspects at the central level of a research consortium or clinical trials group

Studies have shown clear support from patients and their representatives, who, once in receipt of adequate information, are largely in favor that biomaterials not required for diagnostic procedures are made available for research projects [65,66]. However, important ethical, legal, privacy and practical aspects need to be considered in the process of collection, storage, shipment, and sharing of biomaterials. For example, the legal definitions for ownership of biomaterials, and guidelines for informed consent may vary between countries [67], and need to be considered for individual patient care and in the conduct of international clinical trials. Due to the advantages of accurate diagnostic procedures and translational research, it is increasingly accepted that the availability of biomaterials is defined as a mandatory inclusion criterion for patients within clinical trials (e.g. within the SIOPE-PNET5-MB trial [68]). This may not only be justified if biomaterial is a prerequisite for 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 may impact on the understanding of the clinical results of the trials. The main ethical, legal, privacy, and practical aspects of storage, sharing, and shipment of biomaterials are listed in Table 3.

Biobanking

In addition, advantages of central or decentralized (virtual) biobanking need to be considered. Biomaterials can be stored centrally by academic or commercial tumor bank providers, with software systems allowing for maximal transparency about the stored materials. Alternatively, they can be

stored within the respective local tumor banking facilities, and may be shipped according to the requirements of further analyses (diagnostic analyses or collaborative research projects) in batches at later time points. Both central and decentral storage of biomaterials will also allow its use for big data analyses with bioinformatical support, and facilitate a comprehensive cataloguing of biomaterials for collaborative projects between research consortia. In any case, SOPs to control tumor samples for appropriate tissue representation must also be implemented. Storage of biomaterials in aliquots allows the tissue to be used for multiple research projects. Transparent criteria for the access of scientists from local contributing institutions and for independent researchers to the larger biomaterial series may positively impact the cooperation of local centers. Material transfer agreements, SOPs for shipment of materials, and adequate coverage of costs may further facilitate cooperative tumor-banking. In addition, it is important to define coupling of tumor material data to patient data: genomic, transcriptomic, methylomic, metabolomics data from tumor biopsies, as well as data from experiments on patient-derived cell cultures and xenografts, should ideally be stored in an international CNS tumor registry such as the recently established SIOPE DIPG Registry [69], together with comprehensive anonymous clinical, radiological and pathology data of these patients. This will allow for comprehensive Big Data analyses. In this respect, it is of high value to invest in gathering large numbers of retrospective clinical data (baseline characteristics, treatment and survival data) from multiple international groups, and to correlate these with analysis of (epi)genomic data from corresponding banked tumor samples.

National and international research consortia and/or clinical trial groups must consider these aspects and discuss these early in the planning phase of collaborative projects, so that specific national requirements can be implemented in timely manner. Sustainability of data beyond projects and connection of data at overarching levels should be envisioned. Recent large-scale sequencing by International Cancer Genome Consortium and Paediatric Cancer Genome Project has further shown that the genetic and epigenetic repertoire of driver mutations in specific childhood malignancies differs from more common adult-type malignancies. To bring about much needed change, pediatric 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 the SIOPe [70]. These platforms aim to develop mechanism-of-action informed paediatric drug development programmes with aggregated databases of paediatric biological tumour drug targets, to ultimately enable prioritisation and conduct of early phase clinical paediatric trials more rapidly.

~~Access to novel therapies~~

~~Importantly, the SIOPe has called for revisions to the EU Pediatric 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 describing "The state of pediatric medicines in the EU – 10 years of the EU Pediatric Regulation", which concluded that the Pediatric Use Marketing Authorizations (PUMA) concept has so far failed to incentivize the development of pediatric medicines [79]. In North America, the RACE (Research to Accelerate Cures and Equity) for Children Act would require companies to apply PREA (Pediatric Research Equity Act) to any treatment with a molecular target that is relevant in adult and childhood disease.~~

Local institutions need to implement practical solutions according to their structures

It has become evident that the collection and storage of biomaterials can only be achieved successfully, if all relevant steps are solved in each local participating center. As personal and organizational structures are highly different between local participating centers, a general schema may not work in all centers in the same way. The above mentioned aspects rather need to be adapted individually by the local institutions to their structures, ideally by a dedicated local coordinator supported by all other involved disciplines (see table 4). Ultimately, the practical tasks and responsibilities need to be defined and assigned to responsible individuals. ~~As neurosurgical interventions are also undertaken during the night or weekend, SOPs should be established for the adequate storage of tissues outside of regular day time working hours.~~ Specific education and training modules should be developed.

Conclusion

The availability of adequately sampled and stored biomaterial will confer multiple advantages of highest scientific and clinical relevance, such as validation of described and identification of new prognostic factors and drugable targets. This paper aims to stress on the need of biomaterial sampling, and includes also highly relevant practical, ethical, and privacy aspects. Improved sampling of biomaterial is a major prerequisite for the improvement of survival rates for children with CNS tumors, and to reduce treatment-related late-effects.

In addition to increasing knowledge about the roles of conventional treatment modalities in biologically well-defined entities and subgroups, it must be ensured that children are not left behind while precision oncology offers new treatment solutions for adult cancers [71]. As pediatric tumors are clinically and biologically highly distinct from adult cancers, these approaches must be redeveloped in

oncologic diseases, with informative biomaterial. Ideally, data from tumor tissues and biomaterials would be coupled to corresponding anonymous patient data, such as demographics, diagnostic features, radiology and pathology treatment and outcome data, as exemplified by the recently established SIOPE DIPG Registry.

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

In summary, tumor tissue and other biomaterials need to be collected from all children with CNS tumors, and will become increasingly mandatory in prospective pediatric CNS tumor trials. Strong cooperative efforts of 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.

Search strategy and selection criteria

In this review, chairs and representatives of the SIOPe Brain Tumor Group (BTG) (https://www.siope.eu/european-research-and-standards/clinical-research-council/siopecrc/european-clinical-study-groups/siope-brain-tumour-group/) have summarized their views how to efficiently improve biomaterial collection for children with CNS tumours, and why this is urgently required. This has been based on the profound experiences in the conduct of national and international multicenter clinical trials and collaborative research projects. Cited literature for this policy review has been primarily selected by relevance and actuality rather than being object of systematic literature review. SIOPe BTG is a European multidisciplinary association of healthcare professionals which leads in research, treatment (among which international clinical trials) and care of children and young people with tumors of the CNS. It is a subgroup of the European branch (SIOPe) (http://www.siope.eu/) of the International Society of Paediatric Oncology (SIOP) (http://siop-online.org/).

Contributors

The manuscript was mainly written by SR and SWVG. All coauthors contributed to the content of the manuscript from the perspective of the BTG working group they represent, as well as for general aspects of this policy review.

Declaration of interest

CJ reports grants from Roche / Genentech, outside the submitted work; FD reports personal fees from BMS, personal fees from SERVIER, personal fees from TESARO ONCOLOGY, personal fees from CELGENE, outside the submitted work; GS reports personal fees, non-financial support and other from Biomarin Inc, other from Shire Inc, personal fees from Sucampo (now Malinckrodt), outside the submitted work. The other authors declared no conflicts of interest.

References

1. Ostrom QT, Gittleman H, Fulop J, et al. CBTRUS Statistical Report: Primary Brain and Central Nervous System Tumors Diagnosed in the United States in 2008-2012. *Neuro Oncol* 2015; **17 Suppl 4**: iv1-iv62.

2. Kaatsch P, Spix C, Grabow D, Becker C. Annual Report 2016. 2016. http://www.kinderkrebsregister.de/typo3temp/secure_downloads/22605/0/17aa97a18ea4a834424f1eb1a46e6ada9829b582/jb2016_s.pdf2017).

3. Louis DN, Perry A, Reifenberger G, et al. The 2016 World Health Organization Classification of Tumors of the Central Nervous System: a summary. *Acta Neuropathol* 2016; **131**(6): 803-20.

4. Packer RJ. Childhood brain tumors: accomplishments and ongoing challenges. *J Child Neurol* 2008; **23**(10): 1122-7.

5. Pui CH, Gajjar AJ, Kane JR, Qaddoumi IA, Pappo AS. Challenging issues in pediatric oncology. *Nat Rev Clin Oncol* 2011; **8**(9): 540-9.

6. Mertens AC, Yong J, Dietz AC, et al. Conditional survival in pediatric malignancies: analysis of data from the Childhood Cancer Survivor Study and the Surveillance, Epidemiology, and End Results Program. *Cancer* 2015; **121**(7): 1108-17.

7. Armstrong GT, Liu Q, Yasui Y, et al. Long-term outcomes among adult survivors of childhood central nervous system malignancies in the Childhood Cancer Survivor Study. *J Natl Cancer Inst* 2009; **101**(13): 946-58.

8. Bhatia S, Armenian SH, Armstrong GT, et al. Collaborative Research in Childhood Cancer Survivorship: The Current Landscape. *J Clin Oncol* 2015; **33**(27): 3055-64.

9. Gajjar A, Bowers DC, Karajannis MA, Leary S, Witt H, Gottardo NG. Pediatric Brain Tumors: Innovative Genomic Information Is Transforming the Diagnostic and Clinical Landscape. *J Clin Oncol* 2015; **33**(27): 2986-98.

10. Northcott PA, Pfister SM, Jones DT. Next-generation (epi)genetic drivers of childhood brain tumours and the outlook for targeted therapies. *Lancet Oncol* 2015; **16**(6): e293-302.

11. Schwalbe EC, Lindsey JC, Nakjang S, et al. Novel molecular subgroups for clinical classification and outcome prediction in childhood medulloblastoma: a cohort study. *Lancet Oncol* 2017; **18**(7): 958-71.

12. Mack SC, Northcott PA. Genomic Analysis of Childhood Brain Tumors: Methods for Genome-Wide Discovery and Precision Medicine Become Mainstream. *J Clin Oncol* 2017; **35**(21): 2346-54.

13. Liu KW, Pajtler KW, Worst BC, Pfister SM, Wechsler-Reya RJ. Molecular mechanisms and therapeutic targets in pediatric brain tumors. *Sci Signal* 2017; **10**(470).

14. Louis DN, Ohgaki H, Wiestler OD, et al. The 2007 WHO classification of tumours of the central nervous system. *Acta Neuropathol* 2007; **114**(2): 97-109.

15. Thompson EM, Hielscher T, Bouffet E, et al. Prognostic value of medulloblastoma extent of resection after accounting for molecular subgroup: a retrospective integrated clinical and molecular analysis. *Lancet Oncol* 2016; **17**(4): 484-95.

16. Moxon-Emre I, Taylor MD, Bouffet E, et al. Intellectual Outcome in Molecular Subgroups of Medulloblastoma. *J Clin Oncol* 2016; **34**(34): 4161-70.

17. Bull KS, Kennedy CR, Bailey S, Ellison DW, Clifford SC. Improved health-related quality of life outcomes associated with SHH subgroup medulloblastoma in SIOP-UKCCSG PNET3 trial survivors. *Acta Neuropathol* 2014; **128**(1): 151-3.

18. Ellison DW, Kocak M, Dalton J, et al. Definition of disease-risk stratification groups in childhood medulloblastoma using combined clinical, pathologic, and molecular variables. *J Clin Oncol* 2011; **29**(11): 1400-7.

19. Hill RM, Kuijper S, Lindsey JC, et al. Combined MYC and P53 defects emerge at medulloblastoma relapse and define rapidly progressive, therapeutically targetable disease. *Cancer Cell* 2015; **27**(1): 72-84.

20. Morrissy AS, Garzia L, Shih DJ, et al. Divergent clonal selection dominates medulloblastoma at recurrence. *Nature* 2016; **529**(7586): 351-7.

21. Jones C, Karajannis MA, Jones DTW, et al. Pediatric high-grade glioma: biologically and clinically in need of new thinking. *Neuro Oncol* 2017; **19**(2): 153-61.

22. Mackay A, Burford A, Carvalho D, et al. Integrated Molecular Meta-Analysis of 1,000 Pediatric High-Grade and Diffuse Intrinsic Pontine Glioma. *Cancer Cell* 2017; **32**(4): 520-37 e5.

23. Pajtler KW, Witt H, Sill M, et al. Molecular Classification of Ependymal Tumors across All CNS Compartments, Histopathological Grades, and Age Groups. *Cancer Cell* 2015; **27**(5): 728-43.

24. Ramaswamy V, Hielscher T, Mack SC, et al. Therapeutic Impact of Cytoreductive Surgery and Irradiation of Posterior Fossa Ependymoma in the Molecular Era: A Retrospective Multicohort Analysis. *J Clin Oncol* 2016; **34**(21): 2468-77.

25. Pajtler KW, Mack SC, Ramaswamy V, et al. The current consensus on the clinical management of intracranial ependymoma and its distinct molecular variants. *Acta Neuropathol* 2017; **133**(1): 5-12.

26. Sturm D, Orr BA, Toprak UH, et al. New Brain Tumor Entities Emerge from Molecular Classification of CNS-PNETs. *Cell* 2016; **164**(5): 1060-72.

27. Picard D, Miller S, Hawkins CE, et al. Markers of survival and metastatic potential in childhood CNS primitive neuro-ectodermal brain tumours: an integrative genomic analysis. *Lancet Oncol* 2012; **13**(8): 838-48.

28. Johann PD, Erkek S, Zapatka M, et al. Atypical Teratoid/Rhabdoid Tumors Are Comprised of Three Epigenetic Subgroups with Distinct Enhancer Landscapes. *Cancer Cell* 2016; **29**(3): 379-93.

29. Packer RJ, Pfister S, Bouffet E, et al. Pediatric low-grade gliomas: implications of the biologic era. *Neuro Oncol* 2017; **19**(6): 750-61.

30. Hawkins C, Walker E, Mohamed N, et al. BRAF-KIAA1549 fusion predicts better clinical outcome in pediatric low-grade astrocytoma. *Clin Cancer Res* 2011; **17**(14): 4790-8.

31. Qaddoumi I, Orisme W, Wen J, et al. Genetic alterations in uncommon low-grade neuroepithelial tumors: BRAF, FGFR1, and MYB mutations occur at high frequency and align with morphology. *Acta Neuropathol* 2016; **131**(6): 833-45.

32. Mistry M, Zhukova N, Merico D, et al. BRAF mutation and CDKN2A deletion define a clinically distinct subgroup of childhood secondary high-grade glioma. *J Clin Oncol* 2015; **33**(9): 1015-22.

33. Wang L, Yamaguchi S, Burstein MD, et al. Novel somatic and germline mutations in intracranial germ cell tumours. *Nature* 2014; **511**(7508): 241-5.

34. Fukushima S, Otsuka A, Suzuki T, et al. Mutually exclusive mutations of KIT and RAS are associated with KIT mRNA expression and chromosomal instability in primary intracranial pure germinomas. *Acta Neuropathol* 2014; **127**(6): 911-25.

35. Schulte SL, Waha A, Steiger B, et al. CNS germinomas are characterized by global demethylation, chromosomal instability and mutational activation of the Kit-, Ras/Raf/Erk- and Akt-pathways. *Oncotarget* 2016; **7**(34): 55026-42.

36. Ichimura K, Fukushima S, Totoki Y, et al. Recurrent neomorphic mutations of MTOR in central nervous system and testicular germ cell tumors may be targeted for therapy. *Acta Neuropathol* 2016; **131**(6): 889-901.

37. Murray MJ, Bell E, Raby KL, et al. A pipeline to quantify serum and cerebrospinal fluid microRNAs for diagnosis and detection of relapse in paediatric malignant germ-cell tumours. *Br J Cancer* 2016; **114**(2): 151-62.

38. Palmer RD, Murray MJ, Saini HK, et al. Malignant germ cell tumors display common microRNA profiles resulting in global changes in expression of messenger RNA targets. *Cancer Res* 2010; **70**(7): 2911-23.

39. De Mattos-Arruda L, Mayor R, Ng CK, et al. Cerebrospinal fluid-derived circulating tumour DNA better represents the genomic alterations of brain tumours than plasma. *Nat Commun* 2015; **6**: 8839.

40. Brastianos PK, Taylor-Weiner A, Manley PE, et al. Exome sequencing identifies BRAF mutations in papillary craniopharyngiomas. *Nat Genet* 2014; **46**(2): 161-5.

41. Brastianos PK, Shankar GM, Gill CM, et al. Dramatic Response of BRAF V600E Mutant Papillary Craniopharyngioma to Targeted Therapy. *J Natl Cancer Inst* 2016; **108**(2).

42. Hofmann BM, Hoelsken A, Fahlbusch R, Blumcke I, Buslei R. Hormone receptor expression in craniopharyngiomas: a clinicopathological correlation. *Neurosurgery* 2010; **67**(3): 617-25; discussion 25.

43. Martinez-Barbera JP. 60 years of neuroendocrinology: Biology of human craniopharyngioma: lessons from mouse models. *J Endocrinol* 2015; **226**(2): T161-72.

44. Thomas C, Sill M, Ruland V, et al. Methylation profiling of choroid plexus tumors reveals 3 clinically distinct subgroups. *Neuro Oncol* 2016; **18**(6): 790-6.

45. Louis DN, Aldape K, Brat DJ, et al. Announcing cIMPACT-NOW: the Consortium to Inform Molecular and Practical Approaches to CNS Tumor Taxonomy. *Acta Neuropathol* 2017; **133**(1): 1-3.

46. Report from the commission to the European Parliament and the Council. State of Paediatric Medicines in the EU - 10 years of the EU Paediatric Regulation. 2017.

47. Zhang J, Walsh MF, Wu G, et al. Germline Mutations in Predisposition Genes in Pediatric Cancer. *N Engl J Med* 2015; **373**(24): 2336-46.

48. Xu J, Erdreich-Epstein A, Gonzalez-Gomez I, et al. Novel cell lines established from pediatric brain tumors. *J Neurooncol* 2012; **107**(2): 269-80.

49. Dietl S, Schwinn S, Dietl S, et al. MB3W1 is an orthotopic xenograft model for anaplastic medulloblastoma displaying cancer stem cell- and Group 3-properties. *BMC Cancer* 2016; **16**: 115.

50. Girard E, Ditzler S, Lee D, et al. Efficacy of cabazitaxel in mouse models of pediatric brain tumors. *Neuro Oncol* 2015; **17**(1): 107-15.

51. Sanden E, Eberstal S, Visse E, Siesjo P, Darabi A. A standardized and reproducible protocol for serum-free monolayer culturing of primary paediatric brain tumours to be utilized for therapeutic assays. *Sci Rep* 2015; **5**: 12218.

52. Liu Z, Zhao X, Mao H, et al. Intravenous injection of oncolytic picornavirus SVV-001 prolongs animal survival in a panel of primary tumor-based orthotopic xenograft mouse models of pediatric glioma. *Neuro Oncol* 2013; **15**(9): 1173-85.

53. Zhao X, Zhao YJ, Lin Q, et al. Cytogenetic landscape of paired neurospheres and traditional monolayer cultures in pediatric malignant brain tumors. *Neuro Oncol* 2015; **17**(7): 965-77.

54. Hashizume R, Smirnov I, Liu S, et al. Characterization of a diffuse intrinsic pontine glioma cell line: implications for future investigations and treatment. *J Neurooncol* 2012; **110**(3): 305-13.

55. Jansen MH, Lagerweij T, Sewing AC, et al. Bevacizumab Targeting Diffuse Intrinsic Pontine Glioma: Results of 89Zr-Bevacizumab PET Imaging in Brain Tumor Models. *Mol Cancer Ther* 2016; **15**(9): 2166-74.

56. Drost J, Clevers H. Translational applications of adult stem cell-derived organoids. *Development* 2017; **144**(6): 968-75.

57. Peet AC. Magnetic resonance spectroscopy and beyond for pediatric brain tumors. *CNS Oncol* 2014; **3**(3): 195-7.

58. Zarinabad N, Wilson M, Gill SK, Manias KA, Davies NP, Peet AC. Multiclass imbalance learning: Improving classification of pediatric brain tumors from magnetic resonance spectroscopy. *Magn Reson Med* 2017; **77**(6): 2114-24.

59. Kickingereder P, Sahm F, Radbruch A, et al. IDH mutation status is associated with a distinct hypoxia/angiogenesis transcriptome signature which is non-invasively predictable with rCBV imaging in human glioma. *Sci Rep* 2015; **5**: 16238.

60. Andronesi OC, Rapalino O, Gerstner E, et al. Detection of oncogenic IDH1 mutations using magnetic resonance spectroscopy of 2-hydroxyglutarate. *J Clin Invest* 2013; **123**(9): 3659-63.

61. Perreault S, Ramaswamy V, Achrol AS, et al. MRI surrogates for molecular subgroups of medulloblastoma. *AJNR Am J Neuroradiol* 2014; **35**(7): 1263-9.

62. Bluml S, Margol AS, Sposto R, et al. Molecular subgroups of medulloblastoma identification using noninvasive magnetic resonance spectroscopy. *Neuro Oncol* 2016; **18**(1): 126-31.

63. Vu-Han TL, Fruhwald MC, Hasselblatt M, et al. Identifying molecular markers for the sensitive detection of residual atypical teratoid rhabdoid tumor cells. *Cancer Genet* 2014; **207**(9): 390-7.

64. Chakravadhanula M, Tembe W, Legendre C, et al. Detection of an atypical teratoid rhabdoid brain tumor gene deletion in circulating blood using next-generation sequencing. *J Child Neurol* 2014; **29**(9): NP81-5.

65. Mitchell D, Geissler J, Parry-Jones A, et al. Biobanking from the patient perspective. *Research involvement and engagement* 2015; **1**: 1-17.

66. Page SA, Manhas KP, Muruve DA. A survey of patient perspectives on the research use of health information and biospecimens. *BMC Med Ethics* 2016; **17**(1): 48.

67. Kaye J, Briceno Moraia L, Curren L, et al. Consent for Biobanking: The Legal Frameworks of Countries in the BioSHaRE-EU Project. *Biopreserv Biobank* 2016; **14**(3): 195-200.

68. Pizer BL, Clifford SC. The potential impact of tumour biology on improved clinical practice for medulloblastoma: progress towards biologically driven clinical trials. *Br J Neurosurg* 2009; **23**(4): 364-75.

69. Veldhuijzen van Zanten SE, Baugh J, Chaney B, et al. Development of the SIOPE DIPG network, registry and imaging repository: a collaborative effort to optimize research into a rare and lethal disease. *J Neurooncol* 2017; **132**(2): 255-66.

70. Pearson AD, Herold R, Rousseau R, et al. Implementation of mechanism of action biology-driven early drug development for children with cancer. *Eur J Cancer* 2016; **62**: 124-31.

71. The Lancet O. Making paediatric precision oncology a reality. *Lancet Oncol* 2016; **17**(10): 1335.

72. Best MG, Sol N, Kooi I, et al. RNA-Seq of Tumor-Educated Platelets Enables Blood-Based Pan-Cancer, Multiclass, and Molecular Pathway Cancer Diagnostics. *Cancer Cell* 2015; **28**(5): 666-76.

73. Jeurissen S, Vergauwen G, Van Deun J, et al. The isolation of morphologically intact and biologically active extracellular vesicles from the secretome of cancer-associated adipose tissue. *Cell Adh Migr* 2017; **11**(2): 196-204.

74. Consortium E-T, Van Deun J, Mestdagh P, et al. EV-TRACK: transparent reporting and centralizing knowledge in extracellular vesicle research. *Nat Methods* 2017; **14**(3): 228-32.

75. Gautam A, Kumar R, Dimitrov G, Hoke A, Hammamieh R, Jett M. Identification of extracellular miRNA in archived serum samples by next-generation sequencing from RNA extracted using multiple methods. *Mol Biol Rep* 2016; **43**(10): 1165-78.

Figure

Figure 1

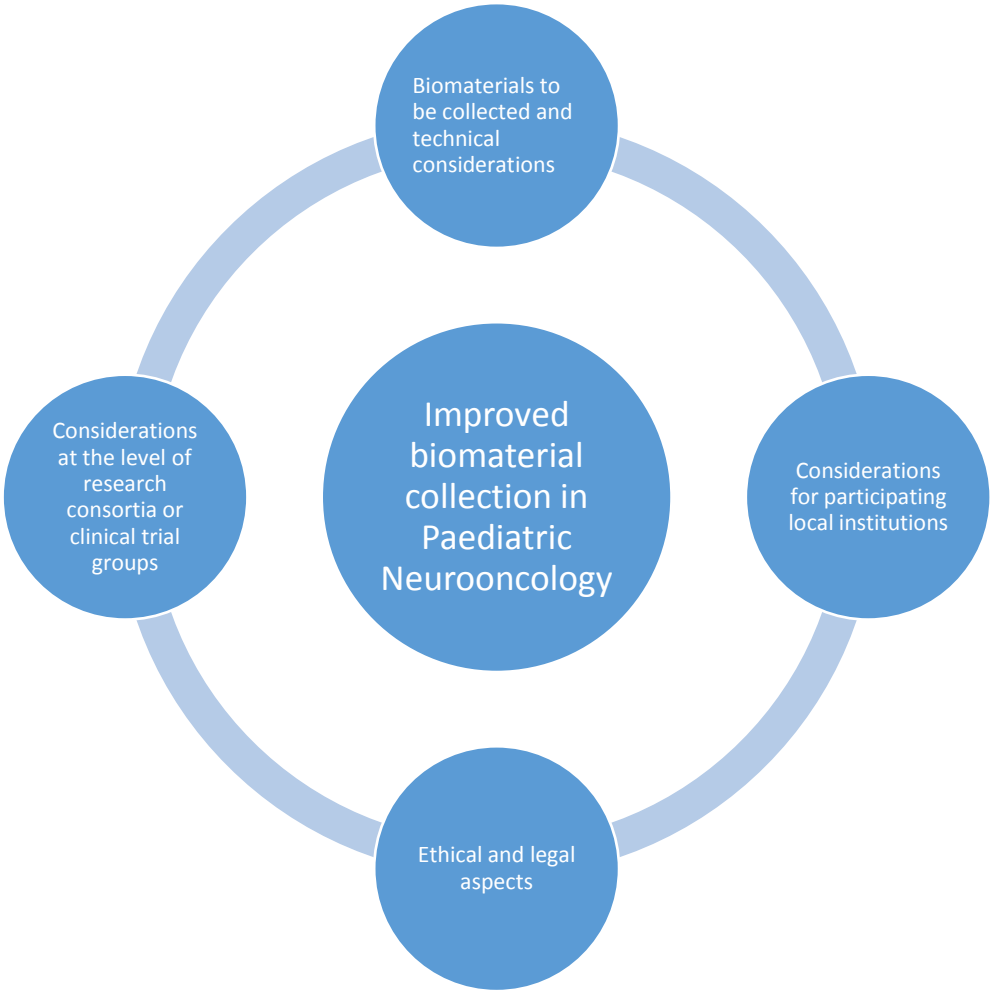

Table 1 unchanged

Table 1. Advantages of adequately sampled and stored biomaterials from tumor and healthy tissue

Adequately sampled and stored biomaterial
is a continuous prerequisite for validation of candidate prognostic factors, and for the discovery of new parameters with clinical significance
is needed 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)
is required for validation of reported targets and the identification of new drugable targets
is the basis for current and future treatment decisions for individual patients, at clinical trials level and for related research directions
allows to identify relevant biopathological mechanisms of tumor etiology, and biological drivers for tumor growth, and resistance to treatments (commonly across pediatric CNS tumors, and within specific entities and subgroups) and will contribute to improve therapeutic solutions
increases the understanding of biological drivers for relapse (especially if paired sets of biological tissues, from time of initial diagnosis and from time of relapse, are available)
contributes to the understanding of the tumor micro-environment, other host-related factors and immunological aspects of tumor control
allows to identify appropriate diagnostic methods for liquid biopsy and may serve to identify future markers for minimal residual disease
facilitates identification and/or validation of associations of genetic polymorphisms with pharmacokinetic assessments and observed treatment-induced acute toxicities and/or late effects (hearing deficits, cognitive impairments, hematological toxicities etc)
will enhance knowledge about the frequency of germline-mutations with treatment-related toxicities, 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 tumor imaging or biomarkers for tumor 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 (e.g. for tumor vaccination strategies)
will ultimately speed up the urgently required scientific progress for children with CNS-tumors as well as early access to new drugs in the context of appropriate clinical trials (targeting of receptors

and signaling pathways, epigenetic alterations, microenvironment and immune system)

Table 2 unchanged

Table 2. Biomaterials to be collected and proposals for collection and storage

Material	Amount	Priority	Processing	Purpose	Assay
Tumor tissue	Min. 1 x 1.5 cm^3 or 3 x 0.5 cm^3. Consider heterogeneity of tumor tissue. When stereotactic biopsy: 2 x 4 needle biopsies are recommended.	1	Formalin-fixed, paraffin embedded of viable tissue sampled early during dissection to obtain viable tumor tissue (avoid crushing)	Diagnostics	IHC/DNA methylation array
	Touch imprint preparation or 1 x 0.5 cm^3	1	Within 20 min from resection snap frozen at -80°C	Diagnostics	FISH, DNA/RNA sequencing
	1 x 0.5 cm^3	2	Within 20 min from resection snap frozen in N2 or at -80°C	Research	DNA/RNA sequencing
	1 x 0.5 cm^3	3	Culture medium (living cells)	Research	Cell culture/PDX models
	1 x 0.5 cm^3	4	Paraformaldehyde	Research	Imaging
	> 1.5 cm^3[1]	1	Sterile, without additives, within 20 min from resection snap frozen at -80°C	Immunotherapy	
CUSA	All available material	2	Culture medium	Research	Cell culture/PDX models
Blood	5 ml EDTA[2]	1	WBC	Diagnostics	DNA
	5 ml EDTA	3	Platelets [72]	Research	RNA
	5 ml EDTA	2	Plasma [73,74]	Research	DNA/RNA/extracellular vesicles[4]
	5 ml serum	2	Serum [75]	Research	DNA/RNA/extracellular vesicles[4]
CSF[3]	All available material	1		Diagnostics	Cytology
	All other available material	2		Research	DNA/RNA/extracellular vesicles[4]
Normal CNS tissue	Obtain during placement of a VP-shunt or subcutaneous intraventricular device or performance of third ventriculoscopy	3	Equal to tumor tissue: FFPE and snap frozen	Research	IHC/DNA methylation array, FISH, DNA/RNA sequencing
Saliva, urine	1 - 5 ml	4		Research	DNA, pharmacokinetics, extracellular vesicles analyses[4]

[1] In case an operation is performed in part with the goal to obtain tumor tissue for preparing dendritic cell vaccines.
[2] Constitutional DNA can eventually be taken via buccal swab.
[3] CSF can be collected during operation. For some treatment schedules, CSF can be taken 14 days after operation.
[4] For analyses of extracellular vesicles, material should be processed within one hour according to specific procedures (http://evtrack.org).

Table 3 unchanged

Table 3. 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, recipients of material and use of anonymized or pseudonymized clinical data, followed by clear forms for informed consent
Ensure that coupling of tumor material data to patient data including treatment and imaging is possible, allowing also for comprehensive 'Big Data' analyses
Ethical approval and permissions from international and/or national and/or local authorities must be obtained
To gain and provide insight in the adherence and availability of biomaterials and to identify potential for further improvements, consider a monitoring system for biomaterials and for accessory informed consents per local hospital
Consider ownership issue for biological tissue and related clinical data, which may be different between countries
Consider advantages of central versus decentralized (virtual) tumor-banking, and procedures to check for appropriate tissue representation for interpretable biological results
Consider if the availability of biomaterial should be defined as a mandatory inclusion criterion for patients within clinical trials
In the context of clinical trials, responsibilities of trial coordinators and local centers should be defined and adapted to applicable laws and regulations.
Adequate coverage of the local costs and shipment of biomaterials by research grants will facilitate the compliance of local institutions
Integrated, reusable tumor box devices may facilitate shipment of frozen and unfrozen materials
To optimize the availability for both local and central research projects, the respective biomaterials should be stored in reasonable 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 for centrally stored material following transparent rules for evaluation of such applications, thus having benefit from their participation wherever appropriate

Table 4. Aspects for standard operation procedures to be considered in local institutions

Staff from all involved disciplines (neurosurgeons and operation room staff, (neuro)pathologists, pediatric oncologists, research nurses, etc.) must know about 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:

Information and consent from patients and their legal representatives: pre-operative oral in case of emergency, later written consent; biobanking, research/trial documents
Amount and types of tissue, blood, and other biomaterials
Neurosurgical considerations (frozen section, debulking, cusa, infiltration zone, healthy material, CSF). Freezing and fixation of maximal amounts of material. As neurosurgical interventions are also undertaken during the night or weekend, SOPs should be established for the adequate storage of tissues outside of regular day-time working hours.
Histopathological diagnosis, reference assessments
MRI sending via digital route or anonymized and coded CDrom
Adequate short term storage of tumor tissue and other materials like blood, mucosa, saliva, urine (labelling of samples, eventually -80° freeze in operation room in due time, labelling of samples)
Transfer to long-term storage, or shipment of samples according to SOP
Trial-specific requirements (e.g. touch imprint preparation for FISH)
Supply for cell culture
File for documentation of collected materials per study
Confirmation of received materials at research institute
Procedure for prioritization of pathology in case of sparse material

Full professors

SR (Rutkowski), CMK (Kramm), UT(Thomale), and CJ (Jones) are full professors.

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To

The Editorial board

The Lancet Oncology

Hamburg, May 8, 2018

Resubmission Policy review Manuscript THELANCETONCOLOGY-D18-00435R1

Dear Alexandra Sklan, dear editors,

Thank you for your feedback to our revised version of our policy review

"Biological material collection to advance translational research and treatment of children with CNS tumors: A position paper and practical considerations from the SIOP-Europe Brain Tumor Group".

The manuscript has been revised according to the referee comments as follows:

1. On page 5 the title "Classification and clinical relevance" would be improved by adding "Tumour classification and clinical relevance".

Reply: This has been added to the title.

2. The paragraphs "The need of tumor tissue for precision medicine" and "The importance of biological assessments with relevance to tumor imaging", should be subheadings within the paragraph "The importance of adequately sampled and stored biomaterial"

Reply: Both parts have been placed with subheadings into the indicated paragraph as suggested (pages 11 and 12).

3. I agree with reviewer 2 (minor comment 2) that the relevance of the paragraph "Access to novel therapies" on p15 is not clear in terms of its relation to biomaterial collection. Its relevance should be explained and incorporated into the paragraph "The importance of biological assessments in pediatric CNS tumors" or deleted.

Reply: The relevance of the paragraph has been explained by adding "Successful biomaterial collection is a key prerequisite for preclinical research projects aiming to identify effective new drugs for children with CNS tumors, and will contribute to their improved access to novel therapies" at the end of this paragraph, and the paragraph has been incorporated as suggested (page 9).

4. On p 15 in the sentence "To bring about much needed change, pediatric platforms such as ACCEL-

ERATE 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 the SIOPe" it should be explained which changes can be achieved by ACCELERATE.

Reply: The changes which can be achieved have been explained by adding the sentence “These platforms aim to develop mechanism-of-action informed paediatric drug development programmes with aggregated databases of paediatric biological tumour drug targets, to ultimately enable prioritisation and conduct of early phase clinical paediatric trials more rapidly.” to the paragraph (page 16).

Although this also refers to access to new drugs, we suggest to keep this part in the biobanking section, as our argument arises in this context from the improved availability of biomaterials.

5. On page 16 the sentence "As neurosurgical interventions are also undertaken during the night or weekend, SOPs should be established for the adequate storage of tissues outside of regular day-time working hours. Specific education and training modules should be developed." is redundant within the text as it has been discussed already extensively on p 12/13, but should be added to table 4.

Reply: As suggested, we have deleted this sentence from the text (page 17) and added it to table 4.

As some paragraphs of the manuscript have been incorporated into other sections (see above), we have revised the order of the literature citations accordingly. All elements of the paper and all relevant information has been provided.

We are convinced that the manuscript has been further improved by the changes made in response to the reviewer’s comments. Therefore, we hope that our manuscript will be accepted for publication in *The Lancet Oncology*.

With kind regards,

Prof. Dr. Stefan Rutkowski, corresponding author,
Chair of the SIOP-Europe Brain Tumor Group