STANDARD CLINICAL PRACTICE RECOMMENDATIONS
FOR PAEDIATRIC HIGH-GRADE GLIOMA

INTRODUCTORY PAGE

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1 BACKGROUND AND RATIONALE

1.1 Rationale

Paediatric high-grade gliomas (pedHGGs) are highly invasive brain tumours that account for approximately 15% of all central nervous system (CNS) tumours in children and adolescents. The general outcome for these tumours is poor with 5-year survival rates of less than 20% (*1*). They present a completely different biology compared to the HGGs that arise in adults, and it is now understood that they represent a heterogenous group of tumours rather than just one entity(*1*), a fact that has recently been further acknowledged in the 5th edition of the WHO CNS tumour classification (2021)(*2*).

Despite the historical existence of a significant number of prospective clinical trials for children with pedHGGs either at diagnosis or recurrence, over the past 4 decades, there has been little improvement in patient outcomes. Until now, after surgery and adjuvant radiotherapy, temozolomide-containing chemotherapeutic approaches have been standard practice in the paediatric neuro-oncology community, and also used as a control arm in clinical trial(*3-5*), with most care providers aiming to ultimately enrol pedHGG patients into investigational clinical trials.

The general challenges for clinical trial design in pedHGGs are 4-fold (*adapted from(6)*):

1. Intratumoral heterogeneity and molecular pathway redundancy.
2. Lack of currently actionable alterations in a large proportion of patients.
3. Small subsets of patients for each given biology and target expression.
4. Issues with drug delivery as a result of poor blood–brain barrier (BBB) passage

1.2 Background

1.2.1 Diffuse midline glioma H3K27M-altered / DIPG

H3K27-altered diffuse midline gliomas (DMGs) represent 10% of brain tumours and 70% of HGG in children. These tumours arise in the midline structures of the brain (pons, thalamus, spinal cord). Most of these tumours carry a mutation or alteration in H3K27 and this genetic mark is associated with a uniformly fatal disease course, independent of tumour location (*7, 8*). H3K27-altered DMGs have been acknowledged as a new entity in the WHO 2016 classification and have been subdivided in the recent 2021 classification into a few different subtypes: a predominant group presenting histone H3 mutations (H3.3 p.K28M (K27M)-mutant, and H3.1 or 3.2 p.K28M (K27M)-mutant), often associated with *PDGFRA* and MYC amplifications. A smaller group of H3K27-altered DMGs are histone H3 wildtype but show EZHIP overexpression resulting in loss of H3K27 trimethylation and often coincide with an ACVR1 mutation. Furthermore, an additional group with H3K27me3 loss and frequent EGFR gene alteration has been described. All H3K27-altered DMGs share Polycomb Repressor Complex 2 (PRC2) inhibition and are therefore classified together in the WHO classification 2021 (*9*). A small subgroup of high-grade gliomas that occur in the midline showing

amplification of MYCN (GBM-MYCN) is now re-classified separately within the group of diffuse paediatric-type high-grade glioma, H3-wildtype, and IDH-wildtype, subgroup pHGG MYCN (*2, 10*).

DMGs have historically been treated in the same way as hemispheric gliomas, although the Children's Oncology Group study ACNS-0126 of temozolomide adjuvant to RT in hemispheric pedHGG and DMG concluded that there is little justification for using TMZ in DIPG (*11*). The study by Cohen et al. was not randomized to radiotherapy only but showed TMZ-RT not to be inferior or superior to the preceding CCG-9941 study that employed intensive pre-radiation chemotherapy and hyper-fractionated. Interestingly, a large analysis of 1130 DIPG patients by the SIOPE and International DIPG registries revealed that any neoadjuvant or adjuvant systemic therapy in addition to RT (which was mostly TMZ-based adjuvant therapy) correlated with longer survival in both univariable and multivariable analyses. This had also previously been observed in retrospective analyses by Wagner et al. where a better median OS was observed in DIPG patients treated with adjuvant chemotherapy after RT (11.3 months) compared with patients treated with RT alone (9.5 months; P = .03). Likewise, in a retrospective analysis by Kebudi et al. patients receiving adjuvant temozolomide or other chemotherapy (CCNU, vincristine) after RT, had a significantly higher survival than those treated with RT only (*12*), this has also been shown in other studies using neo-adjuvant intensive chemotherapy (*13-15*). However, a limitation of all these studies is that none of them addresses a well-defined H3K27-altered DMG subgroup and, as non-randomized studies, might be subject to bias. In the HERBY randomized trial for non-brainstem midline pedHGG, H3K27M was equally poor (8.0 months MOS) with no superiority of bevacizumab added to temozolomide-RT (*5, 16*).

In 2014, for DMGs H3K27-altered, an adaptive design protocol was developed, the BIOMEDE 1.0 trial. In this study, most patients received a treatment assumed to specifically target a biological abnormality identified on the biopsy. The three drugs administered were erlotinib, dasatinib and everolimus (NCT02233049). None of these targeted agents was shown to be superior to the other, with a median OS of 10.0, 10.5 and 11.9 months respectively. Everolimus was chosen as a 'standard arm' for the subsequent BIOMEDE 2.0 trial for showing less side effects (*17*).

Both the SIOP Europe and International Diffuse Intrinsic Pontine Glioma Registries (https://dipgregistry.eu and https://dispgregistry.org) created in 2012 have made a major contribution towards understanding this challenging disease and have been broadened to all DMGs in 2022 and 2019 respectively. The registries ensure prospective data collection and helps developing new approaches to treating DMGs.

1.2.2 Hemispheric pedHGG

Hemispheric pedHGG represent 5% of brain tumours and 30% of HGG in children. These include H3G34R/V diffuse hemispheric glioma, paediatric-type high-grade glioma and infant-type high-grade glioma, as classified in the 2021 WHO classification of CNS tumours. From the United States, the first prospective, randomized clinical trial, CCG-943, for children with HGG was published in 1989 by the Children's Cancer Study Group (CCG) and showed a significant

improvement in outcome of RT followed by procarbazine/chloroethyl-cyclohexyl nitrosourea [CCNU]/vincristine chemotherapy, over radiation therapy (RT) alone, after maximal safe surgery (*18*). 5-year overall survival (OS) rates of 43% (±9%) and 17% (±7%) were reported for RT/PCV vs RT, respectively. In the follow-up RCT CCG-945 study, the RT/PCV regimen was compared to eight-drugs-in-1-day (8-in-1) chemotherapy with no significant difference between the arms, with a 5-year OS of 36% (±6%)(*19*). Gross total resection (GTR, >90%) was found to be an important prognostic marker for survival. Overexpression of O^6-DNA methylguanine-methyltransferase (MGMT) was strongly correlated with adverse outcome in both arms of the CCG-945 study (*20*). Of note, later central review of pathology of the CCG-945 study indicated that 30% of patients were low-grade gliomas misclassified as HGG, resulting in an adjusted overall survival rate of 22% (±3%) for HGG in CCG-945 (*21*). Unfortunately, this neuropathological reanalysis was not performed for the CCG-943 study.

In a pivotal trial that ran from August 2000 to March 2002 in adult glioblastoma, the alkylating agent temozolomide (TMZ) was introduced for adult glioblastoma. Single-agent TMZ, when administered during and after RT, significantly prolonged EFS and OS in adults with glioblastoma compared with RT alone (*22*). While methylation of the *MGMT* promoter was confirmed as a prognostic marker, the predictive value for benefit from TMZ has not been prospectively demonstrated for paediatric patients as in the adult setting (*23, 24*). In analogy to the experience with TMZ in adults, the Children's Oncology Group (COG) study ACNS0126 employed TMZ concurrently with radiotherapy and showed an equal survival outcome to the previous CCG-945 study, with a 3-year EFS and OS of 11 ±7% and 22% ±5% respectively. TMZ treatment showed less toxicity compared to previous CCG trials (*11*). The role of CCNU added to TMZ was investigated in the subsequent ACNS-0423 study that resulted in better 3-year EFS and OS rates of 22 ±8% and 28 ±8% respectively, most pronounced for patients with methylation of the *MGMT* promoter and non-GTR patients (*25*). Likewise, in adult GBM patients with *MGMT* promoter metylated tumors, the CeTeG/NOA-09 study (NCT01149109) showed TMZ combined with CCNU to be superior to TMZ alone.(*26*). This study was performed in a selected group of *MGMT* methylated patients, as a prior pilot study had indicated that no benefit of adding CCNU to TMZ was observed in MGMT methylated, MGMT expressing tumours (*27*). In contrast, another not-randomised phase 2 trail (UKT-03) suggested lomustine-temozolomide plus radiotherapy to be superior to temozolomide chemoradiotherapy in newly diagnosed glioblastoma with methylation of the MGMT promoter (MGMTp). However, the current paediatric trial HIT-HGG_2013 could show over 180 patients that only 8 patients had a confirmed MGMTp hypermethylated tumor and only 22 are in a somehow grey area regarding MGMTp methylation. Thus, confirming that the vast majority of pedHGG are not MGMTp hypermethylated and that this mechanism is not significant for survival in the setting of TMZ-RT-based therapy (C.Kramm personal communication from (*28*))

The most recent COG HGG trial, ACNS0822, compared two different experimental arms with vorinostat or bevacizumab during RT with a control arm with TMZ during RT. The study was initially planned as a "pick-the-winner" phase II design to be advanced into phase III testing, but the study was permanently closed in 2014 during phase II, as no arm showed any clear superiority over TMZ/RT(*29*). The addition of bevacizumab to a backbone of TMZ/RT (Herby trial) failed to improve EFS and OS in non-brainstem pedHGG (*5*). Post-hoc analyses of the

molecular characteristics of the patients included in this trial, however, seem to indicate that the addition of bevacizumab might provide some benefit to certain subgroups of pedHGG, including hypermutated and BRAF-V600E mutated pedHGG (*16*).

The use of pre-irradiation chemotherapy has been evaluated in a phase II approach, where 4 courses of neo-adjuvant ICE chemotherapy were given, followed by hyperfractionated radiotherapy (1.1Gy bid for 30 days) and 4 courses of ICE adjuvant therapy. This study showed low toxicity and 5-year progression free survival (PFS) of 56% and OS of 67%. Brainstem tumours in this study did not benefit from this approach (*30*). Furthermore, in patients with pedHGG treated on the German HIT-GBM-C cooperative group study with intensive chemotherapy during and after RT (cisplatin, etoposide (PE), and weekly vincristine during radio-chemotherapy, with one cycle of cisplatin, etoposide, and ifosfamide (PEI) during the last week of radiation, and subsequent maintenance chemotherapy followed by oral valproic acid), survival was better than that seen in prior HIT-GBM studies in the subgroup of patients with HGG who had undergone gross total resection (5-year OS rate 63% vs 17% for the historical control group, P = .003, log-rank test). Molecular data were not provided, however, rendering the data difficult to interpret (*31*).

The German cooperative group is currently conducting the HIT-HGG-2013 trial (*28*) comparing the combination of temozolomide and valproate with historical data from their previous studies, HIT-HGG-2007 (NCT03243461), using single agent temozolomide. Final data will be published in the very near future.

In parallel, some other phase II studies are exploring the combination of temozolomide with nivolumab and immunotherapy (PNOC007 H3.3K27M Peptide vaccine with nivolumab for children with newly diagnosed DIPG and other gliomas NCT02960230; NIVOGLIO Nivolumab in combination with temozolomide and radiotherapy in children and adolescents with newly diagnosed high-grade glioma NCT04267146).

1.2.3 Hemispheric HGG in infants

Infants with HGG have long been known to show better survival compared with older children and an improved outcome both with chemotherapy after surgery and, if necessary, delayed radiotherapy. Infants with malignant astrocytoma treated with the 8-in-1 regimen used in the CCG-945 study were reported to have a 3-year PFS and OS of 36% and 51% respectively, markedly better than older children treated with this regimen in combination with RT (*32*). In parallel, from 1986-1996, the Baby POG I study reported cases cured with 24 months chemotherapy alone using prolonged alternating chemotherapy consisting of two cycles of cyclophosphamide and vincristine followed by a third cycle of cisplatin and etoposide. The study reported 5-year PFS and OS of 43% and 50% for the 18 HGG patients included (*33*). With the French chemotherapy-only BBSFOP protocol, an 18-month schedule of seven cycles of three drug pairs (carboplatin - procarbazine, cisplatin - etoposide and vincristine - cyclophosphamide) in HGG patients under 5 years of age a 5-year PFS and OS of 35.3% and 58.8% was observed (*34*). In the UKCCSG/SIOP CNS 9204 trial, infants with non-brainstem HGG were treated with courses of carboplatin/vincristine, high-dose methotrexate/vincristine,

cyclophosphamide monotherapy and cisplatin monotherapy, resulting in PFS and OS rates of 13.0% and 30.9% (*35*).

Whether the survival differences compared to older children are caused by a different molecular make-up, drug-delivery differences to the brain or related to the different therapeutic approach were, until recently, unknown. Recent years, however, have seen the emergence of evidence that different biology may be a major contributing factor. A large proportion of infant patients, especially those *under 2 years of age*, were shown to have tumours molecularly distinct from those in older children, and the group 'Infant-type hemispheric glioma' is now recognized in WHO CNS 2021. These studies also indicate a possible role for targeted therapies in this patient group, as driving targetable molecular alterations have been defined such as fusions in ALK, ROS1, NTRK1/2/3 and MET (*36, 37*).

2 PATIENT GROUP

At diagnosis, pedHGG present with a variety of symptoms related to the tumour localization and the age of presentation. Some symptoms can result from increased intracranial pressure. Infants' symptoms are more often non-specific (failure to thrive, irritability) with delayed increased ICP symptoms because of the skull elasticity. The duration of symptoms tends to be shorter since high-grade gliomas develop faster.

For the infant subgroup we choose the age cut-off of 2 years in these guidelines, based on the neuropathology diagnosis (*36, 37*) even though the treatment age groups are usually defined with an age cut-off of 3 years.

Considering infant HGG (in children under 2 years), we will not address in these guidelines the specific case of *infratentorial* infant HGGs. Pontine glioma do exist in children below the age of two (the youngest patient with an H3K27M proven glioma was 6 months old) although in this age group low-grade gliomas are predominant. For these rare cases, diagnosis is an issue since stereotactic biopsy is not feasible due to the thinness of the skull. Open biopsy could be discussed but it could be discussed that LGG treatment is applied in these patients without a biopsy (*38*). We will here focus only on *supratentorial hemispheric* infant HGG.

3 DIAGNOSTIC CRITERIA

3.1 Imaging

Essential MRI sequences for brain and spine imaging, tumour measurement guidelines, post-operative residual tumour definitions and response criteria from the recommendations of the *SIOP-Europe Brain Tumour Imaging Group* (*39*) should be followed and are available in Appendix and in the Standard Clinical Practice Recommendations - Imaging Working Group.

PedHGG are mainly diffusely infiltrating lesions, with ill-defined margins and variable contrast enhancement patterns (absent/inhomogeneous). Diffusion weighted imaging (DWI) is particularly valuable in the assessment of pedHGG as areas of diffusion restriction can identify foci of increased cellularity. This can be very useful in the assessment of non-enhancing lesions including metastasis, disease progression and differentiating it from pseudo-progression.

During the meeting of the SIOPE HGG working group held in Milan (6-7th April 2022), a consensus was achieved that spinal MRI (sagittal T1 complemented with axial T1) is required at diagnosis for staging of all pedHGG before surgery.

This chapter presents the SIOPE imaging guidelines and differences with the RAPNO guidelines can be found in Appendix 1.

3.1.1 Characteristics of DMG imaging

When compared to diffuse hemispheric gliomas, DMG have shown a different radiological phenotype, being well defined with minimal or no perilesional oedema, smaller volumes on presentation and a higher incidence of leptomeningeal metastasis (*40*).

As general neuroimaging consensus, DMGs are T1-hypointense and T2-hyperintense on MRI diffusely infiltrating lesions arising in the ventral aspect of the pons, involving ≥ 50% of its cross-sectional diameter and often encasing the basilar artery. T2-hypointense lesions (*41*) or foci of necrosis may be present within the tumour. DMG in the pons (DIPG) typically shows little or no contrast enhancement on MRI at initial diagnosis, and enhancement patterns can vary considerably. As previously reported in a large DIPG study (*41*), the presence of ring enhancement was an independent predictor of adverse OS but this is not reported in all studies, including only biopsy-proven DMG(*42*).

Routine biopsy in DIPGs remains under debate (*41, 43*) and mainly reserved for cases with an atypical imaging appearance or within research studies/clinical trials. Typical DIPG diagnosis may be established based on MRI and clinical criteria only (duration of symptoms <6 months, multiple cranial neuropathies, long tract signs [hyper-reflexia, clonus, increased tone, presence of a Babinski reflex], and ataxia). The diagnosis of DIPG can pose challenges in cases with atypical findings and central-neuroimaging review can be valuable in increasing the accuracy of diagnosis in prospective trials (*44, 45*). Systematic biopsies, such as in the BIOMEDE 1.0 trial, have however shown that an imaging diagnosis only can be misleading in up to 10 % of cases. Differential diagnoses include pilocytic astrocytoma, gangliogliomas, MYB-rearranged gliomas, HGNET-MycN or even non-tumoral lesions such as MOG encephalomyelitis (manuscript in preparation)(*17*).

3.1.2 Spine imaging in pedHGG

In pedHGG, no standard for obtaining follow-up spine imaging exists, and routine evaluation of spinal disease in the context of clinical trials is frequently inconsistent. The minimum requirement is a sagittal T1-weighted post contrast scan of the entire spinal canal, which may be implemented by additional T1 axials.

Ideally, spinal MRI should be done *before surgery*. If spinal imaging is not performed preoperatively this should be performed on the post-operative scan, within 48-72 hours pots-surgery, with the caveat that repeat imaging may be needed if postoperative subdural collections degrade image quality (*46, 47*).

Since the Milan meeting, the SIOPE HGG working group recommends that spinal MRI (sagittal T1 complemented with axial T1) is required at diagnosis for staging of all pedHGG *before surgery.*

3.2 Role of CSF cytology

CSF collection is not routinely performed for pedHGG. It can potentially become relevant in the context of liquid biopsies, but currently this is not a standard.

For classification of leptomeningeal dissemination see Appendix 2.

A lumbar puncture for analysis of CSF is usually not indicated prior to surgery. Clinical or radiological signs of raised intracranial pressure or a posterior fossa tumour represent contraindications due to the high risk of herniation. CSF collection during surgery or taken from a possible external ventricular drain may result in an inaccurate diagnosis due to contamination of tumour cells but standardized procedures are under discussion in the SIOPE BTG to encourage CSF collection uniform procedures in all brain tumour groups at diagnosis. When performed after surgery, CSF analysis should be done at least 14 days after surgery (e.g. prior to radiotherapy) by lumbar puncture (*28*), to avoid post-surgical artifacts.

3.3 Biopsy in DMG of the pons

The diagnosis of DIPG is conventionally made on the combination of a typical clinical presentation and the well-described radiological findings on MR imaging. This became possible once MRI provided the resolution required to distinguish diffuse gliomas from the less common focal brainstem tumours (*48*). Tumour tissue is now not considered necessary for diagnosis and management unless there are atypical features with respect to patient age, quality, and duration of symptoms, or neuroradiological appearances. Over the years this has led to considerable debate among neurosurgeons and oncologists (*45, 49*). The paucity of biological tissue accounts for the poor understanding of the molecular biology of DIPG, and potentially the lack of therapeutic progress, relative to other tumours (*50, 51*). It has been difficult to ethically reconcile the surgical risk of a biopsy, and the lack of direct benefit to the individual child, with the overall value of obtaining biological tissue and furthering the biological understanding of this tumour.

However, several studies have shown that biopsy of DIPG is safe in experienced hands (*51-53*). A large meta-analysis evaluated 735 biopsy procedures in paediatric brainstem tumours and found an overall diagnostic success rate of 96.1%; the rates for permanent morbidity and mortality were both only 0.6% (*53*). Surgical adjuncts such as navigational robotic technology

increase accuracy and safety (*54, 55*). In studies such as BIOMEDE and INFORM biopsy was mandatory, and the tumour tissue obtained yielded sufficient material for detailed molecular investigation, with very low rates of adverse events (*56*). Currently, however, biopsy is still not considered a standard of care for DIPG by all, as a recent survey amongst paediatric neurosurgeons practicing in Europe showed that a majority (57% of 73 respondents) are still reluctant to consider biopsy of DIPG outside of a clinical trial (*43*). It is hoped that as new clinical trials and potential therapeutic options emerge, the value of biopsy in identifying the molecular subgroups, defining prognosis, and assessing trial eligibility is reappraised (*57*). In the SIOPE-HGG working group survey biopsy was considered as a standard in 15/33 countries and three countries discussed biopsy on an individual basis. Six countries were never performing biopsy.

Outside of clinical trials, biopsy is advised for suspected non-pontine DMG and atypical brainstem cases (duration of symptoms, radiological peculiarities like calcifications). For suspected classical pontine DMG (i.e., DIPG), it is increasingly likely that biopsy will be required as part of clinical trials taking into account the heterogeneity of DIPG in terms of prognosis albeit mostly fatal. Biopsy can be considered outside of a clinical trial where the risk/ benefit needs discussion with the patients and their family to inform on the use of specific targeted therapies. It is now accepted that for most clinical trials a biopsy will be requested for brainstem suspected DMG as per the study protocol since the procedure is now widely disseminated in many neuro-oncology centres without life-threatening complications.

3.4 Neuropathological diagnosis

Neuropathological diagnostic procedures should be applied according to local standards for all children with suspected pedHGG, leading to a diagnosis according to the 5th edition of the WHO Classification of Tumours of the Central Nervous system (*58*). Neuropathological diagnosis of pedHGG should ONLY be made by neuropathologists or pathologists who can demonstrate both specialist training and ongoing professional development in brain tumour diagnosis. They should take part in external quality assurance schemes, and if these are not available, work towards their establishment. Diagnostic procedures should be performed in laboratories accredited according to appropriate local standards (e.g., ISO15195).

For the exact classification of pedHGG and the exclusion of histological mimics the diagnostic methodology should include *immunohistochemical assessment* of cell lineage and surrogate protein markers for genetic alterations (including immunohistochemistry with antibodies against mutant proteins) as well as *molecular pathological technologies* to identify genetic alterations on the DNA and/or RNA level and to establish epigenetic profiles for methylation-based tumour classification. Most pedHGG entities are defined by the presence/absence of specific genetic alterations and can also be identified by their *characteristic methylation profiles.*

The following algorithm from WHO CNS 2021 may help to further molecularly characterize pedHGG:

Histology	DIFFUSE HIGH-GRADE GLIOMA				
Localisation	hemispheric			midline	any location
Pediatric age group	adolescents	adolescents	infants < 2 years	[pre]school children	(pre)school children
IDH status	IDH wildtype	IDH mutant	IDH wildtype	IDH wildtype	IDH wildtype
Histone 3 status	H3.3 G34R/V	H3 wildtype	H3 wildtype	H3 K27me3 loss H3.3 K27M/K27I mutant or H3.1/H3.2 K27M mutant or EZHIP overexpression or EGFR altered (see below)	H3 wildtype
RTK status	PDGFRA mutation / amplification		ALK or ROS1 or MAPK3-5 or MET fusion	EGFR exon20ins mutation or other EGFR alterations (mostly in thalamic / EZHIP cases)	PDGFRA/EGFR/MET amplification
Mismatch repair status (if MMR deficiency suspected)	no PMS2, MLH1,MSH2, MSH6 IHC loss	no PMS2, MLH1,MSH2, MSH6 IHC loss	no PMS2, MLH1,MSH2, MSH6 IHC loss	no PMS2, MLH1,MSH2, MSH6 IHC loss	MMR associated HGG: loss of expression of at least one of mismatch repair proteins
Methylome	Distinct profile	Distinct profile	Distinct methylome profile, also for non-ALK/ROS/MET-NTRK cases	Can help detect EZHIP OE DMGs	For further subtyping (pedRTK1, pedRTK2, MYCN)
Integrated diagnosis	Diffuse hemispheric glioma, H3 G34-mutant, CNS WHO grade 4	Astrocytoma IDH-mutant CNS WHO grade 3/4	infant-type hemispheric glioma, H3-wildtype and IDH-wildtype, no CNS WHO grade	Diffuse midline glioma, H3 K27-altered, CNS WHO grade 4	Diffuse pediatric-type HGG, H3-wildtype and IDH-wildtype, CNS WHO grade 4

For further glioma entities that enter the differential diagnosis of pedHGG or may behave like that, additional molecular tests become necessary. Examples are “adult-type” IDH-mutant astrocytomas which can also occur in older children/adolescents and pleomorphic xanthoastrocytoma for which diagnostic testing for homozygous *CDKN2AB* deletions and *BRAF*V600E and other MAP kinase alterations are recommended. Very recently, a phase II trial in patients with relapsed/refractory BRAF V600–mutant pHGG with dabrafenib plus trametinib demonstrated tolerable safety and frequent and durable responses(*59*). DNA methylation profiling adds an important layer of information to confirm neuropathological diagnoses, provide information on subtypes of H3- and IDH-wildtype HGG, and to identify molecular or histological mimics of pedHGG.

In infant-type hemispheric gliomas (but also in other pedHGG subtypes), appropriate RNA- and/or DNA-based analysis for specific *gene fusions* (such as *ALK, ROS1, MET, NTRK* family) may help to identify possible candidates for targeted therapy.

Molecular/immunohistochemical assessment of *mismatch repair genes* (*MLH1*, *MSH2*, *MSH6*, *PMS2*) or assessment of tumour mutational burden (TMB) may help to identify patients with (germline) mismatch repair deficiency, which could indicate a rationale for immune checkpoint inhibitor treatment. We recommend the use of IHC for the MMR genes in cases with clinical suspicion of a constitutional mismatch repair deficiency syndrome (CMMRD) or routinely in countries where CMMRD is common (*60*). Also, High-grade IDH-WT diffuse glioma may occur in kids with Li-Fraumeni syndrome (*61*).

In H3K27-altered gliomas, *double-mutant entities* can be found (Auffret L. *et al.*, unpublished data) with the BRAFV600E mutation or the FGFR1/2, but they are still rare. Interestingly the prognosis of these tumours seems to be remarkably better. One concern is that (based on histology and even on imaging) it's not always fully clear if a tumour arising in the midline regions is really a diffuse glioma versus another, (more) circumscribed glioma.

The impact of *MGMT* promoter methylation status in pedHGG has not been established yet. Analyses of major clinical trials series, either confirming or discarding a similar impact as for adult GBM with respect to predicting TMZ efficacy, are pending.

Where adequate molecular testing is not available to determine the type of pedHGG, the term 'High-grade gliomas, NOS' should be used.

4 TREATMENT DETAILS

4.1 Treatment

4.1.1 Surgery

The goal of surgery in hemispheric pedHGG is to achieve as an extensive resection as possible without causing neurological deficits. Experience from adult HGG treatment suggests that gross total resections increase the time of progression-free survival (*62, 63*). The surgical strategy follows basic techniques for resection. Advances in surgery are primarily related to other technical developments that enable the surgeon to tailor the resection so that as much tumour tissue as possible can be removed while at the same time avoiding structures that can lead to deficits. Preoperative workup with advanced MRI techniques can help in visualizing vital anatomical structures (white matter) in relation to the tumour and how the tumour has altered the normal anatomy. Neuronavigation is used to plan for safe surgical approaches. With the introduction of intra-operative MRI (ioMRI) it has also become possible to evaluate the degree of resection during surgery and update information on important anatomical structures (*64-66*). Intraoperative ultrasound can also be used during surgery to understand anatomical relations between structures. 5-ALA fluorescence-guided surgery, which enables better differentiation between tumour and normal tissue, is also being introduced in paediatric tumour surgery (*67*). Awake surgery is difficult in the paediatric population but in selected cases it could be used to increase safety when operating near eloquent areas (*68, 69*).

4.1.2 Steroid use and avoidance

Corticosteroids are commonly used in children with symptomatic CNS tumours (*70*). Kofman et al. first reported the beneficial role of glucocorticoids in managing brain tumours in 1957 (*71*). Nowadays corticosteroids are routinely administered in children at the time of diagnosis, to ease symptom with surgery or with radiotherapy, as well as for long term use in palliative situations. Dexamethasone (DXM) is often prescribed *at diagnosis* to reduce tumour-associated vasogenic oedema and perioperative parenchymal swelling in patients with brain tumours and consequently raised intracranial pressure (ICP) (*72, 73*). Some other centres try to operate the tumour within 24 hours but in case of emergency and hydrocephalus, a ventricular derivation is a consensual first procedure. DXM administration is often also continued after biopsy or resection to reduce postoperative oedema, and further continued during RT to reduce oedema associated with radiation (*74*). Finally, in the case of inoperable

tumours, DXM is widely prescribed for long-term use *as* supportive or palliative treatment (*75*).
Despite the lack of randomized and controlled trials, dexamethasone can reduce cerebral oedema, even if the choice of the drug, the timing of administration and the dose applied are still under strong debate (*76*).

Dose and administration schedule

Investigations among neuro-oncologists suggest that different institutions use varying dosing schemes, primarily driven by the clinical context of the individual patient or the physician's personal preferences (*75, 77, 78*). Moreover, a growing discrepancy between DXM schedules used by neurosurgeons and neuro-oncologists has recently been called to attention (*79*).

A recent review of studies investigating steroids in paediatric brain tumour patients (*75*) revealed that most papers describe the use of dexamethasone, with doses ranging from 0.15 mg/kg/day (4.5 mg/m^2/day)(*80*) to 2.0 mg/kg/day (60 mg/m^2/day) (*81*) but no clinical trials investigating optimal dose or regimen could be retrieved in the literature. In some centres betamethasone is chosen preferentially.

The dose is adapted to weight, and the steroids are given orally, or intravenously in case of swallowing defect. A clinical guideline proposal from Glaser *et al.* for the Kids Neuro-Oncology Workshop (KNOWS) proposed sharp bursts of 10 mg/m^2/day dexamethasone for three to five days (*73*). After 5-8 days, it is consensual that these pulses should be followed by a tapering schedule to reach the minimal necessary dose (*82*).

Side effects

The most frequent side-effects can include a rise in serum glucose level, peripheral oedema, psychiatric disorder, and Cushing's syndrome. Life-threatening complications (e.g., gastrointestinal ulcer or thromboembolism) remain rare, even if the side effects of dexamethasone itself can increase over time (*72*). In the study by Palombi et al. no side effects were recorded related to dexamethasone therapy, except for sporadic detections of increased glycaemia in adult patients with GBM receiving dexamethasone upfront, concurrent with RT plus temozolomide in order to prevent the neurological effects related to cerebral oedema (*72*). In children the severe mood and behavioural changes impact the individual child and his and her family (*73*). The development of a moon face also presents a practical problem for the radiotherapist. A well-fitting facemask is required to ensure that the patient's face is correctly positioned for optimal delivery of cranial radiation. Therefore, the beneficial effects of relief of symptoms attributable to raised ICP must be balanced by the frequently observed side effects.
The possibly more important negative effect of immunosuppression, namely a reduction of anti-tumour immunogenicity, is not mentioned in any of the articles from the literature search (*13, 75*). Thus, the corticosteroids indications in children should be restricted to maximise their quality of life (*73*).

In conclusion, the benefit of corticosteroids is recognized in case of raised ICP and in preparation to surgery, under radiotherapy and in long term palliative symptomatic treatment, but their use should be restricted as corticosteroids reduce the permeability of the blood brain barrier (*83*) and might impair the anti-tumour immunity.

4.1.3 Radiotherapy

Infant – type hemispheric glioma

Infant HGG is usually managed with surgery and systemic treatment (chemotherapy and/or target therapy). Radiation therapy is rarely considered in the treatment strategy considering the severe late effects in infant. Furthermore, no benefit of radiation therapy is demonstrated. Radiotherapy is an option at relapse in selected cases (according to patient age, the previous treatment and molecular subtype).
No specific recommendation is therefore available for this rare entity (*34, 35, 84-88*).

Diffuse paediatric-type high-grade glioma, H3-wildtype and IDH-wildtype (*5, 18, 19, 25, 89-91*).

Optimal dose and volume of irradiation have never been studied prospectively or retrospectively.
In the past, recommendations for radiotherapy were based on adult experience despite different radiological presentation, the biology and outcomes.
After maximal safe surgery, a delay of less than 4 – 6 weeks is recommended before start of radiation therapy(*92*).

Dose:
- According to multi-institutional studies, local radiotherapy is proposed to patients older than 6 years, with a dose of 54 Gy +/- a boost of 5,4 Gy (1,8 Gy/fraction) to residual disease (*93*).
- In case of Intensity Modulated Radiation Therapy, a simultaneous boost can be proposed to optimize the dose to healthy tissue (54 Gy/1,8 by fraction and 60 Gy/2 Gy by fraction).

Target Volume (*94*):
- Regarding irradiation volume, Gross Total Volume (GTV) is defined as surgical cavity plus post-operative residual disease on T1gado (or T2 Flair for non-enhanced tumour).
- Clinical Total Volume (CTV) is GTV plus a margin of 15 mm limited by natural anatomic barrier. In case of a non-enhancing tumour, CTV margin could be reduced to 10 mm.
- Planning Target Volume (PTV) is CTV plus geometric expansion of 2 – 5 mm according to institutional policy.

Data regarding **re-irradiation** in patients with non-DIPG are scarce. Retrospective data suggest that re-irradiation is safe and can offer good palliation of symptoms. Optimal dose, fractionation dose and volume are unknown(*95, 96*).
In adults, a median dose of 35 Gy in 10 fractions in association with bevacizumab shown to be safe but without improvement of survival. Another retrospective study from Combs has

showed similar result with 36 Gy (2Gy/fraction) in stereotactic conditions. In these reports, target volume is defined as GTV plus a margin for PTV (*95, 97, 98*).

Diffuse hemispheric glioma H3 G34-mutant

Currently, there is no specific treatment for diffuse hemispheric glioma H3 G34-mutant. This new entity is treated according to the same principles as other non-DMG(*99*).

Diffuse midline glioma H3K27-altered

In childhood, diffuse midline glioma H3K27-altered (DMG) are mainly located in the pons followed by thalamic location and rarely in spinal cord.

For **non-DIPG diffuse midline glioma**, limited data is available about specific treatment.

Currently, recommendations for radiation therapy are the same as for other diffuse paediatric-type high-grade glioma.

For **diffuse intrinsic pontine glioma**, the recommendations are as follows:

Although rapid initiation of radiation therapy is desirable, the 'optimal delay' (if needed) between diagnosis and start of radiotherapy is unknown. Short delay (within 2 weeks) does not improve overall survival (*92, 100*)

Dose and fractionation:
- Standard dose of radiation therapy is 54 Gy in 1,8 Gy by fraction (5 fractions a week).
- Hypo fractionated treatment is an option in aim to reduce treatment burden in this poor prognosis disease. Hypofractionated scheme is proven to be non-inferior to conventional fractionation (*101-106*). The mostly used scheme is 39 Gy in 13 fractions (3 Gy/fraction in 2,5 weeks) without concomitant systemic therapy.
- There is no role of hyper fractionated radiotherapy in the management of diffuse intrinsic pontine glioma(*17, 107, 108*).

Target volume(*109*):
- GTV is defined by a combination of the T1contrast ,T2 and Flair abnormality.
- CTV include GTV plus a margin of 10 mm limited by natural anatomic barrier such as bony calvarium and tentorium.
- PTV is CTV plus geometric expansion of 2 – 5 mm according to institutional policy.

Re-irradiation of DIPG (*95, 97, 109-115*)
There is evidence that re-irradiation in DIPG patients improves survival and symptoms in more than 2/3 of patients. The best candidates are patients with response to initial treatment and after at least 3 months since first irradiation course.

Dose:

Re-irradiation dose, volume and fractionation are variable according to the different institutions. Some data suggest that ≥ 20 Gy (1,8 – 2 Gy/fraction) is slightly more effective in term of symptom improvement.
More data is needed to determine if dose up to 36 Gy could offer additional benefit.

Target Volume:
PTV is usually GTV plus a margin of 2 – 5 mm with a limited margin for CTV, at the discretion of the radiation oncologist, in the absence of consensus.

Spinal Cord Glioma (*25, 89, 116*)

High grade glioma arising from spinal cord is very rare in paediatric patients. H3K27M alteration is very frequent in this location (50-80%) (*86, 117, 118*).
Most of them are not treated according to specific clinical trials. Due to a lower dose tolerance of the spinal cord to radiotherapy, the delivered dose is usually lower compared to intracranial gliomas.

Dose: 45 – 50.4 Gy (1,8 Gy/fraction) according to the length of involved spinal cord and neurological status.

Target Volume: there is no agreement and treatment is based on experience from intracranial high grade glioma with CTV margin up to 20 mm in CC direction.

Metastatic DMG

For all DMGs (DIPG included), CSI with a boost to the primary tumour and macroscopic metastases up to 54 Gy, in case of metastatic disease at time of diagnosis, is an option.

In case of metastatic relapse (up to 50% of patients with thalamic lesion), CSI at 36 Gy could be offered to a patient accordingly to the general status.

4.1.4 Chemotherapy

Despite of biological insight into paediatric high-grade glioma (pedHGG) and the promise of more effective therapies, little progress has been made in the effective treatment and, hence, the outcome of these tumours in the last four decades. Much of the evidence for the use of chemotherapy in pedHGG is extrapolated from adult data, and the evidence for its use in the paediatric population is weak. To date, only a few randomised trials have been performed involving newly diagnosed high-grade glioma (HGG) in children with sizeable patient numbers that have clearly demonstrated a benefit from adjuvant chemotherapy (*18, 19*). Although most children receive adjuvant chemotherapy, the optimal regimen to offer patients with newly diagnosed pedHGG has not been established.

Given there is no clear indication to support one approach over another, SIOPE HGG Working Group conducted a pan-survey aiming to establish the current management approaches of pedHGG in Europe and UK. Based on the practice in 33 countries, an attempt was made to achieve a consensus on management of these tumours using a Delphi method (*119*). Forty-three recognized neuro-oncology experts from 33 countries were invited to participate in the Delphi process between December 2021 and March 2022. Voting and responses were collated using a web-based survey (*120*).

Chemotherapy treatment for diffuse paediatric-type high-grade glioma, H3-wildtype and IDH-wildtype

A concomitant daily administration of Temozolomide (TMZ) with a local radiotherapy followed by adjuvant chemotherapy with TMZ has been widely adopted by the paediatric neuro-oncology community throughout Europe as the preferred treatment option for paediatric patients with newly diagnosed HGG. Sixty percent of the Delphi participants agreed that the recommended treatment regimen is chemoradiation with TMZ followed, after a TMZ treatment break of approximately 4 weeks, by 6-12 cycles of TMZ, irrespective of *MGMT* promotor methylation status.

Treatment should begin approximately 4 weeks after cranial surgery. Alternatively, after irradiation, patients should be enrolled into a clinical trial when available.

Recommended chemoradiation regimen:

During the chemoradiation treatment phase:

- Daily continuous TMZ (75 mg/m^2/d) starting concomitantly with the first radiation fraction and ending with the last radiation fraction (see details in radiotherapy section).

During the TMZ adjuvant treatment phase:

- Temozolomide (150 – 200 mg/m^2/d) x 12 cycles

- 1st cycle 150 mg/m^2/days 1-5, escalated to 200 mg/m^2/days 1-5 from the 2nd cycle onwards depending on the tolerance during the 1st cycle

- Cycle length = 28 days

The above regimen, commonly referred to as the 'Stupp regimen', has been based on the first randomized study to demonstrate significant survival benefit when adjuvant chemotherapy was added to radiotherapy in adult patients with newly diagnosed GBM. This trial demonstrated an improvement in the median and 2-year survival, a benefit that lasted throughout 5 years of follow-up (*121*).

Subsequently, the efficacy, safety, and tolerability data from the completed large single-arm Phase II Children's Oncology Group (COG) study (ACNS0126) provided support for the use of

radiotherapy with concomitant and adjuvant TMZ in paediatric patients with newly diagnosed pedHGG (*11*). Although the efficacy results did not demonstrate a clear advantage of this regimen over other chemotherapy agents in subsequent trials (*19, 90*), the favourable safety profile and excellent tolerability of this regimen have nevertheless resulted in its continued acceptance by both physicians and patients.

Thirty percent of the Delphi participants would support the management of hemispheric high-grade glioma using concomitant and/or adjuvant TMZ as the backbone, but would consider adding Lomustine (CCNU) based on the COG ACNS-0423 trial due to the findings of the difference in survival between the cohort of patients with *MGMT*-overexpressing tumors in ACNS0126 and ACNS0423 (*25*). In this trial, children with a newly diagnosed non-metastatic pedHGG underwent radiotherapy with concurrent TMZ following maximal surgical resection. Adjuvant chemotherapy consisted of up to 6 cycles of CCNU 90 mg/m^2/day on day 1 and TMZ 160 mg/m^2/day days 1-5 every 6 weeks. Cycles were repeated every 28 days upon bone marrow recovery (*25*).

The hypothesis was that the dual-alkylator regimen might help to overcome *MGMT*-mediated resistance by depleting *MGMT*. However, this remains of debate as the study was non-randomized and MGMT immunohistochemistry is controversial in comparison to the *MGMT* methylation status. Moreover, the significance of *MGMT* expression in predicting response to alkylating agents in pediatric high-grade glioma is unknown.

As for pedHGG driven by germline or somatic DNA replication repair deficiency, including both mismatch repair and/or polymerase-proofreading deficiency, focal irradiation is recommended (*122*). TMZ should be avoided in those circumstances, but CCNU can be considered as an adjuvant therapy (*123, 124*). Moreover, immune checkpoint inhibition is well established to improve survival at progression for these hypermutant gliomas (*125*), and may be considered as a frontline treatment for some patients with favorable genomic and immune biomarkers (*99*).

Chemotherapy treatment for recurrent/progressive hemispheric high-grade glioma, H3-wildtype and IDH-wildtype patients

There is currently no standard of care for treatment of recurrent/progressive hemispheric high-grade glioma. All patients should be fully restaged and assessed before considering management options to allow delivery of the most appropriate treatment. Available evidence for selection of specific treatment strategies for the recurrent/progressive hemispheric HGG is limited and mostly based on retrospective cohort studies on heterogeneously treated patients.

Members of the SIOPE-BTG and the GPOH were surveyed on therapeutic options for recurrent/progressive paediatric and adolescents HGG (*126*). Based on the results of this survey SIOPE HGG Working Group recommends surgical resection, if feasible, at the time of relapse/progression combined with molecular pathology to identify potential targeted

therapy, such as BRAF/MEK inhibitor, anti-EGFR therapy, CDK inhibitor, EZH2 inhibitor. Patients should be enrolled into clinical trials if available.

Given lack of international cooperative trials for recurrent/progressive hemispheric high-grade glioma, it is reasonable to combine conventional multimodal treatment concepts, including re-irradiation, with targeted therapy based on molecular genetic findings (*97, 126*).

Advice on the management of individual patients and details of various treatment regimens, can generally be provided by national experts. In addition, advice can be sought among members of the SIOPE HGG Working Group.

Chemotherapy treatment for diffuse hemispheric glioma H3 G34- mutant

Currently, there is no specific treatment for diffuse hemispheric glioma H3 G34-mutant. This new entity is treated according to the same principles as hemispheric high-grade glioma, H3-wildtype, and IDH-wildtype patients (*99*).

Chemotherapy treatment for diffuse midline glioma *H3K27*-altered

Currently, there is no available evidence for selection of specific chemotherapy treatment strategies for diffuse midline glioma *H3K27*-altered. Therefore, the mainstay of treatment is radiotherapy (see Radiotherapy section 4.1.3 of this guidelines).

With regards to chemotherapy, the SIOPE HGG Working Group has not managed to reach consensus on management of these tumours using the Delphi method (*119, 120*). Fifty percent of the Delphi participants agreed that the recommended treatment regimen is chemoradiation with TMZ, followed, after a TMZ treatment break of approximately 4 weeks, by 6- 12 cycles of TMZ, irrespective of *MGMT* promotor methylation status. This management approach is supported by the results of the HIT-HGG-2007 trial (ISRCTN19852453) presented at ISPNO in 2022 (*127*). A sub-group analysis showed a 3-month event-free survival (EFS) and overall survival (OS) benefit for patients with non-pontine pedHGG treated with Temozolomide (median EFS 10.7 versus 7.4 months, and 19.3 versus 16.2 months, respectively). This also confirmed other reports supporting Temozolomide as a better tolerable alternative to other cytostatic therapy (*29*) .

Given that the above results have not been published yet and further subgroup survival analysis is ongoing, the remaining fifty percent of the Delphi participants did not support the role of Temozolomide in the treatment of children with diffuse midline glioma *H3K27*-altered (*120*). We hope that future studies might help to resolve this area of controversy.

If a potential target for therapy is identified following a biopsy, the biological agent should ideally be used within the context of a clinical trial. If enrolment into a clinical trial is not feasible, this is due to discretion of a treating clinician to consider the individual patient, the risk profile of the drug(s) to ensure the risk-benefit-balance is appropriate. Patients and their

parents/guardians should be informed of the experimental nature of the treatment and potential side effects.

Therapy should be primarily based on national therapy guidelines, and each plan should be tailored according to patients' needs. Advice can be sought among members of the SIOPE HGG Working Group.

Chemotherapy treatment for progressive/relapsed diffuse midline glioma *H3K27*-altered

Similarly, to the *de novo* diagnosis of diffuse midline glioma *H3K27*-altered, currently, there is no evaluated and agreed chemotherapy treatment standard for progressive/relapsed diffuse midline glioma *H3K27*-altered.

If a potential target for therapy is identified following a biopsy and considered at the time of tumour progression/relapse, similar principles apply as in *de novo* diagnosis.

Specificities in Chemotherapy treatment for diffuse midline glioma *H3K27*-altered located in the pons (previously known as DIPG)

Numerous studies of systemic chemotherapy have all failed to demonstrate any significant improvement in survival. This failure is, in part, due to the blood–brain barrier (BBB) preventing therapeutic concentrations of drug from penetrating the tumour. Therefore, currently the mainstay of treatment is radiation given with palliative intent (see Radiotherapy section 4.1.3 of this guidelines) (*99*).

A sub-group analysis of the HIT-HGG-2007 trial (ISRCTN19852453), presented at ISPNO this year, showed a 2-month EFS benefit for patients with pontine pedHGG treated with TMZ (median 8.2 versus 6.2 months). However, there was no OS benefit for these patients (median OS 11.4 versus 11.3 months) (*127*).

If a potential target for therapy is identified following a biopsy, the biological agent should ideally be used within the context of a clinical trial. If enrolment into a clinical trial is not feasible, similar principles apply as in management of other pedHGG.

Chemotherapy treatment for progressive/relapsed diffuse midline glioma *H3K27*-altered within pons only (previous DIPG)

Similarly, to the *de novo* diagnosis of intrinsic pontine glioma, if a potential target for therapy is identified following a biopsy and considered at the time of tumour progression/relapse, the therapy must be regarded as an experimental and ideally would be given in the context of a clinical trial. If the enrollment into a clinical trial is not feasible, similar recommendations apply.

Infant-type hemispheric HGG

A chemotherapy only approach has been widely adopted by the paediatric neuro-oncology community worldwide as the preferred treatment option for infant patients with newly diagnosed HGG. Seventy-five percent of the Delphi participants agreed that radiation therapy should be avoided in the management of infant HGG to prevent significant adverse effects to the developing brain. Indeed, it is now worldwide recognized that radiation is not recommended in the young child under the age of 3 years. Poor outcome and late treatment effects have engendered a reluctance to treat those patients with radiation therapy.

The three currently recommended chemotherapy regimens are based on the French chemotherapy-only (BBSFOP) protocol, the German modified HIT-SKK (without intraventricular methotrexate) chemotherapy-only strategy, and the UK-chemotherapy only approach as per UKCCSG/SIOP CNS 9204 trial:

The BBSFOP protocol is a 16-month schedule of 7 cycles of three drug pairs of Carboplatin - Procarbazine, Cisplatin – Etoposide, and Vincristine – Cyclophosphamide (*34*). The drugs selected were a combination of those used with acceptable toxicities in infants and young children with malignant brain tumours (*128*). The aim of this protocol was to develop a mild chemotherapy that could be given for a long period to delay / avoid radiotherapy.

See details of this regimen in appendix 8.2.

The HIT-SKK chemotherapy (Chemotherapie für Säuglinge und Kleinkinder mit Hirntumoren [Chemotherapy for Infants and Toddlers with Brain Tumors]) is the German strategy to delay and avoid radiotherapy in young brain tumor patients. Patients treated by the HIT-SKK multiagent chemotherapy receive three two-month cycles of chemotherapy consisting of intravenous methotrexate, cyclophosphamide, vincristine, carboplatin, and etoposide (*129*). The HIT-SKK chemotherapy – in combination with intraventricular methotrexate for young children with medulloblastoma patients – has been shown to be feasible and well tolerated (*130*). In infant-type hemispheric HGG the modified HIT-SKK chemotherapy (without intraventricular methotrexate) is currently frequently used.

See details of this regimen in appendix 8.3.

In the UK version of this chemotherapy, slightly different but with similar cumulative doses: infants were treated without intraventricular therapy, with courses of Carboplatin-Vincristine, High-dose Methotrexate - Vincristine, Cyclophosphamide - Vincristine and Cisplatin monotherapy (*35*). The drugs chosen have different mechanisms of cytotoxic action in an attempt to prevent the early emergence of drug resistance by alternating courses of myelosuppressive and relatively non-myelosuppressive chemotherapy. The aim was to enhance treatment intensity with chemotherapy given every 2 weeks.

See details of this regimen in appendix 8.4.

Molecular pathology has recently shed light on molecular groups in infant HGG with distinct survival (*36, 37*). If possible molecular analysis should be undertaken to detect common gene fusions in ALK, ROS1, NTRK1/2/3 and MET, which maybe targetable ideally as part of a clinical trial.

CONCLUSION

The levels of evidence for treatment recommendations for children diagnosed with pedHGG are limited. Numerous efforts to generate new evidence by doing prospective studies pointing at this unmet need in neuro-oncology are ongoing. Translating and adapting adult treatment recommendations into paediatric practice can be challenging and might inadvertently lead to inappropriate management. Therefore, the medical community needs to develop research studies for this rare disease group, including investigation into the biology of diseases and treatment options.

4.1.5 Future directions

Numerous studies of systemic chemotherapy have all failed to demonstrate any significant improvement in survival of patients with all HGGs. This failure of systemic therapy is, in part, due to the blood-brain barrier (BBB) preventing therapeutic concentrations of drug from penetrating the tumour (*131, 132*). To overcome these challenges, several novel approaches have been proposed, such as a convection-enhanced drug delivery (CED). The concept of CED emerged as an interesting and innovative technique that describes the infusion of drugs under controlled pressure to the brain parenchyma/tumour via targeted micro-catheters (*133, 134*). This facilitates the delivery of therapeutically effective drug concentrations through clinically relevant volumes of brain tissue with a direct installation into the tumour bed. Higher local concentrations of drugs can be achieved via CED than via the systemic route. Clinical experience with a small cohort of children and young adults with DIPG provided the preliminary evidence of safety and feasibility of this new technique and merits formal evaluation in a clinical trial, which is currently being explored (*133, 134*).

Focused ultrasound (FUS) in combination with microbubbles is another technique that allows non-invasive and localized opening of the blood-brain barrier. This approach has been tested in animal models for over two decades and has recently translated to clinical trials in adults. There are several ongoing clinical trials in Canada, France, and the USA using FUS and microbubbles for BBB opening (*135-139*). Preliminary results indicate that the use of FUS for the treatment of glioblastoma multiforme (GBM) is both safe and efficient in humans. The published clinical study regarding the application of FUS in patients with GBM showed that BBB was disrupted without detectable radiological or clinical adverse effects (*135, 140*). The authors used an implantable unfocused single-element transducer, which was fixed in the skull bone. Patients were exposed to repeated monthly FUS treatments before receiving systemic chemotherapy with carboplatin.

CED and FUS are the two most promising approaches currently in development for circumventing BBB and improving brain drug delivery; the hope is to forward potential future direction in this field.

CAR-T cells is also a promising strategy when targeting oncoproteins or surface markers not found in normal tissue, but they are limited with poor trafficking and tumor infiltration (*141*). Activated immune cells, including T cells and DCs, can cross the BBB (*142*) Repeat administration of CAR-T-cells, perhaps through an Ommaya reservoir (an intraventricular catheter used for aspiration of CSF and for delivery of drugs into the CSF), could increase the efficacy of therapy as compared to either intravenous administration, or single dose intraventricular administration (*143*) and bystander immunity is an important adjuvant to address the immunosuppressive tumor microenvironment (*144, 145*).

5 APPENDIX 1- THE RAPNO GUIDELINES FOR DIPG/HGG

The RAPNO DIPG/HGG guidelines (*146, 147*) present the following differences with the SIOPE imaging guidelines:

1. Imaging protocol: In RAPNO DIPG guidelines, FLAIR images are recommended following contrast administration while in RAPNO HGG and SIOPE guidelines, FLAIR is acquired before contrast; differences regarding 3D T1-weighted sequences.

2. Tumour measurement: According to RAPNO guidelines, measurement of the longest tumour dimension and its perpendicular for each target lesion is recommended [2D measurements], while according to SIOPE guidelines the maximum dimensions in standard planes [i.e., antero-posterior, transverse, cranio-caudal] should be assessed [3D measurements].

3. Cut-off defining partial response (PR): According to RAPNO DIPG guidelines, PR is defined as ≥ 25% decrease in pontine and ≥50% decrease in non-contiguous extrapontine lesions of the largest perpendicular diameters compared with baseline. The cut-off in the SIOPE guidelines for PR is ≥50% reduction in tumour volume. In RAPNO HGG, PR is a ≥ 50% reduction in the sum of the products of the two perpendicular diameters of target lesions, compared with baseline measurement

4. Imaging techniques: In RAPNO HGG guidelines, evaluation of Diffusion Weighted Imaging has been introduced to define treatment response.

6 APPENDIX 2 – LEPTOMENINGEAL DISSEMINATION STAGING

For classification of leptomeningeal dissemination the staging system of Chang (*148*) reviewed for medulloblastomas by employing CSF cytology and cranial/spinal MRI findings will be adopted (*149*):

M 0: *No evidence of gross subarachnoid or haematogenous tumour dissemination*

M 1: *Microscopic presence of tumour cells in CSF obtained by lumbar puncture more than 14 days following any kind of tumour surgery, but no concurrent leptomeningeal enhancement on MRI or CT scan. Additional immunocytochemistry (e.g., for GFAP) may be helpful.*

M 2: *Gross tumour dissemination in the cerebral, cerebellar subarachnoid space or in the ventricles area in form of (i) layered leptomeningeal thickening or (ii) nodular deposits or very thick leptomeningeal layers d.*

M 3: *Gross tumour dissemination in the spinal subarachnoid space in form of (i) layered leptomeningeal thickening or (ii) nodular deposits or very thick leptomeningeal layers*

M 4: *Extraneuroaxial metastasis (related to a ventricular shunt or not).*

7 APPENDIX 3 – IMAGING

7.1.1 Brain imaging, essential MRI sequences

1.5 Tesla MRI scanner

Sequence	Technique	Parameters	Plane
T1W	2D SE, TSE/FSE	Slice thickness ≤ 4 mm and slice gap ≤ 1 mm (10% of slice thickness is desirable)	Axial (along AC-PC axis), sagittal
T2W	2D SE, TSE/FSE	Slice thickness ≤ 4 mm and slice gap ≤ 1 mm (10% of slice thickness is desirable)	Axial
FLAIR	2D TSE/FSE*	Slice thickness ≤ 4 mm and slice gap ≤ 1 mm (10% of slice thickness is desirable)	Axial or coronal
T1W + Contrast	2D SE, TSE/FSE	Slice thickness ≤ 4 mm and slice gap ≤ 1 mm (10% of slice thickness is desirable)	Axial, coronal and sagittal
DWI with ADC	2D EPI	Slice thickness ≤ 4 mm and slice gap ≤ 1 mm (10% of slice thickness is desirable) b=0 and 1000. ADC maps reconstructed online	Axial

3 Tesla MRI scanner

Sequence	Technique	Parameters	Plane
T1W	3D gradient echo (MPRAGE/IR, SPGR/Fats, SPGR/3D, TFE/3D, FFE)	Slice thickness ≤ 1 mm with no slice gap. An isotropic voxel resolution of 1mm x 1mm x 1mm is desirable depending on scanner capability	Axial or sagittal
T2W	2D SE, TSE/FSE	Slice thickness ≤ 4mm and slice gap ≤ 1mm (10% of slice thickness is desirable)	Axial
FLAIR	2D TSE/FSE*	Slice thickness ≤ 4mm and slice gap ≤ 1mm (10% of slice thickness is desirable)	Axial or coronal
T1W + Contrast	2D SE, TSE/FSE	Slice thickness ≤ 4mm and slice gap ≤ 1mm (10% of slice thickness is desirable)	Axial

T1W + Contrast	3D gradient echo (MPRAGE/IR, SPGR/Fats, SPGR/3D, TFE/3D, FFE)	Slice thickness ≤ 1 mm with no slice gap. An isotropic voxel resolution of 1 mm x 1 mm x 1 mm is desirable depending on scanner capability	Axial or sagittal, to match pre-contrast
DWI with ADC	2D EPI	Slice thickness ≤ 4 mm and slice gap ≤ 1 mm (10% of slice thickness is desirable) b=0 and 1000. ADC maps reconstructed online	Axial

*3D FLAIR can be used instead of 2D FLAIR but not if 2D sequences have been used for the same individual on previous occasions.

7.1.2 Spine imaging, essential MRI sequences

Sequence	Technique	Parameter	Plane
T1W + Contrast	2D SE/ TSE	Slice thickness ≤3mm Slice gap <0.5mm	Sagittal whole spine (entire dural sac)
T1W + Contrast	2D SE/TSE or 3D gradient	Slice thickness 4-5 mm No slice gap	Axial –suspicious areas

7.1.3 Tumour measurement

As volumetric measurement tools are not available at all centres, the tumour volume is calculated using the (ellipsoid volume) formula A x B x C x ½ where A, B and C are the maximum dimensions in the standard anteroposterior, transverse and craniocaudal planes.

Imaging 3D-volumetric calculations may be performed additionally. These volume calculations are the basis for follow-up evaluation and every effort should be made to ensure the highest possible accuracy. It is desirable to save the measurements as annotated images if possible.

If there are multiple lesions, the sum of the 5 largest lesions must be obtained. This will need further validation and may change in the future.

Please note that the measurement guidelines may be altered in some trials where 2D measurements in the axial plane or different measurement methods for the 3 dimensions may be employed.

7.1.4 Early post-operative imaging

Optimal evaluation is made within the first 48 hours following surgery. As non-specific intracranial enhancement is often seen after 3 days following surgery the postoperative MRI must be obtained within this time. However, even within this time false positive nodular enhancement can be seen with haemostatic materials and following electrocoagulation. It is therefore prudent to carefully evaluate the pre- and post-contrast T1-weighted images in combination with the signal intensities on the T2-weighted and FLAIR sequences.

With increasing use of intraoperative MRI imaging, the validity of the final intraoperative scan as baseline scan has been debated. Based on a single centre study and consensus it has been agreed that the final intraoperative MRI scan is acceptable as a base line provided it is from a 3T scanner (as it has been only validated on 3T), the SIOPE brain tumour protocol is followed, supervised by radiologist experienced in children's brain tumours and reported in consensus with the operating neurosurgeon. The preoperative and final intraoperative sequences must be comparable. If there has been further resection following the intraoperative scan, this will not qualify as a final intraoperative scan. A further scan after the extended resection according to the full SIOPE protocol should be performed. The final decision to use intraoperative MRI scans rests with the national reference radiologist/ radiology panel as the practices vary in different countries.

Comparability with the preoperative MRI is essential for the detection of residual tumour. The size of a possible residuum must be measured in all three planes. If the residuum is best visible on T2-weighted images a second plane incorporating a T2-weighted or FLAIR sequence must be employed.

A residuum is defined by any area of persisting pathological signal and/or enhancement that is comparable with the appearance of the pre-operative tumour. DWI is helpful to demonstrate any local surgical or ischaemic injury, which may influence enhancement patterns and tumour evaluation on subsequent examinations.

For the evaluation of residual tumour seen on imaging, the surgical report is often valuable and should be available.

7.1.5 Follow-up MRI

Timing for follow-up MRIs should be planned according to the individual trial protocol.

If the tumour enhances uniformly, the post-contrast T1 should be used for the measurement of the diameters. For heterogeneously, poorly, or non-enhancing tumours the dimensions on T2/FLAIR or PD and pre-contrast T1 can be relevant. In some instances, therapy related reduction of enhancement disproportionate to the change in tumour volume may be encountered. The best sequence cannot be predicted at the outset in these tumours. In these circumstances, it is useful to choose the initial sequence on which the tumour was measured or change the sequence (e.g., due to a change in contrast behaviour) and compare the tumour dimensions with the same sequence on the previous staging MRI to assess response.

Differencing true progression from treatment-related changes can be challenging on MRI (*150*). Pseudo progression is an abnormal contrast enhancement soon after completing adjuvant therapy that resolves within the following weeks and does not imply a worsened prognosis, while pseudo response is a prompt and markedly reduced contrast enhancement.

In the paediatric neuro-oncology setting, pseudo response mainly refers to reduction of enhancement following anti-angiogenic therapy and the response assessment in this setting is based on measurement on the T2 and FLAIR sequences.
In the paediatric neuro-oncology setting, pseudo response mainly refers to reduction of enhancement following anti-angiogenic therapy and the response assessment in this setting is based on measurement on the T2 and FLAIR sequences.

In instances where the MRI findings are equivocal for tumour progression/resolution (pseudo progression/pseudo response) another MRI scan within 4-6 weeks may be required to assess true progression/response (*147*). PET imaging has been proposed for distinguishing pseudo progression and pseudo response, but a lack of diagnostic PET assessment often limits this indication (*151*). When true progression is confirmed, the initial scan which showed the new abnormality should be considered as the baseline.

7.1.6 Definition of residual tumour

The evaluation of early postoperative imaging for residual tumour can pose challenges. As very subtle residual tumours may not be visible on imaging the presence/grading of residual tumour should be made in consensus with the neurosurgical report.

Residual tumour will be defined as follows (applies only for early postoperative MRI):

R0: No residual tumour on post-operative MRI in accordance with the neurosurgical report

R1: No residual tumour on MRI but description of a small tumour residuum by the neurosurgeon or if the neurosurgical result is unknown.

R2: Small residual tumour on MRI with the maximum diameter < 5mm in any plane or thin residual tumour covering the surgical margins like a ring or extending beyond the surgical margins.

R3: Residual tumour measurable and ≥ 5mm in all 3 planes.

R4: Size of the residual tumour unchanged or only minimally changed from the pre- operative status (e.g., after biopsy).

A thin line of enhancement can be physiological or reactive on early post-operative MRI and correlation with the non-contrast sequences for evidence of haemorrhage/tissue injury and detailed comparison with pre-operative MRI may be required before considering the presence of residual tumour. If imaging is inadequate or the appearance of the surgical cavity is difficult to interpret the term “unclear” should be used. In some cases, early follow up imaging in 2-4

weeks with additional sequences, better resolution parameters and additional planes may be necessary for further clarification.

7.1.7 Definitions for neuro-radiological response evaluation

Measurable tumours

A measurable lesion is defined as being reliably followed up and allowing for the slight variations of the scan planes. The definition of measurable lesion is based on the historic practice of using 2D measurements on predominantly 2D imaging and mainly based on the RANO criteria. The current definition is based on the assumption that 2D sequences are predominantly used in a number of centres. This may change in the future when the quality of volumetric imaging is more reliable for tumour measurement and performed in all centres.

Measurable lesion:

Lesion visible in the 3 standard planes with a diameter of ≥ 10mm in each plane. This is provided that the 2D image slice thickness + gap is ≤ 5mm. If the slice thickness + gap is > 5mm, then the maximum diameter should be ≥ 2 times the slice thickness + gap.

Non measurable lesion also includes lesions with poorly defined margins.

When there are multiple lesions, sum of the volumes of the 5 largest lesions are used.

Response criteria

CR (complete response): no evidence of residual or recurrent tumour or meningeal dissemination.

PR (partial response): Reduction of tumour volume ≥ 50% compared to the previous staging MRI.

(The extent of meningeal dissemination can only be estimated, and PR means considerable reduction of meningeal disease)

SD (stable disease): Tumour volume between < 50% decrease in size and < 25% increase in size compared to the previous staging MRI (no significant change of meningeal dissemination)

NOTE. MR (minor response); This criterion is used in some trials for 50% to 25% decrease in tumour volume.

PD (progressive disease): increase of tumour volume of ≥ 25% or new lesion.

As previously highlighted, for measurable lesions, the best sequence for measurement cannot be predicted in advance and it may require a comparison between repeated measurements on the most reliable sequence on the current scan and a similar sequence on the prior/baseline scan for accurate response assessment.

Radiotherapy as a primary treatment may be associated with radiation-induced reaction if there is measurable tumour growth after treatment. The radio-chemotherapy combination may temporarily affect the imaging (enlarging contrast enhancing lesion, increased FLAIR/T2 abnormality) in up to 30-40% of cases, which are collectively known as pseudo-progression and may be mistaken for early true progression. If new enhancement or increase in residual tumour size occurs during the first 12 weeks after the end of irradiation and within the irradiated field, this should not be considered a true progression, unless otherwise confirmed (either by histology or on a short interval follow up scan – after at least 4-6 weeks). If there is confirmed growth on the subsequent follow-up MRI, then the date of progression is ascribed to the first time point when tumour growth was documented.

7.1.8 Multi-Modal Advanced MRI

There is increasing experience in the use of several advanced MRI techniques that may add useful information to the conventional MRI. The individual techniques should be thought of as complimentary and as such a multi-modal approach is most appropriate.

We have developed and tested protocols that seek to provide a balance between quality of data and length of acquisition and at the same time give sufficient flexibility so that they can be implemented on most MR scanners. MR spectroscopy (MRS) and Diffusion Tensor Imaging (DTI) methods are well established throughout the age range and the protocols for these techniques are well agreed. However, contrast injection perfusion imaging is less well-established in children. We recommend Dynamic Susceptibility Contrast (DSC) – MRI at present, although there are still some areas of active development in the protocol particularly related to the contrast injection (see section below). The current protocols for these three methods are given in the parameter table below. We do recognise that there are centres that will use more advanced protocols and would encourage anyone who is doing this or considering it to contact the SIOPE – Brain Imaging Group so that we can share experiences and further develop protocols. Examples are: 1) Arterial Spin Labelling for measuring perfusion without injecting contrast. Whilst this has generated considerable interest, we feel that further experience is required in applying this technique to children's brain tumours, in particular its relationship with DSC-MRI, prior to recommending an international protocol. 2) Multi-b value DWI and the IVIM model for measuring perfusion without injecting contrast. 3) MRS imaging to investigate the heterogeneity of tumours. 4) Functional brain connectivity via a steady state fMRI protocol especially in diencephalic syndrome. We are keen to carry out limited centre studies of such techniques.

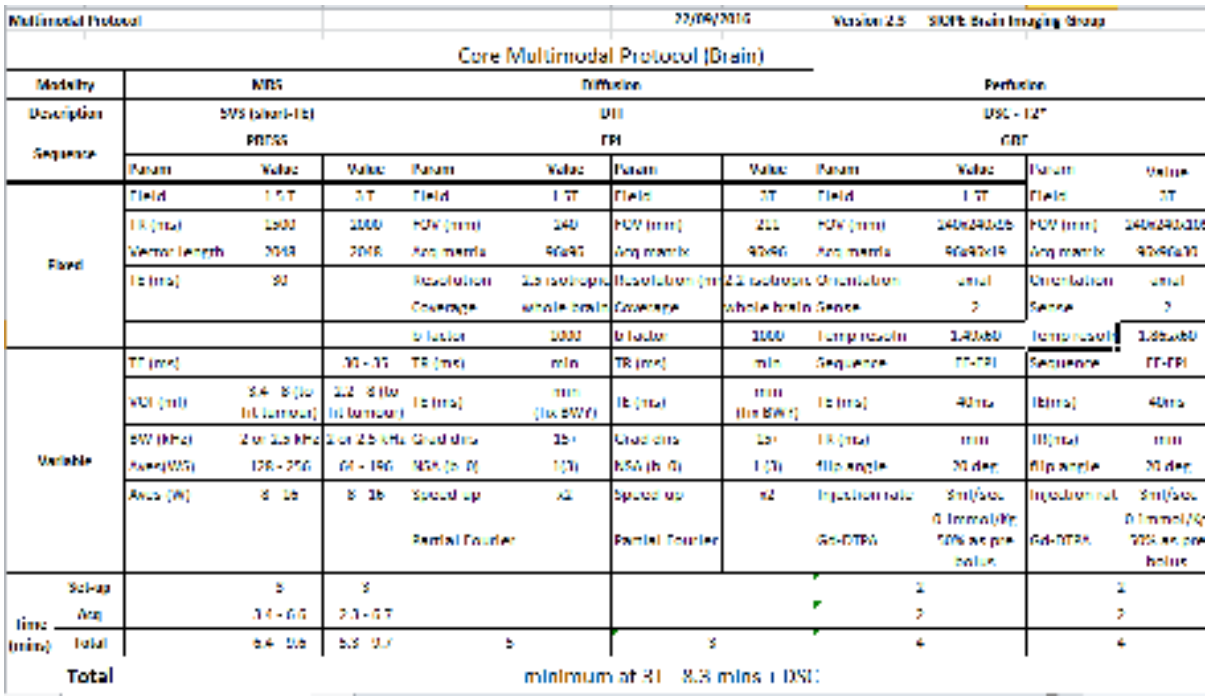
Data Saving

It is important that data is saved in a way that it can be analysed in a quantitative manner. DICOM headers should not be altered in a manner that renders the data uninterpretable, which can happen when images are sent to some PACS systems or anonymised. Please seek advice if you are unsure.

Contrast Injection

For DSC-MRI, contrast (usually Gd-DTPA or Gd-DOTA) injection should be via a pump injector. Most centres will not use these via a central venous line and so injection will need to be via an intravenous cannula placed prior to the scan. In order to reduce the T1 effects we recommend giving a pre-bolus injection which is half of the full amount (i.e.

0.05mmol/Kg Gd) at least 2 minutes prior to the main injection which should also be 0.05mmol/Kg Gd, so that the total dose is 0.1mmol/Kg Gd. The rate of injection is standardised at 3ml/sec. This protocol has been used successfully in infants although there can be problems with the pump injector in very small ones, the protocol is subject to further development particularly in this age group.



7.1.9 PET Imaging

There is growing interest and evidence in the use of PET imaging to assess brain tumours in children at diagnosis and/or surveillance. The following section aims to serve as a guidance for the usage of PET in paediatric brain tumours. PET imaging can supplement MRI using an amino acid tracer as O-(2-[18F] fluoroethyl-L-tyrosine (FET), L-[methyl- 11C]methionine (MET), or 3,4-dihydroxy-6-[18F]fluoro-L-phenylalamine (F-DOPA). 2-deoxy-2-[18F]fluoro-D-glucose (FDG) is a less useful tracer due to the high uptake in normal grey matter and will not be mentioned further. Four hours of fasting is recommended before tracer injection to ensure stable metabolic conditions. The use of a head holder is recommended to avoid motion artifacts.

Tracer	Dose MBq/kg	Examples of Scan times	Background region (healthy tissue)	Tumour-to background ratio	Physiological uptake

FET	3	20-40 min p.i. or 0-40 min p.i. (dynamic)	Cortical non-affected GM and WM	>1.6 (or >1.8)	Vascular structures, cerebellum, skin, basal ganglia, pineal body, venous anomaly
MET	10	10-30 min p.i. /20-40 min p.i.	Cortical non-affected GM and WM	>1.3	
FDOPA	3	15-45 min p.i.	Contralateral striatum	>1	Basal ganglia, pituitary, skin, pineal body, venous anomaly
			Cortical non-affected GM and WM	>1.6	

8 APPENDIX 4-CHEMOTHERAPY

8.1 Stupp Regimen(*22*)

In the EORCT trial for adults, the radiotherapy dose was 60 Gy. However, 54 Gy +/- boost up to 59.4 Gy is the standard for pediatric patient and this regimen is recommended in children as described below:

A total dose of 54 Gy delivered in 30 daily fractions of 1.8 Gy over 6 weeks (see details in radiotherapy section).

During the chemoradiation treatment phase:

- Daily continuous TMZ (75 mg/m^2/d) starting concomitantly with the first radiation fraction and ending with the last radiation fraction.

During the TMZ adjuvant treatment phase:

- Temozolomide (150 – 200 mg/m^2/d) x 12 cycles

- 1st cycle 150 mg/m^2/days 1-5, escalated to 200 mg/m^2/days 1-5 from the 2nd cycle onwards depending on the tolerance during the 1st cycle

- Cycle length = 28 days

8.2 BBSFOP(*34*)

Chemotherapy commenced approximately 4 weeks following maximal safe surgical resection. The protocol is an 18-month schedule of 7 cycles of three drug. Haematological toxicity alone was not an indication to delay treatment.

1 course is 3 weeks **3 courses is one cycle** **7 Cycles in total**	**For children over 3 years of age, doses were calculated in milligrams per square metre**
Course 1 day 1-21	
Carboplatin in a 1-h iv d 1 Procarbazine p.o. d 1–7	15 mg/kg (450 mg/m2/d) 550 mg/m2 4 mg/kg/d (120 mg/m2/d)
Course 2 day 22-42	
Etoposide in a 1-h iv on d 22-23 Cisplatin in a 3-h iv with mannitol plus saline on d 22-23	5 mg/kg/d (150 mg/m2/d) 1 mg/kg/d (30 mg/m2/d)
Course 3 day 43-63	
Vincristine as an iv bolus on day 43 Cyclophosphamide in a 1-h iv with hydration and Mesna (Uromitexan®) on day 43	0.05 mg/kg/d (1.5 mg/m2/d) 50 mg/kg/d (1500 mg/m2)

Mandatory criteria to start a new course of chemotherapy were absolute neutrophil count > 0.8 x 10^9/L, platelets > 120 x 10^9/L. For children over 3 years of age, doses were calculated in milligrams per square metre.

Cumulated doses levels of 21 courses were 3150 mg/m2 of carboplatin, 5880 mg/m2 of procarbazine, 2100 mg/m2 of etoposide, 420 mg/m2 of cisplatin, 10.5 mg/m2 of vincristine and 10.5 g/m2 of cyclophosphamide.

8.3 HIT-SKK chemotherapy without ventricular therapy (*129, 130, 152*)

General recommendation: Before each treatment element (cyclophosphamide/vincristine versus high-dose methotrexate/vincristine versus carboplatin/etoposide), complete blood count, electrolytes, creatinine levels, and liver function parameters were assessed. Serum levels of methotrexate were determined at regular intervals until levels were lower than 0.25 µmol/L.

HIT-SKK chemotherapy: After surgery, HIT-SKK chemotherapy should be started as soon as possible usually within 2-4 weeks. The second and third HIT-SKK cycle will be applied after progressive disease could be excluded by the MRI two weeks after the first or second HIT-SKK cycle, respectively. HIT-SKK chemotherapy consists of intravenous cyclophosphamide, vincristine, methotrexate (followed by leucovorin rescue after 42 hours), carboplatin, and etoposide.

HIT-SKK cycles 1-3 (one cycle is 4 elements of 2 weeks each)

Week 1 10 19	Week 3 12 21	Week 5 14 23	Week 7 16 25
Element (E) IIS	Element (E) IIIS/1	Element (E) IIIS/2	Element (E) IVS
<u>Cyclophosphamide</u> 800 mg/ m²/day 1 h i.v. days 1, 2, 3 <u>Mesna</u> 750 mg/m²/day 24-h-Infusion days 1-4	<u>Methotrexate</u> 5 g/ m² 24 h i.v. <u>CF-rescue</u> 15 mg/m² x6q6h Start h 42	<u>Methotrexate</u> 5 g/ m² 24 h i.v. <u>CF-rescue</u> 15 mg/m² x6q6h Start h 42	<u>Carboplatin</u> 200 mg/m²/day 1 h i.v. days 1, 2, 3
<u>Vincristine</u> 1,5 mg/m² i.v. (max. 2 mg) day 1	<u>Vincristine</u> 1,5 mg/m² i.v. (max. 2 mg) day 1	<u>Vincristine</u> 1,5 mg/m² i.v. (max 2 mg) day 1	<u>Etoposide</u> 150 mg/m²/day ½ h i.v. days 1, 2, 3

CF-rescue, leucovorin rescue; i.v., intravenously. Doses of cyclophosphamide, vincristine, and carboplatin were adjusted for age (6 months of age or less, 66 percent of the full dose; 7 to 12 months of age, 80 percent).

Cumulated doses levels for 3 cycles (totally 25 weeks) were 13.5 mg/m2 of vincristine, 1800 mg/m2 of carboplatin, etoposide 1350 mg/m2, 30 g/m2 of methotrexate, 7,2 g/m2 of cyclophosphamide.

8.4 The UKCCSG/SIOP CNS 9204 trial chemotherapy details (35)

After maximal surgical resection, the chemotherapy schedule comprised blocks of alternating myelosuppressive and non-myelosuppressive drugs repeated at 14-d intervals to produce a high-intensity regimen with modest individual drug-dose intensity (Table). Chemotherapy was to start within 4 weeks of surgery and continued for 1 year unless there was unacceptable toxicity (determined by the treating physician), or until disease progression. Haematological toxicity alone was not an indication to delay treatment.

1 course is 14 days **1 cycle is 56 days** **7 Cycles in total**	**Children up to 10 kg (dose by weight)**	**Children > 10 kg (dose by surface area)**
Course 1; day 0		
Vincristine (iv bolus) Carboplatin (iv over 4 h)	0.05 mg/kg 20 mg/kg	1.5 mg/m^2 550 mg/ m^2
Course 2; day 14		
Vincristine (iv bolus) Methotrexate Folinic acid	0.05 mg/kg 250 mg/kg 15 mg fixed dose	1.5 mg/ m^2 8000 mg/ m^{2}15 mg fixed dose
Course 3; day 28		
Vincristine (iv bolus) Cyclophosphamide Mesna	0.05 mg/kg 50 mg/kg 60 mg/kg	1.5 mg/ m^2 1500 mg/ m^2 1800 mg/ m^2
Course 4; day 42		
Cisplatinum: (continuous infusion for 48 h)	1.3 mg/kg × 2 d	40 mg/ m^2 × 2 d

Cumulated dose levels of 7 cycles were 31.5 mg/m^2 of vincristine, 3850 mg/m^2 of carboplatin, 56 g/m^2 of methotrexate, 10.5 g/m^2 of cyclophosphamide and 560 mg/m^2 of cisplatin.

10% of the total dose of methotrexate was given over the first hour then the remaining 90% was given intravenously over 23 h.

Hydration with 0.18% NaCl + 2.5% dextrose + NaHCO3 50 mmol/L + KCl 20 mmol/L was given before, during and for at least 48 h after the methotrexate infusion was completed.

Methotrexate serum concentration was measured at 24 h, 48 h and 72 h post infusion. Folinic acid rescue was a fixed dose of 15 mg starting 36 h after the beginning of the

methotrexate infusion 3-hourly for five doses, then 6-hourly until serum methotrexate concentration was under 0.1 µmol/L (<1 × 10−7 M).

Mesna was given alongside the cyclophosphamide (1800 mg/ m^2 or 60 mg/kg) and was given intravenously commencing with prehydration, continuing through 4-h cyclophosphamide infusion and ending 12 h after the completion of cyclophosphamide infusion. For cisplatin administration, prehydration included 0.45% saline + 2.5% dextrose, 200 mL/m2 for 3 h. Hydration during and for 6 h post cisplatin was 0.45% saline + 2.5% dextrose + KCl 20 mmol/L + mannitol 12 g/L. Total intravenous infusion rate was equal to 125 mL/m2/h for 48 h.

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