



Brain Tumour Committee
PNET Working Group

**AN INTERNATIONAL PROSPECTIVE STUDY ON
CLINICALLY STANDARD-RISK MEDULLOBLASTOMA
IN CHILDREN OLDER THAN 3 TO 5 YEARS WITH
LOW-RISK BIOLOGICAL PROFILE (PNET 5 MB - LR)
OR AVERAGE-RISK BIOLOGICAL PROFILE (PNET 5
MB -SR)**

SIOP PNET 5 Medulloblastoma

PROTOCOL FINAL VERSION 11
(1st substantial amendment)

2014, Nov 17

Replaces protocol version (protocol history):

SIOP PNET 5 MB Version 10 - 2013, Feb 21

SIOP PNET 5 MB Version 9 - 2012, Sep 27

Amendment history

	Substantial Changes	Page	Rationale	Version
1	WNT subgroup/status replaces β -catenin status	6, 7, 21, 22, 26, 51, 53, 59, 65, 76, 101 Appendix D.1.	The criteria chosen by the SIOP-E PNET biology group are no longer only based on immunohistochemistry, showing stabilisation of nuclear β catenin expression. Current criteria also incorporate molecular diagnosis either with direct β catenin mutation assessment, or indirect WNT subgrouping based on the documentation of somatic monosomy 6, as recommended in a recent international consensus	11 – 2014, Nov 17
	Assessments for obtaining WNT-status updated: <ul style="list-style-type: none"> β-catenin by IHC, mandatory (unchanged) β-catenin mutation, mandatory (was optional) monosomy 6 status, optional (added) 	7, 22, 26, 51, 53, 56, 59, 65-69 Appendix D.1. Appendix I, Form 4B		
	Standard Operating Procedure for DNA and RNA extraction at national reference centres added	Appendix D.5.		
	Diagram for Definition of WNT subgroup status added	6		
2	Definition of upper age limit for LR-arm	7, 21, 51, 55, 65	Recent clinical trials suggest that WNT subgroup patients have a bimodal age distribution and that patients greater than 16 years at diagnosis do not share the good prognosis of younger WNT-subgroup patients	11 – 2014, Nov 17
	Rationale for upper age-limit in LR-arm added	38		
3	Reduction of CSI dose in LR-arm: From 23.4 Gy to 18.0 Gy for brain and spine in 10 daily fraction instead of 13 Primary tumour boost changed from 30.6 to 36.0 Gy in 20 daily fractions instead of 17	7, 21, 23, 51, 80, 105 Appendix C.1	The good prognosis of the WNT-subgroup allow a further de-escalation of craniospinal dose from 23.4 to 18.0 Gy, which is expected to allow a better cognitive function in the years following treatment. A craniospinal dose reduction is currently performed in the ongoing St.-Jude study (SJMB12, opened in 2013) and is planned in the next COG protocol.	11 – 2014, Nov 17
	Rationale for lowering the craniospinal dose in the LR arm of the PNET 5 MB study added	41-42		
	Sentence removed: Radiotherapy doses and technique are the same in PNET 5 MB-LR and both treatment arms of PNET 5 MB-SR.	77		

	Substantial Changes	Page	Rationale	
4	Criteria for a particular per-protocol analysis for patients who started radiotherapy later than 40 days after surgery added.	105	Patients cannot be excluded from study if radiotherapy is delayed after inclusion, but patients will not be included in per-protocol analysis	11 – 2014, Nov 17
5	Possibility and terms of participation for new countries added	18	High interest from further countries to participate	11 – 2014, Nov 17
6	Updated chapter for data aquisition for QoS assessments to enable the use of the “HealthTracker”	71	HealthTracker chosen by QoS study group as database for QoS data	11 – 2014, Nov 17
	Requirements on data management for QoS assessments added	76		
7	Added inclusion criteria:	21ff	See 1.	11 – 2014, Nov 17
	<ul style="list-style-type: none"> New definition of WNT-positivity/ WNT-negativity 	21, 22, 26, 56		
	<ul style="list-style-type: none"> Upper age limit in LR-arm 16 years 	7, 21, 55	See 2.	
	<ul style="list-style-type: none"> Timeline of 14 days for 2nd surgery removed and changed into “if timeline for start of radiotherapy can be kept” Submission of blood and CSF obligatory for all patients, who agree on germline DNA studies. Submission of CSF is recommended. Foreseeable inability to start radiotherapy within 40 days after surgery renders patients ineligible Screening for the compliance with eligibility criteria should be completed, and patient should be included into the study within 28 days after first surgery (in case of second surgery within 35 days after first surgery). Inclusion of patients is not possible later than 40 days after first tumour surgery, or after start of radiotherapy. Information must be provided to the patient on biological studies (tumour and germline), and written informed consent obtained of agreement for participation). 	22, 26, 56, 57	More precise definition of requirements	
8	Added exclusion criteria: <ul style="list-style-type: none"> Ependyoblastoma WNT subgroup status not determinable (replaces β-catenin status) 	22, 26, 57	Was not mentioned before, but necessary to be excluded WNT subgroup needs to be defined to allow inclusion	11 – 2014, Nov 17

SIOP PNET 5 Medulloblastoma

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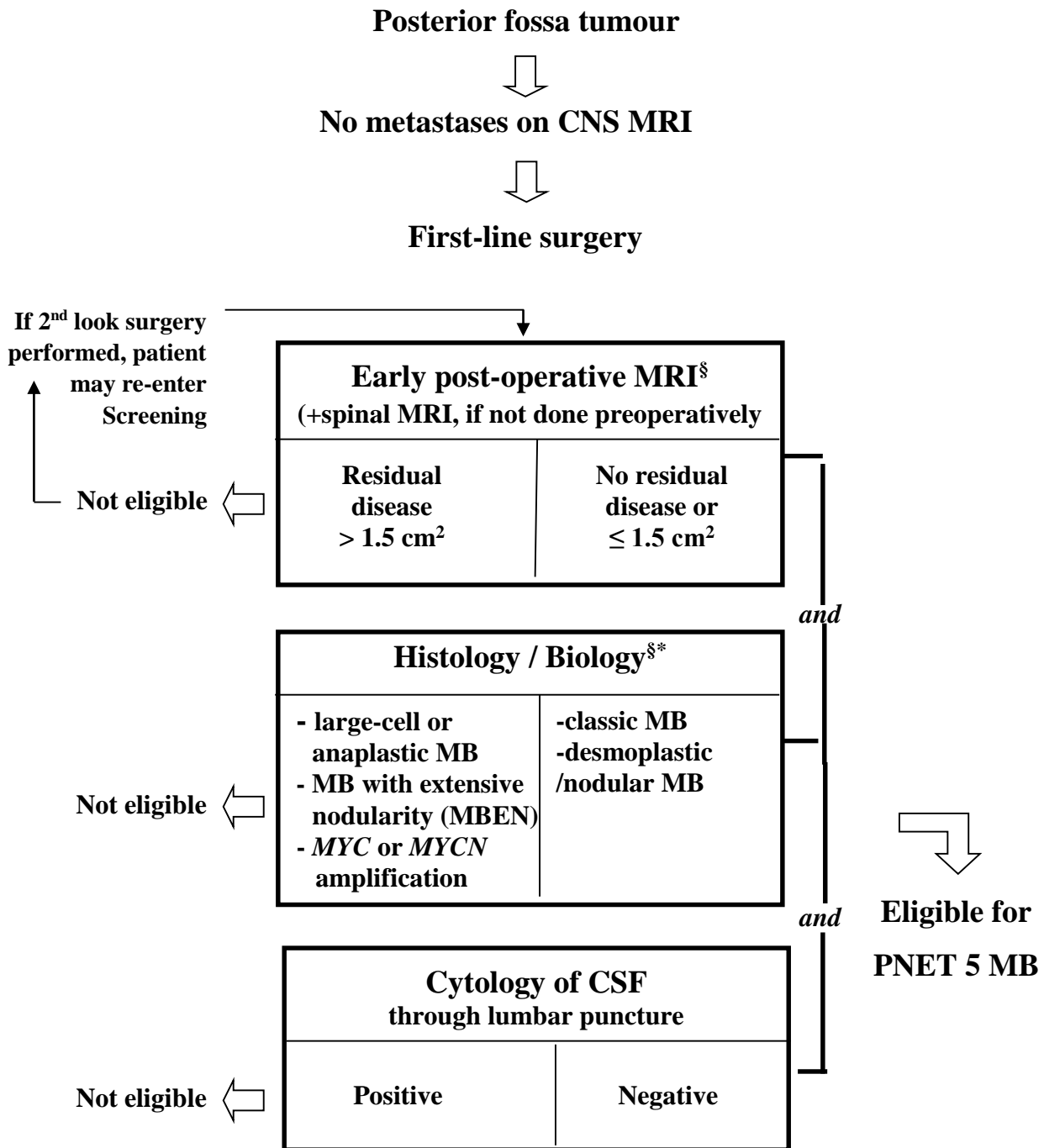
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CONFIDENTIALITY NOTE / IMPORTANT NOTE

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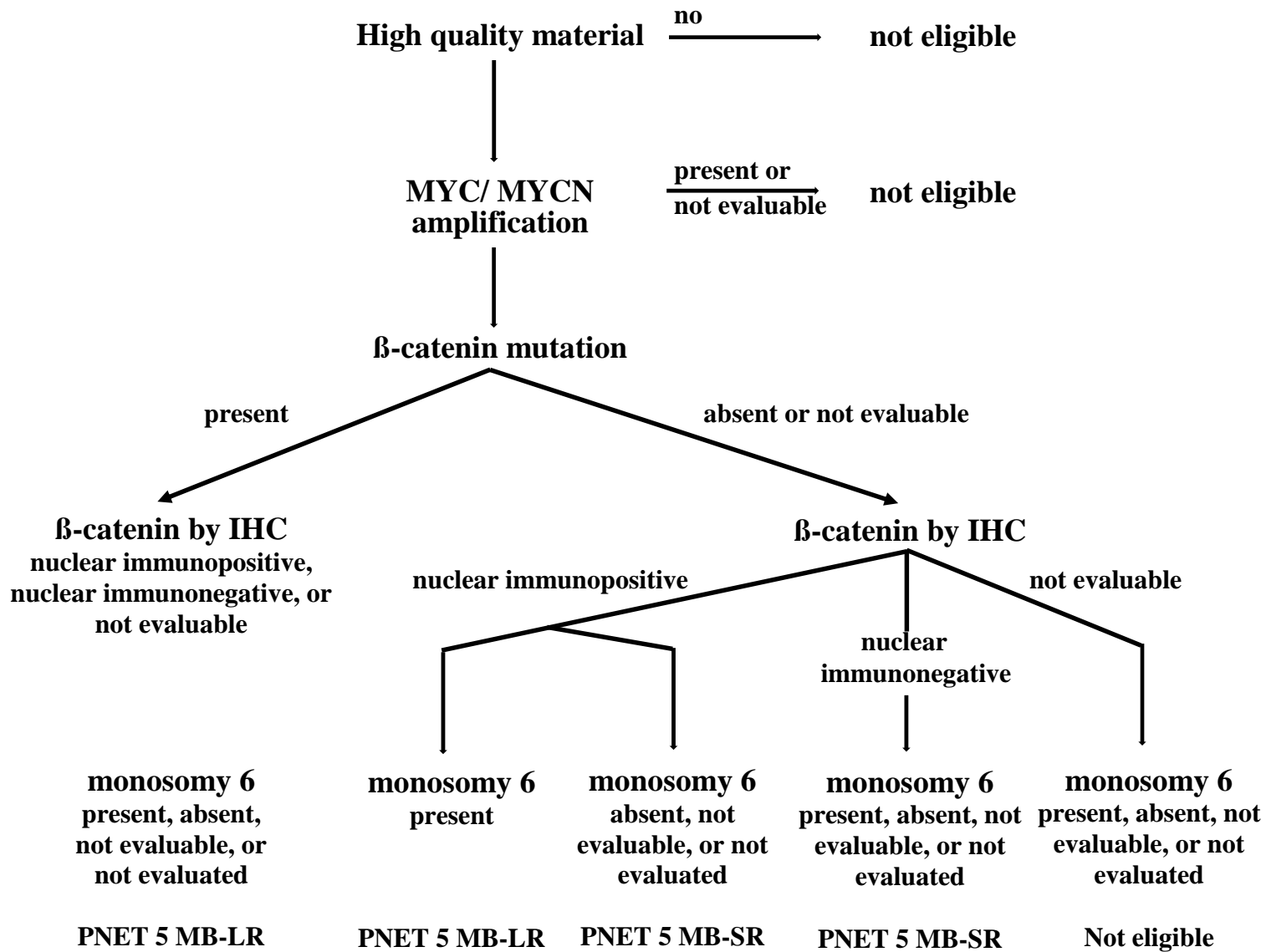
Flow Sheets of Patient Inclusion and Treatment

Patient Inclusion:

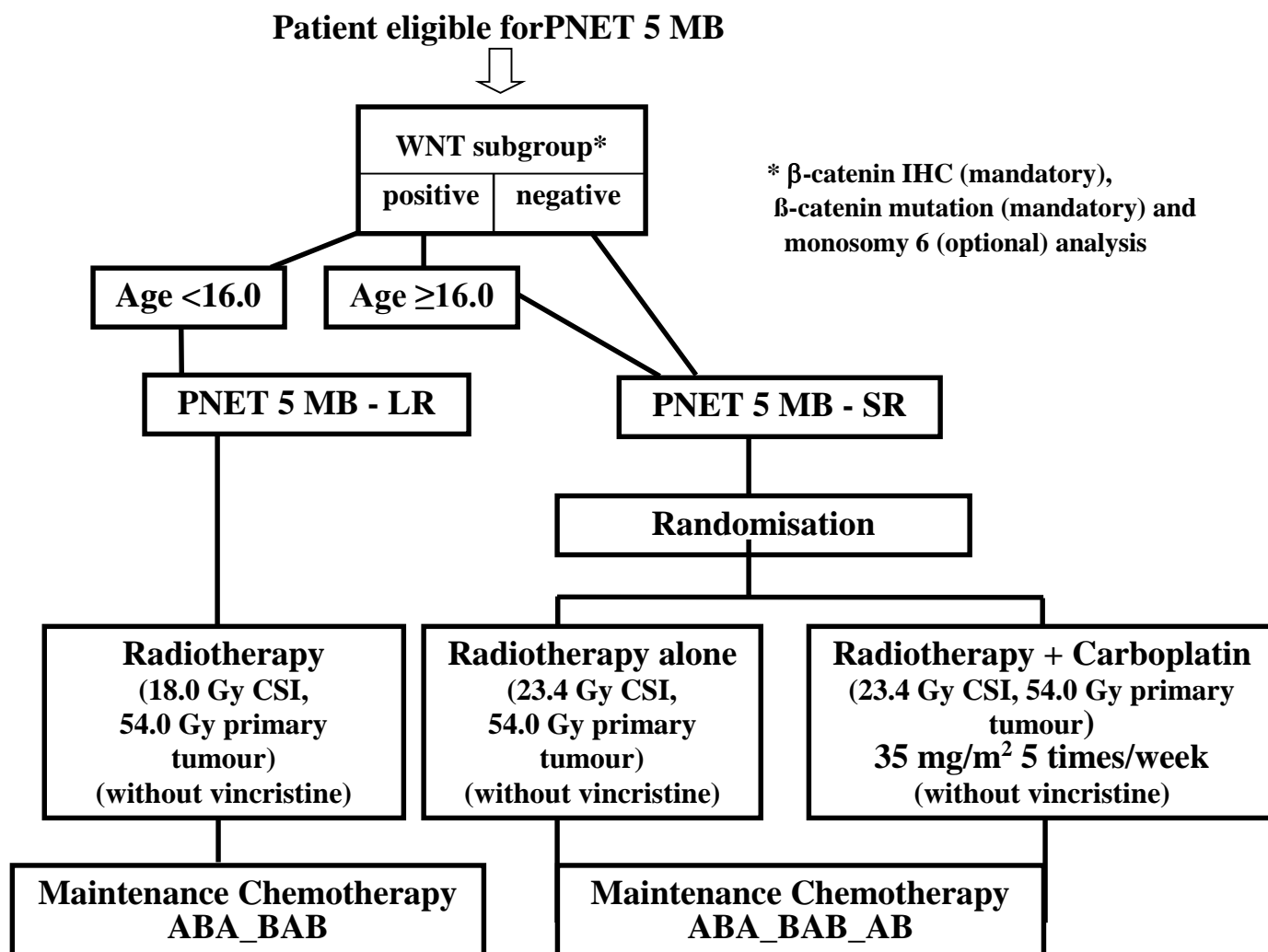


[§] central reference assessment and ^{*}submission of high quality biological materials mandatory

Definition of WNT subgroup status:



Treatment plan:



Radiotherapy:

To be started no more than 28 (maximum 40) days after surgery

LR-arm:

Brain – 18.0 Gy in 10 daily fractions of 1.80 Gy

Spine – 18.0 Gy in 10 daily fractions of 1.80 Gy

Primary tumour boost – 36.0 Gy in 20 daily fractions of 1.80 Gy

(Total dose to primary – 54.0 Gy in 30 daily fractions of 1.80 Gy)

SR-arm:

Brain – 23.4 Gy in 13 daily fractions of 1.80 Gy

Spine – 23.4 Gy in 13 daily fractions of 1.80 Gy

Primary tumour boost – 30.6 Gy in 17 daily fractions of 1.80 Gy

(Total dose to primary – 54.0 Gy in 30 daily fractions of 1.80 Gy)

Maintenance Chemotherapy:

To be started 6 weeks after end of radiotherapy

A: Cisplatin 70 mg/m² day 1, CCNU 75 mg/m² day 1, vincristine 1.5 mg/m² days 1, 8, 15

B: Cyclophosphamide (1 x 1000 mg/m²/d days 1-2), vincristine 1.5 mg/m² (day 1)

Signatures PNET 5 MB

Sponsor / Delegate sponsor:

Univ. Med. Center Hamburg
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Date

Signature

Coordinating Investigator (Trial Chairman):

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The National Trial Coordinators will have primary responsibility for the conduct of the study within their National Group and are members of the Study Committee that will be informed about the progress of the study and approve protocol amendments.

Each National Group will appoint a **National Radiotherapy Coordinator** who will have primary responsibility for Radiotherapy issues within their National Group, and in particular will be responsible for the organisation of Radiotherapy Quality Control procedures.

Each National Group will appoint a **National Reference centre for Biology and Pathology**. The respective Coordinator will have the responsibility to undertake, coordinate, and quality control molecular diagnostics, central pathology review, and translational biological studies.

Each National Group will appoint a **National Reference centre for Neuroradiology**. The respective Coordinator will have the responsibility to conduct, and quality control the central neuroradiological review.

Contact details of the respective Coordinators are listed in the Appendix A.

Further countries may participate in the trial provided that a National Trial Coordinator, a National Radiotherapy Coordinator, a National Coordinator for Biology and Pathology, a National Coordinator for Neuroradiology, and a National Coordinator for Quality of Survival are assigned prior to study participation and accepted by the Study Committee.

2. List of Abbreviations

AMB	Anaplastic Medulloblastoma
APC	Adenomatous-polypsis-coli -Protein
ATRT	Atypical Teratoid Rhabdoid Tumour
BRIEF	Behaviour rating Inventory of Executive Function
BSA	Body surface area
CCNU	Chlorethyl-Cyclohexyl-Nitroso-Urea (Lomustine)
CMB	Classic Medulloblastoma
CNS	Central Nervous System
CR	Complete Response
CRF	Case Record Form
CSF	Cerebrospinal Fluid
CSRT	Cerebrospinal Radiation Therapy
CT	Computed Tomography
CTV	Clinical Target Volume
DMB	Desmoplastic Medulloblastoma
DMSC	Data Monitoring and Safety Committee
DNA	Deoxyribonucleic Acid
EFS	Event-Free Survival
FISH	Fluorescence in situ Hybridization
FSIQ	Full-Scale Intelligence Quotient
GTV	Gross Tumour Volume
Gy	Gray
HFRT	Hyperfractionated Radiation Therapy
HUI	Health Utilities Index
IHC	Immunohistochemistry
LC/A MB	Large Cell / Anaplastic Medulloblastoma
LCMB	Large Cell Medulloblastoma
LEF	Lymphoid Enhancer binding protein Factor
MB	Medulloblastoma
MBEN	Medulloblastoma With Extensiive Nodularity
MEES	Medical Education Employment and Social Questionnaire
MFI	Multidimensional Fatigue Inventory
MRI	Magnetic Resonance Imaging
OR	Organs At Risk
OS	Overall Survival
PCR	Polymerase Chain Reaction

PedsQL	Pediatric Quality of Life Inventory
PF	Posterior Fossa
PFRT	Posterior Fossa Radiation Therapy
PFS	Progression-Free Survival
PNET	Primitive Neuro-ectodermal Tumour
PTV	Planning Target Volume
QC	Quality Control
QLQ-C30	EORTC Quality of Life Questionnaire
QoL	Quality of Life
QoS	Quality of Survival
RNA	Ribonucleic Acid
RT	Radiation Therapy
SDQ	Strengths and Difficulties Questionnaire
SIOP	Société Internationale d'Oncologie Pédiatrique
TCF	T-cell factor
VCR	Vincristine
WNT	Wingless-type

3. PNET 5 MB

The study PNET 5 MB has been designed for children with medulloblastoma of standard risk (according to the risk-group definitions which have been used so far; e.g. in PNET 4). With the advent of biological parameters for stratification into clinical medulloblastoma trials, the WNT subgroup status and the age of the patient will be the criteria according to which study patients will be assigned to either treatment arm PNET 5 MB - LR or to PNET 5 MB - SR, respectively. The initial diagnostic assessments (imaging, staging, histology, and tumour biology) required for study entry are the same for both treatment arms. To facilitate their use by the participating institutions, both treatment arms are described here in separate synopses.

3A. Synopsis - PNET 5 MB - LR

STUDY NAME	SIOP - PNET 5 MB - LR
TITLE	An International Prospective Study in Children Older than 3 to 5 Years with Clinically Standard-Risk Medulloblastoma with Low-Risk Biological Profile
SPONSOR	University Medical Center Hamburg-Eppendorf, Germany
STUDY COORDINATOR	Stefan Rutkowski, Hamburg and Co-PI: François Doz, Paris
STUDY DESIGN	This is an international, prospective, Phase-II, open study in patients between the ages of 3 to 5 years and less than 16.0 years, with ‘standard-risk’ medulloblastoma and a low-risk biological profile.
OBJECTIVES	<p><i>Primary objective:</i></p> <ul style="list-style-type: none"> - to confirm that the 3-year Event-Free Survival (EFS) rate in children and adolescents with standard-risk medulloblastoma having a low-risk biological profile remains in excess of 80% when patients are treated with 18.0 Gy neuraxis irradiation plus boost to the primary tumour, and reduced-intensity chemotherapy. <p><i>Secondary objectives:</i></p> <ul style="list-style-type: none"> - to investigate the Overall Survival (OS) rate, and the pattern of relapse in this patient group, - to study the late effects of the reduced-dose approach, focusing on hearing, endocrine, and neurologic function, and standardized, patients/ parents rated measurements of health status, executive function, behavioural outcome, medical, educational, employment and social situation, and quality of life. - to conduct comprehensive studies in a prospective fashion on the biological basis of WNT-subgroup medulloblastoma, with the aim of identification, investigation and validation of biomarkers and drug targets with therapeutic potential in this disease subgroup. These investigations will focus on (i). detailed analysis of biological pathways and molecular events established to play a role in medulloblastoma, or that are of potential prognostic significance in this disease group, (ii). comprehensive genome-wide investigations of novel medulloblastoma defects, and (iii). defining diagnostic correlates of WNT pathway activation..
NUMBER OF PATIENTS	The expected annual accrual rate is 10 to 12 patients. With a duration of inclusion of 6 years, 60 patients may be included in the study.
STUDY POPULATION	<p><i>Inclusion criteria:</i></p> <ol style="list-style-type: none"> a) Age at diagnosis, at least 3 - 5 years (depending on the country) and less than 16.0 years. The date of diagnosis is the date on which surgery is undertaken; b) Histologically proven medulloblastoma, including the following subtypes, as defined in the WHO classification (2007): <ul style="list-style-type: none"> - classic medulloblastoma, - desmoplastic/nodular medulloblastoma Pre-treatment central pathology review is considered mandatory. c) Standard-risk medulloblastoma, defined as;

	<ul style="list-style-type: none"> - total or near total surgical resection with less than or equal to 1.5 cm² (measured on axial plane) of residual tumour on early post-operative MRI, without and with contrast, on central review; - no CNS metastasis on MRI (cranial and spinal) on central review; - no tumour cells on the cytospin of lumbar CSF - no clinical evidence of extra-CNS metastasis; <p>Patients with a reduction of postoperative residual tumour through second surgery to less than or equal to 1.5 cm² are eligible, if timeline for start of radiotherapy can be kept.</p> <ul style="list-style-type: none"> d) Submission of high quality biological material including fresh frozen tumour samples for the molecular assessment of biological markers (such as the assessment of <i>MYC</i> gene copy number status) in national biological reference centers. Submission of blood is mandatory for all patients, who agree on germline DNA studies. Submission of CSF is recommended. e) No amplification of <i>MYC</i> or <i>MYCN</i> (determined by FISH); f) Low-risk biological profile, defined as WNT subgroup positivity. The WNT subgroup is defined by the presence of (i) β-catenin mutation (mandatory testing), or (ii) β-catenin nuclear <u>immuno-positivity</u> by IHC (mandatory testing) and β-catenin mutation, or (iii) β-catenin nuclear <u>immuno-positivity</u> by IHC and monosomy 6 (optional testing). g) No prior therapy for medulloblastoma other than surgery; h) Radiotherapy aiming to start no more than 28 days after surgery. Foreseeable inability to start radiotherapy within 40 days after surgery renders patients ineligible for the study i) Screening for the compliance with eligibility criteria should be completed, and patient should be included into the study within 28 days after first surgery (in case of second surgery within 35 days after first surgery). Inclusion of patients is not possible later than 40 days after first tumour surgery, or after start of radiotherapy. j) CTC grades < 2 for liver, renal, haematological function k) No significant sensineural hearing deficit as defined by pure tone audiometry with bone conduction or air conduction and normal tympanogram showing no impairment ≥ 20 dB at 1-3 kHz. If performance of pure tone audiometry is not possible postoperatively, normal otoacoustic emissions are acceptable, if there is no history for hearing deficit. l) No medical contraindication to radiotherapy or chemotherapy, such as preexisting DNA breakage syndromes (e.g. Fanconi Anemia, Nijmegen breakage syndrome), Gorlin Syndrome or other reasons as defined by patient's clinician; m) No identified Turcot and Li Fraumeni syndrome. n) Written informed consent (and patient assent where appropriate) for therapy according to the laws of each participating country. Information must be provided to the patient on biological studies (tumour and germline), and written informed consent obtained of agreement for participation. o) National and local ethical committee approval according to the laws of each participating country (to include approval for biological studies). <p><i>Exclusion criteria:</i></p> <ul style="list-style-type: none"> a) One of the inclusion criteria is lacking; b) Brainstem or supratentorial primitive neuro-ectodermal tumour; c) Atypical teratoid rhabdoid tumour; d) Medulloepithelioma; Ependymoblastoma e) Large-cell medulloblastoma, anaplastic medulloblastoma, or medulloblastoma with extensive nodularity (MBEN), confirmed on central pathological review. f) Unfavourable or undeterminable biological profile, defined as amplification of <i>MYC</i> or <i>MYCN</i>, or WNT subgroup status not determinable. g) Metastatic medulloblastoma (on CNS MRI and/or positive cytospin of postoperative lumbar CSF);
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	<p>h) Patient previously treated for a brain tumour or any type of malignant disease;</p> <p>i) DNA breakage syndromes (e.g. Fanconi anemia, Nijmegen breakage syndrome) or other, or identified Gorlin, Turcot, or Li Fraumeni syndrome</p> <p>j) Patients who are pregnant;</p> <p>k) Female patients who are sexually active and not taking reliable contraception;</p> <p>l) Patients who cannot be regularly followed up due to psychological, social, familial or geographic reasons;</p> <p>m) Patients in whom non-compliance with toxicity management guidelines can be expected.</p>
STUDY DURATION	<p>Inclusion period: 6 years.</p> <p>Treatment period: 39 weeks.</p> <p>Follow-up period: 3 years.</p> <p>Total duration of study: 9 years.</p>
TREATMENT	<p><i>Radiation therapy for low risk patients with WNT positive localised tumours:</i></p> <p>Brain – 18.0 Gy in 10 daily fractions of 1.80 Gy</p> <p>Spine - 18.0 Gy in 10 daily fractions of 1.80 Gy</p> <p>Primary tumour boost – 36.0 Gy in 20 daily fractions of 1.80 Gy</p> <p>Total dose to primary – 54.0 Gy in 30 daily fractions of 1.80 Gy</p> <p><i>Chemotherapy:</i></p> <p>Maintenance chemotherapy starts 6 weeks after radiotherapy.</p> <p>6 cycles alternating Regimen A and Regimen B.</p> <p>Regimen A (cycles 1, 3, 5): cisplatin 70 mg/m² day 1, CCNU 75 mg/m² day 1, vincristine 1.5 mg/m² days 1, 8 and 15,</p> <p>Regimen B: (cycles 2, 4, 6): cyclophosphamide 1 x 1000 mg/m² days 1-2, vincristine 1.5 mg/m² day 1.</p> <p>Interval after cycle A: 6 weeks, after cycle B: 3 weeks, for a total duration of 27 weeks.</p>
OUTCOME MEASURES	<p>Event-free survival</p> <p>Overall survival</p> <p>Pattern of relapse</p> <p>Late effects of therapy</p> <ul style="list-style-type: none"> • Endocrine function • Audiology • Neurology • standardized, patients'/ parents' rated measurements of health status, executive function, behavioural outcome, medical, educational, employment and social situation, and quality of life. <p>Prognostic relevance of biological tumour markers</p>
STATISTICAL CONSIDERATIONS	<p>The primary endpoint is the 3-year EFS rate. The aim of the study is to achieve a 3-year rate in excess of 80%.</p> <p>Results will be analysed according to a modified multistage Fleming procedure, in order to be able to stop the trial early only if too many events are observed. Sixty patients are to be included in the trial. Three interim analyses, the third one being the final one, will take place when 20, then 40 and finally 60 patients have been included and these patients will be followed for at least 36 months. Results of the interim analyses will be reviewed by an independent Data Monitoring and Safety Committee (DMSC).</p> <p>Calculations of the boundaries have been performed assuming a 36 months EFS rate less than or equal to 80% as not acceptable (null hypothesis) and controlling the power in order to detect in 88% of cases a 36 months EFS rate equal or higher than 91%. The error risk α to wrongly accept the protocol is limited to 11%.</p> <p>EFS will be estimated from date of first operation. Any progression, any relapse, any occurrence of second malignancy, and any death will be considered as an event.</p> <p>The observed 36 months-rates will be calculated as crude rates, when all the studied patients will have at least 36 months of follow-up. When the results are</p>

	analysed, if all patients do not have the required 36 months minimum follow-up, then the EFS rate will be estimated by Kaplan-Meier and compared to the corresponding Fleming lower boundary.
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3B. Synopsis - PNET 5 MB - SR

STUDY NAME	SIOP - PNET 5 MB - SR
TITLE	An International Prospective Study in Children Older than 3 to 5 Years with Clinically Standard-Risk Medulloblastoma with Average-Risk Biological Profile
SPONSOR	University Medical Center Hamburg-Eppendorf, Germany
STUDY COORDINATOR	Stefan Rutkowski, Hamburg
STUDY DESIGN	This is an international, prospective, Phase-III, randomised study in patients between the ages of 3 to 5 years and less than 22 years, with 'standard-risk' medulloblastoma and an average-risk biological profile.
OBJECTIVES	<p><i>Primary objective:</i></p> <ul style="list-style-type: none"> -to test whether the Event-Free Survival (EFS) in children and adolescents with standard-risk medulloblastoma having an average-risk biological profile is different for patients treated with or without carboplatin concomitantly with radiotherapy (23.4 Gy neuraxis irradiation plus boost to the primary tumour) followed by a modified maintenance chemotherapy. <p><i>Secondary objectives:</i></p> <ul style="list-style-type: none"> -to investigate the Overall Survival rates (OS), the Progression-free survival rates (PFS), and the pattern of relapse in the randomized treatment arms. -to test the feasibility of carboplatin treatment concomitantly with radiotherapy -to study the late effects in the randomized treatment arms, focusing on hearing, endocrine, and neurologic function, and standardized, patients/parents rated measurements of health status, executive function, behavioural outcome, medical, educational, employment and social situation, and quality of life. -to conduct comprehensive studies in a prospective fashion on the biological basis of standard-risk medulloblastoma, with the aim of identification, investigation and validation of biomarkers (diagnostic, prognostic and predictive) and drug targets with therapeutic potential in this disease subgroup. These investigations will focus on (i). detailed analysis of biological pathways and molecular events established to play a role in medulloblastoma, or that have been shown to have potential prognostic significance in this disease subgroup (e.g. chromosome 17 abnormalities), and (ii). comprehensive genome-wide investigations of novel medulloblastoma defects.
NUMBER OF PATIENTS	The expected accrual rate is 50 patients per year. With a duration of inclusion of 6 years, 300 patients may be included in the study.
STUDY POPULATION	<p><i>Inclusion criteria:</i></p> <p>as per PNET 5 MB - LR, with the exception of f):</p> <ol style="list-style-type: none"> Age at diagnosis, at least 3 - 5 years (depending on the country) and less than 22 years. The date of diagnosis is the date on which surgery is undertaken; Histologically proven medulloblastoma, including the following subtypes, as defined in the WHO classification (2007): <ul style="list-style-type: none"> - classic medulloblastoma, - desmoplastic/nodular medulloblastoma Pre-treatment central pathology review is mandatory. Standard-risk medulloblastoma, defined as; <ul style="list-style-type: none"> - total or near total surgical resection with less than or equal to 1.5 cm² (measured in axial plane) of residual tumour on early post-operative MRI, without and with contrast, on central review; - no CNS metastasis on MRI (cranial and spinal) on central review; - no tumour cells on the cytospin of lumbar CSF. - no clinical evidence of extra-CNS metastasis;

	<p>Patients with a reduction of postoperative residual tumour through second surgery to less than or equal to 1.5 cm² are eligible, if timeline for start of radiotherapy can be kept.</p> <p>d) Submission of high quality biological material including fresh frozen tumour samples for the molecular assessment of biological markers (such as the assessment of <i>MYC</i> gene copy number status) in national biological reference centers. Submission of blood is mandatory for all patients, who agree on germline DNA studies. Submission of CSF is recommended.</p> <p>e) No amplification of <i>MYC</i> or <i>MYCN</i> (determined by FISH);</p> <p>f) Average-risk biological profile, defined as WNT subgroup negativity. WNT-negative tumours are defined by (i) β-catenin nuclear <u>immuno-negativity</u> by IHC (mandatory testing), and the absence of β-catenin <u>mutation</u> (mandatory testing) and <u>monosomy 6</u> (optional testing), or (ii) β-catenin nuclear <u>immuno-positivity</u> by IHC (mandatory testing) in the absence of β-catenin <u>mutation and monosomy 6</u>, or (iii) <u>monosomy 6</u> in the absence of β-catenin nuclear <u>immuno-positivity</u> by IHC or β-catenin <u>mutation</u>. WNT subgroup positive tumours arising in patients age ≥ 16.0 years at diagnosis. The WNT subgroup is defined by the presence of (i) β-catenin <u>mutation</u> (mandatory testing), or (ii) β-catenin nuclear <u>immuno-positivity</u> by IHC (mandatory testing) and β-catenin <u>mutation</u>, or (iii) β-catenin nuclear <u>immuno-positivity</u> by IHC and <u>monosomy 6</u> (optional testing).</p> <p>g) No prior therapy for medulloblastoma other than surgery;</p> <p>h) Radiotherapy aiming to start no more than 28 days after surgery. Foreseeable inability to start radiotherapy within 40 days after surgery renders patients ineligible for the study.</p> <p>i) Screening for the compliance with eligibility criteria should be completed, and patient should be included into the study within 28 days after first surgery (in case of second surgery within 35 days after first surgery). Inclusion of patients is not possible later than 40 days after first tumour surgery, or after start of radiotherapy.</p> <p>j) CTC grades < 2 for liver, renal, haematological function</p> <p>k) no significant sensineural hearing deficit as defined by pure tone audiometry with bone conduction or air conduction and normal tympanogram shows no impairment ≥ 20 dB at 1-3 kHz. If performance of pure tone audiometry is not possible postoperatively, normal otoacoustic emissions are acceptable if there is no history for hearing deficit.</p> <p>l) No medical contraindication to radiotherapy or chemotherapy, such as preexisting DNA breakage syndromes (e.g. Fanconi Anemia, Nijmegen breakage syndrome) Gorlin Syndrome or other reasons as defined by patient's clinician;</p> <p>m) No identified Turcot and Li Fraumeni syndrome.</p> <p>n) Written informed consent (and patient assent where appropriate) for therapy according to the laws of each participating country. Information must be provided to the patient on biological studies (tumour and germline), and written informed consent obtained of agreement for participation).</p> <p>o) National and local ethical committee approval according to the laws of each participating country (to include approval for biological studies).</p> <p><i>Exclusion criteria:</i></p> <p>a) One of the inclusion criteria is lacking;</p> <p>b) Brainstem or supratentorial primitive neuro-ectodermal tumour;</p> <p>c) Atypical teratoid rhabdoid tumour;</p> <p>d) Medulloepithelioma; Ependymoblastoma</p> <p>e) Large-cell medulloblastoma, anaplastic medulloblastoma, or medulloblastoma with extensive nodularity (MBEN), centrally confirmed.</p> <p>f) Unfavourable or undeterminable biological profile, defined as amplification of <i>MYC</i> or <i>MYCN</i>, or WNT subgroup status not determinable.</p>
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	<p>g) Metastatic medulloblastoma (on CNS MRI and/or positive cytospin of postoperative lumbar CSF);</p> <p>h) Patient previously treated for a brain tumour or any type of malignant disease;</p> <p>i) DNA breakage syndromes (e.g. Fanconi anemia, Nijmegen breakage syndrome) or other, or identified Gorlin, Turcot, or Li Fraumeni syndrome</p> <p>j) Patients who are pregnant;</p> <p>k) Female patients who are sexually active and not taking reliable contraception;</p> <p>l) Patients who cannot be regularly followed up due to psychological, social, familial or geographic reasons;</p> <p>m) Patients in whom non-compliance with toxicity management guidelines can be expected.</p>
STUDY DURATION	<p>Inclusion period: 6 years.</p> <p>Treatment period: 48 weeks.</p> <p>Follow-up period: 4 years.</p> <p>Total duration of study: 10 years.</p>
TREATMENT	<p><i>Radiation therapy:</i> as per PNET 5 MB - LR: with or without carboplatin 35 mg/m² 5 times/week. Brain – 23.40 Gy in 13 daily fractions of 1.80 Gy Spine - 23.40 Gy in 13 daily fractions of 1.80 Gy Primary tumour boost – 30.6 Gy in 17 daily fractions of 1.80 Gy Total dose to primary – 54.0 Gy in 30 daily fractions of 1.80 Gy</p> <p><i>Chemotherapy:</i> Maintenance chemotherapy starts 6 weeks after radiotherapy. 8 cycles alternating Regimen A and Regimen B. Regimen A (cycles 1, 3, 5, 7): cisplatin 70 mg/m² day 1, CCNU 75 mg/m² day 1, vincristine 1.5 mg/m² days 1, 8 and 15 Regimen B: (cycles 2, 4, 6, 8): cyclophosphamide 1 x 1000 mg/m² days 1-2, vincristine 1.5 mg/m² day 1. Interval after cycle A: 6 weeks, after cycle B: 3 weeks, for a total duration of 36 weeks.</p>
OUTCOME MEASURES	<p>as per PNET 5 MB - LR:</p> <p>Event-free survival</p> <p>Overall survival</p> <p>Pattern of relapse</p> <p>Late effects of therapy</p> <ul style="list-style-type: none"> • Endocrine function • Audiology • Neurology • standardized, patients/ parents rated measurements of health status, executive function, behavioural outcome, medical, educational, employment and social situation, and quality of life. <p>Prognostic relevance of biological tumour markers and:</p> <p>Feasibility of carboplatin treatment concomitantly to radiotherapy</p>
STATISTICAL CONSIDERATIONS	<p>The primary endpoint is event-free survival (EFS). The aim of the trial is the comparison of EFS between patients receiving radiotherapy and 8 cycles of maintenance chemotherapy with and without concurrent carboplatin during radiotherapy. Does the concurrent administration of carboplatin during radiotherapy change the distribution of event-free survival times (EFS_{OP})? Null hypothesis: The distribution of EFS_{OP} between patients with and without concurrent administration of chemotherapy of carboplatin during radiotherapy does not differ.</p> <p>This hypothesis will be tested with a two-sided log-rank test on difference. For descriptive reasons the Kaplan-Meier curves for the EFS_{OP}, the quartiles of the EFS_{OP} with 95%-confidence intervals, and the EFS_{OP}-rates at years 1, 3 and 5 will be given for both arms.</p>

	<p>Three analyses are to be performed to answer the main question, unless the trial is stopped prematurely. The trial will be terminated after an interim analysis, if the main question can already be answered at this interim analysis. After each interim analysis a data-dependent sample size calculation will be performed. Then, the accrual period, the observation time, the schedule of the second interim and final analysis (required number of events) and the number of interim analyses will be adapted taking the data into account. If the 3-step sequential plan according to Wang and Tsatis described above does not need to be changed, the first interim analysis will take place after 21 events, the second interim analysis will take place after 42 events and the final analysis will take place after 105 events.</p> <p>Sample size calculation is based on assumption of 3-year EFS_{OP} of 75% for patients with standard therapy. 3-year EFS_{OP} for patients with additional carboplatin during radiotherapy is assumed to be 10% higher, i.e. 85%. With a significance level of 5%, an accrual rate of 6 years and a follow-up period of 4 years 299 patients will be needed to observe 105 events in both therapy arms and to obtain a power of 80% with a 3-step group sequential design according to Wang and Tsatis with boundary shape parameter of $\Delta = 0.37$ when using the log-rank test. This corresponds to an accrual rate of 50 patients per year.</p>
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4. Background and Rationale

Medulloblastoma is a highly cellular malignant embryonal neoplasm classified as a Primitive Neuroectodermal Tumour [PNET] (Louis, Ohgaki et al. 2007). It is the most common malignant brain tumour in children, accounting for between 15 and 20 % of all childhood primary central nervous system (CNS) neoplasms. Medulloblastoma arises in the posterior fossa, usually from the cerebellar vermis in the roof of the 4th ventricle. As with other PNETs, medulloblastomas have a marked propensity to metastasize via CSF pathways, and evidence of such metastatic spread is present in up to 35 % of cases at diagnosis.

The following histological variants of medulloblastoma are recognised in the WHO classification of CNS tumours (2007):

- Classic medulloblastoma,
- Desmoplastic/nodular medulloblastoma
- Medulloblastoma with extensive nodularity
- Large-cell medulloblastoma
- Anaplastic medulloblastoma

The treatment of medulloblastoma currently involves surgical resection followed by radiotherapy and chemotherapy. While this combined-modality treatment regimen has substantially improved the cure rate, medulloblastoma remains incurable in approximately one-third of patients (Gilbertson 2004), and 20-25% of standard risk patients (Gajjar, Chintagumpala et al. 2006; Packer, Gajjar et al. 2006). Moreover, survivors suffer from long-term toxic side effects related to therapy that often seriously affect their quality of life (Ris, Packer et al. 2001; Gurney, Kadan-Lottick et al. 2003; Maddrey, Bergeron et al. 2005; Mulhern, Palmer et al. 2005).

In the previous trials, prognosis was most commonly assessed based on clinical criteria. Risk-adapted treatment of medulloblastoma was established, using age, extent of resection, and presence of metastases for stratification. However, this stratification is not optimal for a numerically significant subset of patients. Some of them suffer from early relapse although they are stratified as clinically low risk patients, other receive intense and toxic therapy although they might have a good prognosis with less intense therapy.

Recent data suggest that the histological subtype of medulloblastoma as well as biological factors influence the clinical behaviour, and can therefore be used to optimize treatment stratification (Eberhart, Kepner et al. 2002; Pomeroy, Tamayo et al. 2002; Rutkowski, von Hoff et al. 2010).

4.1. Clinical Risk Stratification and the Concept of Standard-risk Medulloblastoma

The prognosis of medulloblastoma is closely related to three main clinical factors; the age of the patient, the extent of disease at diagnosis (presence of metastases) and the extent of residual disease after tumour resection. These factors are again influenced by the biology of the tumour, which also influences the histological appearance.

4.1.1. Age of the Patient

Age less than 3 years is associated with a 2-fold higher risk of disease progression within 5 years of diagnosis in comparison with older patients (Zeltzer, Boyett et al. 1999; Packer, Rood et al. 2003). One of the reasons for this less favourable prognosis is a different biological behaviour. It is known that different histological and biological medulloblastoma subtypes have different age distributions.

In infants, nodular desmoplastic medulloblastomas are frequent, and are associated with a good prognosis. On the reverse side, prognosis of infants with classic medulloblastoma is worse compared to older children with classic medulloblastoma.(Grill, Sainte-Rose et al. 2005; Rutkowski, Bode et al. 2005) Decreased prognosis is also explained by an unwillingness to apply dose-intense radiotherapy in this young age group, as this causes severe damage to the developing brain. (Duffner, Horowitz et al. 1993) Due to these factors, infants are not considered to be “standard risk” patients, and cannot be included into this trial.

4.1.2. Metastatic Disease

Patients with disseminated disease have a much poorer prognosis. The presence of metastatic disease at presentation as diagnosed by the presence of meningeal enhancement on MRI of the brain (Chang Stage M2) or spine (Chang Stage M3) clearly carries a poor prognosis.(Chang, Housepian et al. 1969; Zeltzer, Boyett et al. 1999) Although it was not consistently used for stratification in the early trials, microscopic spread to the CSF has also been shown to be associated with an impaired prognosis, independently of the presence of macroscopic metastases.(Fouladi, Gajjar et al. 1999; Miralbell, Bieri et al. 1999; Verlooy, Mosseri et al. 2006; von Hoff, Hinkes et al. 2009) It is widely accepted that patients must be staged by MRI and CSF analysis to exclude metastases in order to be regarded as standard risk patients. However, in a multicenter trial with a large number of participating centers, technique and quality of MRI imaging differs. Therefore, a standardised imaging technique was defined in PNET 4 and central reference assessment of the MRI was recommended. Within this trial the outcome of patients whose scans had not been centrally reviewed was found to be worse than of patients in which central review of MRI scans of brain and spine had taken place.. This suggests that quality assurance of imaging is a relevant tool for keeping the group of included patients clear of patients with falsely negative metastasis staging. The relevance of MRI review has also been shown by other groups.(Oyharcabal-Bourden, Kalifa et al. 2005; Packer, Gajjar et al. 2006)

4.1.3. Residual Disease

Few studies have demonstrated the prognostic importance of achieving a gross total or near gross total surgical excision(Albright, Wisoff et al. 1996; Zeltzer, Boyett et al. 1999), although the difference was only seen in the subgroup of M0 patients. Consequently, most of the international trials excluded patients with residual tumours larger than 1.5 cm² from “standard-risk” trials, precluding the possibility to further evaluate this factor. However, in the HIT-91 study, and the PNET-3 study no significant impact of residual tumour has been shown on outcome, leading to decision to include incompletely resected patients within the PNET 4 trial.(Taylor, Bailey et al. 2003; von Hoff, Hinkes et al. 2009) Within PNET 4 residual tumour > 1.5 cm² was associated with an impaired prognosis. Therefore, patients with residual tumours > 1.5 cm² are not considered as standard risk patients within PNET 5 MB - LR and PNET 5 MB - SR. If incompletely resected tumours are amenable for second surgery, patients can be included in PNET 5 MB - LR and PNET 5 MB - SR as long as the residual tumour post second surgery is <1.5cm², and if second surgery is performed within 14 days after first surgery.

It is important to note that the definition of 1.5 cm² (on MRI) as the limit for inclusion is arbitrary. Other groups used the definition of “any measurable” tumour on MRI (Oyharcabal-Bourden, Kalifa et al. 2005), and even within groups that use the definition of 1.5 cm² there is no international consensus about the plane in which this area should be measured and calculated. As the early trials were based on CT imaging, the axial plain was commonly used for area calculation. But with the use of MRI, calculation of residual tumour area was also estimated in the maximum cross-sectional area, or 3-dimensionally as volume. To allow comparability to the earlier trials, it has been decided to use 1,5 cm² on axial plane in PNET 5 MB - LR and PNET 5 MB - SR.

For estimation of residual disease patient's pre-operative MRI imaging is compared with that obtained post-operatively. To allow proper comparison, adherence to the standard MRI technique described in the protocol is important (see chapter 9.2. Recommendations for imaging and central MRI review, page 62)

It is accepted that postoperative imaging is best performed within 72 hours of surgery, after which post-operative changes render interpretation of residual disease difficult. Therefore for the purposes of this trial all patients should have post-operative MRI imaging before and after contrast injection within 72 hours of surgery.

4.2. Stratification based on histological subtypes of medulloblastoma

Histological classification of medulloblastoma has improved over the last years. The following histological variants of medulloblastoma are recognised in the WHO classification of CNS tumours (2007): classic medulloblastoma, desmoplastic/nodular medulloblastoma, medulloblastoma with extensive nodularity, large-cell medulloblastoma, anaplastic medulloblastoma.(Louis, Ohgaki et al. 2007) According to this classification, tumours with myogenic or melanocytic differentiation are no longer regarded as separate entities but as features that may occur in the different variants described above. Extensive immunohistochemical evaluation and reticulin staining is mandatory to define these entities in diagnostic workup and to exclude differential diagnoses such as astrocytic and ependymal neoplasms, atypical teratoid/rhabdoid tumours, plexus carcinomas and other entities.

Several studies have shown that these variants have different clinical behaviours. Therefore, current treatment strategies use histology as a tool for patients' stratification.

4.2.1. Large cell medulloblastoma, anaplastic medulloblastoma

These variants were formerly grouped together as anaplastic/large cell medulloblastomas. However, the current classification separates these entities.

Large cell medulloblastoma was been defined as a separate entity, after its initial description in 1992.(Giangaspero, Rigobello et al. 1992) It is characterized by monomorphic cells with large round vesicular nuclei, single prominent nucleoli and variable amounts of eosinophilic cytoplasm. The cells often lack cohesiveness and mitotic as well as apoptotic figures are frequent. These cells have to be predominant in the specimen. Their focal occurrence does not qualify for the diagnosis of this variant. Large cell medulloblastomas frequently show a dot-like synaptophysin reactivity.

The clinical impact of this rare variant, with a highly aggressive behaviour was described in several reports. (Brown, Kepner et al. 2000; McManamy, Lamont et al. 2003)

Severe cytological anaplasia was recognised to be a negative prognostic factor. (Eberhart, Kepner et al. 2002) In the WHO classification of 2007 anaplastic medulloblastoma has been accepted as a histological subgroup.(Louis, Ohgaki et al. 2007) The cytological signs of severe anaplasia such as marked nuclear and cellular pleomorphism, nuclear moulding (wrapping) and high mitotic and apoptotic activity have to be predominant to qualify for this diagnosis. Focal severe anaplasia or diffuse moderate anaplasia do not confer the diagnosis of an anaplastic variant of medulloblastoma. On the molecular level, it has been shown that both, large cell and anaplastic medulloblastoma are associated with *MYC* amplification(Lamont, McManamy et al. 2004) and expression. (Eberhart, Kratz et al. 2004; Stearns, Chaudhry et al. 2006) But not all large cell and anaplastic medulloblastoma show *MYC* amplification, and the prognostic relevance of histology in the absence of *MYC* abnormalities is not clear. (von Hoff, Hartmann et al. 2009)

However, due to their negative prognostic significance, large cell and anaplastic medulloblastoma were excluded from randomisation in the PNET-4 trial. Patients with large cell or anaplastic

medulloblastoma cannot be considered as standard risk patients. Further research may further define this group and allow for the risk adapted treatment.

Patients with large cell or anaplastic medulloblastoma are not eligible for treatment within this protocol.

4.2.2. Desmoplastic / nodular Medulloblastoma and Medulloblastoma with extensive Nodularity

This medulloblastoma variant is characterized by nodular, reticulin-free tumour islands surrounded by areas with densely packed, proliferative tumour cells which produce a dense, intercellular reticulin fibre network. Even if this typical pattern is present only focally, a medulloblastoma specimen has to be classified as desmoplastic nodular medulloblastoma. The islands of desmoplastic / nodular medulloblastomas frequently show a lower mitotic and proliferative activity and signs of early neuronal differentiation. Cases with nodular appearance but without typical fibre network do not qualify for the diagnosis of desmoplastic / nodular medulloblastoma. Medulloblastomas only showing an increased fibre content without the typical biphasic (island) pattern are not classified as desmoplastic / nodular medulloblastomas. Such (unspecific) fibre induction may occur as desmoplastic reaction when tumours grow superficially within leptomeningeal areas.

The related variant “Medulloblastomas with extensive nodularity” show a similar biphasic pattern as desmoplastic nodular medulloblastomas. However, the reticulin-free islands with lower cellularity are enlarged and contain isomorphic neurocyte-like tumour cells with round nuclei and low mitotic activity embedded in a neuropil-like matrix. Immunohistochemically, these cells express markers of advanced neurocytic differentiation such as NeuN. These areas of advanced differentiation have to dominate the histology to qualify a case for the diagnosis of medulloblastoma with extensive nodularity. The cells of the reticulin-rich areas resemble their counterparts in desmoplastic/nodular medulloblastomas.

Medulloblastomas with extensive nodularity usually only occur in very young children, and are associated with a good prognosis.(Rutkowski, Bode et al. 2005; Garre, Cama et al. 2009) Patients with medulloblastoma with extensive nodularity are not eligible for treatment within this protocol.

4.2.3. Classic Medulloblastoma

Classic medulloblastomas are composed of densely packed small undifferentiated cells characterized by round to oval or ‘carrot-like’ hyperchromatic nuclei and scant cytoplasm. The mitotic activity is significantly elevated but may vary between different tumour areas. Some cases show the formation of neuroblastic (Homer-Wright) rosettes. Classic medulloblastoma lack areas with the typical biphasic reticulin / island pattern of desmoplastic / nodular medulloblastomas or medulloblastomas with extensive nodularity. Some cases show immunohistochemical evidence of early neuronal differentiation such as synaptophysin expression. In addition, cells with astrocytic, myogenic or melanocytic differentiation may be found. However, advanced astrocytic or ependymal differentiation would be very unusual for classic medulloblastomas. Marked cytological anaplasia such as high nuclear pleomorphism may occur focally. However, diffuse and severe cytological anaplasia is not compatible with the diagnosis of classic medulloblastoma but qualifies for the diagnosis of anaplastic medulloblastoma. Similarly, groups of tumour cells may show the typical cytological features of large cell medulloblastomas. However if the histological picture is dominated by these cells, the diagnosis of a large cell medulloblastoma has to be made.

4.3. Molecular Markers and the Concept of Biological Risk Profile

Medulloblastoma (MB) is a heterogeneous disease at the molecular level and no diagnostic cytogenetic or molecular abnormality has been identified. Nonetheless, a series of major non-random molecular genetic abnormalities have been identified in the human disease, which (i) have informed our understanding of the molecular mechanisms underlying its pathogenesis and (ii) offer significant potential for improved treatment stratification and/or the identification of novel therapeutic targets. In particular, a number of consistent chromosomal abnormalities have been identified, as have critical oncogenes and tumour suppressor genes, and an involvement for specific molecular pathways.

4.3.1. Aberrations in Developmental Cell-signalling Pathways

The most significant insights into biological pathways involved in medulloblastoma pathogenesis have come from the investigation of rare familial syndromes that predispose to medulloblastoma development. Although only a small proportion of medulloblastomas (<5%) are associated with an inherited familial predisposition, many of the genetic defects that cause these syndromes have subsequently been shown to play a more extensive role in sporadic medulloblastoma development. Mutations of *TP53*, *APC* and *PTCH* in sporadic medulloblastoma have been uncovered through their causative roles in Li-Fraumeni, Turcot and NBCC syndromes, respectively. (Malkin, Li et al. 1990; Evans, Farndon et al. 1991; Cogen, Daneshvar et al. 1992; Hamilton, Liu et al. 1995; Hahn, Wicking et al. 1996; Johnson, Rothman et al. 1996; Kleihues, Schauble et al. 1997; Paraf, Jothy et al. 1997; Pietsch, Waha et al. 1997; Raffel, Jenkins et al. 1997; Vorechovsky, Tingby et al. 1997; Wolter, Reifenberger et al. 1997; Huang, Mahler-Araujo et al. 2000; Koch, Waha et al. 2001) The *APC* and *PTCH* genes represent key components of the Wnt/Wingless (Wnt/Wg) and Sonic hedgehog (SHH) developmental cell signalling pathways (Figure 1), respectively, and subsequent studies have identified extensive roles for both signalling cascades in the disease. Both pathways are essential in normal neural and cerebellar development and become aberrantly activated in subsets of medulloblastomas. (Gilbertson and Ellison 2008)

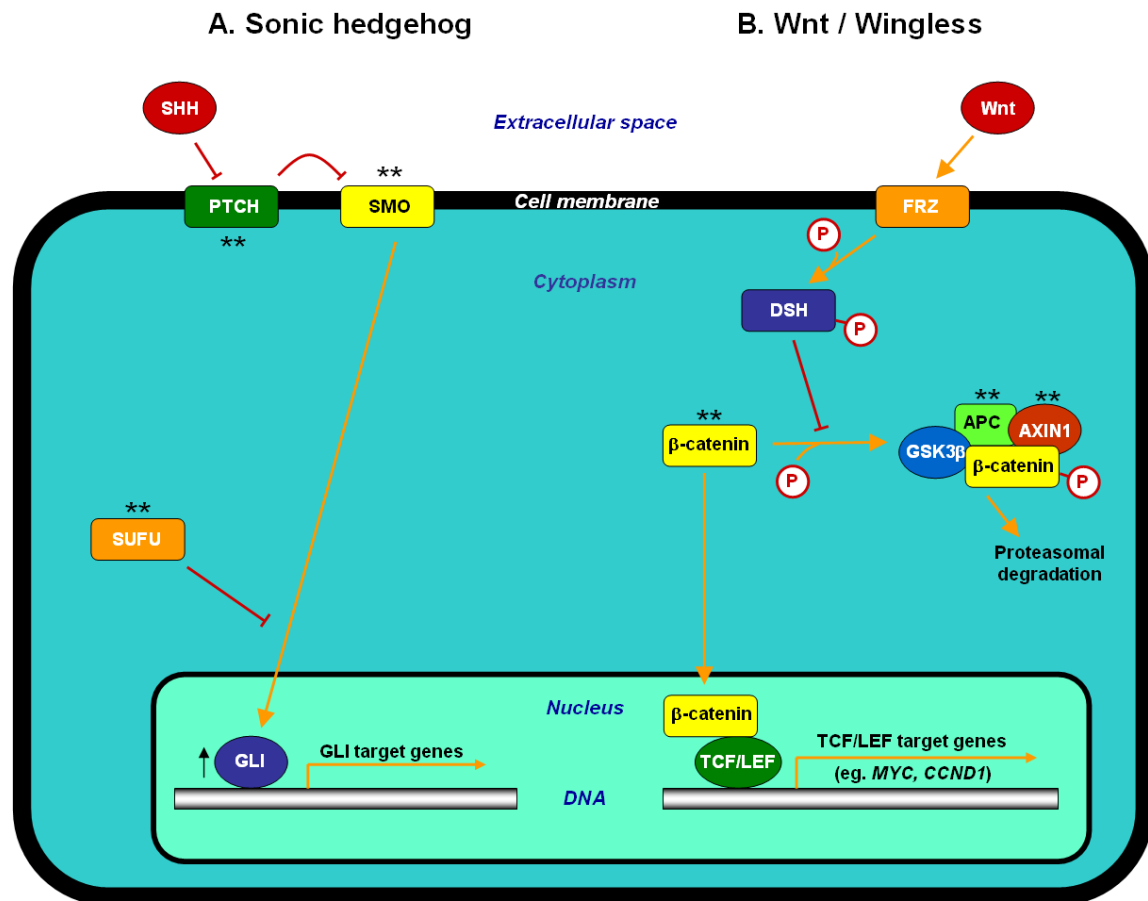


Figure 1. The Sonic hedgehog (A) and Wnt/Wingless (B) cell signalling pathways. Yellow pointed arrows = stimulatory effect, red blunted arrows = inhibitory effect. P, phosphorylation. See text for detailed descriptions of each signalling cascade. Mutations in pathway components marked ** have been reported in medulloblastoma, associated with aberrant pathway activation. SHH, sonic hedgehog; PTCH, patched; SMO, smoothened; SUFU, suppressor of fused; GLI, GLI family of transcription factors; FRZ, frizzled; DSH, dishevelled; GSK3 β , glycogen synthase kinase 3 beta; APC, adenomatous polyposis coli protein; AXIN1, axis inhibition protein 1; TCF/LEF, TCF/LEF transcriptional complex; CCND1, cyclin D1. From Gajjar A and Clifford SC (2010). Embryonal brain tumours. In: Clinical Paediatric Oncology and Hematology. (Gilbertson, Estlin & Wynn, Eds.). Blackwell Publishing. pp34-51

Wingless (WNT) Signalling

APC is an essential component of the canonical Wnt/Wg signalling pathway, which is necessary for normal development, including roles in the determination of neural cell fates.(Taipale and Beachy 2001; Marino 2005) Briefly, the canonical Wnt/Wg pathway regulates intra-cellular localisation of β -catenin, a key transcriptional activator (Figure 1). Stimulation of Wnt/Wg signalling leads β -catenin to translocate to the nucleus, transactivate TCF/LEF transcriptional complexes, and up-regulate specific downstream pro-tumourigenic target genes including cyclin D1 and MYC.(Morin 1999; Clevers 2000; Taipale and Beachy 2001)

Aberrant Wnt/Wg activation characterises a significant sub-set of medulloblastomas. At the mutational level, approximately 10% of cases contain oncogenic mutations in the *CTNNB1* gene (which encodes β -catenin). Mutations of alternative pathway components (*APC*, *AXIN1* and *AXIN2*) each affect a further ~2.5% of cases, but *GSK3- β* mutations have not been detected where investigated.(Zurawel, Chiappa et al. 1998; Eberhart, Tihan et al. 2000; Huang, Mahler-Araujo et al. 2000; Dahmen, Koch et al. 2001; Koch, Waha et al. 2001; Baeza, Masuoka et al. 2003) *CTNNB1*

mutations target the GSK-3 β phosphorylation domain of its protein, resulting in its constitutive stabilization, nuclear accumulation, and contribution to tumourigenesis. Nuclear β -catenin protein stabilisation thus provides a comprehensive indication of Wnt/Wg pathway activation in medulloblastoma, and affects 18-30% of cases overall.(Eberhart, Tihan et al. 2000; Ellison, Onilude et al. 2005) A strong relationship is observed between *CTNNB1* mutations and nuclear β -catenin accumulation (by immunohistochemistry) in clinical samples, with *CTNNB1* mutations detected in 60-70% of β -catenin nucleopositive cases.(Eberhart, Tihan et al. 2000; Koch, Waha et al. 2001; Ellison, Onilude et al. 2005; Clifford, Lusher et al. 2006; Fattet, Haberler et al. 2009) While rarer *AXIN2* mutations have been associated with β -catenin stabilization(Koch, Hrychyk et al. 2007), any relationship between the mis-sense *APC* or *AXIN1* mutations that have been reported, and nuclear β -catenin accumulation, is less clear. Mutations of the *APC* mutation cluster region have not been observed in β -catenin nucleopositive sporadic medulloblastoma cases which lack *CTNNB1* mutations.(Ellison, Onilude et al. 2005) Mutations of further pathway components and/or alternative mechanisms of gene inactivation are thus likely to underlie β -catenin stabilisation in cases which display protein accumulation in the absence of a detectable *CTNNB1* mutation.

Wnt/Wg pathway activation defines a unique molecular sub-group of medulloblastomas, which display distinct gene expression profiles, patterns of genomic abnormalities and clinical outcome. Wnt/Wg medulloblastomas exhibit a characteristic gene expression signature, which is mutually exclusive from expression signatures displayed by the SHH sub-group of tumours (below) and other medulloblastoma expression sub-groups.(Thompson, Fuller et al. 2006). Genomically, Wnt/Wg-active medulloblastomas appear to be exclusively associated with the loss of an entire copy of chromosome 6 in the majority of cases, while this sub-group is independent of chromosome 17 aberrations, the most common chromosomal alterations detected in medulloblastoma.(Clifford, Lusher et al. 2006; Thompson, Fuller et al. 2006) Moreover, the Wnt/Wg-active medulloblastoma sub-group displays an idiosyncratic clinical behaviour, and β -catenin status has been shown to be an independent marker of favourable clinical outcome (greater than 90% overall survival) across independent clinical trials-based biological studies.(Ellison, Onilude et al. 2005; Gajjar, Chintagumpala et al. 2006) Although Wnt/Wg-positive medulloblastomas tend to display classic or large-cell/anaplastic histology and arise in older children, they cannot be readily distinguished from other tumours that display equivalent clinical and histological features, and require identification at the molecular level.(Ellison, Onilude et al. 2005; Gajjar, Chintagumpala et al. 2006; Thompson, Fuller et al. 2006)

Sonic Hedgehog (SHH) Signalling

The SHH pathway plays a key role in normal cerebellar development, where the SHH ligand is secreted by Purkinje neurons and promotes mitogenesis in external granule layer (EGL) progenitor cells during early development.(Taipale and Beachy 2001; Wechsler-Reya and Scott 2001) Response to the SHH signal is controlled through two trans-membrane proteins, PTCH and its associated protein 'smoothed' (SMO) (Figure 1). In the absence of SHH ligand, PTCH suppresses SMO activity. Upon SHH stimulation, this inhibition is removed, leading to a SMO-induced transcriptional response, mediated by the activation and repression of target genes by the 'GLI' family of zinc-finger containing transcription factors (GLI-1, GLI-2, GLI-3). In human cells, suppressor of fused (SUFU), co-operates with BTRCP, to inhibit GLI-1-mediated transcription, however the mechanisms by which SMO activation is coupled to nuclear proteins in the mammalian SHH pathway are otherwise not well understood.(Stone, Murone et al. 1999; Taipale and Beachy 2001; Wechsler-Reya and Scott 2001)

Aberrant SHH pathway activation by genetic mutation occurs in at least 15% of medulloblastomas, based on estimates from genetic data, and arises through mutations affecting multiple alternative

pathway components. In addition to *PTCH1* mutations (~10% of cases), *SMO* activating mutations have been reported in ~5% of cases. *SUFU* mutations have also been described, although their incidence and involvement are likely to be lower than initially reported (0-10% of cases). (Pietsch, Waha et al. 1997; Raffel, Jenkins et al. 1997; Vorechovsky, Tingby et al. 1997; Wolter, Reifenberger et al. 1997; Reifenberger, Wolter et al. 1998; Taylor, Liu et al. 2002; Koch, Waha et al. 2004) Notably, only specific SHH pathway components are affected by mutations, and mutations in other pathway components (e.g. *SHH*, *BTRCP* and other genes) have not been found where investigated. (Zurawel, Allen et al. 2000; Taylor, Liu et al. 2002; Wolter, Scharwachter et al. 2003) Genetic disruption of the SHH pathway results in inappropriate constitutive activation of the signalling cascade, and downstream mitogenic effects most likely mediated through over-expression of GLI proteins, GLI-dependent target genes (including *PTCH* itself) and downstream response mediators (e.g. *BMI-1*, *MYCN*, cyclin D1). (Taipale and Beachy 2001; Leung, Lingbeek et al. 2004) A recent study using unsupervised hierarchical cluster analysis of expression microarray data from a series of medulloblastomas identified a sub-group of cases associated with a distinct SHH-related gene expression profile, characterized by the over-expression of pathway members and targets. This expression cluster was consistent with SHH pathway activation in ~25% of medulloblastomas, and was highly associated with genetic mutations in SHH pathway components. (Thompson, Fuller et al. 2006) The role of SHH in medulloblastoma tumorigenesis is further supported by observations that mice in which the SHH pathway is aberrantly activated develop cerebellar tumours that mimic human medulloblastoma at both the histological and gene expression level. (e.g. *Ptch*^{+/-} or *Ptch*^{+/-}, *Tp53*^{-/-} mice; *Sufu*^{+/-}, *Tp53*^{-/-} mice; *ND2:SmoA1* mice) (Goodrich, Milenkovic et al. 1997; Wetmore, Eberhart et al. 2000; Hallahan, Pritchard et al. 2004; Lee, Kawagoe et al. 2007)

Aberrant SHH pathway activation appears to be associated with development of the nodular/desmoplastic medulloblastoma histological sub-type. The majority of medulloblastomas in NBCCS correspond to the nodular/desmoplastic variant. (Schofield, West et al. 1995) Similarly, *PTCH* mutations, deletion of chromosome 9q elements, and SHH-associated gene expression profiles occur preferentially and in a significant proportion (30-40%) of sporadic nodular / desmoplastic medulloblastomas. (Schofield, West et al. 1995; Pietsch, Waha et al. 1997; Nicholson, Ross et al. 1999) *SUFU* mutations have also been reported to segregate preferentially with nodular / desmoplastic tumours. (Taylor, Liu et al. 2002). However, the relationship between SHH defects and nodular/desmoplastic medulloblastomas is not absolute and pathway activation is also observed in classic and large cell/anaplastic tumours. (Wolter, Reifenberger et al. 1997; Thompson, Fuller et al. 2006) Ongoing studies of tumours isolated from patients treated in the PNET 5 MB - LR and PNET 5 MB - SR prospective clinical studies will aim to better define the relationship between histological subtype and molecular alterations.

Additional Aberrant Cell Signals in Medulloblastoma

Mutations in *PIK3CA*, a member of the family of phosphatidylinositol 3'-kinase (PI3K) signalling pathway, have been reported in ~5% of medulloblastomas (Broderick, Di et al. 2004), and suggest that PI3K, its pathway components and effectors may play a role in medulloblastoma tumorigenesis. Other specific genetic targets have not been reported in the human disease. Abrogation of additional signalling networks, including those regulated through the neurotrophin, PDGFR and ERBB2 receptor families, have been implicated by gene expression studies in human primary medulloblastomas, however a genetic basis for their disruption has not yet been uncovered. (Grotzer, Janss et al. 2000; MacDonald, Brown et al. 2001; Gilbertson and Clifford 2003; Hernan, Fasheh et al. 2003; Gilbertson, Langdon et al. 2006)

4.3.2. Chromosomal Abnormalities and Gene Amplifications

Abnormalities of chromosome 17 are the most common chromosomal aberrations in MB: isochromosome 17q (iso(17q)) is observed in approximately 40% of cases.(Bigner, Mark et al. 1988; Biegel, Rorke et al. 1989; Reardon, Michalkiewicz et al. 1997; Nicholson, Ross et al. 1999; Lamont, McManamy et al. 2004). Isolated loss of 17p is observed in an additional ~20% of cases.(Reardon, Michalkiewicz et al. 1997; Nicholson, Ross et al. 1999; Gilbertson, Wickramasinghe et al. 2001; Lamont, McManamy et al. 2004). Gain of chromosome 7 also occurs commonly and affects ~40% of cases. Extensive non-random losses of chromosomes 8, 9, 10q, 11 and 16q are each observed in ~30% of cases.(Reardon, Michalkiewicz et al. 1997; Avet-Loiseau, Venuat et al. 1999; Nicholson, Ross et al. 1999) The specific genetic targets of most of these common chromosomal abnormalities remain to be identified. Mutations in *PTCH* (at 9q22.3), *SUFU* (10q24.3) and *TP53* (17p13.1) have each been described in ~10% of tumours.(Cogen, Daneshvar et al. 1992; Pietsch, Waha et al. 1997; Raffel, Jenkins et al. 1997; Taylor, Liu et al. 2002) Since mutation of these genes occurs far less frequently than loss of the corresponding chromosomal locus, then these genes might be haplo-insufficient, inactivated by alternative mechanisms e.g., methylation, or located close to alternative tumour suppressor genes (TSGs).

Double minute chromosomes have been observed in cytogenetic studies of MB, and gene amplification is a feature of a small subset of cases.(Bigner, Mark et al. 1988) *MYCN* (at 2p24) and *MYC* (at 8q24) genes are the most commonly amplified loci, which have been verified across multiple studies. Each occurs in 5 to 15% of medulloblastoma cases, and has been associated with the large-cell/anaplastic medulloblastoma variant and an adverse clinical prognosis.(Aldosari, Bigner et al. 2002; Eberhart, Kratz et al. 2002; Lamont, McManamy et al. 2004; Pfister, Remke et al. 2009) Additional gene amplifications affecting other loci are rare in MB, but have been documented in either single studies or in isolated cases. Genes affected include *OTX2*, *NOTCH2*, *hTERT*, *MYCL1*, *PDGFRA*, *KIT*, *MYB*, *PPM1D* and *CDK6*.(Fan, Wang et al. 2003; Fan, Mikolaenko et al. 2004; Boon, Eberhart et al. 2005; Di, Liao et al. 2005). The incidence and biological and clinical significance of these events require clarification.

4.3.3. The Relationships between Biological Factors, Histology and Prognosis

Only clinical variables are currently used in the therapeutic stratification of patients with medulloblastoma. Molecular markers and histopathological subclassification do not currently influence therapeutic strategy. However, variability in outcome exists within current clinical risk groups. The accurate identification of disease risk remains a major goal, as a more robust stratification of disease-risk would facilitate the targeted use of adjuvant therapies; intensive regimens for aggressive tumours and reduced long-term side effects for patients with more readily curable tumours.

Assessment of the prognostic significance of molecular defects in medulloblastoma has frequently been limited by the retrospective analysis of individual markers in small, heterogeneously treated cohorts. Nonetheless, a range of molecular markers with prognostic potential have now been identified in trial-based studies (Table 1). Nuclear immunoreactivity of β -catenin, indicative of Wnt/Wg pathway activation, has been consistently associated with a favourable outcome.(Ellison, Onilude et al. 2005; Gajjar, Chintagumpala et al. 2006) Amplification of the *MYC* oncogene has been associated with a poor prognosis in multiple studies(Lamont, McManamy et al. 2004; Rutkowski, von Bueren et al. 2007), while defects of chromosome 17(Lamont, McManamy et al. 2004), and expression of the ERBB2 receptor tyrosine kinase(Gajjar, Hernan et al. 2004), have each been associated with an adverse outcome in isolated studies. Other defects, such as expression of the *MYC* oncogene or the *TRKC* neurotrophin receptor, and amplification of the *MYCN* oncogene, have shown prognostic significance in some studies, but have not had demonstrable value in others.(Gajjar, Hernan et al. 2004; Lamont, McManamy et al. 2004; Rutkowski, von Bueren et al. 2007) Initial studies in

individual cohorts indicate activation of the SHH pathway or associated defects (i.e. 9q loss) do not appear to have prognostic significance.(Lamont, McManamy et al. 2004; Gajjar, Chintagumpala et al. 2006)

	Disease feature	Method of detection	Prevalence	Survival (risk-group vs. others)	Statistical analysis	Clinical trial	Cohort age range	Reference
Favourable risk	Wnt/Wg pathway activation (β-catenin nuclear stabilization)	IHC	27/109 (25%)	92% vs 65% (5 year OS)	$p=0.006^m$	PNET3	3 - 16.8 yrs	(Ellison, Onilude et al. 2005)
			10/69 (14%)	100% vs 68% (5 year EFS)	$p=0.03^u$	SJMB96	3.1 – 20.2 yrs	(Gajjar, Chintagumpala et al. 2006)
	Desmoplasia (in infants ≤3yrs)	Histopathological assessment	20/43 (47%)	85% vs 34% (7 year PFS)	$p<0.001^m$	HIT-SKK'92	<3 yrs	(Rutkowski, Bode et al. 2005)
			17/28 (61%)	53% vs 17% (5 year OS)	NR	CNS9204	<3 yrs	(McManamy, Pears et al. 2007)
Adverse risk	MYC gene amplification	FISH	5/84 (6%)	All dead at 5 yrs**	$p<0.001^m$	PNET3	>3 yrs	(Lamont, McManamy et al. 2004)
		qPCR	5/111 (4.5%)	40% vs 66% (7 year OS)	NS	HIT '91	3 - 18 yrs	(Rutkowski, von Bueren et al. 2007)
	Large-cell / anaplastic Histology	Histopathological assessment	23/116 (20%)	57% vs ~80% (5 year EFS)	$p=0.04^u$	SJMB96	3.1 – 20.2 yrs	(Gajjar, Chintagumpala et al. 2006)
			52/315 (17%)	~55% vs ~75% (5 year OS)	$p=0.024^m$	PNET3	2.7 – 16.4 yrs	(McManamy, Pears et al. 2007)

Table 1. Molecular and histopathological markers of disease-risk in medulloblastoma, showing disease features which display consistent associations with prognosis in ≥2 clinical trials-based studies. IHC, immunohistochemistry; FISH, fluorescence in situ hybridization; qPCR, quantitative polymerase chain reaction; OS, overall survival; EFS, event-free survival; PFS, progression-free survival; ^mmultivariate analysis; ^uunivariate analysis; NR, not reported; NS, not significant; COG, Children's Oncology Group. **Cases showing high-level gene amplification in >25% of tumour nuclei. From Gajjar A and Clifford SC (2010). Embryonal brain tumours. In: Clinical Paediatric Oncology and Hematology. (Gilbertson, Estlin & Wynn, Eds.). Blackwell Publishing. pp34-51

Clinical studies in patients from the SIOP-UKCCSG PNET3 clinical trial which first established the prognostic significance of the WNT subgroup were undertaken on patients aged <16.0 years at diagnosis (Ellison et al, 2005; Ellison et al 2011) and a recent large retrospective study suggested WNT subgroup patients have a bimodal age distribution and that patients greater than 18 years at diagnosis do not share the same favourable outlook (Korshunov et al, 2010). These data are supported by initial findings from the HIT-SIOP PNET4 trial, in which WNT subgroup patients aged <16.0 years at diagnosis had significantly better outcomes than those aged ≥16.0 years (Events in 3/42 vs. 3/10 patients respectively, $p=0.01$, log-rank test)

Among the histopathological variants of medulloblastoma, an aggressive biological behaviour has been established for the overlapping large-cell and anaplastic variants, which have been associated with a poor outcome in several clinical trial cohorts.(Brown, Kepner et al. 2000; Eberhart, Kepner et al. 2002; McManamy, Lamont et al. 2003; Gajjar, Chintagumpala et al. 2006; McManamy, Pears et al. 2007) In contrast, the MBEN, which is a tumour of infancy, is associated with a good prognosis(Giangaspero, Perilongo et al. 1999), and the wider desmoplastic variant (including MBENs) has consistently been associated with a more favourable outcome in trials-based studies in

infants.(McManamy, Pears et al. 2007; Rutkowski, von Hoff et al. 2010) The desmoplastic variant has been reported to confer a more favourable(McManamy, Pears et al. 2007) or no difference(Gajjar, Chintagumpala et al. 2006) in prognosis, in trials studies of older children.

While there is clearly overlap between high-risk clinical, pathological and molecular variables, schema for stratifying medulloblastomas on the basis of combinations of these variables, which are predictive of high-, standard- and low-risk cases, are likely to allow a more targeted approach to the use of adjuvant therapy in the future. Such stratification systems now require development and prospective validation in large group-wide clinical trials, to establish any applications in routine diagnostic practice. Moreover, the identification of additional prognostic disease features, through ongoing biological studies, is likely to facilitate the further refinement of the precision of disease stratification systems.

4.3.4. Rationale for Biological Testing

In this study, the best validated biological prognostic criteria, based on current understanding, will be utilised alongside clinical and histopathological indices, in order to define the low-risk biological profile among standard-risk medulloblastomas, as well as to exclude cases with high-risk biological profiles from the standard-risk and low-risk medulloblastoma groups. In addition, biological studies attached to PNET 5 MB - LR and PNET 5 MB - SR will investigate the molecular pathology of MB, its prognostic significance and clinical associations, encompassing investigations of established biological features of medulloblastoma and the characterisation of novel disease features. The principal aim of these investigations will be (i) to improve our understanding of the molecular basis of MB, (ii) the identification of independent and informative prognostic markers for a more efficient patient stratification in future SIOP clinical trials, and (iii) the identification of targets for therapeutic exploitation. Studies in PNET 5 MB - LR will focus on the detailed biological characterisation of the low-risk biological sub-group.

4.4. Risk-adapted Therapy

4.4.1. Neurosurgery

The importance of the role of surgical resection in patients with medulloblastoma is widely recognised. Neurosurgeons, aided by modern technological adjuncts, make considerable efforts to achieve complete resection or near complete resection. Such surgery can bring an increased risk of neurological deficits of a temporary or even a permanent nature. Post-operative complications and neurological deficits resulting from surgery not only impact upon quality of survival, but can also contribute to delay in commencing adjuvant therapy. This may be compounded when neurosurgery takes place in one department and the adjuvant therapies are delivered in other departments, or even different institutions. There are, however, still few data on the toxicity of surgery, especially as patient related factors as the local anatomy, the behaviour of the tumour, and the presence of hydrocephalus and other surgical factors (such as surgical intent, technique, and the use of high-technology instrumentation) are often overlapping. A commonly encountered clinical problem after surgery is the posterior fossa syndrome. Its frequency is described to be up to 29% of medulloblastoma patients.(Robertson, Muraszko et al. 2006; Gudrunardottir, Sehested et al. 2011) There is growing evidence that presence and severity of posterior fossa syndrome after surgery is associated to the neurological and neurocognitive long term outcome.(Palmer, Hassall et al. 2010; Wells, Khademian et al. 2010) Therefore, this problem will be assessed within this study in addition to the standard neurosurgical questionnaire. .

4.4.2. Radiotherapy in Standard-risk Medulloblastoma

Radiotherapy remains a key modality in the curative treatment approach of patients with medulloblastoma (MB). Until recently, the standard therapeutic approach for standard-risk medulloblastoma has consisted of complete or near total surgical resection followed by post-operative craniospinal radiation therapy (CSRT). Given the propensity of MB to disseminate via the CSF current standard practice in non-infants with confirmed MB requires adjuvant craniospinal irradiation with a boost to the posterior fossa/ primary tumour bed as part the curative treatment strategy. In Europe and the US the conventional doses of radiotherapy for standard risk patients in the past were 35-36 Gy to the craniospinal axis followed by a whole posterior fossa boost up to a total dose of 54-55.8 Gy. Recent studies have demonstrated that survival rates in patients who receive reduced-dose CSRT (in the range of 23.4 to 25 Gy) in combination with chemotherapy, are very high and often better than those for patients on previous studies who received a higher CSRT dose without chemotherapy. Between 1990 and 1994 the CCG 9892 pilot study recruited patients to receive 'reduced dose' craniospinal irradiation (23.4 Gy) with concurrent vincristine chemotherapy followed by adjuvant lomustine, vincristine and cisplatin chemotherapy. PFS rates of 86% at 3 years and 79% at 5 years were promising and hence this trial formed the basis for a Phase III trial (CCG A9961). This randomised trial included 379 standard risk MB patients treated with 23.4 Gy of craniospinal irradiation followed by a whole posterior fossa boost to 55.8 Gy.(Packer, Gajjar et al. 2006) All patients received concurrent vincristine chemotherapy and were then randomised to receive one of two adjuvant chemotherapy regimens: lomustine, vincristine and cisplatin or cyclophosphamide, vincristine and cisplatin. EFS at 5 years was 81% and OS 86% with no difference between the two chemotherapy arms. This was the first prospective, randomised study to demonstrate an excellent clinical outcome with a combined modality treatment and 'reduced-dose' craniospinal irradiation, and based on these data this radiotherapy schedule is now accepted as current 'standard' treatment. These results have been reproduced in Europe. In the M-SFOP 93 study, 136 patients with standard-risk medulloblastoma were treated with CSRT to 25 Gy and posterior fossa radiation therapy (PFRT) to 55 Gy followed by chemotherapy.(Oyharcabal-Bourden, Kalifa et al. 2005) Five-year recurrence-free survival was $64.8\% \pm 8.1\%$ and overall survival was $73.8\% \pm 7.6\%$. The HIT-SIOP PNET 4 was designed to compare HFRT 1.0 Gy x 2 to 36, 60, 68 and 36 Gy respectively to the head, posterior fossa, tumour, and spine and conventional RT to 23.4, 54 and 23.4 Gy in patients with standard-risk MB. All patients also received adjuvant chemotherapy with cisplatin, CCNU and Vincristine. At a medium follow-up of 58 months, the overall 3 year EFS rate for 340 patients was $0.83 \pm 0.02\%$. Based on these data at present the current recommended dose of RT for standard risk patients is 23.4 Gy to the CSA and 30.6 Gy to the posterior fossa.

Late sequelae of RT

It is well established that long-term survivors treated with radiotherapy for MB are at risk to suffer from significant late sequelae, including cognitive and endocrine deficits as well as hearing loss. Although some of these late effects are related to the tumour itself, tumour associated hydrocephalus and the complications of both surgery and chemotherapy, RT is an important contributing factor in the pathogenesis of these late sequelae. Of most concern are the well-recognised neuropsychological sequelae of children receiving cranial irradiation. Several studies have demonstrated marked losses of IQ of up to 30 points or more which are most predominant in young children, particularly those less than seven or eight years of age.(Riva, Pantaleoni et al. 1989; Hoppe-Hirsch, Renier et al. 1990; Lannering, Marky et al. 1990) In a retrospective study, Grill et al showed that there is a significant correlation between the full-scale IQ score (FSIQ) and the CSRT dose, with mean FSIQ scores at 84.5, 76.9 and 63.7 for 0 Gy (i.e. posterior fossa radiotherapy alone), 25 Gy and 35 Gy of CSRT respectively.(Grill, Renaux et al. 1999) An analysis of the neuropsychological sequelae reported in the literature(Miralbell, Lomax et al. 1997) has been used to construct a dose response curve, which relates to the probability of neuropsychological sequelae to the brain RT dose. This pooling of data

suggests a dose-response effect with greater morbidity seen with increasing cranial RT dose. Only one study has attempted to examine the dose effect in the context of a randomised control trial. Mulhern examined the neuropsychological functioning of survivors of children with medulloblastoma entered in the POG 8631/CCG 923 study.(Mulhern, Kepner et al. 1998) This showed that children treated with 23.4 Gy CSRT experience less neuropsychological toxicity than those treated with 36 Gy CSRT. However, the number of patients studied was small, the individual patient IQ changes varied considerably and the results of the cross-sectional analysis were not confirmed on a longitudinal basis. In the recent CCG 9892 study, the neuropsychological effect of 23.4 Gy CSRT was reported to be a decline of 4.3 Full Scale IQ points per year.(Ris, Packer et al. 2001) The median interval between radiotherapy and the patient's most recent evaluation in that study was 2.5 years. The declines in IQ were reported to be relatively more marked in females, children with higher baseline scores and children aged less than 7 years. The authors considered that their findings were suggestive of some degree of intellectual preservation compared to the effect of conventionally dosed radiotherapy but also stated that the estimated decline in IQ of 20.8 points in their younger group did not clearly support an advantage to these patients for the reduced radiotherapy regimen.

In addition, it is well documented that the majority of survivors suffer growth and endocrine dysfunction predominately due to irradiation of the pituitary gland and hypothalamic axis in combination with the effects of whole spine radiotherapy.(Pasqualini, Diez et al. 1987; Schmiegelow, Lassen et al. 1999; Adan, Sainte-Rose et al. 2000) Although exact dose effect relationships are not known, there is evidence to suggest that dose reduction might decrease the risk for such hypothalamic-pituitary dysfunctions as well as for decreasing the risk for growth retardation of the spine. Moreover, radiotherapy of the spinal axis may contribute to a risk of gonadal dysfunction in young girls caused by scatter irradiation.

Rationale for lowering the craniospinal dose in the LR arm of the PNET 5 MB study.

The current 5y EFS of standard risk medulloblastoma in children above 3-5 years is around 80% with the current treatment using 23.4 Gy craniospinal radiotherapy, local boost up to 54 Gy and standard chemotherapy regimen (Lannering et al. J Clin Oncol 2012, Packer et al. J Clin Oncol 2006). Among this standard risk medulloblastoma group of patients, the very good prognosis of the WNT medulloblastoma subgroup was already reported (Ellison et al., J Clin Oncol 2005, Thompson J Clin Oncol 2006) and has been confirmed in several recently published studies and reviews (Kool, Acta Neuropathol. 2012, Robinson et al., Nature 2012, Taylor et al. Acta Neuropathol. 2012). This is also prospectively confirmed in the HIT-SIOP-E PNET 4 study (Clifford et al. submitted), for children younger than 16 years old. However, the good prognosis of the WNT medulloblastoma subgroup does not seem to be confirmed in adults (Korshunov J Clin Oncol 2010). The good prognosis of WNT medulloblastoma in children younger than 16 years allows the initiation of a prospective study evaluating reduced intensity treatments in order to reduce late effects in this specific subgroup.

Previous studies of the toxicity of craniospinal dose on neurocognitive functions have shown a clear dose-effect relationship. This has been the rationale to decrease the craniospinal dose from 36 to 23.4 Gy in standard risk medulloblastoma, establishing better cognitive outcome after craniospinal dose reduction (Mulhern J Clin Oncol 2005, Grill Int J Radiat Oncol Biol Phys 1999). Further de-escalation of craniospinal dose from 23.4 to 18 Gy is also expected to allow a better cognitive function in the years following treatment.

Finally, the current methods to attest the WNT medulloblastoma subgrouping in the context of a prospective multicentric international trial have improved, which allows to increase the specificity of WNT subgrouping. Indeed, the criteria chosen by the SIOP-E PNET biology group are no longer only based on immunohistochemistry, showing stabilisation of nuclear β catenin expression. Current

criteria also incorporate molecular diagnosis either with direct β catenin mutation assessment, or indirect WNT subgrouping based on the documentation of somatic monosomy 6, as recommended in a recent international consensus (Gottardo, *Acta Neuropathol* 2013).

Thus, reduced intensity treatment using a reduced craniospinal dose in well characterised WNT medulloblastoma subgroup is highly justified within the context of the multicentric international prospective clinical phase II PNET5 LR trial. Such a craniospinal dose reduction is currently performed in the ongoing St.-Jude study (SJMB12, opened in 2013) and is planned in the next COG protocol. More experimental strategy trying to avoid any radiation on the WNT medulloblastoma patients has not been chosen by the SIOP-E medulloblastoma/PNET working group because the WNT subgrouping could be in fact a marker of radiosensitivity, and because there is no demonstration of the efficacy of salvage therapy in case of relapse after first line treatment without radiation.

Radiotherapy Technique

Given the continuing refinement of treatment techniques quality control is a major factor in ensuring that the improved survival rates are maintained, since both patient evaluation and standards of radiotherapy applied have been shown to correlate with outcome. In the HIT'91 study, patients who were fully assessable, i.e. with central histopathological review and complete staging, had higher EFS and overall survival rates.(von Hoff, Hinkes et al. 2009) The above-mentioned study by the Children's Oncology Group similarly found a higher EFS rate in patients who had central pathological review, including determination of tumour sub-type, and cytology of lumbar CSF, as well as neuro-imaging, to evaluate extent of disease and post-operative residual tumour.(Packer, Gajjar et al. 2006) In the M-SFOP 93 study described above, quality control of RT, performed a posteriori, found that relapse occurred in 67% of patients who had three or more major deviations. In the M-SFOP 98 study, quality control of RT was used prospectively to detect major protocol deviations and to correct them prior to treatment start, so as to limit the rate of relapse due to targeting deviations.(Carrie, Grill et al. 2009) Nine of the 14 deviations detected on QC were corrected before treatment, leading to a marked reduction in the number of relapses. The same approach to quality control of radiotherapy was used in the HIT-SIOP PNET 4 study and will be used in PNET 5 MB. Given the pivotal role of radiotherapy quality control of RT is of particular importance when complex treatment techniques are involved and hence the intended upfront RT quality control of the CSRT.

Reducing the risk of late sequelae associated with radiotherapy in patients with MB can be achieved by either reducing dose or target volumes. In the past the boost volume of the posterior fossa was defined as the whole posterior fossa. Increasingly more conformal approaches are employed. In conformal PF RT, as reported e.g. by Merchant et al., the radiation beam is shaped to "conform" to the profile of the primary tumour bed with a defined additional margin, thereby reducing toxicity to surrounding normal tissues (e.g. cochlea and mesotemporal lobe). Strict targeting guidelines in the study allowed for a reduction in the volume of the posterior fossa irradiated of, on average, 13%.(Merchant, Kun et al. 2008) The cumulative incidence of local failures at 5 years was 4.9%, which is similar to the rate in patients treated with irradiation to the entire posterior fossa. In addition, the same group suggested a dose response relationship for ototoxicity in patients receiving RT alone.(Hua, Bass et al. 2008) The data of 78 patients treated for posterior fossa/ suprasellar localised brain tumours with focal RT suggested a beginning high frequency hearing loss at doses over 30 Gy and intermediate to low frequency loss starting at doses over 40 Gy. High tone hearing loss was 2 % at 35 Gy and 5% at 45 Gy, with intermediate tone hearing loss 1 and 1.5% at 45 Gy respectively. In the M-SFOP 98 study, reduced-dose CSRT was not used, however the conformal approach was similarly employed to reduce the boost volume. No relapses were observed within the posterior fossa outside the boost volume. Similar findings have been reported by other groups (Wolden, Dunkel et al. 2003; Douglas, Barker et al. 2004; Carrie, Grill et al. 2009), suggesting that adequate local control can be achieved by a primary tumour boost with a target volume being less than the previously used

whole posterior fossa. The current phase 3 POG study compares in a prospective randomised fashion if the conventional whole posterior fossa boost can be reduced to a focal approach defining the planning target volume as tumour bed plus a defined 3D margin.

To maximise the outcome for patients with MB, radiotherapy should be delivered within 45-50 days in order to avoid a negative impact on event-free and overall survival.

The close relationship between EFS and a prolonged total treatment time of RT has been firstly described by del Charco *et al.* (del Charco, Bolek *et al.* 1998) This finding has been confirmed by the HIT-SIOP PNET-3 and 4 studies. (Taylor, Bailey *et al.* 2003). Hence treatment interruptions due to e.g. haematological toxicity should be avoided if patients can be appropriately supported with blood products or haematological growth factors.

The role of hyperfractionated radiation therapy (HFRT) in the treatment of MB is currently debated. The M-SFOP 98 study was designed to explore HFRT in the context of prospective quality control. Conventional dose fractionation generally uses one fraction per day, five days per week, with a daily dose fraction in the range of 1.5 to 2.0 Gy. HFRT involves giving a smaller dose per fraction, with fractions administered at least twice a day, usually 6 to 8 hours apart. The total radiotherapy dose is increased, but in smaller doses, and the therapeutic ratio is improved, either by enhancing the anti-tumour effect, with no increase in late effects, or by maintaining the anti-tumour effect and reducing late effects. In the M-SFOP 98 study, both CSRT (36 Gy) and conformal PFRT (32 Gy) were administered in fractions of 1 Gy, twice per day, with good results in terms of EFS and cognitive outcomes, although it is not possible to determine whether the latter are related to hyperfractionation, reduced boost volume through the conformal approach, or the absence of adjuvant chemotherapy. (Carrie, Grill *et al.* 2009) At present, the available data from HIT-SIOP PNET 4 do not suggest any overall or event-free survival advantage of the hyperfractionated arm. (Lannering, Rutkowski *et al.* 2012) Hence the basis for the current study will be conventional fractionated CSRT as described above.

Nowadays, innovative treatment techniques are increasingly explored to lower the burden of late toxicity. Proton beam therapy seems to be particularly of interest. Early evaluation report promising results of early and late toxicity and comparative planning studies demonstrate lower dose to normal brain and inner ear for posterior fossa irradiation when compared to photon modalities. However, prospective data, quality of life analysis and long term evaluation are needed to prove clinical superiority. (Kirsch and Tarbell 2004; Yuh, Loreda *et al.* 2004; Bjork-Eriksson and Glimelius 2005; Lee, Bilton *et al.* 2005; Cochran, Yock *et al.* 2008; MacDonald, Safai *et al.* 2008)

4.4.3. Chemotherapy in Standard-risk Medulloblastoma

Medulloblastoma is clearly a chemosensitive tumour, as demonstrated in numerous phase-II studies for relapse (Allen, Walker *et al.* 1987; Gaynon, Ettinger *et al.* 1990; Lefkowitz, Packer *et al.* 1990; Gentet, Doz *et al.* 1994; Ashley, Meier *et al.* 1996) or in the initial treatment of metastatic disease. (Kovnar, Kellie *et al.* 1990; Strauss, Killmond *et al.* 1991)

Over the last 25 years, a number of multicentre studies have addressed the role of adjuvant chemotherapy in order to improve survival in medulloblastoma and, more recently, to facilitate a reduction in the dose of CSRT. The North American CCG-942 (1976-1981) study compared RT alone (craniospinal dose 35-40 Gy) with RT followed by chemotherapy (vincristine, CCNU, and prednisolone). Overall, for the 223 children entered in the study, 5-year progression free survival (PFS) was 55%. (Evans, Jenkin *et al.* 1990) Although there was no statistical difference in survival

between the two treatment groups, in the group of patients with high-risk disease, those children randomised to receive post-RT chemotherapy had a PFS of 46% and an overall survival (OS) of 57% as compared to those treated with RT alone who had a PFS of zero and OS of 19%.

In the SIOP 1 study (1975-1980), which was similar in design, 286 patients were randomised to RT alone (CRST dose 30-35 Gy) or RT plus chemotherapy with CCNU and vincristine. As with the CCG 942 study, there was no significant difference in survival between the two groups, however a benefit for chemotherapy in terms of improved survival was noted for a subgroup of patients who had metastatic disease, subtotal resection, brain stem invasion and Chang Stage T3 and T4 disease. (Tait, Thornton-Jones et al. 1990) In summary, both these first-generation randomised studies, as well as non-randomised studies (Packer, Sutton et al. 1991; Bouffet, Gentet et al. 1994; Gentet, Bouffet et al. 1995; Pezzotta, Cordero di Montezemolo et al. 1996), suggested a benefit for chemotherapy given after radiotherapy in patients with high-risk features.

The next generation of studies focused on investigation of the timing of chemotherapy, particularly the use of pre-RT chemotherapy or so called ‘sandwich chemotherapy’. In the SIOP 2 study described above, no benefit was seen for sandwich chemotherapy prior to 35 Gy CRST, and a decrease in survival was seen for patients treated with chemotherapy before reduced dose CRST of 25 Gy. (Bailey, Gnekow et al. 1995)

In the SIOP PNET 3 study (1992-2000), patients with standard-risk medulloblastoma were randomised to receive immediate radiotherapy alone or ‘sandwich chemotherapy’ consisting of a twelve-week regimen of four pulses of chemotherapy, two courses each of carboplatin and etoposide alternating with cyclophosphamide and etoposide. The radiotherapy dose was 35 Gy CSRT, which was based on the early results from SIOP 2 that suggested an overall benefit for conventional as opposed to reduced-dose radiotherapy. The results of PNET 3 again showed the benefit of chemotherapy. (Taylor, Bailey et al. 2003) A significant difference in EFS was demonstrated for patients treated by chemotherapy and RT at 3 and 5 years with an EFS of 78.7% and 73.4% respectively compared with 64.2% and 60.0% for RT alone ($p=0.0419$). The 3-year and 5-year OS for the two arms were 82.1% and 76.1% for patients treated with chemotherapy and RT, compared with 75.8% and 66.5% for treatment with RT alone ($p = 0.1662$). For patients who had undergone total resection, event-free survival was significantly better with chemotherapy + RT than RT alone ($p=0.0346$).

Further randomised studies have compared pre-RT with post-RT chemotherapy. The German study HIT '91 compared ‘sandwich chemotherapy’ to immediate RT followed by maintenance chemotherapy with the ‘Packer regimen’ of CCNU, vincristine and cisplatin. (Kortmann, Kuhl et al. 2000) This regimen, consisting of eight doses of vincristine, given during RT, followed by eight cycles of all three drugs given every six weeks, commencing six weeks after the end of RT, had previously been investigated in the context of reduced-dose RT CRST of 23.4 Gy with a boost of 31.8 Gy (total dose to posterior fossa 55.2 Gy) in carefully-staged standard-risk patients. PFS was $86\% \pm 4\%$ at 3 years and $79\% \pm 7\%$ at five years. (Packer, Goldwein et al. 1999) In the HIT '91 study, the CSRT dose was 35.2 Gy and the posterior fossa dose was 55.2 Gy. There was an advantage for maintenance post-RT chemotherapy as compared to the ‘sandwich chemotherapy’ with relapse-free survival of 78% versus 65% at three years for non-metastatic patients.

Despite initial enthusiasm for “sandwich chemotherapy”, no studies to date have shown a benefit for this approach as compared to post-RT chemotherapy. In addition, studies such as SIOP 2 show a possible detrimental effect of delaying radiotherapy. Subsequent interest has focused on the role of post-RT chemotherapy in the context of reduced-dose RT.

The “Packer regimen” was compared to cisplatin, vincristine and cyclophosphamide in a recent study in children with standard-risk MB treated with reduced-dose CSRT.(Packer, Gajjar et al. 2006) Five-year EFS and overall survival were found to be similar in the two groups at $82\% \pm 2.8\%$ and $87\% \pm 2.69\%$ compared to $80\% \pm 3.1\%$ and $85\% \pm 2.8\%$ in the CCNU-containing and cyclophosphamide-containing arms respectively. Toxicity, probably due to cisplatin, was again a major concern, as almost one quarter of patients in both arms experienced Grade 3 or 4 audiological toxicity.

This study and others confirmed the role of post-RT chemotherapy in what has now become the treatment standard for patients with standard-risk MB, i.e. CSRT in the range of 23.4 to 25 Gy followed by PFRT. Indeed, coverage with post-RT chemotherapy may be a requisite part of the new treatment standard.

Post-RT maintenance chemotherapy has also been studied in the context of fractionated and hyperfractionated RT regimens as an integral part of the overall therapeutic strategy. In the PNET 4 study, comparing conventionally fractionated RT and HFRT, all patients received chemotherapy with vincristine during RT, followed by eight courses of cisplatin, CCNU and vincristine.(Lannering, Rutkowski et al. 2012) Significant acute toxicities were mainly reported during chemotherapy and were not related to the type of RT received. In PNET 5 MB - LR, post-RT maintenance chemotherapy will include only six treatment cycles, three cycles of cisplatin, CCNU and vincristine in alternation with three cycles of cyclophosphamide and vincristine.

The use of HFRT alone, without additional chemotherapy, also showed promising results, in the context of strict upfront quality control of radiotherapy.(Carrie, Muracciole et al. 2005) However, there was only a limited number of patients treated. The rates of 6 year EFS and OS were 75% and 78%, respectively, showing promising but slightly inferior long term results than comparable studies with chemotherapy.(Carrie, Grill et al. 2009)

4.4.4. Rationale for Chemotherapy

Rationale for Carboplatin as concomitant treatment to radiotherapy in PNET 5 MB - SR

According to the results from the PNET 4 study, 5-year EFS for children with standard medulloblastoma without a favourable biological profile may be around 75%. At relapse, possible salvage strategies after primary therapy have yielded relatively poor results. Therefore, a randomised study of intensification of primary therapy is appropriate. Considering possible strategies to intensify treatment, the following aspects have to be taken into account. Firstly, radiotherapy may be placed most effectively as the first adjuvant treatment element after tumour resection, since a randomised comparison with sandwich chemotherapy in HIT’91 revealed significantly better survival rates for postoperative radiotherapy followed by maintenance chemotherapy.(Kortmann, Kuhl et al. 2000; von Hoff, Hinkes et al. 2009) Secondly, eight cycles of maintenance chemotherapy with CCNU, vincristine and cisplatin cannot be administered without dose modifications in a significant portion of children(von Hoff, Hinkes et al. 2009) and therefore an intensification of maintenance chemotherapy may not be feasible. Thirdly, the relapse pattern in PNET 4 was predominantly metastatic, suggesting that intensification of the neuroaxis treatment may contribute to improve survival rates. And finally, an increase in the craniospinal dose from 23.4 Gy to higher doses, which could potentially increase the associated neurocognitive deficits, should be avoided. All these aspects support the potential role of addition of chemotherapy within the radiotherapy phase as a method of moderate treatment intensification.

The anti-tumour activity of cisplatin has been reported in childhood medulloblastoma.(Packer 1999) However, drug-induced toxicities have limited its clinical application. In order to further improve the therapeutic index, a number of platinum analogues, including carboplatin, have been synthesised. The

use of carboplatin provides the opportunity to reduce nephro- and ototoxicities. However, carboplatin does have dose-limiting haematological toxicities.

Carboplatin has been suggested as a potential agent for treatment intensification additional to radiotherapy.(Douple, Richmond et al. 1985; Groen, Sleijfer et al. 1995)

Comparing cisplatin with carboplatin from a pharmacokinetic point of view, carboplatin is less rapidly bound to plasma proteins, has a longer free platinum half-life and penetrates more efficiently into brain tissue, thus representing an attractive platinum analogue for the treatment of brain tumours.(Doz and Pinkerton 1994) Following IV administration of maximally tolerated doses of carboplatin and cisplatin in mice, the platinum concentration and the tissue-to-plasma ratio in the brain was higher after carboplatin than after cisplatin.(Siddik, Jones et al. 1988)

Based on these findings, phase I/II studies were conducted using carboplatin alone (Bacha, Caparros-Sison et al. 1986; Allen, Walker et al. 1987) and showed mild toxicities, with a lower potential for auditory, renal, and emetic toxicities. Two phase I/II studies, analysing the feasibility of carboplatin combined with radiotherapy in malignant brain stem gliomas demonstrated the feasibility of administration of carboplatin once or twice per week. However, response and survival rates were not different compared to other approaches.(Allen, Siffert et al. 1999; Doz, Neuenschwander et al. 2002)

The phase I/II study COG 99701, open to recruitment of patients from March 1998 to November 2004 for children with newly diagnosed high-risk PNET (3.1-18.2 years), investigated the feasibility of carboplatin given 5 days a week simultaneously to dose-intensive craniospinal and local radiotherapy along with weekly vincristine, followed by maintenance chemotherapy. Myelotoxicity was determined as the dose-limiting toxicity, and 35 mg carboplatin/m²/dose x 30 was the maximum tolerated dose. Ototoxicity was acceptable (Jakacki, Burger et al. 2012) and other toxicities were moderate and, of note, no unacceptable rates of hearing deficits during or after chemoradiotherapy have so far been reported in that study. Preliminary survival rates reported for children with metastatic medulloblastoma treated with this regimen were promising. Thus, daily administration of carboplatin investigated in a randomised setting appears to be a suitable strategy in patients with medulloblastoma without a favourable biological profile, where moderate intensification of treatment is deemed appropriate. In this cohort of patients with non-metastatic medulloblastoma, it was decided to choose a daily dose of 35 mg carboplatin/m²/dose x 30 with radiotherapy, a dose that was shown to achieve cytotoxic drug levels in preclinical models and identified as the MTD in the 99701 trial with more intensive radiotherapy doses. During radiochemotherapy, G-CSF support is not recommended routinely, but may be required if WBC falls below the levels indicated.

Carboplatin given concomitantly to radiotherapy might potentially increase the rate of ototoxicity, neurological toxicities and neurocognitive deficits. Trials which used the cisplatin-containing 'Packer' chemotherapy reported high to low rates of relevant ototoxicity (Packer 1994: 47% Grade II/IV (Packer, Sutton et al. 1994); COG 9961: 23%, and 28%, respectively Grade III/IV (Packer, Gajjar et al. 2006); HIT 91: 9% Grade III/IV (Kortmann, Kuhl et al. 2000)) Due to the use of different scales for grading of ototoxicity, different dose modification schemes, and different timepoints of evaluation, the results cannot be well compared. However, a proactive recommendation for dose-modifications (substitution of cisplatin by carboplatin or stopping further use of any platinum-based compounds) should be instituted.

In PNET 4, 12% of patients had III/IV ototoxicity within the first year after treatment, confirming the observation that early dose modification of cisplatin chemotherapy may reduce the rate of ototoxicity. In PNET 5 MB - SR, significantly lower cumulative doses of cisplatin will be given during maintenance chemotherapy. Therefore, a randomised study of the addition of carboplatin is deemed acceptable. However, guidelines for monitoring of hearing function before, during, and after

treatment, and a stopping rule for an unacceptable rate of ototoxicity has been implemented in the statistical design of PNET 5 MB - SR. For grading of ototoxicity the Chang grading will be used (see 9.4.2. Audiology, page 72).

Due to the potential enhancement of neurological toxicities of the radiotherapy, there will be a prospective evaluation of leucencephalopathy, based on estimation of national reference neuroradiologists. Furthermore, there will be a structured evaluation of clinical neurological follow-up.

Rationale for Maintenance Chemotherapy

It has been shown that eight cycles of maintenance chemotherapy with CCNU, vincristine and cisplatin cannot be applied without dose modifications in a significant portion of children. (von Hoff, Hinkes et al. 2009) In HIT'91, this maintenance chemotherapy was administered to 62 fully assessable patients with M0 or M1 disease. Overall survival of patients with and without dose reductions due to toxicity was not different. Ten-year OS rates were 80% (\pm 11%) for 16 patients without any dose reduction and 88% (\pm 5%) for 40 patients with dose reduction ($p=0.585$). In addition, the introduction of carboplatin in PNET 5 MB - SR with radiotherapy has prompted us to reduce the cumulative dose of cisplatin in the maintenance chemotherapy in order to avoid an unacceptable rate of ototoxicity. Consequently, it was decided to reduce the cumulative doses of cisplatin, CCNU and vincristine given during maintenance chemotherapy in PNET 5 MB - LR (6 cycles ABABAB) and PNET 5 MB - SR (8 cycles ABABABAB). A backbone of cycles with cisplatin, CCNU and vincristine (Regimen A, 3 or 4 cycles, respectively) was chosen. Cyclophosphamide is an active agent in medulloblastoma. (Friedman, Mahaley et al. 1986) Cycles with cyclophosphamide and vincristine (Regimen B), well tolerated in the HIT-SKK protocols since the early 1990s (Rutkowski, Bode et al. 2005), have been adopted as alternating B cycles in PNET 5 MB - LR and PNET 5 MB - SR. As shown in the table below, the cumulative doses of cisplatin, CCNU and vincristine are significantly lower compared to PNET 4 and HIT91 maintenance chemotherapy, aiming to improve the feasibility of applying this maintenance chemotherapy without further significant dose-reductions.

	PNET 4	PNET 5 MB - LR (3 x AB)	PNET 5 MB - SR (4 x AB)
Cisplatin	560 mg/m ²	210 mg/m ²	280 mg/m ²
CCNU	600 mg/m ²	225 mg/m ²	300 mg/m ²
Vincristine	45 mg/m ² (incl 6 times with RT)	18 mg/m ²	24 mg/m ²
Cyclophosphamide		6 g/m ²	8 g/m ²
Carboplatin			1050 mg/m ² (randomised patients)

Table 2 Cumulative Doses of Chemotherapy compared to PNET 4

4.5. Late Effects and Quality of survival

It is widely recognized, that long term survivors after diagnosis and therapy for medulloblastoma are suffering from multidimensional impairments. Survivors typically experience neurological,

endocrine and other health problems (Ribi, Relly et al. 2005; Laughton, Merchant et al. 2008; Benesch, Spiegl et al. 2009; Boman, Hoven et al. 2009; Frange, Alapetite et al. 2009). They also have school difficulties during childhood (Ribi, Relly et al. 2005). In adulthood they experience psychosocial difficulties related to employment, driving, independent living and marital status (Maddrey, Bergeron et al. 2005; Frange, Alapetite et al. 2009). There is also evidence of social isolation (Ribi, Relly et al. 2005); neurocognitive problems (Maddrey, Bergeron et al. 2005; Mulhern, Palmer et al. 2005; Ribi, Relly et al. 2005; Merchant, Kiehna et al. 2006; Nagel, Delis et al. 2006; Reeves, Palmer et al. 2006; Benesch, Spiegl et al. 2009) ; neurobehavioural difficulties (Roncadin, Dennis et al. 2008); and compromised QoL (Ribi, Relly et al. 2005; Benesch, Spiegl et al. 2009).)

Craniospinal irradiation (CSI) is an important determinant of health status and QoS following medulloblastoma but post-operative status prior to adjuvant therapy and the addition of chemotherapy to CSI may compromise further late effects in older children and young adults (Bull, Spoudeas et al. 2007). It is therefore important that the appraisal of the effect of allocation to treatments in RCTs includes systematic assessment of health status, quality of life and related aspects of QoS that may be affected including social and emotional difficulties, and cognitive and executive function. These outcomes are in turn likely to predict the extent to which survivors can function independently in society.

In order to achieve the numbers required for the necessary power to draw reliable conclusions, the methods used to collect outcome data need to be applicable in multiple centres across Europe. The over-riding concern is to keep assessments short and simple and the burden for families and clinicians low. The strategy for achieving this is to rely principally on short questionnaires to assess outcome in a systematic way that includes the full range of types of difficulties and disabilities considered relevant by families: this will document health status, behaviour, endocrine and audiological function, and the subjective experience of the child and family.

A modification of the assessment proposed by Glaser et al in 1999 proved feasible in a previous cross-sectional multicentre study of UK survivors enrolled in PNET3 and detected important differences between treatment arms in children treated for medulloblastoma.(Glaser, Kennedy et al. 1999) In particular, use of chemotherapy ‘sandwiched’ between neurosurgery and cranio-spinal irradiation (CSI) was associated with substantially poorer health status seven years later.(Bull, Spoudeas et al. 2007) The same method was also applied longitudinally in seven languages across all centres participating in PNET 4 although there was fall-off in ascertainment for later (2-3 year) time points after diagnosis. In PNET 5 MB - LR and PNET 5 MB - SR, we expect to improve ascertainment, compared to PNET 4, by making a similar panel of questionnaire assessments accessible to families on-line via personal computers, either in their homes or at the centres where they receive treatment. Online documentation can be performed within HealthTracker® (HT). For patients with limited online access also documentation within paper booklets will be possible. HealthTracker is an online site (<http://www.healthtracker.co.uk/Welcome.aspx?c=19>) that has been specifically developed to capture a wide range of health outcomes including, symptoms, side-effects, quality of life and quality of survival.(Gringras, Santosh et al. 2006) HealthTracker has been developed through workshops with children, young people and their parents and uses an engaging and fun collection of questionnaires and games to give it a ‘bedside manner’. The use of this on-line adaptation of the method was piloted in 2013 and found to be acceptable to users.

Much of the variation in neuro-cognitive outcome following acquired brain injury is unexplained, and part of this is attributable to host genetic factors. There is an extensive literature on the predictive value of allelic variation in the ApoE gene, Interleukin (IL) genes, that determine a pro- or anti-inflammatory phenotype, and other DNA polymorphisms in traumatic brain injury,(Dardiotis, Fountas et al.) but it is only recently that investigators have begun to explore the role of genetic factors in neuro-cognitive outcome following childhood brain tumours.

Dopamine and dopamine degrading enzyme catechol-o-methyltransferase (COMT) are critically important for functions mediated by frontal brain regions in healthy individuals. In 50 childhood brain tumour survivors treated with conformal radiotherapy and 40 healthy siblings, COMT genotype predicted performance on the verbal self-ordered search task measure of working memory on the Behavior Rating of Executive Function (BRIEF): the MET/Val genotype predicted better performance than Val/Val or Met/Met genotype.(Conklin, Meton et al. 2010)

In medulloblastoma patients, polymorphisms in glutathioneS-transferases (GSTs), which catalyze the glutathione conjugation of alkylating agents, platinum compounds and free radicals formed by radiation, may be another important genetic determinant of neuro-cognitive outcome: compared with all others, patients with at least one null genotype had on average 27.2 (p=0.0002) lower full-scale intelligence quotient scores.(Barahmani, Carpentieri et al. 2009) GSTM1 polymorphism indicating lower free radical scavenging enzyme function were also associated with higher anxiety, more depression and more global distress in medulloblastoma survivors.(Brackett, Krull et al. 2010)

It is very likely that further genetic polymorphisms explaining variation in neuro-cognitive outcome in medulloblastoma survivors will be identified, including some with implications for a pharmacogenomics approach. This may have a clear impact in determining optimal therapeutic doses, tailored to the patient's genotype, and minimizing unwanted effects. Therefore it is planned, to collect within an associated study patient DNA for evaluation of the genetic polymorphisms, for better understanding of the role of gene-treatment interactions in determining neuro-cognitive outcome in medulloblastoma survivors

Endocrinology:

Between 60 and 95% of children with brain tumours experience growth hormone insufficiency within 2-5 years of fractionated (1.8Gy) neuraxial radiation and tumour boost delivering estimated pituitary doses in excess of 40Gy (Livesey, Hindmarsh et al. 1990; Schmiegelow, Lassen et al. 2000), but this is usually the only pituitary deficit if the tumour is in the posterior fossa - distant from the central hypothalamo-pituitary axis (HPA).(Spoudeas, Charmandari et al. 2003) Growth hormone insufficiency is evident as early growth failure or arrest with disturbed growth hormone secretion even in children with posterior fossa tumours assessed prospectively from diagnosis to 2 years post radiation with a potentiating effect of additional chemotherapy.(Spoudeas, Hindmarsh et al. 1996) Scattered skeletal irradiation from the exit dose of the spinal beam predisposes to vertebral damage and further statural shortening (Olshan, Gubernick et al. 1992) and a range of thyroid (Ogilvy-Stuart, Shalet et al. 1991) and gonadal (Livesey and Brook 1988) dysfunction over time, including early puberty.(Ogilvy-Stuart, Clayton et al. 1994) These effects seem potentiated by adjuvant chemotherapy after older treatment protocols and are known to be treatment, dose-, time- and (in the case of the gonad) sex-dependent, but their evolution has not been previously studied prospectively in the context of a large brain tumour trial. They are significant ranging from asymptomatic compensated hypothyroidism or hypogonadism - manifest early only as simple TSH or FSH elevation, progressing to frank hypothyroidism and second thyroid cancers if the TSH is not appropriately and timely suppressed by substitutive therapy(Bhatia, Yasui et al. 2003), and infertility (in males), or delayed puberty, pubertal arrest or future premature menopause in girls.(Gurney, Kadan-Lottick et al. 2003) Long term infertility is a major cause of distress, affecting 15 to 30% of all childhood cancer survivors but especially post adolescent males and adult females receiving high cumulative doses of alkylating agents such as the nitrosoureas (CCNU) and cyclophosphamide used in this treatment protocol.(Thomson, Critchley et al. 2002) With high survival rates from cancer, and increasing possibilities for assisted fertilization and pre-treatment fertility preservation (Lee, Schover et al. 2006) it is increasingly important to fully understand the likely evolution of gonadotoxicity after each treatment protocol especially in those treated prepubertally for whom there is little age-appropriate data and for whom preservation treatment options may be unavailable or unethical.(von Wolff, Donnez et al. 2009)

Hormone status has important effects on health-related well-being, bone and muscle mass, QoL, cognition, and second tumour prevention. This is exemplified by the need for adult growth hormone replacement (Carroll, Christ et al. 1998), recognised benefits of sex steroids in maintaining bone and uterine health, pubertal progress, sexuality and reproductive function (Critchley, Bath et al. 2002), and the importance of thyroxine therapy to treat frank or compensated hypothyroidism, in the latter case to suppress tumorigenesis. (Schmiegelow, Feldt-Rasmussen et al. 2003) All also have important effects on emotional and cognitive state and should be replaced in a timely fashion.

To screen for potential infertility / gonadal dysfunction it is possible to assess elevations in the plasma gonadotropin FSH: levels above 15 IU/l are well outside the normative range for both sexes at all age ranges, particularly high for adult women in the follicular phase (D2-6) of a spontaneous cycle and, compared to other biomarkers, likely to indicate subfertility. (Stewart and Turner 2005) Even in early infancy there are sex steroids which suppress gonadotropins, so that gonadal patients such as those with Turner syndrome, exhibit high (castrate) amplitude serum gonadotropins with normal pulsatility. (Conte, Grumbach et al. 1980; Nathwani, Hindmarsh et al. 1998) These, restrained by the CNS, reach a nadir in midchildhood (7 years of age) before rising again towards puberty. (Bridges, Matthews et al. 1994) This CNS restraint may be disrupted in children with brain tumours, tending to an early puberty. It is thus possible with serum FSH levels to screen for dysfunction of gonadal axes even in the young prepubertal child. This will not only ensure the appropriate and timely hormone replacement required for mental and emotional wellbeing but also assess any differential toxicity between two different intensity treatment regimens

To assess dysfunction of other hormone axes, regular endocrinological follow-up is recommended (see chapter 12.3. Post-treatment Follow-up, page 92) and any hormone replacement needs to be documented within PNET 5 MB.

Neurology

Patients with medulloblastoma are at risk for neurologic morbidity, which in some cases persists lifelong. A relation between neurological and neuropsychological outcome has been suggested. (Puget, Boddaert et al. 2009)

Comprehensive surveys of neurological function are time consuming to document, and meaningful analyses are only possible based on focussed and standardised evaluations. Therefore, although comprehensive clinical neurological examinations are necessary for the clinical care of every patient, documentation will be limited to tumour location specific items.

Documentation of postoperative neurological functions will focus on posterior fossa syndrome (see chapter 4.4.1. Neurosurgery, page 39). A previously published scale (Robertson, Muraszko et al. 2006) will be used for grading.

Within follow-up focus of neurologic documentation will be on cerebellar dysfunction. As a short and easy to perform scale, the brief ataxia rating scale will be used. (Schmahmann, Gardner et al. 2009) It depicts localisation specific ataxia (inclusive oculomotor abnormalities) with a standard grading. However, as cerebellar function has not completely developed within young ages, it is necessary to consider age appropriate normal values. These are not yet available currently, but it is planned to evaluate them in parallel, so that they will be available for analysis at the end of the PNET 5 MB study.

5A. AIM AND OBJECTIVES OF PNET 5 MB - LR

5A.1. Aim

The aim of the study is to confirm the high rate of event-free survival in patients between the ages of 3 to 5 years and less than 16.0 years, with ‘standard risk’ medulloblastoma with a low-risk biological profile. Patients eligible for the study will be those with non-metastatic medulloblastoma (by CSF cytology and centrally reviewed MRI imaging) at diagnosis and low-risk biological profile, defined as (i) WNT subgroup positivity, assessed by β -catenin IHC (mandatory), β -catenin mutation (mandatory) and monosomy 6 (optional) testing, and (ii) an absence of high-risk biological features (*MYC* or *MYCN* amplification). Patients will have undergone total or near-total tumour resection and will receive conventionally fractionated (once a day) radiotherapy with a dose of 54.0 Gy to the primary tumour and 18.0 Gy to the craniospinal axis. Following radiotherapy, patients will receive a reduced-intensity chemotherapy with a total of 6 cycles of chemotherapy consisting of 3 courses of cisplatin, CCNU and vincristine alternating with 3 courses of cyclophosphamide and vincristine.

5A.2. Objectives

5A.2.1. Primary Objective of PNET 5 MB - LR

The primary objective of the study is to confirm that the rate of 3-year Event-Free Survival (EFS) in children and adolescents with standard-risk medulloblastoma having a low-risk biological profile remains in excess of 80% when patients are treated with 18.0 Gy neuraxis irradiation plus boost to the primary tumour, and reduced-intensity chemotherapy.

5A.2.2. Secondary Objectives of PNET 5 MB - LR

The secondary objectives are:

- a) To investigate the Overall Survival (OS) rate, progression free survival (PFS), and pattern of relapse in this patient group.
- b) To study the late effects of the reduced-dose approach, focusing on hearing, endocrine, and neurologic function, and standardized, patients/ parents rated measurements of health status, executive function, behavioural outcome, and quality of life.
- c) To conduct comprehensive studies in a prospective fashion on the biological basis of WNT-subgroup medulloblastoma, with the aim of identification, investigation and validation of biomarkers and drug targets with therapeutic potential in this disease subgroup. These investigations will focus on (i). detailed analysis of biological pathways and molecular events established to play a role in medulloblastoma, or that are of potential prognostic significance in this disease group, (ii). comprehensive genome-wide investigations of novel medulloblastoma defects, and (iii). defining diagnostic correlates of WNT pathway activation. .

5A.3 Outcome Measures

5A.3.1. Primary Outcome Measure in PNET 5 MB - LR

The primary outcome measure is the rate of event-free survival (EFS).

An “event” is considered to be any progression or relapse of disease, any deaths, and any occurrence of a secondary neoplasm.

“Relapse” is defined as the appearance of local disease, metastasis, or both following documented complete resection, or previous complete response. “Progression” is defined as tumour growth > 25% (based on the three-dimensional measurement on the MRI) in the case of residual tumour. “Secondary neoplasm” is defined as any diagnosed neoplasm that was distinct from medulloblastoma. The percentage of patients in whom no event has occurred at 36 months of follow-up will be the measure for EFS.

5A.3.2. Secondary Outcome Measures in PNET 5 MB – LR

The rate of overall survival (OS), and progression free survival (PFS), estimated by the Kaplan-Meier method, are secondary outcome measures.

Pattern of relapse is a secondary outcome measure. The site and time to local progression will be the measures for local tumour control. Particular attention will be given to posterior fossa relapse, i.e. relapse within or outside the tumour bed. The time period begins on the date of surgery and ends on the date of appearance of relapse/progression. The appearance of metastases will not be regarded as local progression.

Indirect measures of quality of survival (QoS) are a secondary outcome measure. Standardized, patients/ parents rated scales for measurement of health status (HUI3), executive function (BRIEF), behavioural outcome (SDQ), medical, educational, employment and social situation (MEES), Fatigue (PedsQL Multidimensional Fatigue Scale and, in adults, the MFI), and quality of life (PedsQL and, in adults, the QLQ-C30) will be the indirect measures for QoS.

Audiological toxicity is a secondary outcome measure. The extent of ototoxicity based dose modifications of maintenance chemotherapy, as well as the results of Pure Tone Audiometry (PTA) graded by the Chang criteria (see chapter 9.4.2. Audiology, page 72) evaluated 2 years after diagnosis will be the measures for audiological toxicity.

Endocrine function is a secondary outcome measure. FSH levels (cut-off level >15 IU/l) will be used as biomarker for subfertility. Growth retardation will be calculated as the difference in height standard deviation score (sds) from diagnosis, and the need for, time to, and duration of hormone supplementation will be used as surrogate markers for endocrine deficits. All measures will be evaluated 2 and 5 years from diagnosis/surgery and again in adulthood at 18 years.

Neurological function is an outcome measure. The occurrence and severity of posterior fossa syndrome (as measured by the cerebellar mutism syndrome survey, Robertson et al. Journal of Neurosurgery 2006(Robertson, Muraszko et al. 2006)), and the occurrence and severity of persisting cerebellar symptoms (measured by the brief ataxia rating scale (Schmahmann, Gardner et al. 2009)) will be the measures for neurological function.

The prognostic value of biological tumour markers is an outcome measure. Results of protein expression (including immunohistochemistry), RNA expression, and DNA analysis assays undertaken on tumour, blood or CSF material will be the measures for biological properties.

5B. Aim and Objectives of PNET 5 MB - SR

5B.1. Aim

The aim of the study is to test whether concurrent carboplatin during radiotherapy followed by 8 cycles of maintenance chemotherapy in patients with ‘standard risk’ medulloblastoma with an average-risk biological profile may improve outcome. Patients eligible for the study will be those with non-metastatic medulloblastoma (by CSF cytology and centrally reviewed MRI imaging) at diagnosis and average-risk biological profile, defined as (i) WNT subgroup negativity, assessed by β -catenin IHC (mandatory), β -catenin mutation (mandatory) and monosomy 6 (optional) testing, or (ii) WNT subgroup positivity in patients aged ≥ 16.0 years at diagnosis, and (iii) an absence of high-risk biological features (*MYC* or *MYCN* amplification). Patients will have undergone total or near-total tumour resection and will receive conventionally fractionated (once a day) radiotherapy with a dose of 54.0 Gy to the primary tumour and 23.4 Gy to the craniospinal axis. Following radiotherapy, patients will receive a modified-intensity chemotherapy with a total of 8 cycles of chemotherapy consisting of 4 courses of cisplatin, CCNU and vincristine alternating with 4 courses of cyclophosphamide and vincristine.

5B.2. Objectives

5B.2.1. Primary Objective of PNET 5 MB - SR

The primary objective of the study is to assess whether the concurrent administration of carboplatin during radiotherapy has an effect on the Event-Free Survival (EFS) in children and adolescents with standard-risk medulloblastoma having an average-risk biological profile.

5B.2.2. Secondary Objectives of PNET 5 MB - SR

The secondary objectives are:

- a) To investigate the Overall Survival rates (OS), the Progression-free survival rates (PFS), and the pattern of relapse in the randomized treatment arms.
- b) To test the feasibility of carboplatin treatment concomitantly to radiotherapy
- c) To study the late effects in the randomized treatment arms, focusing on hearing, endocrine, and neurologic function, and standardized, patients/ parents rated measurements of health status, executive function, behavioural outcome, and quality of life.
- d) To conduct comprehensive studies in a prospective fashion on the biological basis of standard-risk medulloblastoma, with the aim of identification, investigation and validation of biomarkers (diagnostic, prognostic and predictive) and drug targets with therapeutic potential in this disease subgroup. These investigations will focus on (i). detailed analysis of biological pathways and molecular events established to play a role in medulloblastoma, or that have been shown to have potential prognostic significance in this disease subgroup (e.g. chromosome 17 abnormalities), and (ii). comprehensive genome-wide investigations of novel medulloblastoma defects.

5B.3 Outcome Measures

5B.3.1. Primary Outcome Measure in PNET 5 MB - SR

The primary outcome measure is event-free survival (EFS).

An “event” is considered to be any progression or relapse of disease, any deaths, and any occurrence of a secondary neoplasm.

“Relapse” is defined as the appearance of local disease, metastasis, or both following documented complete resection, or previous complete response. “Progression” is defined as tumour growth > 25% (based on the three-dimensional measurement on the MRI) in the case of residual tumour. “Secondary neoplasm” is defined as any diagnosed neoplasm that was distinct from medulloblastoma.

EFS will be calculated by the Kaplan-Meier Method.

5B.3.2. Secondary Outcome Measures in PNET 5 MB - SR

The rate of overall survival (OS), and the rate of progression-free survival (PFS) are secondary outcome measures. OS and PFS will be calculated by the Kaplan-Meier Method.

Pattern of relapse is a secondary outcome measure. The site and time to local progression will be the measures for local tumour control. Particular attention will be given to posterior fossa relapse, i.e. local relapse within the tumour bed, or metastatic relapse to the posterior fossa outside the tumour bed. The time period begins on the date of surgery and ends on the date of appearance of relapse/progression. The appearance of metastases will not be regarded as local progression.

Feasibility of carboplatin treatment concomitantly to radiotherapy is a secondary outcome measure. The timely delivery of maintenance chemotherapy, the number of interruption days, and the grade of weight change, dysphagia and esophagitis, transfusion requirement, haematological toxicities, and infection during therapy, as well as ototoxicity are measures of the feasibility of additional carboplatin.

Indirect measures of quality of survival (QoS) are a secondary outcome measure. Standardized, patients/ parents rated scales for measurement of health status (HUI3), executive function (BRIEF), behavioural outcome (SDQ), medical, educational, employment and social situation (MEES), Fatigue (PedsQL Multidimensional Fatigue Scale and, in adults, the MFI), and quality of life (PedsQL and, in adults, the QLQ-C30) will be the indirect measures for QoS.

Audiological toxicity is a secondary outcome measure. The extent of ototoxicity based dose modifications of maintenance chemotherapy, as well as the results of Pure Tone Audiometry (PTA) graded by the Chang criteria (see chapter 9.4.2. Audiology, page 72) evaluated 2 years after diagnosis will be the measures for audiological toxicity.

Endocrine function is a secondary outcome measure. FSH levels (cut-off level >15 IU/l) will be used as biomarker for subfertility. Growth retardation will be calculated as the difference in height standard deviation score (sds) from diagnosis, and the need for, time to, and duration of hormone supplementation will be used as surrogate markers for endocrine deficits. All measures will be evaluated 2 and 5 years from diagnosis/surgery and again in adulthood at 18 years.

Neurological function is an outcome measure. The occurrence and severity of posterior fossa syndrome (as measured by the cerebellar mutism syndrome survey, Robertson et al. Journal of Neurosurgery 2006(Robertson, Muraszko et al. 2006)), and the occurrence and severity of persisting

cerebellar symptoms (measured by the brief ataxia rating scale (Schmahmann, Gardner et al. 2009)) will be the measures for neurological function.

The prognostic value of biological tumour markers is an outcome measure. Results of protein expression (including immunohistochemistry), RNA expression, and DNA analysis assays undertaken on tumour, blood or CSF material will be the measures for biological properties.

6A. Design of the PNET 5 MB - LR Arm

PNET 5 MB - LR is an international, prospective, Phase-II open study in patients between the ages of 3 to 5 years and less than 16.0 years, with 'standard-risk' medulloblastoma with a low-risk biological profile.

6B. Design of the PNET 5 MB – SR Arm

PNET 5 MB - SR is an international, prospective, Phase-III randomised study in patients between the ages of 3 to 5 years and less than 22 years, with 'standard-risk' medulloblastoma with an average-risk biological profile.

7. Eligibility

7.1. Inclusion Criteria

Note: with the exception of criterion f), the Inclusion Criteria are the same for PNET 5 MB - LR and PNET 5 MB - SR.

To be eligible for inclusion in either study, patients must meet all of the following criteria:

- a) Age at diagnosis, at least 3 - 5 years (depending on the country) and less than 16.0 years (LR-arm) or 22.0 years (SR-arm).

The date of diagnosis is the date on which first surgery/biopsy is undertaken.

- b) Histologically proven medulloblastoma, including the following subtypes, as defined in the WHO classification (2007):
 - Classic medulloblastoma
 - Desmoplastic/nodular medulloblastoma

Pre-treatment central pathology review is considered mandatory.

- c) Standard risk medulloblastoma, defined as:

- total or near total surgical resection with less than or equal to 1.5 cm² (measured on axial plane) of residual tumour on early postoperative MRI, without and with contrast, on central review;
- no CNS metastasis on MRI (cranial and spinal) on central review;
- no tumour cells on the cytopsin samples of lumbar CSF (see chapter 8.1.2. Post-operative Period, page 58), according to national regulations a CSF review might be required.
- no clinical evidence of extra-CNS metastasis.

In patients with significant residual tumour ($> 1.5 \text{ cm}^2$) after first surgery, secondary surgery should be considered. Patients with a reduction of postoperative residual tumour through second surgery to less than or equal to 1.5 cm^2 are eligible, if timeline for start of radiotherapy can be kept.

- d) Submission of high quality biological material including fresh frozen tumour samples for the molecular assessment of biological markers (such as the assessment of *MYC* gene copy number status) in national biological reference centers. Submission of blood is mandatory for all patients, who agree on germline DNA studies. Submission of CSF is recommended.
- e) No amplification of *MYC* or *MYCN* (determined by FISH).
- f) **For PNET 5 MB - LR, low-risk biological profile, defined as WNT subgroup positivity, in patients aged <16.0 years at diagnosis. The WNT subgroup is defined by the presence of (i) β -catenin mutation (mandatory testing), or (ii) β -catenin nuclear immuno-positivity by IHC (mandatory testing) and β -catenin mutation, or (iii) β -catenin nuclear immuno-positivity by IHC and monosomy 6 (optional testing).**

For PNET 5 MB - SR, average-risk biological profile, defined as WNT subgroup negativity. WNT-negative tumours are defined by (i) β -catenin nuclear immuno-negativity by IHC (mandatory testing), and the absence of β -catenin mutation (mandatory testing) and monosomy 6 (optional testing), or (ii) β -catenin nuclear immuno-positivity by IHC (mandatory testing) in the absence of β -catenin mutation and monosomy 6, or (iii) monosomy 6 in the absence of β -catenin nuclear immuno-positivity by IHC or β -catenin mutation.

OR

WNT subgroup positive tumours arising in patients age ≥ 16.0 years at diagnosis. The WNT subgroup is defined by the presence of (i) β -catenin mutation (mandatory testing), or (ii) β -catenin nuclear immuno-positivity by IHC (mandatory testing) and β -catenin mutation, or (iii) β -catenin nuclear immuno-positivity by IHC and monosomy 6 (optional testing).

- g) No prior therapy for medulloblastoma other than surgery.
- h) Radiotherapy aiming to start no more than 28 days after surgery. Foreseeable inability to start radiotherapy within 40 days after surgery renders patients ineligible for the study.
- i) Screening for the compliance with eligibility criteria should be completed, and patient should be included into the study within 28 days after first surgery (in case of second surgery within 35 days after first surgery). Inclusion of patients is not possible later than 40 days after first tumour surgery, or after start of radiotherapy.
- j) CTC grades < 2 for liver, renal, and haematological function.
- k) no significant sensineural hearing deficit as defined by pure tone audiometry with bone conduction or air conduction and normal tympanogram shows no impairment $\geq 20 \text{ dB}$ at 1-3 kHz. If performance of pure tone audiometry is not possible postoperatively, normal otoacoustic emissions are acceptable, if there is no history of hearing deficit.
- l) No medical contraindication to radiotherapy or chemotherapy, such as preexisting DNA breakage syndromes (e.g. Fanconi Anemia, Nijmegen breakage syndrome) Gorlin Syndrome or other reasons as defined by patient's clinician;

- m) No identified Turcot and Li Fraumeni syndrome.
- n) Written informed consent (and patient assent where appropriate) for therapy according to the laws of each participating country. Information must be provided to the patient on biological studies (tumour and germline), and written informed consent obtained of agreement for participation.
- o) National and local ethical committee approval according to the laws of each participating country (to include approval for biological studies).

7.2. Exclusion Criteria

Note: the Exclusion Criteria are the same for PNET 5 MB - LR and PNET 5 MB - SR.

To be eligible for inclusion in either study, patients must meet none of the following criteria:

- a) One of the inclusion criteria is lacking.
- b) Brainstem or supratentorial primitive neuro-ectodermal tumour.
- c) Atypical teratoid rhabdoid tumour.
- d) Medulloepithelioma Ependymoblastoma.
- e) Large-cell medulloblastoma, anaplastic medulloblastoma, or medulloblastoma with extensive nodularity (MBEN), centrally confirmed.
- f) Unfavourable or undeterminable biological profile, defined as amplification of *MYC* or *MYCN*, or not determinable *MYC* or *MYCN* or WNT subgroup status.
- g) Metastatic medulloblastoma (on CNS MRI and/or positive cytospin of postoperative lumbar CSF).
- h) Patient previously treated for a brain tumour or any type of malignant disease.
- i) DNA breakage syndromes (e.g. Fanconi anemia, Nijmegen breakage syndrome) or other, or identified Gorlin, Turcot, or Li Fraumeni syndrome.
- j) Patients who are pregnant.
- k) Female patients who are sexually active and not taking reliable contraception.
- l) Patients who cannot be regularly followed up due to psychological, social, familial or geographic reasons.
- m) Patients in whom non-compliance with toxicity management guidelines can be expected.

8. Screening Investigations and Consent

8.1. Screening investigations

(see also chapter 14.2. Registration of Patients and Patient Identification, page 101)

Patients eligible for the study will be those with non-metastatic medulloblastoma, confirmed by CSF cytology and centrally reviewed MRI imaging at diagnosis. Patients will have undergone total or near-total resection of the tumour.

8.1.1. Pre-operative Period

The following assessments are to be performed in the pre-operative period:

- a) Physical examination with full neurological examination.
- b) Whole brain MRI imaging pre- and post-contrast.
- c) MRI of the spine with complete visualisation of the dural sac (can also be performed early postoperatively).

Central review of preoperative MRI imaging is mandatory.

Please see the respective chapter on imaging recommendations (see 9.2. Recommendations for imaging and central MRI review, page 62)

If there is no evidence for metastases in the centrally review of cranial and spinal MRI the patient may be included in the study (see inclusion criterion c).

8.1.2. Post-operative Period

The following assessments are to be performed in the post-operative period:

- a) Cerebral MRI pre- and post-contrast injection.

To be performed within 72 h, preferentially on day 1 or 2 after surgery but not on the day of surgery (= day 0)

The size of any post-operative residual tumour will be recorded.

Central review of post-operative MRI imaging is mandatory.

Please regard the respective chapter on imaging recommendations (see 9.2. Recommendations for imaging and central MRI review, page 62)

If there is a centrally confirmed gross total resection, or residual tumour is less than or equal to 1.5 cm² (area in the axial plane), the patient may be included in the study (see inclusion criterion c). In patients with significant residual tumour (> 1.5 cm²) after first surgery, secondary surgery should be considered. Patients with a reduction of postoperative residual tumour through second surgery to less than or equal to 1.5 cm² are eligible, if second surgery is performed within 14 days after first surgery.

- b) MRI of the craniospinal axis with complete visualisation of the dural sac (if not performed preoperatively).

c) Lumbar puncture for CSF cytology:

Lumbar puncture showing a CSF free of tumour cells is mandatory before randomisation. It is allowed (and recommended for keeping time lines for stratification and to start radiotherapy, if the clinical situation allows lumbar puncture) to perform lumbar puncture within the first 2 weeks after surgery. If a lumbar puncture is performed within 14 days after last surgery and is negative for tumour cells, then this will be taken as evidence of non-metastatic disease. If, however, the CSF is positive for tumour cells on lumbar puncture taken within 14 days after last surgery, the lumbar puncture must be repeated at day 15 or later.

In case of equivocal CSF cytology, performance of a second lumbar puncture is also recommended.

Involvement of CSF pathways by tumour is defined as the unequivocal identification of primitive neuro-ectodermal cells, either on cytological grounds or with a combination of cytological and immunocytochemical features (e.g. reactivity for GFAP or a neuronal marker, such as NSE or synaptophysin). According to national regulations a CSF review might be required.

If the cytospin of lumbar CSF is negative for tumour cells, the patient may be included in the study (see Inclusion Criterion c).

d) Histopathological diagnosis of classic or desmoplastic medulloblastoma in accordance with WHO classification (2007), confirmed by central reference histology.

e) WNT subgroup status (by β -catenin IHC: immunoreactivity +/- (mandatory), β -catenin mutation analysis: +/- (mandatory) and monosomy 6 status (optional)) and *MYC* and *MYCN* amplification status (mandatory).

f) Clinical history and evaluation, including:

- Full neurological examination
- Brief ataxia rating scale (Schmahmann, Gardner et al. 2009), see chapter 9.4.4. Neurology, page 74
- Cerebellar mutism syndrome survey (Robertson, Muraszko et al. 2006)), see chapter 9.4.4 Neurology, page 74
- Endocrine status, including height, weight, and pubertal assessment staged according to Tanner.
- Age appropriate patients and parents QoL questionnaires. To be performed after registration/randomisation and before the start of radiotherapy.
- Information concerning the onset and therapy of hydrocephalus symptoms, birth weight and parental heights, and timing of puberty (where applicable).

g) Audiology – Pure Tone Audiometry, air conduction if needed combined with tympanogram, or bone conduction.

h) Full blood count.

i) Blood biochemistry – electrolytes (ionogram), urea, creatinine, ALT, AST, alkaline phosphatase, bilirubin, albumin, magnesium, calcium, phosphate.

8.2. Consent

Informed consent for therapy and for data handling will be obtained in accordance with national requirements. For children and adolescents who are too young to give informed consent, depending on national definitions, informed consent will be obtained in written form from the parents. Patient assent may also be obtained, depending on national requirements.

It is recommended to obtain a separate consent for the submission of tumour material, MRI, and patient data at the time when the pathological diagnosis of medulloblastoma has been made locally, as these need to be sent to the respective reference centres before eligibility for PNET 5 MB - LR and PNET 5 MB - SR can be checked.

Written informed consent should also be obtained for the biological studies, in accordance with national guidelines. These should include consent for the link-anonymised investigation of all biological materials collected (i.e. tumour biopsy, CSF, blood), including germline DNA.

Consent Forms have to be adapted to the national requirements by each participating national group.

9. Detailed Guidelines for Radiological, Biological, and QOS Investigations

9.1. Overview of study relevant diagnostic assessments

Assessments	Pre-OP	Post-OP	Post-OP before RT	After RT before chemotherapy	Before each course of chemotherapy	During chemotherapy	6 weeks after last chemotherapy	2 years after diagnosis	5 years after diagnosis	Age of 18	Other
MRI	✓	✓		✓		✓ after 3 and 6 courses	✓	Every 4 months during the first year after treatment, every 6 months during the second year after treatment, every year until 5 th year after treatment			
Histology		✓									
Biology (myc, mycn, β-catenin)		✓									
CSF		✓									
Glomerular filtration rate				✓	✓ before course containing cisplatin		✓				
Full blood count		✓		✓	✓	✓ every 2 weeks					
Blood chemistry		✓		✓	✓		✓				
Pure tone audiometry	(✓)		✓	✓	✓ before course containing cisplatin or carboplatin		✓	✓	✓		
Posterior fossa syndrome			✓								
Neurology/ BARS			✓					✓	✓	✓	
Hydrocephalus symptoms	✓	✓									
Auxiology and endocrine evaluations		✓					✓	✓	✓	✓	
QoL assessments			✓					✓	✓	✓	
Neuropsychological outcome*			✓					✓	✓	✓	

For assessments during RT (with or without carboplatin) see specific guidelines

*Associated study

9.2. Recommendations for imaging and central MRI review

Evaluation of the primary tumour, the size of a residual tumour after resection and its possible dissemination within the CSF are crucial for the identification of patients eligible to the PNET 5 MB - LR and PNET 5 MB - SR studies. Therefore the minimum requirements for the imaging in study patients must be adhered to. The most important issue is comparability of MRIs pre- and postoperatively and during follow up. So, if the baseline MRI did not follow these requirements either repeat the imaging preoperatively or perform the postoperative imaging in a way (e.g. additional sequences to your standard protocol) that sequences comparable with the preoperative MRI are available. This is especially important in medulloblastomas which show little or no enhancement. In these cases the T2, PD, FLAIR and precontrast T1 images must be comparable.

In case of very small either primary, residual or recurrent tumour the exact measurement of such a small structure will necessitate smaller slice lengths than usual. In plane resolution is an essential factor of image quality and therefore a 256 (or 512) matrix for the imaging of the brain and a 512 resolution for spinal canal is necessary. The FOV should be restricted to about 230 mm for the brain and max. 350 mm for the spinal MRI.

The tumour and possible postoperative residue should be measurable in the 3 planes for the calculation of tumour volume ($a \times b \times c/2$). D-volume calculations may be performed additionally. Every effort should be put to reach in the highest accuracy achievable.

Please discuss this protocol with your local (neuro-) radiologist to avoid problems during the study. For discussion do not hesitate to contact the national representative for the central imaging review.

Cranial MRI:

The standard imaging plane for the brain should be the axial plane (aligned to the AC-PC axis). Slice thickness should not exceed 4mm and must be adapted to the individual problem. As the signal of a tumour depends on the field strength of the MRI machine the field strength must not be changed during the study.

For 1-1.5 Tesla MR-machines:

Sequences:

Axial T2 and PD or FLAIR

Coronal FLAIR

Axial T1 +/- contrast

Coronal and sagittal T1 (-)/+ contrast

Axial DWI with ADC (3 directions ; b-value = 1000)

Optional but not replacing the standard given above: 3D gradient echo T1 + contrast and functional imaging (e.g. perfusion, MRS, DTI and any other individual local imaging protocols).

For 3 T-MRIs the T1 imaging usually will be performed as 3D-gradient echo sequence without and with contrast. If possible (depending on the individual scanner) additional to the 3D volume sequence T1-SE, T1-gradient echo or T1-IR sequences (e.g. in the axial plane) should be acquired. Please don't change your selection during follow-up.

Spinal MRI:

Avoid 3T MRI for spinal imaging as the image quality is often inferior to that of 1.5T MR-machines and more unpredictable. The dural sac has to be fully visualized.

As only meningeal disease is of interest only sagittal postcontrast T1-weighted sequences are necessary. Sagittal slice thickness must not exceed 3 mm. The physiological veins of the cord can be mistaken for nodules of dissemination and therefore axial slices without gaps (SL can be chosen

individually) are essential for all suspicious areas. As fat suppression often leads to artefacts and is not necessary for the delineation of meningeal disease it should not be used routinely.

Optional:

T2 TSE sequences or fat suppression techniques. They can be added easily after the T1 postcontrast series if necessary.

Early postoperative imaging:

As non-specific intracranial enhancement is often seen after 3 days following surgery the postoperative MRI must be obtained within this time.

It is strongly recommended to perform post operative MRI scans within 72 h, preferentially on day 1 or 2 after surgery but not on the day of surgery (= day 0)

Even within this time, false positive nodular enhancement can be seen with haemostatic materials and after electrocoagulation and therefore not only the T1-weighted images before and after contrast need to be evaluated but also the signal intensities on the T2-weighted and FLAIR series. Comparability with the preoperative MRI is essential for the detection of residual tumour. The size of a possible residue has to be measured in three planes. If the residue is best visible on T2-weighted images a second plane incorporating a T2-weighted sequence must be employed.

A residue is considered to be any area of pathological signal and/or enhancement comparable with the appearance of the preoperative tumour.

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

Sequences for cranial and spinal imaging see prescriptions for cranial and spinal MRI (page 62).

Please note if spinal MRI is performed postoperatively:

Non-specific subdural enhancement may be identified on postoperative imaging of the spine and must not be mistaken for meningeal dissemination. In any case of doubt or if intense subdural enhancement is seen repeat the spinal MRI after about 2 weeks to clarify the situation.

Follow-up MRIs:

Timing for follow-up MRIs should be planned according to the protocol (see Chapter 12. Summary of Follow-up, page 92).

Measurement of tumour size:

Multiply the largest diameters in the three planes according to the formula $(a \times b \times c)/2$. Additionally volume calculations of a 3D-dataset can be calculated if available for comparison. If the tumour takes up contrast completely then the postcontrast T1 can be used for the measurement of the diameters. But in only partly or non-enhancing tumours also the size on T2/FLAIR or PD and T1 without contrast can be relevant and the best sequence cannot be predicted. For follow-up it is useful to choose the same sequence or if you would have to change the sequence e.g. due to a change in contrast behaviour than measure the tumour sizes in the same sequence also on the previous examination for comparison.

Definitions of residual tumour:

As very subtle residual tumours may not be visible on imaging it is encouraged to compare the results of imaging with the neurosurgical report. A thin line of enhancement can be physiological on early postoperative MRI in the absence of a residual tumour and must not be considered tumour.

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

Due to historical reasons, for medulloblastomas the postoperative classification system according to Chang will be used. Previous studies found a worse prognosis for residual tumours after resection

larger than 1.5 cm² in area (in the axial plane to achieve the comparison to imaging in studies during the preceding CT era).

S0: no residual tumour.

S1: residual tumour below or equal to 1.5 cm².

S2: residual tumour larger than 1.5 cm².

S3: residual tumour infiltration the brain stem irrespective of size.

S4: residual tumour leaving the posterior cranial cavity.

If imaging is inadequate or the surgical cavity is very confusing also the term “unclear” will be used. Sometimes the presence of blood can be ruled out and distinguished from tumour if the MRI is repeated after some days.

Definitions for neuroradiological response evaluation:

The staging of a possible residual tumour follows the guidelines of the PNET 4 study:

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

PR (partial response): Reduction of tumour volume equal or more than 50% compared to the previous staging MRI.

IMP (improvement or minor response): Reduction of tumour volume between 50% and equal or more than 25% (and minor reduction of a meningeal dissemination).

SD (stable disease): Tumour volume between +25% and -25% compared to the previous staging MRI (no real change of a meningeal dissemination).

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

Evaluation of leucencephalopathy:

Two years after diagnosis a MRI should be sent for central evaluation of leucencephalopathy.

Grading of leucencephalopathy will be based on T2 or FLAIR images as follows (Fazekas, Chawluk et al. 1987):

Grade 0: no pathology

Grade 1: punctated or small T2-hyperintensities max up to 25% of the frontoparietal white matter

Grade 2: confluent T2-hyperintensities in between 25 and max 50% of the area of the frontoparietal white matter

Grade 3: more than 50% of the frontoparietal white matter

Additionally, the presence of white matter cysts, and white matter lesion enhancement will be recorded.

Evaluation in case of suspected relapse:

In case of suspected relapse a MRI should also be sent for central evaluation.

9.3. Biological Investigations

The overall strategy for biological investigations within the PNET 5 MB - LR and PNET 5 MB - SR studies is two-fold; (i) to stratify patients for therapy on the basis of molecular diagnostics undertaken using well-defined biological markers, and (ii) to conduct comprehensive studies on the biological basis of medulloblastoma, with the aim of identification, investigation and validation of biomarkers and drug targets with therapeutic potential in the disease.

Biological investigations in PNET 5 MB - LR and PNET 5 MB - SR will be undertaken in parallel; studies in PNET 5 MB - LR will focus on investigation of favourable-risk WNT-subgroup cases, aged <16.0 years at diagnosis, while PNET 5 MB - SR will investigate the remaining WNT-negative standard-risk cases. Together, these combined data will provide a comprehensive investigation of the biological basis of non-high-risk medulloblastoma in patients aged 3 to 5 years and less than 22 years at diagnosis.

Note: Timely results (within 22 days of surgery) of histopathology, *MYC* and *MYCN* amplification, and WNT subgroup status (β -catenin immunohistochemistry, β -catenin mutation and monosomy 6 (monosomy 6 testing is optional)) are necessary for inclusion of the patient into the PNET 5 MB - LR and PNET 5 MB - SR studies.

A series of significant logistical challenges exist to the routine assessment of biomarkers and histopathological indices as a basis for therapeutic stratification in MB. Foremost, the collection of fresh-frozen tumour material is essential for the robust assessment of the majority of established MB biomarkers. Patients with medulloblastoma across Europe are treated across a large number of centres in different countries, and the success of these studies will depend on the instigation of standardised systems for the collection, storage and shipping of frozen and formalin-fixed tumour material from multiple centres, followed by its centralised molecular genetic analysis and histo-pathological review, prior to the commencement of adjuvant therapy. This must take place within a 22 days following initial surgical resection of the tumour, to allow the stratification of adjuvant chemotherapy and radiotherapy received, based on the results obtained. The additional challenge exists of standardisation of approach and results between countries, and the requirement to work within the research ethics and governance frameworks mandated by law within each participating country (reviewed in Pizer & Clifford, 2009).

9.3.1. The SIOP PNET Biology Group

A biology committee has been established within the SIOP-E PNET group to undertake, coordinate and quality control molecular diagnostics, central pathology review, and translational biological studies within SIOP-E PNET clinical trials. The committee has biology / pathology representatives from all countries. One central coordinating centre has been designated for each country. The representatives of each national group are named within the “Contacts” section (see Appendix B.2. National Reference centres and Coordinators for Biology and Pathology).

9.3.2. Collection and Storage of Biological Material

The collection and storage of high-quality biological material from study cases is essential for the conduction of the molecular diagnostics and biological research components of the PNET 5 MB - LR and PNET 5 MB - SR trials. All tissue collection, preparation and storage must be undertaken according to the standard operating procedures (SOPs) defined within this protocol (see Appendix D-Standard Operating Procedures (SOP)).

A. Submission of the following materials, immediately following a diagnosis of medulloblastoma at the treating centre, is required for molecular diagnostic assessments

and is mandatory for study entry. Materials should be submitted to the National coordinating centre as described in the attached standard operating procedure (see Appendix D1 sample preparation, central pathology review and molecular diagnostics).

Snap-frozen tumour material

To be used for 'touch imprint' preparation (for FISH investigations, including the assessment of *MYC* and *MYCN* gene amplification and monosomy 6 status), extraction of nucleic acids (for DNA/RNA extraction, β -catenin (*CTNNB1*) mutation analysis and research studies) and proteins.

Formalin-fixed, paraffin embedded tumour material

To be used for histopathology review, β -catenin status assessment by IHC, and preparation of materials (e.g. tissue microarray and unstained sections) for research studies (e.g. FISH and IHC investigations).

- B. To allow further prospective biologic research** evaluation (see chapter 5A. Aim and Objectives of PNET 5 MB - LR, page 51, and chapter 5B. Aim and Objectives of PNET 5 MB - SR, page 53), collection and submission of the following materials to the national biology and pathology coordinating centre, using procedures described in the attached standard operating procedures (see Appendix D1 Sample preparation, central pathology review and molecular diagnostics by National Reference Laboratories) is also required.

Blood samples

Whole blood (for DNA extraction) & serum samples should be submitted.

Cerebrospinal fluid (CSF) sample

A 14- or 15-day post-surgical lumbar CSF sample should be submitted.

- C.** In addition, in relapsing patients, second biopsy status should be monitored and, where available, second biopsy material submitted (as above) for the biological studies.

All research samples and derivatives will be stored at the national biology and pathology coordinating centres, prior to their distribution to participating laboratories, for use in the biological studies described in this protocol.

9.3.3. Biological Investigations 1: Molecular Diagnostics, Relevant for Stratification

The following assessments should be undertaken on tumour material received at the national coordinating centre, and results reported within 22 days of initial surgical resection. These results will be used as the basis of therapeutic stratification in PNET 5 MB - LR and PNET 5 MB - SR:

A. Histopathological review

To be undertaken according to WHO (2007) criteria.

B. *MYC* and *MYCN* gene amplification (both mandatory) and monosomy 6 (optional) status

Assessments will be undertaken on fresh-frozen material. Gene amplification is defined using FISH methods as where >5% of nuclei within the sample (of 200 counted) have a strong speckled staining pattern (indicative of the formation of double-minutes or homogeneously-staining regions), and a test probe copy number ≥ 4 times copy number of the reference signal, showing clear evidence of amplification of *MYC* or *MYCN*. *MYC* and *MYCN* amplification status may also be assessed by array-CGH methods, as described in Appendix D. Monosomy

6 is defined as (i) a single signal for the p- and q-arms of chromosome 6 in >50% nuclei within the sample (of 200 counted) by FISH methods, or (ii) an array-CGH result consistent with the presence of monosomy 6 (see Appendix D). The number of nuclei counted and the number of positive nuclei should be recorded.

Note: FISH methods are the standard option for the assessment of MYC and MYCN amplification status. During the course of the PNET 5 MB trial, alternative methodologies for the detection of gene amplification and monosomy (e.g. SNP arrays, MIP, MLPA) will be assessed by the SIOP-E PNET Biology group. If validated methods of equivalent sensitivity and specificity to FISH are developed, these may be introduced as a second testing option through protocol amendments during the course of the trial (see 9.3.4. Section C (iv)).

C. β -catenin protein status (assessed by IHC; mandatory)

Assessments will be undertaken on formalin-fixed material. The cut-off to determine positivity for β -catenin is defined as the presence of >10% nuclear positive tumour cells within a tissue section. The proportion of positive cells should be recorded.

D. β -catenin mutation status (assessed by direct DNA sequence analysis; mandatory)

Assessments will be undertaken on DNA extracted from snap-frozen material. Positive results are those cases displaying confirmed non-synonymous missense mutations in the mutation cluster region. The nature of the mutation should be recorded.

All biological investigations should be undertaken using the standard reagents and methodologies defined in the attached SOPs (see Appendix D-Standard Operating Procedures (SOP)).

9.3.4. Biological Investigations 2: Additional Prospective Biological Studies

Scope and Aims

The collection of fresh-frozen tumour biopsy material, alongside blood and CSF samples, will enable the conduct of comprehensive biological studies alongside the PNET 5 MB - LR and PNET 5 MB - SR studies. The respective analyses to be performed and time of analyses will be defined and regularly updated by the study committee in cooperation with the PNET biology working group, and consensus will be fixed in a separate document.

The aims of these studies are to:

1. Undertake a detailed characterisation of the established clinical, molecular and histological disease features of medulloblastoma.
2. Identify, characterise and validate novel molecular disease features, using strategies based on unbiased genome-wide investigations of transcriptomic, genomic, epigenomic and proteomic alterations.

The integrated biological datasets obtained will then be used to:

- A. Establish the clinical and pathological relevance of molecular disease features identified, and any inter-relationships that exist between individual disease features.
- B. Identify and/or validate independent prognostic biomarkers for the improved prediction of disease course.
- C. Develop models for the optimal prediction of disease risk, using combined clinical, pathological and molecular indices.
- D. Prioritise potential therapeutic targets for further investigation and validation.

Plan of Investigation

Investigations will focus on (i). detailed analysis of biological pathways and molecular events established to play a role in medulloblastoma, or that are of potential prognostic significance in the disease, and (ii). comprehensive genome-wide investigations of medulloblastoma.

A. Investigation of established medulloblastoma pathways / features

Specific analyses will include:

- (a). *Activation of the WNT pathway*, will be assessed by β -catenin IHC and transcriptomic analysis. Investigations will include mutational analysis of pathway components including *CTNNB1* (encoding β -catenin), *APC*, *AXIN1*, *AXIN2*, and the assessment of chromosome 6 status by FISH and array-based methodologies.
- (b). *Activation of the SHH pathway*, will be assessed by methods including transcriptomic analysis and IHC for the expression of SHH pathway components and target genes, which we have previously identified to be associated with pathway activation. Additionally, mutational analysis of pathway components including *PTCH1*, *SMO* and *SUFU* will be undertaken, alongside assessment of chromosome 9q and 10q status by FISH and array-based methodologies.
- (c). *Disruption of the TP53 pathway* will be assessed by IHC analysis of TP53 stabilisation using the D07 antibody. If indicated, mutational analysis of *TP53* exons 4-9 by PCR-based direct DNA sequence analysis will be performed, alongside analysis of *MDM2* amplification and *P14ARF* deletion status by FISH and array-based methodologies, to assess any role as determinants of pathway disruption.
- (d). *Established medulloblastoma genomic features of potential prognostic importance* will be assessed by FISH and array-based methodologies. These will include investigation of *MYC* gene family amplification status (as previously described), chromosome 17 status, and loci of interest from other studies.
- (e). *Additional medulloblastoma biological disease features*, including those identified within this study, will be investigated using appropriate methodologies (see next section).

B. Comprehensive genome-wide investigations of medulloblastoma

A series of core genome-wide datasets will be established to facilitate the wider investigation of the biological basis of medulloblastoma. These will be undertaken in the primary tumour material, CSF and blood samples, as appropriate, and will include:

- (i). *High-density assessment of genomic defects and variations*, using SNP array (e.g. Affymetrix SNP 6.0 arrays or similar methodologies).
- (ii). *Comprehensive transcriptomic analysis of mRNA expression and splice-variants*, using Affymetrix exon arrays or similar methodologies.
- (iii). *Assessment of micro-RNA expression patterns*.
- (iv). *Assessment of genome-wide epigenetic (i.e. DNA methylomic) patterns*, using methodologies including Illumina Infinium arrays.
- (v). *Assessment of the medulloblastoma proteome*.
- (vi). *Next-generation DNA and RNA sequencing technologies* are expected to become available within the timescale of this project and, where indicated, will be applied to investigate the genomic, transcriptomic and epigenomic features of medulloblastoma, with the aim of identification of gene-specific events in disease development.
- (vii). *Additional candidate medulloblastoma defects*. Additional biological features and/or investigative technologies which are identified in preliminary studies, published in the

literature, or become available over the course of this trial will be considered for investigation in the PNET 5 MB - LR and PNET 5 MB - SR cohorts'.

C. Data analysis:

Molecular features identified in A and B will be assessed to determine their nature and overall incidence at diagnosis. These data will then be used for:

- (i). *Integration and analysis of bioinformatic data.* The bioinformatic datasets generated will be analysed and integrated, and used to identify and characterise the nature of novel and established molecular disease features and sub-groups, and their critical biological determinants.
- (ii). *Establishment of the clinical and pathological relevance of molecular disease features identified, and any inter-relationships that exist between individual disease features.* Molecular disease features will be related to each other, and to clinical and pathological indices, using statistical methods as previously described, including corrections for multiple testing. Emphasis will be placed on the definition of MB molecular disease sub-groups, focussing on investigation of WNT subgroup cases within the PNET 5 MB - LR cohort.
- (iii). *Identification of prognostic biomarkers for the improved prediction of disease course.* Univariate and multivariate analyses of overall (OS) and event-free (EFS) will be undertaken for disease features identified, as previously described. Clinical, pathological and molecular features that are independently predictive of disease outcome will be used to investigate combined models for the optimal prediction of disease risk.
- (iv). *Validation of genome-wide data and marker optimisation.* Molecular disease features which demonstrate associations with molecular or clinical disease features, or disease outcome, will be prioritised for validation and further characterisation using independent methods including IHC, FISH, real-time PCR, direct DNA sequencing and bisulphite sequencing, as appropriate. These validations will also be used to define optimal methodologies and cut-offs for the routine testing of biomarkers identified. Optimal methodologies for the assessment of established biomarkers (e.g. WNT activation, *MYC* amplification) will also be assessed. Particular emphasis will be placed on the investigation and validation of alternative methodologies for the assessment of *MYC* and *MYCN* gene amplification status (e.g. SNP arrays, array-CGH, MIP, MLPA) which could be applied in routine molecular diagnostics (see section 9.2.3.B).
- (v). *Prioritisation of molecular events and potential therapeutic targets for further investigation.* Based on the data generated, further studies will be undertaken to investigate the functional relevance (e.g. *in vitro* / *in vivo* modelling) of molecular, pathological and clinical disease features identified and, where relevant, their potential as therapeutic targets for future exploitation.

9.4. Study on quality of survival (QoS)

9.4.1. Indirect measures for QoS

In PNET 5 MB - LR and PNET 5 MB - SR patients will be assessed on four occasions (post surgery before RT, at two and five years after diagnosis and again at age 18 years) using several brief questionnaires (see Table below). For patients who are 18 years of age at two or five years after diagnosis, the coincidence of these time points will mean that only three assessments are required. These will be completed by parents of all participants with age < 18 years and also by patients themselves when age-appropriate self-complete versions are available.

Proxy-report and self-report and measures of Quality of Survival in PNET 5 MB

Age at assessment	Source of questionnaire responses	Questionnaires used	Assessment time point			
			After surgery, before RT	Two years after diagnosis	Five years after diagnosis	At age 18 yrs ¹
5-17 yrs	Parent proxy-report	HUI, SDQ, PedsQL+fatigue module, BRIEF, MEES	√	√	√	
11-17 yrs	Child self-report	HUI, SDQ, PedsQL+fatigue module	√	√	√	
≥18 yrs	Parent proxy-report	HUI, BRIEF	√	√	√	√
≥18 yrs	Adult self-report	HUI, QLQ-C30, MFI, BRIEF, MEES	√	√	√	√

¹ In participants diagnosed at age 13-18 years, this assessment may replace one of the assessments at one of the other three time points in this Table. For those diagnosed at age 19 or older, this assessment will not be relevant.

Health Status, including neuro-cognitive and behavioural outcome

The Health Utilities Index (HUI)(Feeny, Furlong et al. 1995) is a 15-item plus one ‘global’ question, wide ranging measure of health status, which allows comparison of global Health Status or of the ‘attributes’ of vision, hearing, speech, dexterity, ambulation, cognition, emotion, and pain. This has been found to be sensitive to clinical problems (excepting behavioural problems) in populations of children who have been treated for brain tumours (Kennedy and Leyland 1999), including medulloblastoma.(Le Gales, Costet et al. 1999)

The Strengths and Difficulties Questionnaire (SDQ)(Goodman 1999) is a 25-item questionnaire with subscales for hyperactivity, emotional symptoms, conduct problems, peer relationships, and pro-social behaviour. These problems are very prevalent among children with brain tumours.(Kennedy and Leyland 1999) Both the HUI and the SDQ are available in many European languages and provide a concise description of the health status and behaviour of children involved in clinical trials.

The Behavior Rating Inventory of Executive Function (BRIEF) parent questionnaire (Gioia 2000) contains 86 items in eight non-overlapping clinical scales and two validity scales. These theoretically and statistically derived scales form two broader Indexes: Behavioral Regulation (three scales) and Meta-cognition (five scales), as well as a Global Executive Composite score. Factor analytic studies and structural equation modeling provide support for the two-factor model of executive functioning as encompassed by the two Indexes. Validity scales measure Negativity and Inconsistency of responses.

Perception of Health and Well-being

QoL evaluations will be undertaken Paediatric Quality of Life Inventory(Varni, Seid et al. 1999) and the PedsQL Fatigue scale.(Varni, Burwinkle et al. 2002) The PedsQL is a self administered multi-dimensional measure of HRQL in healthy children and adolescents and those with acute and chronic

health conditions. It is designed for use with children as reported by parents of children aged between two and 18 years, and for children themselves aged between five and 18 years. It consists of 23 items, takes approximately five minutes to complete, and provides information about functioning in four dimensions: physical (eight items), emotional (five items), social (five items) and school (five items).

In place of the PedsQL, adult participants, aged more than 18 years, will complete the European Organisation for the Research and Treatment of Cancer-C30 (EORTC QLQ-C30) questionnaire.(Aaronson, Ahmedzai et al. 1993) Instead of the PedsQL-Fatigue Module, the Multidimensional Fatigue Inventory (Smets, Garssen et al. 1995) is used to assess the amount of fatigue in patients aged more than 18 years.

Socio-Demographic, Educational and Employment Questions

Relevant information on socio-demographic, educational and employment issues will be assessed by the Medical Educational Employment and Social Questionnaire (Glaser, Kennedy et al. 1999), which was also used and well-accepted by patients and parents in PNET 4. For PNET 5, the questionnaire was slightly modified and shortened, the modifications were agreed in expert-discussions in 2010 and 2011. Parallelized self- and proxy versions are available. Translations and backward translations in languages of all incorporated European countries were provided. The questionnaire needs about 10-15 minutes for completion.

Data acquisition

The on-line neuro-oncology module of HealthTracker (HT) was created in the UK and translated into seven other European languages in 2011-13. It is comprised of a 'Patient Portal' and an 'Administration Portal'. On-line documentation within HT is performed automatically by the patients and parents providing their questionnaire responses on HT, whether in patient's homes, or in hospitals, schools or public libraries.

For patients with limited on-line access or for whom on-line data entry is judged inappropriate by the national QoS leads, documentation of their paper-and-pencil responses is an alternative method of collecting patient-reported baseline and outcome data. In this case, the entry of these data into the HT database can be achieved, either by the PNET5 national QoS lead team or by the PNET5 QoS international data coordinator, using the Patient Portal. In countries where paper booklets are used to collect the questionnaire responses of some trial participants, national QoS leads will agree with the PNET5 QoS international data coordinator their preference between entry of these data by PNET5 QoS international data coordinator or entry at a national level by the national QoS lead team. This use of HT to enter paper-and-pencil responses should be agreed by the national QoS-lead and the international study PIs.

QoS on-line data thus obtained, entered either as patients/parents on-line responses or as paper-and-pencil responses subsequently entered into the HT database as described above, will be downloaded from HT at regular intervals and included in the central PNET5 SAS or SPSS data base on which all trial analyses will be based.

Communication with and reminders to families about the need to provide data on HealthTracker, whether from a national QoS centre or from the centre at which the patient's care is delivered, can be done either via the national postal system or via email but use of email would require the patient's families' email address to be available and is not essential for the use of HealthTracker. Consent for the use of email addresses for this purpose would be required.

9.4.2. Audiology

Monitoring of hearing loss will be a fundamental and mandatory part of the study.

Ear-specific pure-tone audiometry should be performed by licensed audiologists experienced in paediatric populations. Sensorineural Hearing Threshold (dB HL) should be evaluated using bone conduction or air conduction with normal tympanogram.

Pure-tone audiometry should be performed **before the onset of postoperative therapy:**

Patients can only be included into the PNET 5 MB - LR or PNET 5 MB - SR study, if there is no significant sensorineural hearing deficit as defined by pure tone audiometry, separately performed for both ears, with bone conduction or air conduction and normal tympanogram shows no impairment ≥ 20 dB at 1-3 kHz (best ear). If performance of pure tone audiometry is not possible postoperatively, normal otoacoustic emissions are acceptable, if there is no history of hearing deficit. (see inclusion criterion k, chapter 7.1. Inclusion Criteria, page 55).

Note that for surveillance of ototoxicity on maintenance chemotherapy a PNET-4 based grading system is used for recommendations of dose modifications.

For evaluation of ototoxicity after chemotherapy as secondary outcome measure the Chang grading will be used.

Chemotherapy dose modifications:

Pure-tone audiometry is mandatory **before every cisplatin (or carboplatin) containing maintenance** chemotherapy cycle (course A):

In case of sensorineural hearing deficit, dose modifications of cisplatin, or substitution with carboplatin should be performed according to the recommendations in chapter 11.3.6. Administration of Chemotherapy – Regimen A, page 87. These dose modifications are based on the modification rules used in the HIT 91, HIT 2000 and PNET 4 studies

Please note, that **dose modifications should be performed based on the highest grade, i.e. the “worst ear”**. Documentation of ototoxicities and possible dose modifications will be performed on the respective CRF.

Ototoxicity as secondary outcome measure:

After end of therapy, it is recommended to perform pure-tone-audiometry 2 and 5 years after diagnosis (in case of hearing deficit, a closer follow-up might be appropriate). See chapter 12.3. Post-treatment Follow-up, page 92.

For assessment of treatment-induced ototoxicity, one original audiogram performed 2 years after diagnosis should be sent to the national coordinator.

Grading will be performed according to the Chang ototoxicity grading, based on the lowest grade, i.e. the “best ear”, as follows (Chang and Chinosornvatana 2010):

Sensorineural hearing threshold, evaluated by bone conduction or air conduction with normal tympanogram:

Chang Grade 0:	≤ 20 dB at 1,2, and 4 kHz
Chang Grade 1a:	≥ 40 dB at any frequency 6 to 12 kHz
Chang Grade 1b:	> 20 and < 40 dB at 4 kHz
Chang Grade 2a:	≥ 40 dB at 4 kHz and above
Chang Grade 2b:	> 20 and < 40 dB at any frequency below 4 kHz
Chang Grade 3:	≥ 40 dB at 2 or 3 kHz and above
Chang Grade 4:	≥ 40 dB at 1 kHz and above

9.4.3. Endocrine Function

Hormone Status is important for interpretation of Quality of Survival data and should be collected at the same time points. Those patients already 18 years of age at 2 or 5 years after diagnosis, will have reduced assessments due to the co-incidence of those time points.

Data to be collected: Height prior to radiotherapy, weight, Age at menarche in girls, Date of starting and discontinuing supplemental hormone therapies, birth weight, gestational age at birth, parental heights. Blood FSH will be collected as a biomarker for subfertility, (cycletimed to D2-5 in spontaneously cycling females).

Timing of Measures of Endocrine Function to co-incide with Quality of Survival Data Acquisition.

	Post surgery before RT	2 years post surgery	5 years post surgery	Aged 18 years
Height	√	√	√	√
Weight	√	√	√	√
Pubertal status	√	√	√	√
Serum FSH	√	√	√	√
Hormone Therapy		√	√	√
Menses-rhythm and spontaneity	√	√	√	√

The above described data only present the endocrinological measures which will be analysed within the PNET 5 MB study.

For appropriate support of the patients, more regular comprehensive endocrinological evaluations and referral to paediatric endocrinologist or paediatrician with endocrine expertise will be needed (see chapter 12.3. Post-treatment Follow-up, page 92)

9.4.4. Neurology

Presence and severity of posterior fossa syndrome will be evaluated **before the onset of radiotherapy and at least 3 weeks after surgery** by the cerebellar mutism syndrome survey. (Robertson, Muraszko et al. 2006).

Cerebellar mutism syndrome survey

Robertson PL, J Neurosurg (6 Suppl Paediatrics) 105:444–451, 2006

time of onset	1 = immediately postop 2 = Days 1–2 3 = Days 2–4 4 = Day 4
mutism	1 = mild (mutism ,1 wk) 2 = moderate (mutism 1–4 wks) 3 = severe (mutism .4 wks)
ataxia	1 = mild (persists ,1 wk) 2 = moderate (persists 1–4 wks) 3 = severe (persists .4 wks)
hypotonia (quadriparesis)	1 = mild (can sit or stand by ,1 wk) 2 = moderate (can sit or stand, 1–4 wks) 3 = severe (can't sit or stand, .4 wks)
irritability	1 = mild (persists ,1 wk) 2 = moderate (persists 1–4 wks) 3 = severe (persists >4 wks)

Presence and severity of ataxia will be evaluated in timely analogy to endocrinology and QoS reports, **before radiotherapy, 2 and 5 years after diagnosis, and at age 18.** The brief ataxia rating scale will be used for evaluation.(Schmahmann, Gardner et al. 2009).

The Brief Ataxia Rating Scale (BARS)

Schmahmann JD, Movement Disorders, Vol. 24, No. 12, 2009, pp. 1820–1828

Gait 0: Normal 1: Almost normal naturally, but unable to walk with feet in tandem position 2: Walking without support, but clearly abnormal and irregular 3: Walking without support but with considerable staggering; difficulties in half turn 4: Walking without support not possible; uses support of the wall for 10-meter test. 5: Walking possible only with one cane 6: Walking possible only with two canes or with a stroller 7: Walking possible only with one accompanying person 8: Walking impossible with one accompanying person (2-person assist; wheelchair)			Knee-tibia test (decomposition of movement and intention tremor) 0: Normal 1: Lowering of heel in continuous axis, but movement is decomposed in several phases, without real jerks, or abnormally slow 2: Lowering jerkily in the axis 3: Lowering jerkily with lateral movements 4: Lowering jerkily with extremely long lateral movements, or test impossible		
Score:			Score:	Left:	Right:
Finger-to-nose test (decomposition and dysmetria of arm and hand) 0: Normal 1: Oscillating movement of arm and/or hand without decomposition of the movement 2: Segmented movement in 2 phases and / or moderate dysmetria in reaching nose 3: Segmented movement in more than 2 phases and / or considerable dysmetria in reaching nose 4: Dysmetria preventing the patient from reaching nose			Dysarthria 0: Normal 1: Mild impairment of rate/rhythm/clarity 2: Moderate impairment of rate/rhythm/clarity 3: Severely slow and dysarthric speech 4: Speech absent or unintelligible		
Score:	Left:	Right:	Score:		
Oculomotor abnormalities 0: Normal 1: Slightly slowed pursuit, saccadic intrusions, hypo/hypermetric saccade, nystagmus 2: Prominently slowed pursuit, saccadic intrusions, hypo/hypermetric saccade, nystagmus					
Score:					

10. Study Entry

Patients will be considered as included in the PNET 5 MB study after informed consent has been signed and eligibility criteria have been verified.

Any eligible patient who is not included in the study, due to the decision of patient, parent or physician, should receive treatment in accordance with the recommendations of the appropriate National Group.

It is recommended to register (anonymously) the number of non-included eligible patients and reasons for exclusion. Due to different national policies, this cannot be organised centrally. National centers are asked to establish methods for registration.

Eligible patients will be assigned to either the PNET 5 MB - LR or PNET 5 MB - SR study arm on the basis of their WNT subgroup status.

PNET 5 MB - LR is an open, non-comparative study, and patients will therefore not be randomised.

PNET 5 MB - SR is a randomised, comparative study. Patients will be randomised to receive either radiotherapy alone or radiotherapy and carboplatin 35 mg/m² 5 times per week. All patients will then receive maintenance chemotherapy.

The National Data Centre will make the following information available to the national QoS lead at the time that registration on Marvin is completed, including

- the patient's Marvin number,
- date of birth or, if this is not compatible with national rules, decimal age at first tumour surgery,
- date of first tumour surgery,
- gender,
- treatment allocation,
- hospital, and
- name of local investigator.

These details will be manually entered into HT either by the national PNET QoS lead team or, if preferred by that team, by the PNET 5 QoS international data coordinator in Southampton.

Finally, patients' families have the option to give consent to provide their contact details including **email address** and to be contacted by the national QoS team. Contact details will be stored separately from study data by the national QoS coordinator.

Randomisation in PNET 5 MB - SR

The randomisation lists will be provided to the randomisation centres by the reference center for Biostatistics (A. Faldum and R. Kwiecien, Münster, Germany). A block randomisation will be used and randomisation will be stratified by gender, age and residual tumour.

This study will follow the principles of "Good Clinical Practice (GCP)" provided by the International Conference on Harmonisation (ICH) as well as the Declaration of Helsinki.

11. Treatment Details

11.1. Supportive Care

Supportive care should be applied as to the standard of the treating centre.

- Steroids:** Patients should not receive steroid therapy, e.g. dexamethasone, during radiotherapy and chemotherapy, if at all possible. If symptoms of raised intra-cranial pressure develop during treatment the cause, e.g. hydrocephalus, should be actively sought. Steroids should be used as a short-term measure prior to definitive treatment of the raised pressure. Use of dexamethasone should be documented prospectively.
- Pneumocystis:** During radiotherapy and during chemotherapy pneumocystis carinii prophylaxis is recommended according to local practice.
- Fungal proph.:** A local fungal prophylaxis is recommended during radiotherapy, e.g. with Nystatin, Amphotericin B or Natamycin. A systemic fungal prophylaxis might be considered according to national standards.
- Anti-emesis:** For antiemesis, a 5HT3 antagonist should be used (mandatory for anti-emesis for cisplatin-containing chemotherapy). Dexamethasone should not be used as an anti-emetic unless other therapies fail.
- Fertility:** As infertility is a possible consequence of the treatment, treating physicians are asked to recommend cryoconservation of semen or other measures, whenever appropriate.

11.2. Radiotherapy Phase

11.2.1. Timing of Radiotherapy (RT)

Following definitive surgery - all patients shall begin RT within 28 days after surgery. Starting radiotherapy more than 40 days post surgery renders the patient ineligible for the study.

11.2.2. Equipment

Patients enrolled in the study must be treated using conformal radiation therapy treatment planning and delivery techniques. IMRT techniques will be allowed assuming that appropriate departmental QA procedures are available and prospectively approved by the national study co-ordinator. A primary IMRT approach (including arcing techniques e.g. tomotherapy, VMAT, RapidArc) must ensure adequate irradiation of the target volume allowing for tissue heterogeneity and the junction between the cranial fields and spinal field can be precisely calculated and implemented and a sufficient dose gradient is employed over the vertebral bodies to ensure symmetrical bone growth arrest. All patients must be treated on isocentric linear accelerators with a minimum source-to-axis distance (SAD) of 80 cm. Megavoltage photons with a nominal energy ≥ 4 MV must be used. Treatment with ^{60}Co is not permitted.

The use of electron spinal fields will be acceptable provided a beam of sufficient energy is available to ensure adequate irradiation of the target volume allowing for tissue heterogeneity and the junction

between the photon cranial fields and spinal electron field can be precisely calculated and implemented.

Equally primary proton therapy (for both phases) is acceptable assuming an adequate coverage of the entire vertebral body to minimise the risk of asymmetrical bone growth arrest.

11.2.3. Energy

The cranial (whole brain) fields shall be treated with megavoltage photons with energies in the range of 4-6 MV. Energies higher than 6 MV should be avoided due to the risk of under-dosing the lateral meninges. The tumour bed RT can be given with a higher energy if deemed dosimetrically beneficial. Photons of 4-6 MV will generally be used for spinal irradiation but electrons of suitable energy or protons can be used as an alternative.

11.2.4. Treatment position

Patients should be immobilised using an immobilisation device according to departmental policies. The patient should be maintained in the same position for the cranial and spinal components of CSRT for the duration of this treatment phase.

11.2.5. RT planning

A planning CT is mandatory for the definition of the target volumes of both craniospinal axis and tumour bed. It is strongly recommended that the CT slice thickness should be no greater than 0.5 cm in the region of the cribriform fossa, base of skull, posterior fossa and cranio-cervical field junction (ideally 2.5 mm or smaller), and no greater than 1.0 cm elsewhere within the craniospinal axis.

If the spinal field is treated with electron beams the dose along the entire spinal axis should be calculated with an appropriate correction for tissue heterogeneity.

11.2.6. Treatment volume, anatomical description and dose

Target Volume

Target volumes will be defined according to ICRU 50/62 guidelines. Delineation of all target volumes is based on a planning CT with i.v. contrast and/ or CT-MR image fusion and will be outlined on each slice of the planning scan.

Craniospinal Axis:

The Clinical Target Volume (CTV) for CSRT comprises the whole brain as well as the spinal cord and thecal sac to the dura.

Whole Brain Volume:

The whole brain CTV should extend anteriorly to include the entire frontal lobe and cribriform plate region. In order to include the cribriform fossa within the CTV, and allowing an additional appropriate margin for PTV, the edge of the field (i.e. the geometric edge of the shielding block) would in many cases include the lenses.

The geometric edge of the shielding should extend at least 0.5 cm inferiorly below the cribriform plate and at least 1 cm elsewhere below the base of the skull. The margin between the shielding and the anterior border of the upper cervical vertebrae should be 0.5 cm. The lower border of the cranial fields should form a precise match with the upper border of the spinal field.

The CTV must include any herniation of the meninges through the craniotomy scar

Cervical Spinal Volume

The spinal field should extend superiorly to form an accurate match with the border with the lower borders of the cranial fields.

Dorso-Lumbar Spine Volume

The inferior limit of the spinal CTV must be determined by imaging the lower limit of the thecal sac on a spinal MR performed as part of the staging process. The treatment field edge will set 1 cm below the lowest point of the thecal sac as visualised on MRI.

Width of the Spinal Volume

The aim is to include the entire subarachnoid space including the extensions along the nerve roots as far as the intervertebral foramina. Thus the spinal CTV should extend laterally to cover the intervertebral foramina. An additional margin, generally 1.0 cm on either side should be added for PTV, and an appropriate field width chosen to allow for this. The use of a 'spade' shaped field to treat the lumbo-sacral spine is not recommended.

Tumour Bed Volume

The GTV includes all gross residual tumour and/or the walls of the resection cavity at the primary site, based on the initial imaging examination that defines the tissue initially involved with disease anatomically and the post-operative and pre-irradiation neuro-imaging examinations. The GTV will have to take into account any anatomical shift or changes after surgery.

The CTV includes the GTV with an added margin that is meant to treat sub-clinical microscopic disease and is anatomically confined (i.e. the CTV is limited to the confines of the bony calvarium and tentorium where applicable). The CTV is defined as the GTV plus a 1.0 cm margin except at bone or tentorial interface where it remains within the confines of the posterior fossa.

The PTV is defined as the CTV plus an additional 0.3 - 0.5 cm margin. The size of the required margin will depend on the quality of the immobilisation device chosen and the departmental reproducibility records for the patient position and chosen device. If a local investigator feels that a 0.5 cm margin is insufficient as a CTV/PTV margin, the radiotherapy principal investigator should be informed and the case should be discussed. CAVE: The final PTV should not extend beyond the boundaries of a "classical" PTV when the entire posterior fossa is defined as CTV unless clinically indicated.

A field arrangement using 3D conformal planning is a mandatory requirement. At least the use of posterior oblique fields is strongly recommended. The purpose of this is to minimise the RT dose to the middle ears and temporal lobes. A beam arrangement of a parallel opposed pair is not permitted.

Organs at risk (OR)

The following minimum number of OR must be defined for 3-D conformal radiation therapy or IMRT planning:

- Supratentorial brain
- Posterior fossa (infratentorial brain)
- Cochlea (left and right)
- Optic chiasm
- Temporal lobes (left / right)
- Hippocampus (left / right)
- Thyroid gland
- Pituitary

The supratentorial volume for OR definition is defined as whole brain volume (down to the foramen magnum) minus the posterior fossa volume.

The posterior fossa volume for OR definition is defined as:

- Superiorly - the tentorium
- Inferiorly - the extension of the spinal meninges 2 cm below the lower limit of the tumour as defined on the pre-operative scan. The resulting inferior field edge should at least include the outer table of the skull at the foramen magnum.
- Anteriorly - the anterior edge of the brain stem.
- Posteriorly - the posterior extension of the meninges to the inner table of the skull.
The CTV does not need to include any herniation of the meninges through the craniotomy defect.
- Laterally - the lateral extension of the meninges around the cerebellum

The study will prospectively record the mean and maximum dose to OR in order to allow future analysis of late toxicity.

Dose Specification

Dose Definition: All doses will be specified according to ICRU 50/ICRU 62.

Reference Point

Brain

If the brain is treated by a pair of parallel opposed fields, the dose should be defined at the midpoint of the central axis.

Spine

The dose to the spine should be prescribed along the central axis at a depth representing the posterior margin of the vertebral bodies.

In the case of electron RT to the spine the anterior border of the target volume (posterior aspect of the vertebral bodies) must be encompassed within the 85% isodose.

Tumour bed boost

The primary tumour bed should be treated, using a suitable technique that allows for the least amount of normal brain tissue and organs to be at risk from exposure to high dose irradiation. The prescription point should be the isocentre unless an IMRT technique is used.

Dose prescription

PNET 5 MB - LR:

Brain – 18.0 Gy in 10 daily fractions of 1.80 Gy

Spine - 18.0 Gy in 10 daily fractions of 1.80 Gy

Primary tumour boost – 36.0 Gy in 20 daily fractions of 1.80 Gy

Total dose to primary – 54 Gy in 30 daily fractions of 1.80 Gy

PNET 5 MB - SR:

Brain – 23.40 Gy in 13 daily fractions of 1.80 Gy

Spine - 23.40 Gy in 13 daily fractions of 1.80 Gy

Primary tumour boost – 30.60 Gy in 17 daily fractions of 1.80 Gy

Total dose to primary – 54 Gy in 30 daily fractions of 1.80 Gy

The mean dose to both cochleas will be limited to 30 Gy. In case of a conflict of the mean cochlea dose constraint with the GTV and CTV dose prescription, priority has to be given to cover the GTV and CTV. The CTV to PTV margin may be compromised in selected cases if deemed clinically acceptable.

Fractionation

All fields should be treated daily (conventional RT) 5 days per week.

Dose uniformity

Dose variations across the target volume should be within + 7% and – 5% of the prescription point according to ICRU 50/62 recommendations. If technically achievable, the dose variation should preferentially be kept within $\pm 5\%$. An effort should be made to spare the cochlea and middle ear in view of the subsequent platinum based consolidation chemotherapy.

Field shaping

The use of customised divergent beam blocks or multi-leaf collimators using beam's eye view facilities is mandatory.

Treatment verification

Regular treatment verification according to institutional policies is required. As a minimum standard, weekly portal images must be performed and the set-up variations recorded.

Rests

There will be no planned rests. Delays due to machine services and bank holidays should be avoided wherever possible.

11.2.7. Treatment Technique**Cranial RT**

The cranial fields will be treated with lateral opposed fields.

Spine Irradiation

If possible the spinal volume should be treated with a single posterior field. If necessary the spinal field can be treated at an extended FSD. The exit from the spinal field should not include the teeth and jaw.

Junctions

Junctions of abutting fields should be moved on a regular basis either intra fractionally, daily or by other predefined time points (moving junction technique).

Primary Tumour Bed Volume

It is mandatory that this volume is treated conformally. The field arrangement will be chosen to provide a high conformity index and to minimise the RT dose to OARs.

Intensity Modulated Radiotherapy (IMRT)

It is likely that during the duration of this study, IMRT planning and delivery techniques will be increasingly employed. As an example, this may be used as an option for reducing the radiation dose to the cochlea. IMRT has also been used to improve homogeneity of spinal RT. If centres employ IMRT then it will be essential to observe strict criteria for immobilisation and departmental quality assurance.

Proton Beam Therapy

Proton Beam Therapy will be increasingly available and is attractive to reduce dose to normal tissues i.e. cochlea, lenses, non-involved brain or pituitary. Due to smaller volume receiving low and medium dose, theoretically also the secondary malignancies risk may decrease. Proton technology is still evolving and the delivery of craniospinal treatment will be provided only in few places worldwide. For posterior fossa treatment rotating gantries seem to be advisable as compared to horizontal beam lines only capable of achieving lateral beam arrangements. As there is some uncertainty about increased RBE (relative biological effectiveness) at the distal Bragg peak, weighting of spots and Bragg peaks need to be carefully evaluated. The use of multiple field techniques might be preferable if high weighted spots cumulate in critical areas. As with conventional treatment, organ tolerances as well as target coverage are to be respected.

11.2.8. Quality control of radiotherapy

Radiotherapy for patients with the diagnosis of a medulloblastoma requires a complex treatment technique. It has been previously clearly demonstrated that the relapse risk is closely related to the quality of radiotherapy.

Thus in PNET 5 quality control (QC) of the radiation technique is considered a fundamental and mandatory component of the study. QC will be principally performed prior to the start of radiotherapy.

General Organisation of Radiotherapy QC

- Radiotherapy QC will be organised and undertaken on a National basis. This should include a procedure to reproduce and check target volumes and dose prescription.
- Each National Group will appoint a National Radiotherapy Coordinator
- The National Radiotherapy QC Coordinator will work in close co-operation with two to three named radiotherapy colleagues forming the National Radiotherapy QC panel. This will ensure the constant availability of a QC assessor without delay. Submitted films will be assessed and returned (if originals were submitted) within 72 hours following receipt. Otherwise the submitting center will be informed via fax or e-mail of the QC assessment result. Once the individual clinician submitting the case has received either by e-mail or fax a copy of the QA review highlighting a major deviation detected he/she will be responsible for addressing the recommendation of the QA review in the final treatment plan delivered.
- Submission of planning documentation – The use of DICOM RT compatible data entailing the treatment plan, conventional imaging or computer generated dose distributions may vary from country to country. Each national group will decide on the most appropriate way of submitting films and plans (i.e. either electronically or via courier). Guidance can be obtained from the National Radiotherapy Coordinator.

- Radiotherapy QC committees will meet three times a year. They will review the current status of compliance with the aim of prospective QC control and review films or scans submitted during the last 4 months. They will provide an annual report that will include any targeting deviations. This report will be sent to the National Study Coordinator, the International Radiotherapy Coordinator and the International Data Centre.
- Countries who do not wish to set up their own QC panel should at the start of the study identify the QC panel of a National Group of their choice, which - if agreed - will provide the prospective QC for them.

Copies of the following imaging for treatment planning (e.g. simulator films, digitally reconstructed radiographs –DRR’s-) should be sent to the National Radiotherapy QC panel (preferably by electronic transfer of digitised images):

1. Whole brain field
2. Spinal field/fields

Any targeting deviations will be defined as either minor or major (Carrie et al. IJROBP 1999;45: 435– 439).

The deviation is defined as a margin between the field edge and the CTV of less than 5 mm for the cribriform fossa and less than 10 mm for field edges elsewhere within the whole brain.

For the cribriform fossa a minor deviation is defined as a margin of 3-5 mm and a major deviation a margin of less than 3 mm.

For the other regions a minor deviation is defined as a margin of 5-10 mm and a major deviation a margin of less than 5 mm. For details see radiotherapy data forms.

For all other cases the full planning DCOM-RT dataset will be made available to the national QC panel who will review it in accordance with the designed QA CRF.

QC review of posterior fossa and tumour bed

QC review of posterior fossa and tumour bed target volumes will be offered by the national RT QC committees.

- Radiotherapy QC of the tumour bed component is not mandatory unless the patient experiences a posterior fossa relapse (see below) in which case a retrospective QC review is mandatory.
- Some National groups may, however, wish to conduct prospective QC of tumour bed radiotherapy. If such prospective QC is undertaken, then the National Radiotherapy QC panel will be responsible for determining the criteria by which posterior fossa treatment techniques are evaluated.
- All patients who relapse within the posterior fossa either alone or in combination with other sites will undergo a separate radiotherapy QC. Treating centers will be requested to provide the documentation of their posterior fossa and/or tumour bed boost together with the diagnostic imaging at diagnosis and relapse within 3 months from the reported time of relapse. The radiotherapy QC panel, in collaboration with appropriate neuro-radiologists if indicated, will determine the exact site of relapse as a function of the irradiation volume.

11.2.9. Treatment Modifications due to Haematological Toxicity

In all patients blood cell count should be controlled twice a week during radiotherapy (3 times per week, if carboplatin is administered; see guidelines on G-CSF administration in chapter 11.3.2.). Treatment will not be interrupted for anaemia, leucopaenia or thrombocytopaenia unless life threatening. Blood product or growth factor support should be instituted according to institutional guidelines or according to protocol recommendations. Irradiated blood products should be used at all times. Transfusions are recommended when the haemoglobin levels fall below 10 g/l. Platelets should be transfused as clinically indicated, or when counts are $\leq 25 \times 10^9$. In case of low absolute neutrophil count ($\leq 0.5 \times 10^9$) growth factors should be considered and given preferably during the weekends (see chapter 11.3.2.). Any treatment interruption should be compensated according to national or institutional policies.

11.3. Chemotherapy

All chemotherapy doses given are based on Body Surface Area (BSA) and calculation of reference values in MARVIN database use the Mosteller formula:

$$S \text{ (surface) [m}^2\text{]} = \sqrt{(L * M)/3600}$$

L = length [cm]

M = weight [kg]

There will be no dose adjustment for cachexic or obese patients.

11.3.1. Carboplatin concomitant to Radiotherapy (PNET5 MB-SR only)

All patients included in the PNET 5 MB - SR study will be randomized to either radiation therapy only or to radiation therapy and **carboplatin at 35 mg/m²/day intravenously (IV) over 15-60 minutes, 5 times a week (Monday through Friday), 1-4 hours before radiation, for 6 weeks (30 applications=total dose)**. Note that vincristine will not be given during radiotherapy in either arm. If radiation treatment is not given, carboplatin should not be given either. If a dose of carboplatin is administered and radiation therapy is not applied due to problems such as sedation, the carboplatin dose should not be made up, i.e. no more than 30 doses of carboplatin should be administered. The weight of the patient should be measured and documented on a weekly basis to allow carboplatin dose adjustments due to weight changes.

Recommendations on G-CSF administration

Generally we recommend the use of G-CSF over the weekends and only in cases with severe and significant neutropenia during the week (i.e. in parallel with radiotherapy and chemotherapy).

Complete blood counts will be obtained every Monday, Wednesday, and Friday. If absolute neutrophil count is $\leq 1000/\mu\text{L}$ on any Friday, G-CSF will be administered subcutaneously or IV on Friday, Saturday, and Sunday. G-CSF should not be given until after the radiation treatment on Friday has been delivered. If the absolute neutrophil count is $>1000/\mu\text{L}$, no G-CSF will be administered that weekend. On any Monday or Wednesday, if the absolute neutrophil count is $<500/\mu\text{L}$, G-CSF will be administered on that day and the following day after the radiation treatment. If the absolute neutrophil count is $\geq 500/\mu\text{L}$ on Monday or Wednesday, G-CSF will not be administered (see Table below). Family members should be taught to administer G-CSF. G-CSF guidelines are tabulated below:

Guidelines for G-CSF administration during radiotherapy

DAY	CBC with diff, plts	ANC 1000-500	ANC < 500
Friday	x	5 mcg/kg/day Fri., Sat., Sun.	5 mcg/kg/day Fri., Sat., Sun.
Monday	x	No G-CSF	5 mcg/kg/day Mon., Tues.
Wednesday	x	No G-CSF	5 mcg/kg/day Wed., Thur.

There is to be no dose escalation of G-CSF for neutropenia alone. In the event of a potentially life-threatening infection with neutropenia, G-CSF may be increased to 5 mcg/kg 2x/day.

Significant myelosuppression is likely to occur, particularly during the last two-three weeks of radiation. **However, radiation and carboplatin treatment should not be withheld for myelosuppression alone. Radiation should be continued even if the patient is hospitalized with fever and neutropenia as long as the patient is clinically stable. Administration of the boost should not be substituted for craniospinal radiation in the face of low counts.**

Non-hematological toxicities during radiotherapy with concomitant carboplatin treatment

The most common non-hematological toxicity encountered for radiochemotherapy containing carboplatin treatment in COG 99701 was esophagitis, followed by loss of skin integrity, and nausea / vomiting. However, interruptions of radiochemotherapy due to these reasons were uncommon, therefore no specific recommendations for interruptions due to non-hematological toxicities are defined within PNET 5 MB - SR. Please contact coordinator in any case of severe non-hematological toxicity which might necessitate treatment interruption.

11.3.2. Investigations before the Maintenance Chemotherapy Phase

The following investigations should be performed just before 6 weeks after the end of radiotherapy:

- Clinical exam with neurological exam.
- Cranial MRI with and without contrast injection.
- MRI of spine to include visualisation with complete visualisation of the dural sac.
- Audiology – Pure Tone Audiometry, air conduction if needed combined with tympanogram, or bone conduction.
- Full blood count.
- Blood biochemistry – electrolytes (ionogram), urea, creatinine, ALT, AST, alkaline phosphatase, bilirubin, albumin, magnesium, calcium, phosphate.
- Glomerular filtration rate - by clearance of radioisotope or creatinine clearance.

11.3.3. Maintenance Chemotherapy Phase

11.3.3.A. Summary of the Chemotherapy Regimen (PNET 5 MB - LR)

Maintenance-chemotherapy starts 6 weeks after the end of radiotherapy

- 6 cycles in the order of A-B-A-B-A-B:

A (cycles 1, 3, 5): Cisplatin 70 mg/m² day 1, CCNU 75 mg/m² day 1, vincristine 1.5 mg/m² days 1, 8 and 15

B (cycles 2, 4, 6): Cyclophosphamide (1 x 1000 mg/m² days 1-2), vincristine 1.5 mg/m² (day 1)

Interval after cycle A: 6 weeks, after cycles B: 3 weeks => total duration: 27 weeks

See Appendix C.1. Treatment Overview PNET 5 MB – LR

11.3.3.B. Summary of the Chemotherapy Regimen (PNET 5 MB - SR)

Maintenance-chemotherapy starts 6 weeks after the end of radiotherapy

- 8 cycles in the order of A-B-A-B-A-B-A-B:

A (cycles 1, 3, 5, 7): Cisplatin 70 mg/m² day 1, CCNU 75 mg/m² day 1, vincristine 1.5 mg/m² days 1, 8 and 15

B (cycles 2, 4, 6, 8): Cyclophosphamide (1 x 1000 mg/m²/d days 1-2), vincristine 1.5 mg/m² (day 1)

Interval after cycle A: 6 weeks, after cycles B: 3 weeks => total duration: 36 weeks.

See Appendix C.2. Treatment Overview_ PNET 5 MB - SR

11.3.4. Investigations Before Each Course of Chemotherapy

- a) Clinical exam with neurological exam.
- b) Full blood count.
- c) Blood biochemistry – electrolytes (ionogram), urea, creatinine, ALT, AST, alkaline phosphatase, bilirubin, albumin, magnesium, calcium, phosphate, creatinine).

11.3.5. Investigations Before Alternate Courses of Chemotherapy

All of the above plus:

- a) Audiology (mandatory before cisplatin or carboplatin containing courses) – Pure Tone Audiometry, air conduction if needed combined with tympanogram, or bone conduction.
- b) Glomerular filtration rate (GFR) estimated according to local standards before cisplatin containing courses

Requirements for start of the courses are described in the chapter 11.3.7. Modification of Dose of Chemotherapy – Regimen A, page 87, and chapter 11.3.9. Modification of Dose of Chemotherapy – Regimen B, page 90, respectively for specific requirements for start of chemotherapy.

11.3.6. Administration of Chemotherapy – Regimen A

Chemotherapy for Regimen A consists of cisplatin, CCNU and vincristine, at the following doses:

- **Cisplatin:** 70 mg/m² intravenously (6 hour infusion) - day 1
- **CCNU (lomustine):** 75 mg/ m² orally - day 1 (Whenever possible patients should be encouraged to swallow the capsules whole, without chewing or crushing. If this is not possible capsules may be opened and the content administered according to local practice. Mixtures of lomustine in water are unstable and should be administered immediately after preparation.)
- **VCR (vincristine):** 1.5 mg/m² intravenously (max. dose 2 mg) - day 1, 8 and 15 (VCR may be given as a bolus or short infusion as necessary to comply with local or national guidelines to prevent inadvertent intrathecal administration)

In the context of a multicentre international study, it is appreciated that different national groups and individual centres have varying but well established methods of administering cisplatin.

The following considerations are, however, considered mandatory for the PNET 5 MB - LR and PNET 5 MB - SR studies:

- Cisplatin to be given as an infusion over 6 hours
- Hyperhydration to be used to maintain an adequate urine output
- The use of mannitol to enhance urine output
- The addition of calcium, magnesium and potassium to hydration fluids
- The use of 5HT3 antagonists for anti-emesis
- Careful monitoring of urine output with appropriate guidelines for treatment of insufficient urine output

Administration of carboplatin (if indicated as substitute for cisplatin during maintenance chemotherapy)

Carboplatin 400 mg/m² is to be given as a 1-hour infusion.

The choice of fluid for administration and any pre- or post-chemotherapy fluids will be at the discretion of the centre/group.

11.3.7. Modification of Dose of Chemotherapy – Regimen A

The following guidelines on the monitoring of toxicity of chemotherapy in Regimen A and modification of the dose of chemotherapy do not replace individual responsibility for patient care!

For additional advice, contact the national coordinator.

Before each course of chemotherapy, the patient's overall clinical status should be good.

Haematology

A full blood count (FBC) should be performed at least every 2 weeks after the start of each course of chemotherapy.

Before each course:

WBC $< 2 \times 10^9/L$ **or**
Neutrophils $< 0.5 \times 10^9/L$ **or**
Platelets $< 100 \times 10^9/L$

Delay chemotherapy for at least one week.

**Platelet/WBC recovery
delays therapy > 2 weeks**

Omit CCNU for next course and reduce CCNU to 50mg/m^2 in all subsequent courses.

If further episode

Omit CCNU for next and all subsequent courses (give full dose cisplatin).

Nadir after course:

WBC $< 0.5 \times 10^9/L$ **or**
Neutrophils $< 0.05 \times 10^9/L$
and after episode of neutropaenic fever

Reduce CCNU to 50mg/m^2 in the next and all subsequent courses.

**If further episode
(with or without G-CSF administration)**

Reduce cisplatin to 50mg/m^2 in the next and all subsequent courses.

Platelets $< 30 \times 10^9/L$

Reduce CCNU to 50mg/m^2 in the next and all subsequent courses.

If further episode

Omit CCNU for next and all subsequent courses (give full dose cisplatin).

Neurotoxicity of Vincristine

Epileptic seizure **or**
Ileus

Stop vincristine in this course and reduce vincristine to 1mg/m^2 in the next course.

After recovery

Aim to give vincristine at 100% doses as tolerated

**Significant dysaesthesia, muscle weakness
or abdominal pain**

Omit vincristine until recovery

After recovery

Aim to give vincristine at 100% doses as tolerated

Nephrotoxicity

- Nephrotoxicity is a major toxicity concern with cisplatin. Both glomerular and tubular toxicity must be monitored during treatment with cisplatin.
- Dose modification is based on glomerular toxicity i.e. a reduction in Glomerular Filtration Rate (GFR).

- In the context of a multicentre international study, it is appreciated that different national groups and individual centres have varying but well-established methods of measuring or estimating GFR. These include methods based on blood clearance of radioisotope, e.g. MAG3 and clearance of ^{51}Cr EDTA or $^{99\text{m}}\text{Tc}$ DTPA, estimation of creatinine clearance from the plasma creatinine level (e.g. using the Schwartz formula) or by direct measurement of urinary creatinine clearance.
- Any well established method of estimating GFR as detailed above may be used prior to chemotherapy.
- The estimation of GFR must be performed before every cisplatin containing course (course A).

Serum creatinine > 1.2 mg/dL (100μM)	or	Delay chemotherapy for 1 week.
> 1.5 ULN	or	
GFR/Creatinine clearance < 80 ml/min per 1.73 m²		

If no recovery	Perform estimation of GFR by clearance of radioisotope.
-----------------------	---------------------------------------------------------

Isotope GFR \geq 60 and < 80 ml/min per 1.73 m²	Use carboplatin 400 mg/m ² instead of cisplatin for next course.
	Perform estimation of GFR by clearance of radioisotope before next course.

Isotope GFR < 60 ml/min per 1.73m²	Omit any platinum for next course.
	Perform estimation of GFR by clearance of radioisotope before next course.

Ototoxicity

Due to the ototoxic potential of the platin analogues, a Pure Tone Audiometry is mandatory before each cisplatin (or carboplatin) containing course. It should be performed either by air conduction, if necessary combined with a tympanogram to exclude problems with air conduction, or by bone conduction.

Dose modification of cisplatin in the event of ototoxicity is based on the system used in the HIT 91, HIT 2000 and PNET 4 studies, as follows:

(Note: dose modification is performed based on the highest grade, i.e. the “worst ear”).

<u>Hearing – PTA</u>	<u>Dose Modification</u>
< 16 dB at 1000-3000 Hz or \leq 40 dB at 4000-8000 Hz	None
16-30 dB at 1000-3000 Hz or > 40 dB at 4000-8000 Hz	Substitute carboplatin 400 mg/m ² for cisplatin
> 30 dB at 1000-3000 Hz	Omit any platinum.

Body Weight

A significant amount of patients will require feeding via nasogastric tube or gastostomy. Consider supplemental feeding if nutrition is becoming compromised.

Loss of body weight greater than 20 % compared to body weight at the end of radiotherapy (or earlier if long term corticosteroids were needed)

Reduce CCNU in next course to 50 mg/m²

If further loss of body weight

Omit CCNU in all subsequent courses

11.3.8. Administration of Chemotherapy – Regimen B

Chemotherapy for Regimen B consists of cyclophosphamide and vincristine at the following doses:

- **cyclophosphamide**: 1x 1000 mg/m²/d given as IV infusion, over one hour, once daily, days 1-2
- **vincristine**: 1.5 mg/m² intravenously (max. dose 2 mg) - day 1 (VCR may be given as a bolus or short infusion as necessary to comply with local or national guidelines to prevent inadvertent intrathecal administration)
- **MESNA**: The use of MESNA to reduce the risk of urothelial toxicity is at the discretion of local centres, who may follow their established practice. If gross hematuria occurs on Day 1 MESNA should be increased in the first instance if previously administered, or added if not previously given. Cyclophosphamide may be omitted on day 2 at the discretion of the treating physician. In the following course MESNA should be given in a dose of at least 120% of the dose of cyclophosphamide with hyper-hydration. The dose of cyclophosphamide may be halved (500 mg/m²) on both days 1 & 2 if this is considered necessary. If well tolerated the standard dose should be administered in subsequent courses with MESNA and hyper-hydration.

Recommendation: 250mg/m² intravenously before first cyclophosphamide infusion. MESNA 750 mg/m²/24 hour, day 1-2

11.3.9. Modification of Dose of Chemotherapy – Regimen B

The following guidelines on modification of the dose of chemotherapy in Regimen B do not replace individual responsibility for patient care!

Before each course:

WBC	< 2 x 10⁹/L	or	Delay chemotherapy for at least one week.
Neutrophils	< 0.5 x 10⁹/L	or	
Platelets	< 80 x 10⁹/L		

Nadir after course:

WBC	< 0.5 x 10⁹/L	or	Consider G-CSF application after following cycles
Neutrophils	< 0.05 x 10⁹/L		

and after episode of neutropenic fever.

If further episode
(with or without G-CSF administration)

Do not administer cyclophosphamide on day 2

Nephrotoxicity:

If there is a known renal toxicity due to previous platin chemotherapy, renal function should be assessed prior to cyclophosphamide.

Contact national coordinator in case of $\text{GFR} < 60 \text{ ml/min per } 1.73\text{m}^2$

Bladder Injury

If gross hematuria occurs on day 1 omit cyclophosphamide application on day 2. Give half dose on both days ($2 \times 500 \text{ mg/m}^2/\text{d}$) in the following course and if well tolerated use normal dose again afterwards.

The use of MESNA to reduce the risk of urothelial toxicity is at the discretion of local centres, who may follow their established practice. If gross hematuria occurs on Day 1 MESNA should be increased in the first instance if previously administered, or added if not previously given. Cyclophosphamide may be omitted on day 2 at the discretion of the treating physician. In the following course MESNA should be given in a dose of at least 120% of the dose of cyclophosphamide with hyper-hydration. The dose of cyclophosphamide may be halved (500 mg/m^2) on both days 1 & 2 if this is considered necessary. If well tolerated the standard dose should be administered in subsequent courses with MESNA and hyper-hydration.

11.3.10. Investigations after Three and Six Courses of Chemotherapy and After Final Course of Chemotherapy

- a) Cranial MRI with and without contrast injection.
- b) Spinal MRI with complete visualization of the dural sac.

11.4. Description of investigational medicinal products

All of the above named drugs are defined as investigational medicinal products (IMPs): Carboplatin, Cisplatin, Vincristine, CCNU, and Cyclophosphamide.

The use of these drugs is current standard of care for paediatric patients with medulloblastoma, (Kortmann, Kuhl et al. 2000; Taylor, Bailey et al. 2003; Packer, Gajjar et al. 2006; Jakacki, Burger et al. 2012; Lannering, Rutkowski et al. 2012) (see also introduction section) and the design of the two study arms is experimental only regarding to the treatment schedule and the cumulative dosing and not in respect of the use of the substances.

Details on administration and dose modifications are given in chapter 11.

All of these drugs are licensed medicinal products which are commercially available within the EU. Therefore labelling is not required.

Information Sheets for Health Professionals (SmPCs in the UK) are included in appendix K of the protocol.

12. Summary of Follow-up

12.1. Follow-up during Treatment

For estimation of efficacy of treatment:

- **Cranial MRI with and without contrast injection**
- **Spinal MRI**

should be performed before the start of maintenance chemotherapy (see chapter 11.3.2. Investigations before the Maintenance Chemotherapy Phase, page 85), and after three, and six courses of maintenance chemotherapy (see chapter 11.3.10. Investigations after Three and Six Courses of Chemotherapy and After Final Course of Chemotherapy, page 91).

Monitoring for therapy associated toxicity should be performed according to the recommendations given in the chapters 11.3.4. Investigations Before Each Course of Chemotherapy, page 86, 11.3.7. Modification of Dose of Chemotherapy – Regimen A, page 87, and 11.3.9. Modification of Dose of Chemotherapy – Regimen B, page 90, and the respective criteria for dose modification should be applied.

12.2. Evaluations at the end of treatment

For estimation of efficacy of treatment a **cranial MRI with and without contrast injection** should be performed about 6 weeks after the last cycle of maintenance chemotherapy.

Monitoring for therapy associated toxicities should include:

- **Audiology– Pure Tone Audiometry, air conduction if needed combined with tympanogram, or bone conduction.**
- **Glomerular filtration rate (GFR)**
- **Auxiology and endocrinological evaluations**
- **Additional individual evaluations, as due to clinical indication (thorough clinical and full neurological examination always necessary)**

12.3. Post-treatment Follow-up

For estimation of efficacy of treatment, **cranial MRI with and without contrast injection** should be performed every 4 months during the first year after treatment, at least every 6 months during the second year after treatment, and at least every year until the 5th year after treatment.

In any case of suspected or proven relapse a complete staging including spinal MRI and possibly also CSF cytology should be performed.

After 5 years after end of treatment, cranial MRI should be performed at clinical indication / suspicion of relapse, or according to national practice. (see Table below)

As disease and therapy related factors may lead to chronic, multi-system late effects, long term follow-up through a specialized, multidisciplinary team is mandatory.

A thorough **clinical and full neurological examination** is the mainstay of patient care and should be used for the selection of appropriate additional investigations.

Hearing function should be evaluated at least 2 years and 5 years after diagnosis, as it can worsen after the end of treatment. It should always be done by pure tone audiometry (air conduction if needed

combined with tympanogram, or bone conduction). The respective grading for trial documentation is described in chapter 9.4.2. Audiology, page 72.

Endocrinological function should be evaluated at least once per year. Because of the high prevalence of growth failure and/or GH insufficiency at 2 years after diagnosis, and the need to standardize detection and treatment of any hormonal dysfunction, we recommend referral to a paediatric endocrinologist or paediatrician with endocrine expertise by 2 years after diagnosis, or in the presence of any of the biochemical or auxological criteria below.

A. Biochemical criteria for endocrine referral:

- Elevated TSH, and/or low fT4.
(Thyroxine treatment [at about 100ug/m²] to maintain TSH in normal range and avoid its carcinogenicity in the irradiated thyroid gland).
- Elevated LH and FSH pre- or post-pubertally, +/- low pubertal estradiol or testosterone according to reference for age and sex
- Confirming growth hormone disturbance (likely at 2-5 years) should be done in an endocrine department familiar with the hazards and interpretation of these tests.

B. Auxological criteria for mandatory endocrine referral and investigation:

- Less than 4cm annual increment in height at any age.
- Less than 8 cm annual increment in puberty spurt (testes 10-12ml or breast buds)
- Sustained growth at the expense of weight gain and/or early puberty
- Early Puberty onset (breast buds < 9y, female; 4ml testes <10y male)
- Delayed Pubertal onset (>12 y in female, > 13 y in male)
- Pubertal Arrest (no pubertal progress, according to Tanner, in one year).
- Secondary amenorrhoea of > 3 months, or primary amenorrhoea after 13.5 y.

Renal function should be evaluated at least once per year, and should include measurement of GFR and tubular function.

Neurocognitive impairments should be evaluated according to local / national standards. For trial documentation a set of patient and parent rated questionnaires are to be filled out 2 years, and 5 years after diagnosis (i.e. 1 and 4 years after end of therapy), as well as at 18 years of age.

The following evaluations should be performed for post treatment follow-up

	1. year after treatment	2. year after treatment	3-5. year after treatment	> 5 years after treatment
Clinical / Neurological evaluation	Every 4 month	At least every 6 month	At least Every year	
Cranial MRI			At least every year	at suspicion
Audiometry	2 and 5 years after diagnosis			individually
Auxiology/ Endocrinology	Refer to endocrinology by 2 years- then at least 6monthly			6monthly to 18years (adult) then 1-2yearly
Renal function	At least 1/year			individually
QoL	2 and 5 years after diagnosis and at 18 years of age			None after 18years
additional individual evaluations, as due to clinical indication				

Additional individual evaluations should be performed as due to clinical indication. Physicians should also take into account screening for the possibility of **secondary neoplasm** (eg thyroid with 1-2 yearly TSH), which needs to be strictly documented. Possible sub/infertility should also be considered, and appropriate opportunities for reproductive assessment and support should be offered.

In addition to the medical aspects, assistance of the family through a psychological team and direct neuropsychologic diagnostics are highly recommended.

12.4. Documentation of efficacy and Quality of Survival data

After treatment, information on the disease status of the patient should be documented at least once per year. Any disease progress, relapse, death, or secondary neoplasm needs to be documented within 1 month.

Note that any death occurring before 30 days after end of treatment will be regarded as SAE, and need to be documented immediately (see chapter 13, Serious Adverse Events and Toxicity Reporting, page 95)

Study relevant late effects / Quality of survival data should be evaluated and documented 2 years, and 5 years after diagnosis, and at age 18, as described in chapter 9.4. Study on quality of survival (QoS), page 69.

12.5. Transition to Adult Care

As this study includes patients up to 21 years at diagnosis, a relevant number of patients will be transferred to adult care while on post-treatment follow-up. For the evaluation of late effects within this trial it is necessary to make sure that the required follow-up investigations and the respective documentation are maintained.

It is recommended that patients are transitioned to specialized, multidisciplinary teams, as some late effects may only appear after many years and once present may persist life-long, with a need for specialized support (e.g. hormone replacement, neurorehabilitation and career advice, second tumour awareness, fertility advice, and late relapses).

To facilitate assessments and documentation, a patient information sheet on necessary follow-up investigations is provided in the appendix.

13. Serious Adverse Events and Toxicity Reporting

13.1. Toxicity and Toxicity monitoring, Definition of terms

The NCI Common Toxicity Criteria for Adverse Events (CTCAE), version 4 will be used for purposes of toxicity grading (see Appendix F – Common Toxicity Criteria).

Adverse event (AE)

An adverse event is any untoward medical occurrence in a patient administered a medicinal product or medical therapy, which does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavourable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medical therapy, whether or not related to the medical therapy.

In order to monitor the safety of all trial participants throughout their trial participation this definition has been extended to include any treatment provided in the trial (e.g. radiotherapy).

The legal definition does not need extension to untoward medical occurrences before start of trial treatment, because any procedures at that time are standard procedures for these patients and do not subject them to any trial specific additional risk.

Adverse reaction (AR)

An adverse reaction is an AE which is usually judged by the responsible physician as having a reasonable suspected causal relationship to an investigational medicinal product administered in any dose. The definition covers also medication errors and uses outside what is foreseen in the protocol. The definition implies a reasonable possibility of a causal relationship between the event and the investigational medicinal product. This means that there are facts (evidence) or arguments to suggest a causal relationship.

‘No reasonable possibility’ means that firstly the time relationship to drug administration is improbable (with the knowledge at the time), and/or another explanation is more likely.

Unexpected Adverse Reaction (UAR)

An unexpected adverse reaction is an AR the nature or severity of which is not consistent with the applicable drug information. Reports which add significant information on the specificity, increase of occurrence, or severity of a known, already documented serious adverse reaction constitute unexpected reactions. Examples of UARs:

- Unexpected outcome (e.g. fatal) of an expected AR
- Increase in the rate of occurrence of an expected AR, which is judged to be clinically important, is considered as unexpected
- New report of more specific disease (e.g. interstitial nephritis) instead of a labelled, more general AR (such as acute renal failure)

Serious adverse event (SAE) or reaction (SAR)

A serious adverse event (SAE) or serious adverse reaction (SAR) is any untoward medical occurrence or effect that at any dose (see chapter 13.2.):

- Results in death
- Is life-threatening
- Requires inpatient hospitalisation or prolongation of existing hospitalisation
- Results in persistent or significant disability/incapacity
- Is a congenital anomaly/birth defect.

Suspected unexpected serious adverse reaction (SUSAR)

A SUSAR is every suspected adverse reaction which is both unexpected and serious.

Diagram for definition of serious adverse events:

Relatedness to an investigational medicinal product	Expected	Unexpected	Expectedness not applicable
Resonable possibility	SAR	SUSAR	
No reasonable possibility			SAE

13.2. Serious Adverse Events Requiring Immediate Reporting on SAE Form

Any of the following AEs occurring after the first day of protocol defined treatment must be reported, as long as no exception criterion for immediate SAE reporting according to 13.3 is met.

From 30 days after end of trial treatment throughout the follow-up period SAEs only have to be immediately reported on the SAE form as long as the investigator suspects a causal relationship of the SAE to the protocol defined treatment.

- AE which results in death:

Death is an outcome of an AE and must be reported together with the cause of death on the SAE form. Note that **every death occurring from first day of treatment until 30 days after end of treatment** has to be reported on a SAE form irrespective of the cause of death. Later than 30 days after end of trial treatment death has only to be reported on SAE form, as long as the investigator suspects a causal relationship to the protocol defined treatment.

- AE which is life-threatening:

The term “life-threatening” refers to an event where the patient is at **immediate risk of death** at the time of the event (e.g. requires *immediate* intensive care treatment). It does not refer to an event which hypothetically might cause death if it were more severe.

Note that haematological toxicity (CTCAE Grade IV) is expected, and not to be immediately reported as an SAE, although life threatening. All other CTCAE Grade IV toxicities need to be immediately reported as SAE.

- AE requiring hospitalisation or AE requiring prolongation of hospitalisation:

Hospitalisation is defined as at least one overnight admission.

Hospitalisation without underlying adverse event is not an SAE and hence does not require SAE reporting. Examples are:

- Hospitalisation for protocol procedures, e.g. chemotherapy or radiotherapy
- Elective hospitalisation for a pre-existing condition that has not worsened
- Admission to a rehabilitation centre or hospice
- Hospitalisation for social reasons (e.g. due to anxiety but otherwise treatable on an outpatient basis)

Expected side effects of chemotherapy which are listed in the drug information will not be reported immediately on an SAE form unless in the opinion of the responsible physician they unexpectedly prolonged the hospitalisation or required intensive care therapy.

Hospitalisation due to signs and symptoms associated with disease progression are only considered immediately reportable as SAE when outcome leads to death during protocol treatment and for 30 days after the last protocol therapy. (see definitions of exeptions to SAE reporting in 13.3.)

- AE or AR resulting in persistent or significant disability or incapacity:

Disability is defined as a substantial disruption in a person's ability to conduct normal life functions e.g. persistent blindness, deafness.

-A congenital anomaly or birth defect

Pregnancy and its outcome should be reported on an SAE form in order to identify and follow up on outcome of pregnancy and on any congenital abnormalities, also births from fathers under chemotherapy are to be reported on the SAE form. However, pregnancy itself is per definition not serious.

- Other medically important conditions

Medical judgement should be exercised in deciding whether an AE is serious in other situations. Important AEs that are in the opinion of the investigator clinically unexpected and not immediately life-threatening or do not result in death or hospitalisation but may jeopardise the subject or may require intervention to prevent one of the other outcomes listed in the definition above, should also be considered serious and are reportable on an SAE form.

13.3. Protocol-Specific Exceptions to immediate SAE Reporting

The use of all investigational drugs is current standard of care for paediatric patients with medulloblastoma,(Kortmann, Kuhl et al. 2000; Taylor, Bailey et al. 2003; Packer, Gajjar et al. 2006; Jakacki, Burger et al. 2012 Lannering, Rutkowski et al. 2012) (see also introduction section) and the design of the two study arms is experimental only regarding to the treatment schedule and the cumulative dosing and not in respect of the use of the substances. The safety profile of these drugs is well established. Exceptions to immediate SAE reporting obligations for certain expected or anticipated events therefore do not compromise patients' safety.

All SAE which do not require immediate reporting will be documented on the electronic or paper CRF forms. This applies from first day of trial treatment until end of the follow-up period defined by the protocol. The investigators' SAE reporting obligations to the sponsor are thereby fulfilled. All SAE, including those not requiring immediate reporting, are regularly seen by the Data Monitoring and Safety Committee.

The following SAE do not require immediate reporting on the SAE form:

- Expected hospitalisation for procedures such as blood transfusion for haematological toxicity CTCAE°1-4 without complications, antibiotic treatment of neutropenic fever or CTCAE°1-3 infections, nutritional support for weight loss CTCAE°1-3 or other expected toxicity CTCAE°1-3 (e.g. bleeding or haematuria due to thrombopenia) is not to be immediately reported on an SAE form. It will be documented on the respective therapy / toxicity CRF.
- SAE that occur within the follow-up period defined by the protocol, but later than 30 days after end of trial treatment, and for which the investigator does not suspect a causal relationship to the protocol defined treatment is not to be immediately reported on an SAE form. It will be documented on the respective follow-up CRF.
- Hospitalisation due to signs and symptoms of disease progression as long as the outcome does not lead to death during protocol treatment and for 30 days after last protocol therapy is not to be immediately reported on an SAE form. It will be documented on the respective CRF for documentation of relapse / progression.

13.4. Serious Adverse Event Reporting, Assessment and Regulatory Requirements

The investigator is responsible for reporting to the sponsor all SAEs in relation to subjects treated by him in the clinical trial from first day of protocol defined treatment until end of the follow-up phase as defined by the protocol. The investigator does not need to actively monitor subjects for adverse events once the trial has ended.

The investigator has to report any SAE requiring immediate reporting (see 13.2) within 24 hours after knowledge by fax on the SAE form to the Safety Desk, Münster, Germany. Personal data have to be replaced by the MARVIN ID before forwarding any information.

Safety Desk Contact:
University Hospital Münster
Centre for Clinical Trials (ZKS) Münster
Von-Esmarch-Str. 62
48129 Münster
Germany
Phone: +49 (0)251 83 57109
Fax: +49 (0)251 83 57112
Email: mssd@ukmuenster.de

The **local investigator** is responsible for the assessment of seriousness, and relatedness of the SAE to the medical therapy defined in the protocol, and/ or concomitant therapy. The SAE form should be completed with as much information as possible. Where possible, a diagnosis rather than a list of symptoms should be given. The local investigator should not wait for full details before making the initial report. This should include at least the following information: MARVIN ID, description of SAE, seriousness criterion, details about medical therapy, causality assessment, current outcome, and identification of reporting investigator. SAEs must be followed up until the condition resolves or stabilises. Any follow-up information should be reported as soon as possible. The investigator should answer queries concerning an SAE as soon as possible.

The causal relationship to the medical therapy should be judged as follows:

- *Reasonable possibility* = there are facts or arguments to suggest a causal relationship (e.g. a clear or reasonable time sequence to administration of the drug).
- *No reasonable possibility* = clinical event and/or lab abnormality, with an improbable time sequence to drug administration and/or in which other drugs, chemicals or underlying disease provide plausible explanation.

The Safety Desk will document each SAE, check it and query additionally required information. The Safety Desk will immediately inform the Coordinating Investigator. He will reassess the relatedness of the event. He will also assess whether an SAR is expected or unexpected (SUSAR) according to the reference safety information, which is included in the selected Summary of Product Informations for the investigational medicinal products as in the appendix to the protocol. Relevant version is the version at the time of occurrence of the SAE.

The coordinating investigator will also assess whether an SAE might influence the benefit-risk-ratio or might require changes in the conduct of the trial.

Note, that neither a national trial coordinator nor the Coordinating Investigator and/or the sponsor can downgrade a local investigator's causality assessment. If the Coordinating Investigator disagrees that the event is related to the drug, clarification may be sought from the local investigator. If the

international coordinator still disagrees both opinions must be provided with the report. However, up-grading is possible.

In case of a **SUSAR** the Coordinating Investigator together with the Safety Desk is responsible to inform – by submitting an expedited report a.s.a.p. - the national competent authorities, the Ethics Committees, the respective legal EU authorities, the DMSC and the National Coordinators within the time limits specified by law and in accordance with local requirements. These time limits are:

- **7 days in case of death or life-threatening SUSAR, and**
- **15 days otherwise.**

The National Trial Coordinators are responsible for informing all investigators in their countries according to local legal requirements. They are also responsible for providing any support in SUSAR or other safety submissions to their national competent authority or Ethics Committee according to local requirements.

In case of **SAE or SAR** (not SUSAR), the case will be recorded and included within the annual safety / toxicity reports.

The Coordinating Investigator is responsible for the ongoing safety evaluation of the trial. The Safety Desk will inform him immediately about any relevant safety information coming to its knowledge as will the Coordinating Investigator inform the Safety Desk. In case of safety relevant issues (besides SUSAR) which require immediate reporting, the Safety Desk will support the Coordinating Investigator to submit an appropriate report in due time. This includes issues which might materially alter the current benefit-risk assessment of an investigational medicinal product or that would be sufficient to consider changes in the investigational medicinal products administration or in the overall conduct of the trial as well as urgent safety measures to protect the subjects against any immediate hazard.

Annual safety report

According to the legal requirements, annual safety reports (Development Safety Update Reports, DSUR) will be prepared and submitted. The Coordinating Investigator is responsible for providing the updated benefit-risk assessment of the trial for the Annual Safety Report (passages requiring medical assessment) and for the accuracy of the report. The Coordinating Data Centre is responsible for supplying information from the central database. The Safety Desk is responsible for preparing the template, adding the other parts of the annual safety report, finalizing it and distributing it the National Trial Coordinators for submission in a timely manner.

An annual safety report of all study groups will be included in the annual report to the independent Data Monitoring and Safety Committee (DMSC). If the DMSC have any concerns regarding the safety of patients, they will report these concerns to the PNET 5 MB study coordinator, respectively.

13.5. Adverse Event Documentation

All AEs as required for endpoint analyses are to be reported after the respective treatment element on the paper or electronic toxicity CRF.

All immediately reportable SAE are to be reported on the SAE form

All SAE which are exempted from immediate reporting are to be documented on the respective CRFs as described in chapter 13.3..This applies from first day of trial treatment until end of the follow-up period defined by the protocol. The investigators' SAE reporting obligations to the sponsor are thereby fulfilled.

14. Data Management

14.1. Data Collection

In PNET 5 MB, data will be captured by a certified Remote Data Entry (RDE) system, e.g. MARVIN software (Xclinical). Data collection and management will be done by participating institutions, national data centres, and coordinating data centres.

14.1.1. Participating Institutions

As to national policy, patient data might be documented in the participating institutions directly via internet based RDE, or on case record forms (CRFs) which are provided and put into the RDE database by the national data centres.

Completion of the CRF / RDE files may be delegated by the Investigator in each centre to an authorised member of the site research team. In all cases, it remains the responsibility of the Investigator in each centre to ensure that the CRF /RDE file has been completed correctly and that the data recorded are accurate.

14.1.2. National data centres

Data management will be performed by national group data centre. The responsibilities of the national data centres include:

- Validation of eligibility of patient
- Registration of patients
- Randomisations of patients (where appropriate)
- Shipment of documents required at the trial sites (investigator site file, CRFs, etc.)
- Providing information on treatment according to protocol
- According to national policy: Collection of paper CRFs, or data entry in Marvin
- Clarification of implausible or missing data
- Relaying queries to the investigators

14.1.3. Coordinating data centre

The coordinating data centre (CDC) for PNET 5 MB - LR is located at the Institute Curie, France. The coordinating centre for PNET 5 MB - SR is located at the University Medical Center of the University of Hamburg (respective adressess, see chapter 1. Principal Coordinating Investigators , page 13)

Programming of the Marvin software, and management of the electronic data will be supported by the GPOH (German Paediatric Hematology and Oncology) central data management, Hannover, Germany.

The responsibilities of the coordinating data centres include:

- Implementation of randomisation lists within the Marvin System
- Defining plausibility checks
- Generating annual reports for the Independent Data and Safety Monitoring Committee
- Generating annual reports for the Study Committee
- Providing information for the annual safety report
- Interim analysis as defined in the protocol
- Closing of database
- Analysis of the randomised arms
- Preparation of final trial report

14.2. Registration of Patients and Patient Identification

Screening for eligibility:

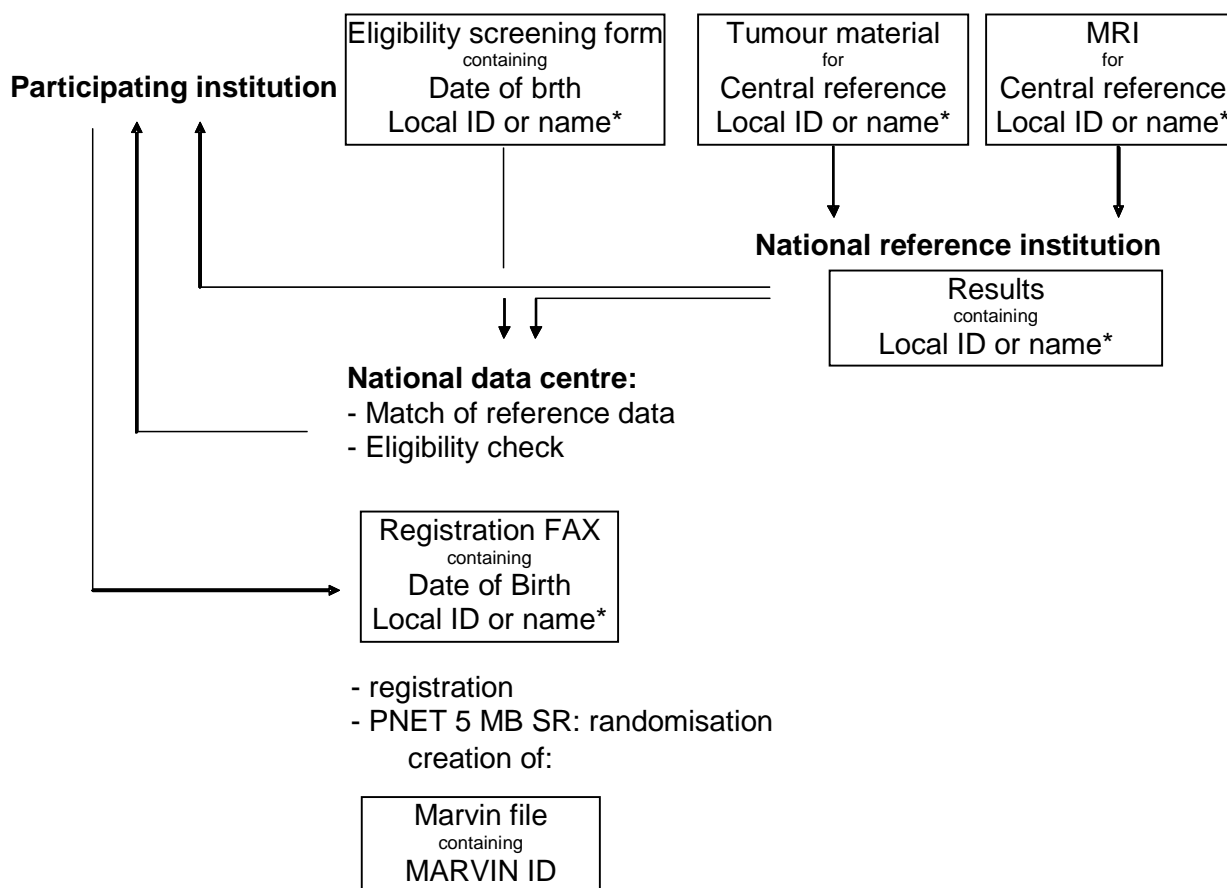
Participating institutions will inform national data centres about the patient, by fax using the eligibility screening form. This form contains information about inclusion and exclusion criteria of the patient, and the sending of material for mandatory central reference evaluations will be recorded. In order to enable the exact calculation of decimal age at first surgery, and verification of inclusion criterion a, (age at diagnosis 3-5 years –depending on the country – and less than 22 years) the birth date of the patient will be recorded.

As central national pathology/ biology / and neuroradiology review are prerequisite for inclusion of patients, tumour material and MRI need to be sent to the respective national reference institutions. Results of these reviews will be reported to the local institution as well as to the national data centre. For sending of these materials and matching to the data at the national data centre, either a local patient ID or the name of the patient can be used, according to the national policy and laws. In the national groups, where patient names are recorded, the authorisation to record the names will be included in the appropriate informed consent form. If local patient IDs are used, the local investigator is obliged to keep a confidential list of patients connecting local patient IDs with the name.

Registration:

After eligibility of the patient is verified in the national data centre with respect to the results of the central review evaluations, a registration fax will be prepared by the national data centre and will be sent to the participating institution for confirmation of signing of the requested informed consents, and in PNET 5 MB - SR the agreement to randomization. If this is confirmed (via the prepared Fax) by the participating institution, the patient will be registered, and in PNET 5 MB - SR be randomized. A patient file will be generated in Marvin on a screening platform by the participating institution. Hereby, a unique patient ID (MARVIN ID) will be generated. Neither names, initials nor birth dates will be documented in Marvin. Identification in the international RDE (Marvin) will solely be based on the MARVIN ID. Decimal age at first surgery will be calculated based on the reported date of birth, but only the decimal age will be recorded in Marvin. Eligibility data will be entered by the national data centre and in the remote data entry system the eligibility criteria will again be automatically checked with the input of registration data. If all entered data match the eligibility criteria, patients will be stratified into PNET 5 MB - LR or PNET 5 MB - SR, according to the WNT subgroup Status. If a patient is stratified as a PNET 5 MB - SR patient, randomisation will be performed on demand in the RDE system, based on the predefined randomisation lists (see chapter 10. Study Entry, page 76).

The result of the stratification / randomisation as well as the MARVIN ID will be reported via Fax on the registration form to the participating institution and to the national QoS coordinator. As to the policy of the national group, further documentation will be done by the participating institution directly into Marvin, or in written form on the CRFs to the national data centre.



Flow of patient data preceding patient inclusion.

* Use of local ID (allocated by local investigator) or patient name, according to national policy

14.3. Collected data

The master versions (in English) of the case record forms (CRFs) are contained in the appendix. All data will be collected by remote data entry, and will be put into the internet based platform <https://gpoh-1.xclinical.net> either directly by the participating institutions, or by the national data centres. The items recorded by RDE are closely based on the CRFs, so that they can be used alternatively for transmission of data.

The following rules for RDE documentation have to be observed:

- Corrections of data are only possible with the explanation of a reason. An audit trail will record the data input and any changes
- Input of data is only possible for authorized persons who will have been trained in the use of the system

The following rules for completing paper CRFs have to be observed:

- CRFs are to be filled in with a black ballpoint pen. Pens or pencils are not allowed.
- Script must be clear and legible.
- Mistakes are to be cancelled by a simple horizontal line and correction is to be written above or next to it. The correction has to be signed and dated.
- Data fields which cannot be completed due to missing information have to be marked and commented.

- CRFs have to be completed in a timely fashion and finally checked and signed by the investigator.
- In case of major corrections and/or missing data, the reason is to be given.
- All requested data fields should be answered completely; this applies also if there is no major change from a previous examination.
- All primary data (e.g. diagnostic findings) which are lodged in the CRF have to be signed and dated by the responsible local investigator.

The following data are regarded as source data:

- All data contained in the patient's medical records.
- Pathology/reference pathology.
- Images.
- Surgical reports.

PLEASE NOTE: If additionally to the CRF / RDE documentation, parts or the individual patient record (as e.g. patient reports) are sent to the national data centre, the patient identification on these papers must be replaced by the local patient ID where applicable. An exception is defined, if national laws and policy allow the recording of the name of the patient, and the patient has given its explicit informed consent on this issue.

14.4. Archiving

Archiving and protection of the RDE data will be performed by XClinical. Data exports will be created for analyses at the national and coordinating data centres.

The archiving of all study relevant documents at the trial site, at the trial offices, and at the coordinating investigator's site will be handled according to national law.

14.5. Confidentiality and Data Protection

Individual data of all participating patients, i.e. data regarding the disease, treatment and follow-up will be collected in the database. If it is according to national laws, and a patient and/or his/her parents/legal guardian have given informed consent, the patient's name and date of birth may be held at the national trial office. This is needed for central treatment planning and in case of direct patient contact. The data will be stored separate from the database and handled confidentially.

All study relevant data will be stored electronically and handled confidentially. For statistical analysis and documentation, patients will be identified only by the central patient number.

The investigators and all members of a trial centre or other persons involved in the trial are obliged to keep study data and information confidential and to grant access only to individuals who are involved in the study. An exception to this rule applies only to representatives of the sponsor or regulatory authorities.

All legal requirements concerning safety, confidentiality and prevention of data loss will be respected. All involved individuals are sworn to secrecy. All databases will be backed up every day. Access to data is strictly limited to authorised persons. Anonymity of data in the scope of biometrical analysis is guaranteed.

15. Quality Management

Quality control of primary diagnostic procedures through central reference evaluations are a central element of the PNET 5 MB trials and are described in chapter 9. Detailed Guidelines for Radiological, Biological, and QoS investigations, page 61.

Quality control of radiotherapy is described in chapter 11.2.8. Quality control of radiotherapy, page 82)

Names and contact data of the respective national reference institutions are given in the appendix B – International Contact Details.

Analyses of the trials will include per-protocol analyses. Per-protocol criteria are mentioned in chapter Statistical Considerations in PNET 5 MB-LR, page 104, and chapter Statistical Considerations in PNET 5 MB-SR, page 105, respectively.

16A. Statistical Considerations in PNET 5 MB - LR

Study Analysis - LR

The primary endpoint is the 3 year-event free survival (EFS) rate, the aim of the study being to achieve a 3 year rate in excess of 80%. Any progression, any relapse, any occurrence of second malignancy, and any death will be considered as an event. EFS will be calculated from date of first operation.

The expected annual accrual in this phase 2 study is around 10-12 patients. With a duration of inclusion of 6 years, 60 patients could be included in this study. Final evaluation would be then possible 9 years after trial initiation.

Results will be analysed according to a modified multistage Fleming procedure, in order to be able to stop early the trial only if too many events are observed. Sixty patients are to be included in the trial. Three interim analyses, the third one being the final one, will take place when 20, then 40 and at last 60 patients will have been included and followed at least 36 months.

Calculations of the boundaries have been performed assuming a 36 months EFS rate less than or equal to 80% as not interesting (null hypothesis) and controlling the power in order to detect in 88% of cases a 36 months EFS rate equal or higher than 91% (error risk β of 11.6% of rejecting wrongly the new protocol, the true event rate being $\geq 91\%$). The error risk α to wrongly accept the protocol is limited to 11.1% (probability of accepting the new protocol with a true EFS rate $\leq 80\%$).

The first 20 patients included in the study will be analysed when their minimum follow-up will be 36 months. The stopping rule will be reached if 5 or more than 5 events are observed during those 36 months (observed EFS rate = 75%).

If less than 5 events are observed, then a second analysis will be performed when further 20 patients have been included and followed at least 36 months. The stopping rule will be reached if 7 or more than 7 events are observed during those 36 months among these 40 patients (observed EFS rate = 82.5%).

Once again if less than 7 events are observed, then a third and final analysis will be performed when further 20 patients have been included and followed at least 36 months. If 9 or more than 9 events have been observed during those 36 months (observed 3 year- EFS $\leq 85\%$), then we will conclude to the inefficacy of the treatment, that is a 3 year EFS equal or lower to 80%. If 8 or less than 8 events have been observed (observed 3 year- EFS $\geq 86.7\%$), then results will be consistent with a 3 year EFS superior to 80%.

In the final analysis, the precision of the observed 3-year event free survival rate will be then estimated by calculating the lower limit of the 89% one-sided exact confidence interval.

When analysing results, if all patients do not have the 36 months required minimum follow-up, then the event free survival rate will be estimated by Kaplan-Meier. This estimation will be compared with the Fleming lower boundary, in order to conclude if it is equal or lower to the corresponding lower rejection boundary,

The design means that inclusion in the trial will continue before the assessment of the primary endpoint of the previous patients. It is then anticipated that by the time the first analysis will be performed, most of the patients will have been already included in the trial.

Group sequential design due to Fleming is regarded in the framework of inverse normal adaptive method. Therefore sample size adjustments are allowed.

Analyses will be performed according to the intention to treat principle.

During the interim and final analyses, the 3 year EFS rate will also be estimated on per-protocol patients for exploratory purposes. Per-protocol patients are defined as the fraction of analysed patients fulfilling inclusion and non inclusion criteria and receiving radiotherapy and chemotherapy protocol-conform, including treatment modifications defined in the protocol. A patient receives radiotherapy protocol conform if radiotherapy starts within 40 days post surgery, if treatment planning is performed via 3D planning system, if dose to CSA is 18.0 Gy, if primary tumour bed dose is 36.0 Gy, and if completion of radiotherapy treatment is within 50 days. A patient receives maintenance chemotherapy protocol-conform, if he received 3 courses of chemotherapy A and 3 courses of chemotherapy B (\pm dose modifications due to toxicities according to defined standard).

Even if exploratory, the results of the the per-protocol analysis will have to be carefully investigated by the DMSC, together with the ITT analysis for decision of closure or continuation of the trial. Kaplan Meier estimation of the 3 year EFS, excluding patients who do not fulfil per-protocol criteria, will be calculated and compared with the Fleming lower boundary. In order to take into account the delayed start of radiotherapy, which can not be checked as an eligibility criteria at the time of inclusion, but may increase the risk of relapse if longer than 40 days, a particular per-protocol analysis excluding only patients for whom this criteria is violated will be performed and considered as very important.

Results of the interim analyses will be reviewed by the DMSC; the decision to close or continue the trial is essentially a medical one, the statistical stopping rules will be used as a guideline to inform this decision.

16B. Statistical Considerations in PNET 5 MB - SR

Study Analysis - SR

The aim of the study is the comparison between radiotherapy and 8 cycles of maintenance chemotherapy with and without concurrent carboplatin during radiotherapy. Patients will be randomly assigned to one of the two therapies. Inclusion and exclusion criteria are described in chapter 7. Eligibility, page 55.

This is a multicenter, prospective, non-blinded, two-arm, randomised, controlled clinical study. The study schedule is determined by the number of events pooled over both therapy groups. The final analysis is intended to be performed after 105 events. An accrual period of 6 years and an additional follow-up time of 4 years are expected to observe 105 events. Randomisation will be performed block by block. The randomisation will be stratified by residual tumour (defined by central MRI review), sex and age group to achieve the following 5 strata:

- Stratum 1: Patients with unequivocal residual tumour ($< 1.5\text{cm}^2$) (expected: 12%)
- Stratum 2: Female patients without unequivocal residual tumour and age ≤ 7 years at diagnosis (expected: 9%)
- Stratum 3: Female patients without unequivocal residual tumour and age > 7 years at diagnosis (expected: 13%)
- Stratum 4: Male patients without unequivocal residual tumour and age ≤ 7 years at diagnosis (expected: 26%)
- Stratum 5: Male patients without unequivocal residual tumour and age > 7 years at diagnosis (expected: 40%)

The randomisation lists will be provided by the reference center for Biostatistics (A. Faldum and R. Kwiecien, Münster, Germany).

The study will follow the principles of “Good Clinical Practice” (GCP) provided by the International Conference on Harmonisation (ICH) as well as the Declaration of Helsinki.

16B.1. Endpoints

Primary endpoint

The primary endpoint is event-free survival (EFS_{OP}) defined as time from first operation to

- a) First progression
- b) First relapse of disease
- c) Occurrence of secondary malignancy
- d) Death of any cause
- e) Or to date of last contact for patients without event (a-d) (censoring variable)

Secondary endpoints

Secondary endpoints are the following:

- 1) **Event-free survival (EFS_{end})** defined as time from end of maintenance chemotherapy to
 - a) first progression
 - b) first relapse of disease
 - c) occurrence of secondary malignancy
 - d) death of any cause
 - e) or to date of last contact for patients without event (a-d) (censoring variable)
- 2) **Overall survival (OS_{OP})** defined as time from first operation to
 - a) death of any cause
 - b) or to date of last contact for patients without event (a) (censoring variable)
- 3) **Overall survival (OS_{end})** defined as time from end of maintenance chemotherapy to
 - a) death of any cause
 - b) or to date of last contact for patients without event (a) (censoring variable)
- 4) **Progression-free survival (PFS_{OP})** defined as time from first operation to
 - a) worsening of disease (PD)
 - b) first relapse of disease
 - c) death of any cause
 - d) or to date of last contact for patients without event (a-b) (censoring variable)

Definition of PD is given in chapter 9.2. recommendations for imaging and central MRI review, page 62.

5) Feasibility of carboplatin treatment

- a) defined as timely delivery of chemotherapy: begin of maintenance chemotherapy within 7 weeks after end of radiotherapy, yes or no
- b) defined as number of interruption days during radiotherapy
- c) defined by toxicities within 8 weeks after end of radiotherapy. The following toxicities will be evaluated:
 - i) Weight change between begin and end of radiotherapy treatment.
 - Grade 0: weight change < 5%
 - Grade 1: $5\% \leq \text{weight change} < 10\%$
 - Grade 2: $10\% \leq \text{weight change} < 20\%$
 - Grade 3: weight change > 20%
 - ii) Dysphagia and/or esophagitis: according to CTC criteria: grade 0-4
 - iii) Ototoxicity according to Chang criteria: grade 0-4 (see chapter 9.4.2. Audiology, page 72)
 - iv) Transfusion requirement: yes vs. no.
If transfusion: application of erythrocytes and/or thrombocytes: yes vs. no.
If application: number of applications.
 - v) Hematotoxicity (PLT, HGB, ANC) according to CTC criteria: grade 0-4 (see Appendix F)
 - vi) Infection according to CTC criteria: grade 0-4 (see Appendix F)
- 6) **Residual tumour** (<1.5 cm²) will be estimated by central MRI review postoperatively
- 7) **Relapse pattern** will be evaluated at the end of therapy and is defined as categorical variable: no relapse, local relapse (within or outside the boost field), distant relapse, local and distant relapse or death of any cause.
- 8) **Indirect measures for quality of survival** will be evaluated with the following standardized questionnaires / scores at 4 defined time points (post surgery/before RT, at two, and at five years after diagnosis, and where appropriate at age 18 years):
 - HUI3 (health status)
 - BRIEF (executive function)
 - SDQ (behavioural outcome)
 - PedsQL (quality of life)
 - QLQ-C30 (quality of life)
 - MEES
 - MFI
- 9) **Ototoxicities** will be categorised
 - a) the extent of ototoxicity based dose modifications of maintenance chemotherapy
 - b) according to SIOP grading criteria (grade 0-4, see chapter 9.4.2. Audiology, page 72). Evaluation will be performed based on an original pure tone audiometry audiogram, to be performed and sent to the national data centre 2 years after diagnosis.
- 10) **Endocrine function:**
 - a) subfertility (as indicated by FSH > 15 IU/l)
 - b) endocrine deficits (need for, time to, and duration of supplementation of TSH, GH, hydrocortisone, GnRH, sex steroids)
 - c) growth retardation (calculated as the difference in height standard deviation score (sds) from diagnose)Endocrine function will be evaluated 2 and 5 years after diagnosis, and at age 18 years.
- 11) **Neurologic function**
 - a) presence (yes/no)/ duration / and therapy (steroids/EVD/ventriculostomy/shunt) of hydrocephalus symptoms (estimated pre and postoperatively)

- b) presence of fossa posterior syndrome, measured by the cerebellar mutism survey (estimated after surgery, before the onset of radiotherapy)
 - c) cerebellar symptoms, measured by the brief ataxia rating scale (postoperative, at two, and at five years after diagnosis, and where appropriate at age 18 years)
 - d) presence of symptoms for brain nerve dysfunction (yes/no/respective nerve nr.) or limb stiffness/weakness
- 12) **Biological tumour markers**
- a) detailed analysis of biological pathways and molecular events established to play a role in medulloblastoma, or that have been shown to have potential prognostic significance in this disease subgroup (e.g. chromosome 17 abnormalities).
 - b) comprehensive genome-wide investigations of novel medulloblastoma defects
- 13) **Leukoencephalopathy (LEP)** grade 0, 1, 2, 3, 4 as defined in chapter 9.2. Recommendations for imaging and central MRI review, page 62.
- 14) **Audit of compliance with protocol defined therapy:**
- a) Administration of radiotherapy will be categorised according to days to start of radiotherapy, total radiotherapy treatment time, primary tumour bed dose, and presence of major uncorrected protocol treatment deviations
 - b) The administration of concomitant carboplatin will be evaluated as number of doses given
 - c) Administration of maintenance chemotherapy will be categorised in three groups: no dose modification (100% of recommended dose), less than 100% of recommended dose but at least 3 courses of chemotherapy A and 3 courses of chemotherapy B (\pm dose modifications due to toxicities according to defined standard), and less than 3 courses of chemotherapy A or less than 3 courses of chemotherapy B, or dose modifications deviant from protocol recommendations.

16B.2. Statistical Analyses

The statistical analyses will be performed by the reference center for biostatistics (Prof. Dr. A. Faldum, Dr. R. Kwiecien, Münster, Germany). The analyses will be performed according to the intention-to-treat principle. A per-protocol analysis will be performed for exploratory purposes.

Per-protocol patients are defined as all patients fulfilling the inclusion and exclusion criteria (see chapter 7. Eligibility, page 55), and who received radiotherapy and chemotherapy protocol-conform.

A patient received radiotherapy conforming to protocol if:

- Radiotherapy was started within 40 days post surgery
- Treatment planning was performed via 3D planning system
- Dose to CSA 23.4 Gy
- Primary tumour bed dose 30.6 Gy
- Completion of radiotherapy treatment within 50 days

A patient received chemotherapy with carboplatin (concomitant to radiotherapy) conforming to protocol, if he/she received all 30 doses.

A patient received maintenance chemotherapy conforming to protocol, if he/she received at least 3 courses of chemotherapy A and 3 courses of chemotherapy B (\pm dose modifications due to toxicities according to defined standard).

For the analysis of toxicities, all patients who received at least 5 days of radiotherapy will be included.

The main question will be tested according to the intention to treat principle with an overall significance level of $\alpha = 5\%$. All p-values corresponding to the secondary questions and the per-protocol analysis will be regarded as explorative.

Missing Values

For the analysis of survival times, missing values will be regarded as censored data. For all other analyses, only patients with available data will be included.

16B.3. Questions of the Study

The following questions will be answered by the study:

Main question

Does the concurrent administration of carboplatin during radiotherapy change the distribution of event-free survival times (EFS_{OP})?

Secondary questions

- 1) Does the concurrent administration of carboplatin during radiotherapy change the distribution of event-free survival times defined as time from end of maintenance chemotherapy (EFS_{end})?
- 2) Does the concurrent administration of carboplatin during radiotherapy change the distribution of overall survival times (OS_{OP})?
- 3) Does the concurrent administration of carboplatin during radiotherapy change the distribution of overall survival times defined as time from end of maintenance chemotherapy (OS_{end})?
- 4) Does the concurrent administration of carboplatin during radiotherapy change the distribution of progression-free survival times (PFS_{OP})?
- 5) **Feasibility of carboplatin treatment:**
 - a) Does the timely delivery of chemotherapy (begin of maintenance chemotherapy within 7 weeks after end of radiotherapy) depend on the therapy?
 - b) Does the number of interruption days during radiotherapy depend on the therapy?
 - c) Does the grade of weight change depend on the therapy?
 - d) Does the grade of dysphagia and/or esophagitis depend on the therapy?
 - e) Does the grade of ototoxicities depend on the therapy?
 - f) Does the transfusion requirement depend on the therapy?
 - g) Does the grade of hematotoxicities depend on the therapy?
 - h) Does the grade of infection depend on the therapy?
- 6) Does the presence of a **postoperative residual tumour < 1.5 cm²** change the distribution of event-free (EFS_{OP}) or overall survival times (OS_{OP})?
- 7) Does the **relapse pattern** depend on the therapy?
- 8) **Indirect measures for quality of survival:**
 - a) Do indirect QoS measures (PedsQL plus Multidimensional Fatigue Scale or QLQ-C30 plus MFI for patients >18 years, HUI3, BRIEF, SDQ, MEES) depend on therapy?
 - b) Are indirect QoS measures (PedsQL plus Multidimensional Fatigue Scale or QLQ-C30 plus MFI for patients >18 years, HUI3, BRIEF, SDQ, MEES), estimated 2 and 5 years after diagnosis, and at age 18, influenced by postoperative neurological function or health status (measured by cerebellar syndrome survey, BARS, hydrocephalus symptoms, brain nerve involvement and HUI)?

- c) Do indirect QoS measures (PedsQL plus Multidimensional Fatigue Scale or QLQ-C30 plus MFI for patients >18 years, HUI3, BRIEF, SDQ, MEES) differ between patients treated within PNET 5 MB - SR and PNET 5 MB - LR?
 - d) Do indirect QoS measures (PedsQL plus Multidimensional Fatigue Scale or QLQ-C30 plus MFI for patients >18 years, HUI3, BRIEF, SDQ, MEES) differ from the equivalent scores obtained in PNET 4 survivors?
- 9) **Ototoxicities**
- a) Does the extent of ototoxicity based dose modifications of maintenance chemotherapy depend on the therapy?
 - b) Does the ototoxicity evaluated 2 years after diagnosis depend on the therapy?
 - c) Does the ototoxicity grade correlate to the total platinum dose and mean cochlear radiation dose
- 10) **Endocrine function:**
- a) Does the frequency of subfertility (estimated by FSH > 15 IU/l) depend on therapy?
 - b) Does the need for, time to, and duration of hormone supplementation (TSH, GH, hydrocortisone, GnRH, sex steroids) depend on therapy?
 - c) Does the growth retardation calculated as the difference in height standard deviation score (sds) from diagnosis depend on therapy?
- 11) **Neurological function:**
- a) What is the incidence of neurological dysfunctions (presence and therapy for hydrocephalus; posterior fossa syndrome; primary and persisting cerebellar symptoms; brain nerve involvement; hemiparesis)?
 - b) Does the frequency of persisting neurologic dysfunctions depend on therapy?
 - c) Does the presence of preoperative or postoperative neurological dysfunctions have an influence on EFS_{OP} and OS_{OP}?
 - d) Does the presence of preoperative or postoperative neurological dysfunctions have an influence on persisting cerebellar symptoms (measured by BARS 2 and 5 years after diagnosis, and at age 18)
 - e) Does the presence of neurological dysfunctions have an influence on indirect QoS measures?
- 12) **Biological tumour markers:**
- a) Identification, investigation and validation of biomarkers (diagnostic, prognostic and predictive)
 - b) Identification, investigation and validation of drug targets with therapeutic potential in this disease subgroup
- 13) Does the grade of **Leukoencephalopathy (LEP)** evaluated 2 years after diagnosis depend on therapy?
- 14) **Audit of compliance with protocol defined therapy:**
- a) Radiotherapy:
 - i) Does the time to start of radiotherapy and duration of radiotherapy change the distribution of event-free survival times (EFS_{OP})?
 - ii) Does the dose administered to the primary tumour bed change the distribution of event-free survival times (EFS_{OP})?
 - iii) Does the presence and severity of major uncorrected radiotherapy treatment deviations change the distribution of event-free survival times (EFS_{OP})?
 - b) Does the dose of concomitant carboplatin change the distribution of event-free survival times (EFS_{OP})?
 - c) Does the dose of maintenance chemotherapy delivered change the distribution of event-free survival times (EFS_{Send})?
- 15) **Multivariable Cox regression analysis:**
- The following variables will be tested for their prognostic relevance on **EFS_{OP} and OS_{OP}**:

- a) Age at diagnosis
- b) Sex (female vs. male)
- c) Histology (desmoplastic/nodular vs. classic medulloblastoma)
- d) Therapy arm (additional carboplatin vs. no standard radiotherapy)
- e) Other potentially influencing factors (from univariate analyses)

16) **Multivariable Cox regression analysis:**

The following variables will be tested for their prognostic relevance on **EFS_{end}** and **OS_{end}**:

- a) Time to start of radiotherapy
- b) Total radiotherapy treatment time
- c) Dose administered to primary tumour bed
- d) Presence of major uncorrected treatment deviations
- e) Therapy arm (radiotherapy with/without carboplatin)
- f) Dose of concomitant carboplatin administered
- g) Dose of maintenance chemotherapy administered
- h) Other potentially influencing factors (from univariate analyses)

16B.4. Null Hypotheses and Statistical Tests

Main question

Null hypothesis: The distribution of **event-free survival times (EFS_{OP})** between patients with and without concurrent administration of carboplatin during radiotherapy does not differ.

This hypothesis will be tested with a two-sided log-rank test on difference. For descriptive reasons the Kaplan-Meier curves for the EFS_{OP}, the quartiles of the EFS_{OP} with 95%-confidence intervals, and the EFS_{OP}-rates at years 1, 3 and 5 will be given for both arms.

Secondary questions

- 1) Null hypothesis: The distribution of **event-free survival times defined as time from end of maintenance chemotherapy (EFS_{end})** between patients with and without concurrent administration of carboplatin during radiotherapy does not differ.
This hypothesis will be tested with a two-sided log-rank test. For descriptive reasons the Kaplan-Meier curves for the EFS_{end}, the quartiles of the EFS_{end} with 95%-confidence intervals, and the EFS_{end}-rates at years 1, 3 and 5 will be illustrated for both arms.
- 2) Null hypothesis: The distribution of **overall survival times (OS_{OP})** between patients with and without concurrent administration of carboplatin during radiotherapy does not differ.
This hypothesis will be tested with a two-sided log-rank test. For descriptive reasons the Kaplan-Meier curves for the OS_{OP}, the quartiles of the OS_{OP} with 95%-confidence intervals and the OS_{OP}-rates at years 1, 3 and 5 will be illustrated for both arms.
- 3) Null hypothesis: The distribution of **overall survival times defined as time from end of maintenance chemotherapy (OS_{end})** between patients with and without concurrent administration of carboplatin during radiotherapy does not differ.
This hypothesis will be tested with a two-sided log-rank test. For descriptive reasons the Kaplan-Meier curves for the OS_{end}, the quartiles of the OS_{end} with 95%-confidence intervals, and the OS_{end}-rates at years 1, 3 and 5 will be illustrated for both arms.
- 4) Null hypothesis: The distribution of **progression-free survival times (PFS_{OP})** between patients with and without concurrent administration of carboplatin during radiotherapy does not differ.
This hypothesis will be tested with a two-sided log-rank test. For descriptive reasons the Kaplan-Meier curves for the PFS_{OP}, the quartiles of the PFS_{OP} with 95%-confidence intervals and the PFS_{OP}-rates at years 1, 3 and 5 will be illustrated for both arms.

5) **Feasibility of carboplatin treatment:**

- a) Null hypothesis: There is no difference in rates of timely delivery of chemotherapy of more than 7 weeks between both arms.

This hypothesis will be tested with a two-sided Fisher's exact test. For descriptive reasons the corresponding cross-table will be given.

- b) Null hypothesis: There is no difference in the number of interruption days during radiotherapy between both arms.

This hypothesis will be tested with a two-sided Mann-Whitney U-test. For descriptive reasons boxplots with the corresponding medians, quartiles, minima and maxima will be illustrated for both arms.

- c) Null hypothesis: There is no difference with regard to increase or decrease of rates in the grade of weight change between both arms.

This hypothesis will be tested with a two-sided Cochran-Armitage test for trend. For descriptive reasons the corresponding cross-tables will be given.

- d) Null hypothesis: There is no difference with regard to increase or decrease of rates in the grade of dysphagia and / or esophagitis between both arms.

This hypothesis will be tested with a two-sided Cochran-Armitage test for trend. For descriptive reasons the corresponding cross-table will be given.

- e) Null hypothesis: There is no difference with regard to increase or decrease of rates in the grade of ototoxicities between both arms.

This hypothesis will be tested with a two-sided Cochran-Armitage test for trend. For descriptive reasons the corresponding cross-table will be given.

- f) Null hypothesis: There is no difference in rates of transfusion requirement between both arms.

This hypothesis will be tested with a two-sided Fisher's exact test. For descriptive reasons the corresponding cross-table will be given.

- g) Null hypothesis: There is no difference with regard to increase or decrease of rates in the grade of hematotoxicities between both arms.

This hypothesis will be tested with a two-sided Cochran-Armitage test for trend. For descriptive reasons the corresponding cross-table will be given.

- h) Null hypothesis: There is no difference with regard to increase or decrease of rates in grade of infection between both arms.

This hypothesis will be tested with a two-sided Cochran-Armitage test for trend. For descriptive reasons the corresponding cross-table will be given.

- 6) The distribution of event-free (overall) survival times (EFS_{OP}, OS_{OP}) between patients with and without **postoperative residual tumour < 1.5 cm²** does not differ.

This hypothesis will be tested with a two-sided log-rank test. For descriptive reasons the Kaplan-Meier curves for the EFS_{OP} and OS_{OP}, the quartiles of the EFS_{OP} and OS_{OP} with 95%-confidence intervals, and the EFS_{OP}- and OS_{OP}-rates at years 1, 3 and 5 will be illustrated for both arms.

- 7) Null hypothesis: The pattern of relapse does not differ between both arms.

This hypothesis will be tested with a two-sided Cochran-Armitage test for trend. For descriptive reasons the corresponding cross-table will be given.

- 8) **Indirect measures for quality of survival:**

- a) Null hypothesis: There is no difference in scores of indirect QoS measures (PedsQL plus Multidimensional Fatigue Scale or QLQ-C30 plus MFI for patients >18 years, HUI3, BRIEF, SDQ, MEES) between both arms.

This hypothesis will be tested with a two-sided Mann-Whitney U-Test. For descriptive reasons boxplots with the corresponding medians, quartiles, minima and maxima will be illustrated for both arms.

- b) Null hypothesis: There is no difference in scores of indirect QoS measures (PedsQL plus Multidimensional Fatigue Scale or QLQ-C30 plus MFI for patients >18 years, HUI3, BRIEF, SDQ, MEES) according to postoperative neurological function and/or health status.

This hypothesis will be tested, fitting a proportional Odds model (ordinal logistic regression)(McCullagh 1980)

- c) Null hypothesis: There is no difference in scores of indirect QoS measures (PedsQL plus Multidimensional Fatigue Scale or QLQ-C30 plus MFI for patients >18 years, HUI3, BRIEF, SDQ, MEES) between patient treated within PNET 5 MB - LR and PNET 5 MB - SR.

This hypothesis will be tested with a two-sided Mann-Whitney U-Test. For descriptive reasons boxplots with the corresponding medians, quartiles, minima and maxima will be illustrated for both arms.

- d) Null hypothesis: There is no difference in scores of indirect QoS measures (PedsQL plus Multidimensional Fatigue Scale or QLQ-C30 plus MFI for patients >18 years, HUI3, BRIEF, SDQ, MEES) between PNET 5 MB - SR patients and the equivalent scores obtained in PNET 4 survivors.

This hypothesis will be tested with a two-sided Mann-Whitney U-Test. For descriptive reasons boxplots with the corresponding medians, quartiles, minima and maxima will be illustrated for both arms.

9) **Ototoxicities**

- a) Null hypothesis: The extent of ototoxicity based dose modifications does not differ with regard to increase or decrease of rates between both arms.

This hypothesis will be tested with a two-sided Cochran-Armitage test for trend. For descriptive reasons the corresponding cross-table will be given.

- b) Null hypothesis: The grade of ototoxicity evaluated 2 years after diagnosis does not differ with regard to increase or decrease of rates between both arms.

This hypothesis will be tested with a two-sided Cochran-Armitage test for trend. For descriptive reasons the corresponding cross-table will be given.

- c) Null hypothesis: The grade of ototoxicity evaluated 2 years after diagnosis correlates with the total platinum dose and mean cochlear dose.

This hypothesis will be tested fitting a proportional Odds model (ordinal logistic regression) (McCullagh 1980)

10) **Endocrine function:**

- a) Null hypothesis: The frequency of subfertility does not differ between both arms.

This hypothesis will be tested with Fisher's exact test (two-sided). For descriptive reasons the corresponding cross-table will be given

- b) Null hypotheses: The need for, time to, and duration of hormone supplementation (TSH, GH, hydrocortisone, GnRH, sex steroids) do not differ between both arms.

These hypotheses will be tested with Fisher's exact test (two-sided), and two sided Mann-Whitney U-Test. For descriptive reasons the corresponding cross-table will be given.

- c) Null hypothesis: The growth retardation does not differ between both arms.

This hypothesis will be tested with two sided Mann-Whitney U-Test. For descriptive reasons boxplots with the corresponding medians, quartiles, minima and maxima will be illustrated for both arms

11) **Neurological function:**

- a) The incidence (score and the frequency of primary neurologic dysfunctions) will be analysed by computing mean, standard deviation, median, interquartile range, and frequency tables with 95% confidence intervals.

Null hypotheses: The score or the frequency of primary neurologic dysfunctions does not differ between both arms. These hypotheses will be tested with two sided Mann-Whitney U-Test, and Fisher's exact test (two-sided). For descriptive reasons boxplots with the corresponding medians, quartiles, minima and maxima will be illustrated for both arms, and the corresponding cross-table will be given. b

- b) The score and the frequency of persisting neurologic dysfunctions will be analysed by computing mean, standard deviation, median, interquartile range, and frequency tables with 95% confidence intervals.

Null hypotheses: The score or the frequency of persisting neurologic dysfunctions does not differ between both arms. These hypotheses will be tested with two sided Mann-Whitney U-Test, and Fisher's exact test (two-sided). For descriptive reasons boxplots with the corresponding medians, quartiles, minima and maxima will be illustrated for both arms, and the corresponding cross-table will be given

- c) Null hypothesis: The distribution of event-free (overall) survival times (EFS_{OP}, OS_{OP}) between patients with and without presence of preoperative or postoperative neurological dysfunctions does not differ.

This hypothesis will be tested with a two-sided log-rank test on difference. For descriptive reasons the Kaplan-Meier curves for the EFS_{OP} and OS_{OP}, the quartiles of the EFS_{OP} and OS_{OP} with 95%-confidence intervals, and the EFS_{OP}- and OS_{OP}-rates at years 1, 3 and 5 will be illustrated for both arms.

- d) Null hypothesis: The presence of preoperative or postoperative neurological dysfunctions does not influence the persisting cerebellar symptoms (measured by BARS 2 and 5 years after diagnosis and at age 18). These hypotheses will be tested with two sided Mann-Whitney U-Test. For descriptive reasons boxplots with the corresponding medians, quartiles, minima and maxima will be illustrated for both arms.
- e) Null hypothesis: The presence of neurological dysfunctions does not influence the indirect QoS measures. This hypothesis will be tested with two-sided Mann-Whitney U-Test. For descriptive reasons boxplots with the corresponding medians, quartiles, minima and maxima will be illustrated for both arms.

- 13) Null hypothesis: The therapy does not influence the grade of **Leukoencephalopathy (LEP)** evaluated 2 years after diagnosis. This hypothesis will be tested, fitting a proportional Odds model (ordinal logistic regression) (McCullagh 1980)

14) **Audit of compliance with protocol defined therapy:**

- a) Administered radiotherapy:

- i) Null hypothesis: The time of start of radiotherapy and duration of radiotherapy does not influence the distribution of event-free survival times (EFS_{OP}). This hypothesis will be tested, fitting a multivariate Cox-Regression model.
 - ii) Null hypothesis: The administered primary tumour bed dose does not influence the distribution of event-free survival times (EFS_{OP}). This hypothesis will be tested, fitting a Cox-Regression model.
 - iii) Null hypothesis: The presence and severity of major uncorrected radiotherapy treatment deviations does not influence the distribution of event-free survival times (EFS_{OP}). This hypothesis will be tested, fitting a multivariate Cox-Regression model.
- b) Null hypothesis: The dose of concomitant carboplatin does not influence the distribution of event-free survival times (EFS_{OP}). This hypothesis will be tested, fitting a Cox-Regression model.
 - c) Null hypothesis: The dose of administered maintenance chemotherapy does not influence the distribution of event-free survival times (EFS_{Send}). This hypothesis will be tested, fitting a Cox-Regression model.

- 15) Null hypothesis: The variables listed in secondary question 15 do not have an influence on EFS_{OP} and OS_{OP}.

This hypothesis will be tested fitting a multivariate Cox-Regression model. Model building will be forward stepwise variable selection and backward stepwise variable selection (inclusion criterion: p-value of the Score test $\leq 5\%$, exclusion criterion: p-value of the likelihood ratio test $\geq 10\%$).

- 16) Null hypothesis: The variables listed in secondary question 16 do not have an influence on EFS_{end} and OS_{end}.

This hypothesis will be tested fitting a multivariate Cox-Regression model. Model building will be forward stepwise variable selection and backward stepwise variable selection (inclusion criterion: p-value of the Score test $\leq 5\%$, exclusion criterion: p-value of the likelihood ratio test $\geq 10\%$).

16B.5. Interim Analyses and Final Analysis

Three analyses are intended to be performed to answer the main question, unless the trial is stopped prematurely. The trial will be terminated after an interim analysis, if the main question can already be answered at this interim analysis.

The criteria for stopping the trial after an interim analysis are given by the inverse normal method corresponding to a 3-step group sequential plan according to Wang & Tsatis with boundary shape parameter of $\Delta = 0.37$, with the possibility to stop the trial in favour of the alternative hypothesis and without the possibility to stop for futility. (Wang and Tsatis 1987) The bounds of the 3-step group sequential design result from a two-sided test, $\alpha=5\%$, power=80% and 3-year-EFS_{OP} rates of 75% and 85% for the two groups, a sample size allocation of 1 and an information rate of 1/5 for the first and 2/5 for the second interim analysis. This Δ minimizes the average sample size under the alternative hypothesis.

After each interim analysis a data dependent sample size calculation may be performed. Then, the accrual period, the observation time, the schedule of the second interim and final analysis (required number of events) and the number of interim analyses can be adapted amongst others.

If the 3-step sequential plan according to Wang and Tsatis described above will not be changed, the first interim analysis will take place after 21 events, the second interim analysis will take place after 42 events and the final analysis will take place after 105 events.

The numbers of events of both therapy arms are added up to evaluate the number of occurred events.

16B.6. Sample Size Calculation

Aim of the trial is to investigate the additional administration of carboplatin during radiotherapy. The 3-year EFS_{OP} for patients with standard therapy is assumed to be 75%, the 3-year EFS_{OP} for patients with additional carboplatin during radiotherapy is assumed to be 10% higher, i.e. 85%. We assume exponential EFS_{OP}-times and a Weibull-distributed drop-out-rate in both groups with a drop-out-rate of 5% after 3 years and a shape parameter of $\gamma = 2.4113$ (assumptions according to the data of the previous trial PNET 4). With a significance level of 5%, an accrual rate of 6 years and a follow-up period of 4 years 299 patients will be needed to observe 105 events in both therapy arms and to obtain a power of 80% with a 3-step group sequential design according to Wang and Tsatis with boundary shape parameter of $\Delta = 0.37$ when using the log-rank test. This corresponds to an accrual rate of 50 patients per year. The number of events was calculated with ADDPLAN 4.0.

16B.7. Stopping rules for Ototoxicities and Death

A comparison of the distributions of grade-III and -IV ototoxicities and treatment associated death between the two therapy arms will be performed with a one-sided Fisher's exact test. A 4-step group sequential design according to Pocock has been chosen.

Ototoxicity will be graded according to Chang criteria.

Treatment associated death is defined as any death which occurs while the patient receives therapy, until 1 month after therapy.

Analyses will be performed after 2, 3, 4 and 5 years unless the study was stopped before.

With an accrual period of 6 years, a follow-up period of 3 years, an accrual rate of 50 patients per year, an ototoxicity rate of 12% for patients with standard therapy, a sample size allocation of 1 and an overall significance level of 5% we will obtain a power of

- a) 15% if the ototoxicity and treatment associated death rate in the experimental arm is 15%
- b) 47% if the ototoxicity and treatment associated death rate in the experimental arm is 20%
- c) 79% if the ototoxicity and treatment associated death rate in the experimental arm is 25%
- d) 95% if the ototoxicity and treatment associated death rate in the experimental arm is 30%

If one of the interim analyses shows a significant difference in the rates of ototoxicities and treatment associated death between the two therapy arms, the DMSC will be informed. They will decide whether the study will be stopped due to ototoxicities.

STOPPING RULES FOR DEATH

A comparison of the distributions of overall survival between both therapy arms will be done with a two-sided log-rank test. A 3-step group sequential design according to Wang and Tsatis has been chosen with boundary shape parameter of $\Delta = 0.28$.

Analyses will be performed after 10, 15 and 20 events occurred unless the trial was stopped before.

With a two-sided test, $\alpha=10\%$, an accrual period of 5 years with no further follow-up, an accrual rate of 50 patients per year, a 5-year-OS_{OP} rate of 92% in the standard arm (estimated according to PNET 4), a sample size allocation of 1 and an information rate of 1/2 for the first and 3/4 for the second interim analysis, we will have a power of:

- a. 59% if the 5-year OS_{OP} rate in the experimental arm is 82% (10% lower than the standard arm)
- b. 79% if the 5-year OS_{OP} rate in the experimental arm is 77% (15% lower than the standard arm)
- c. 91% if the 5-year OS_{OP} rate in the experimental arm is 72% (20% lower than the standard arm)

If one of the interim analyses shows a significant difference in the distribution of overall survival between the two therapy arms the DMSC will be informed. They will decide whether the trial will be stopped due to overall survival.

16B.8. Modifications to the Statistical Design

The design of the study may be changed, if necessary, in the event of important new discoveries. Modifications to the protocol will be made only in form of written amendments and with the agreement of the Study Committee. The respective Ethic Committees have to be informed about the modifications. The patient information has to be changed according to the modifications of the protocol.

If an adaptation of the adaptive group sequential design is necessary – e.g. because of a low recruitment rate – the respective changes of the time points, number of interim analyses, maximal sample size and α -spending function may be done according to the conditional rejection error probability method by Schäfer and Müller.(Schäfer and Müller 2001) The modifications can be done during a planned or unplanned interim analysis on the basis of the observed data collected so far. The corresponding conditional rejection error probability functions are defined by Schäfer.(Schäfer and Müller 2001) If a design change is made the time point, the data file of the trial, all calculations and the description of the new group sequential design have to be recorded in the amendment.

17. Study Duration and End of Study

17A Study Duration PNET 5 MB - LR

The study will be closed after accrual of 60 patients. If the annual accrual rate is 10-12 patients per year as expected, the study duration will be as follows.

Inclusion period: 6 years,
Treatment period: 39 weeks
Follow-up period: 3 years
Total duration of study: 9 years

17B Study Duration PNET 5 MB - SR

The study will be closed after accrual of 300 patients. If the annual accrual rate is 50 patients per year as expected, the study duration will be as follows.

Inclusion period: 6 years
Treatment period: 48 weeks
Follow-up period: 4 years
Total duration of study: 10 years

If the main question can already be answered at an interim analysis, the trial will be terminated after this interim analysis (see 16B.5. Interim Analyses and Final Analysis, page 115). The statistical design might be modified (see chapter 16B.5. Interim Analyses and Final Analysis, page 115)

18. Study Organisational Aspects

18.1. Sponsor

The Sponsor of the PNET 5 MB study is the Universitätsklinikum Hamburg-Eppendorf.

18.2. Study Coordinator

The Study Coordinator for PNET 5 MB - LR is Professor Stefan Rutkowski of the Universitätsklinikum Hamburg-Eppendorf and Professor François Doz of the Institut Curie.

The Study Coordinator for PNET 5 MB - SR is Professor Stefan Rutkowski of the Universitätsklinikum Hamburg-Eppendorf.

18.3. Study Committee

All national coordinators are members of the study committee. They are listed in chapter 1.2. National Trial Coordinators , page 15.

Additionally, each participating working group/discipline will be represented by its coordinator in the study committee. These members are:

Frank Saran (Radiotherapy)

Monika Warmuth Metz (Neuroradiology)

Steve Clifford (Biology)

Torsten Pietsch (Pathology)

Colin Kennedy, (Quality of Survival, Endocrinology, Late Effects)

Matthieu Vinchon (Neurosurgery)

Andreas Faldum and Veronique Mosseri (Statistics)

The study committee will be informed about the progress of the study and approve protocol amendments.

18.4. Study Board

The study board consists of the coordinating investigator, co-investigator, and discipline coordinators as mentioned in 18.3. The study board will oversee and monitor the progress of the study and make suggestions for amendments of the study protocol.

18.5. Data Monitoring and Safety Committee

An independent Data Monitoring and Safety Committee (DMSC) composed of 3 international experts will monitor the progress of the study from an ethical and scientific standpoint. The role of the DMSC will be to review the accrual rate and to examine interim analyses.

Each interim analysis will be reported to the DMSC. The interim analyses will remain confidential. On the basis of the analyses, the DMSC will recommend whether the study can continue, or whether it should be changed or prematurely terminated.

In order to monitor toxicity, the statistician for the study will circulate to the members of the DMSC a report on the toxicity of treatment every 12 months. The DMSC will review the interim toxicity data and any relevant information will be forwarded to each Study Co-ordinator. This procedure is intended to detect and prevent problems of major toxicity.

The DMSC will also be asked to review any major modification to the study proposed by the Co-ordinators prior to its implementation.

Members of the DMSC are:

Eric Bouffet, Neurooncology, Toronto, Canada

Carolyn Freeman, Radiation Oncology, Montreal, Canada

Martin Zimmermann, Biometrics, Hannover, Germany

18.6. Quality control and quality assurance

18.6.1. Monitoring

The National Coordinating Centre is responsible for the organization of an adequate monitoring process in the respective country. It is the responsibility of the National Coordinating Centre to ensure the quality and accuracy of all data submitted for their country. Minimum standard and reporting requirements will be defined in a Monitoring Manual.

18.6.2. Audits

To guarantee that the conduct of the study is in accordance with ICH-GCP and the national laws, the Sponsor or his legal representatives reserves the right to audit selected participating institutions. The auditor will be independent from the staff involved in the proceedings of this clinical trial.

18.6.3. Inspections

Inspections of participating institutions maybe performed by the competent authorities at any time during or after the completion of the clinical trial.

19. Ethical and Regulatory Considerations

19.1. Ethical Considerations

The study will be conducted in accordance with the ethical principles set out by the 18th World Medical Assembly in Helsinki in 1964 and all subsequent amendments made by the World Medical Assemblies, in accordance with European Directive 2001/20/EC and with Good Clinical Practice as set out in ICH-E6.

19.2. Regulatory Considerations

The study will be conducted in compliance with all international laws and regulations pertaining to the conduct of biomedical research and with all national laws and local regulations in effect in the countries in which the study is being performed.

The trial is conducted in accordance with the Declaration of Helsinki, revised version of 2013 (see Appendix G – Ethical Considerations And Declaration Of Helsinki).

The trial is conducted in accordance with the internationally established guidelines on the implementation of Good Clinical Practice (ICH-GCP).

19.3. Independent Ethics Committee/Institutional Review Board

The protocol of the study will be submitted to a duly constituted Independent Ethics Committee or Institutional Review Board (IEC/IRB) for approval in each of the countries in which the study is to be performed. Where required by national laws and local regulations, the Informed Consent Form and Patient Information Sheet, as well as the curriculum vitae of participating investigators will also be submitted to the IEC/IRB.

Any amendments to the protocol will be submitted to the IEC/IRB for approval prior to being implemented, unless over-riding concern for the safety of patients require that the amendment be implemented forthwith. If protocol amendments entail changes to the Informed Consent Form and/or Patient Information Sheet, and where required by national laws and local regulations, the revised versions of these documents will also be submitted to the IEC/IRB for approval prior to being implemented.

19.4. Informed consent

The Investigator (according to applicable regulatory requirements), or a person designated by the Investigator, and under the Investigator's responsibility, should fully inform the legal guardians of the Patient of all pertinent aspects of the Clinical Trial including the written information giving approval/favourable opinion by the Ethics Committee (IRB/IEC). All parents / legal guardians should be informed to the fullest extent possible about the study, in language and terms they are able to understand.

Prior to a patient's participation in the clinical trial, the written Informed Consent Form should be signed, name filled in and personally dated by the patient's legally acceptable representative, and by the person who conducted the informed consent discussion. A copy of the signed and dated written Informed Consent Form will be provided to the legal guardians of patient.

If new safety information results in significant changes in the risk/ benefit assessment, the consent form should be reviewed and updated as necessary.

20. Obligations Regarding Authorisation

20.1. Ethics committee's opinion

The trial will not be commenced until the leading ethics committee has issued a favourable opinion on the trial. Additional trial sites may not commence accepting patients into the trial until the leading ethics committee has issued a favourable opinion and the competent local ethics committee has confirmed the qualification of the clinical investigator(s) and the quality of the trial site.

20.2. Sponsorship

The Sponsor of the international clinical trial in the legal sense as defined in the Directive 2001/20/EC of the European Parliament and of the Council April 2001 is:

The Sponsor transfers its duties for every participating country to an authorised institution by written agreement. This authorised institution will be the National Coordinating Centre for that country. The National Coordinating Centre will fulfil the transferred duties for the sponsor and warrants the compliance with all the statutory provisions relevant for the Sponsor. The Sponsor reserves the right to audit the National Coordinating Centre to control adherence to all legal requirements.

20.3. Competent Health Authority approval

The Sponsor is responsible for registering the study in the EUDRACT database.

The National Coordinating Centre is responsible, on behalf of the Sponsor, for ensuring that every participating institution within that country has gained approval for conducting the clinical trial from an appropriate competent authority. Copies of the approval should be sent to the Sponsor.

Authorisation from the competent authority must be obtained before study initiation.

20.4. Investigators / sponsors responsibilities

The national coordinators of each country will provide insurance or indemnity in accordance with the applicable regulatory requirements for all patients within that country.

Any investigator or co-investigator who signed this protocol agrees to carry out this research in accordance with the protocol approved by the ethic committee, GCP and regulatory requirements.

Study personnel involved in conducting this trial will be qualified by education, training, and experience to perform their respective task(s).

The investigator should provide the Sponsor with his Curriculum Vitae and the co-investigator's ones. The Coordinating Investigator is obliged to observe the following requirements regarding reports and documents to be filed with the federal regulatory authorities and the leading ethics committee:

- Fileable amendments requiring approval or favourable opinion must be submitted.
- Measures taken to eliminate imminent danger must be reported without delay (as soon as possible thereafter an appropriate amendment must be submitted for approval /favourable opinion).
- Any Suspected Unexpected Serious Adverse Reaction (SUSAR) must be reported within legal time limits.
- Any condition requiring re-assessment of the benefit-risk ratio must be reported.
- Annual (safety) reports must be submitted.
- The regular or premature termination of the trial must be reported.
- A summary of the final trial report giving all essential results of the trial must be submitted.

The above reportability requirements also pertain to the authorities of participating member states. The responsibilities regarding information of the national authorities and ethics committees are delegated to the national coordinators. SUSAR reporting obligations to the competent authorities remain with the Coordinating Investigator being supported by the ZKS Münster Safety Desk.

20.5. Insurance

The National Coordinating Centre of participating countries are responsible for the provision of insurance or indemnity to cover the liability of the National Coordinator, Local Principal Investigators, Local Co-investigators and the Sponsor in the respective country, as required by the

directive 2001/20/EC of the European Parliament and of the Council of 4 April 2001 and the corresponding national laws.

Insurance must be obtained before the initiation of the study.

21. Indemnity

Patients will not receive any indemnity or financial compensation for their participation in the study.

22. Publication Policy

Participating centres may publish information relating to their own cases, but agree to allow the PNET 5 MB committee exclusive rights to publish the results of the study in part or in total. All such publications will be presented on behalf of the PNET 5 MB committee and will acknowledge the contribution of the participating centres. Authorship of such publications will recognise those members of the committee, and others, who were involved in the preparation of the data and the manuscript. Authorship and time of publication will be discussed with the full PNET 5 MB committee before preparation of publications (abstracts or manuscripts) and requires the approval of the Study Coordinators (S. Rutkowski for PNET 5 MB – SR, and Francois Doz for PNET 5 MB – LR).

23. Writing Committee

Stefan Rutkowski, Katja von Hoff, Regine Riechers (Univ. Med. Center Hamburg – Eppendorf)

Francois Doz, Veronique Mosseri, Yann De Rycke, Sandra Pelissier (Institut Curie)

Andreas Faldum, Robert Kwiecien (University Münster)

Steve Clifford (Newcastle University)

Frank Saran (Royal Marsden NHS Foundation Trust)

24. References

- Aaronson, N. K., S. Ahmedzai, et al. (1993). "The European Organization for Research and Treatment of Cancer QLQ-C30: a quality-of-life instrument for use in international clinical trials in oncology." *J Natl Cancer Inst* **85**(5): 365-76.
- Adan, L., C. Sainte-Rose, et al. (2000). "Adult height after growth hormone (GH) treatment for GH deficiency due to cranial irradiation." *Med Pediatr Oncol* **34**(1): 14-9.
- Albright, A. L., J. H. Wisoff, et al. (1996). "Effects of medulloblastoma resections on outcome in children: a report from the Children's Cancer Group." *Neurosurgery* **38**(2): 265-71.
- Aldosari, N., S. H. Bigner, et al. (2002). "MYCC and MYCN oncogene amplification in medulloblastoma. A fluorescence in situ hybridization study on paraffin sections from the Children's Oncology Group." *Arch Pathol Lab Med* **126**(5): 540-4.
- Allen, J., J. Siffert, et al. (1999). "A phase I/II study of carboplatin combined with hyperfractionated radiotherapy for brainstem gliomas." *Cancer* **86**(6): 1064-9.
- Allen, J. C., R. Walker, et al. (1987). "Carboplatin and recurrent childhood brain tumors." *J Clin Oncol* **5**(3): 459-63.
- Ashley, D. M., L. Meier, et al. (1996). "Response of recurrent medulloblastoma to low-dose oral etoposide." *J Clin Oncol* **14**(6): 1922-7.
- Avet-Loiseau, H., A. M. Venuat, et al. (1999). "Comparative genomic hybridization detects many recurrent imbalances in central nervous system primitive neuroectodermal tumours in children." *Br J Cancer* **79**(11-12): 1843-7.
- Bacha, D. M., B. Caparros-Sison, et al. (1986). "Phase I study of carboplatin (CBDCA) in children with cancer." *Cancer Treat Rep* **70**(7): 865-9.
- Baeza, N., J. Masuoka, et al. (2003). "AXIN1 mutations but not deletions in cerebellar medulloblastomas." *Oncogene* **22**(4): 632-6.
- Bailey, C. C., A. Gnekow, et al. (1995). "Prospective randomised trial of chemotherapy given before radiotherapy in childhood medulloblastoma. International Society of Paediatric Oncology (SIOP) and the (German) Society of Paediatric Oncology (GPO): SIOP II." *Med Pediatr Oncol* **25**(3): 166-78.
- Barahmani, N., S. Carpentieri, et al. (2009). "Glutathione S-transferase M1 and T1 polymorphisms may predict adverse effects after therapy in children with medulloblastoma." *Neuro Oncol* **11**(3): 292-300.
- Benesch, M., K. Spiegl, et al. (2009). "A scoring system to quantify late effects in children after treatment for medulloblastoma/ependymoma and its correlation with quality of life and neurocognitive functioning." *Childs Nervous System* **25**(2): 173-181.
- Bhatia, S., Y. Yasui, et al. (2003). "High risk of subsequent neoplasms continues with extended follow-up of childhood Hodgkin's disease: report from the Late Effects Study Group." *J Clin Oncol* **21**(23): 4386-94.
- Biegel, J. A., L. B. Rorke, et al. (1989). "Isochromosome 17q in primitive neuroectodermal tumors of the central nervous system." *Genes Chromosomes Cancer* **1**(2): 139-47.
- Bigner, S. H., J. Mark, et al. (1988). "Structural chromosomal abnormalities in human medulloblastoma." *Cancer Genet Cytogenet* **30**(1): 91-101.
- Bjork-Eriksson, T. and B. Glimelius (2005). "The potential of proton beam therapy in paediatric cancer." *Acta Oncol* **44**(8): 871-5.
- Boman, K. K., E. Hoven, et al. (2009). "Health and persistent functional late effects in adult survivors of childhood CNS tumours: a population-based cohort study." *European Journal of Cancer* **45**(14): 2552-2561.
- Boon, K., C. G. Eberhart, et al. (2005). "Genomic amplification of orthodenticle homologue 2 in medulloblastomas." *Cancer Res* **65**(3): 703-7.
- Bouffet, E., J. C. Gentet, et al. (1994). "Metastatic medulloblastoma: the experience of the French Cooperative M7 Group." *Eur J Cancer* **30A**(10): 1478-83.
- Brackett, J., K. Krull, et al. (2010). "Anti-oxidant enzyme polymorphisms and neuropsychological outcomes in medulloblastoma survivors: a report from the Childhood Cancer Survivor Study (CCSS). Abstract from the International Society of Paediatric Oncology SIOP XXXXII Congress Boston, United States October 21-24, 2010." *Pediatr Blood Cancer* **55**: 937.
- Bridges, N. A., D. R. Matthews, et al. (1994). "Changes in gonadotrophin secretion during childhood and puberty." *J Endocrinol* **141**(1): 169-76.

- Broderick, D. K., C. Di, et al. (2004). "Mutations of PIK3CA in anaplastic oligodendrogliomas, high-grade astrocytomas, and medulloblastomas." Cancer Res **64**(15): 5048-50.
- Brown, H. G., J. L. Kepner, et al. (2000). "'Large cell/anaplastic' medulloblastomas: a Pediatric Oncology Group Study." J Neuropathol Exp Neurol **59**(10): 857-65.
- Bull, K. S., H. A. Spoudeas, et al. (2007). "Reduction of health status 7 years after addition of chemotherapy to craniospinal irradiation for medulloblastoma: A follow-up study in PNET 3 trial survivors - On behalf of the CCLG (formerly UKCCSG)." Journal of Clinical Oncology **25**(27): 4239-4245.
- Bull, K. S., H. A. Spoudeas, et al. (2007). "Reduction of health status 7 years after addition of chemotherapy to craniospinal irradiation for medulloblastoma: a follow-up study in PNET 3 trial survivors on behalf of the CCLG (formerly UKCCSG)." J Clin Oncol **25**(27): 4239-45.
- Carrie, C., J. Grill, et al. (2009). "Online quality control, hyperfractionated radiotherapy alone and reduced boost volume for standard risk medulloblastoma: long-term results of MSFOP 98." J Clin Oncol **27**(11): 1879-83.
- Carrie, C., X. Muracciole, et al. (2005). "Conformal radiotherapy, reduced boost volume, hyperfractionated radiotherapy, and online quality control in standard-risk medulloblastoma without chemotherapy: results of the French M-SFOP 98 protocol." Int J Radiat Oncol Biol Phys **63**(3): 711-6.
- Carroll, P. V., E. R. Christ, et al. (1998). "Growth hormone deficiency in adulthood and the effects of growth hormone replacement: a review. Growth Hormone Research Society Scientific Committee." J Clin Endocrinol Metab **83**(2): 382-95.
- Chang, C. H., E. M. Housepian, et al. (1969). "An operative staging system and a megavoltage radiotherapeutic technic for cerebellar medulloblastomas." Radiology **93**(6): 1351-9.
- Chang, K. W. and N. Chinosornvatana (2010). "Practical grading system for evaluating cisplatin ototoxicity in children." J Clin Oncol **28**(10): 1788-95.
- Clevers, H. (2000). "Axin and hepatocellular carcinomas." Nat Genet **24**(3): 206-8.
- Clifford, S. C., M. E. Lusher, et al. (2006). "Wnt/Wingless pathway activation and chromosome 6 loss characterize a distinct molecular sub-group of medulloblastomas associated with a favorable prognosis." Cell Cycle **5**(22): 2666-70.
- Cochran, D. M., T. I. Yock, et al. (2008). "Radiation dose to the lens during craniospinal irradiation-an improvement in proton radiotherapy technique." Int J Radiat Oncol Biol Phys **70**(5): 1336-42.
- Cogen, P. H., L. Daneshvar, et al. (1992). "Involvement of multiple chromosome 17p loci in medulloblastoma tumorigenesis." Am J Hum Genet **50**(3): 584-9.
- Conklin, H., A. Meton, et al. (2010). "COMT polymorphisms and self-ordered search – evidence for genetic protection against cognitive late effects in childhood brain tumour survivors. Abstract from the International Society of Paediatric Oncology SIOP XXXXII Congress Boston, United States October 21–24, 2010" Pediatr Blood Cancer **55**: 939-40.
- Conte, F. A., M. M. Grumbach, et al. (1980). "Correlation of luteinizing hormone-releasing factor-induced luteinizing hormone and follicle-stimulating hormone release from infancy to 19 years with the changing pattern of gonadotropin secretion in agonadal patients: relation to the restraint of puberty." J Clin Endocrinol Metab **50**(1): 163-8.
- Critchley, H. O., L. E. Bath, et al. (2002). "Radiation damage to the uterus -- review of the effects of treatment of childhood cancer." Hum Fertil (Camb) **5**(2): 61-6.
- Dahmen, R. P., A. Koch, et al. (2001). "Deletions of AXIN1, a component of the WNT/wingless pathway, in sporadic medulloblastomas." Cancer Res **61**(19): 7039-43.
- Dardiotis, E., K. N. Fountas, et al. "Genetic association studies in patients with traumatic brain injury." Neurosurg Focus **28**(1): E9.
- del Charco, J. O., T. W. Bolek, et al. (1998). "Medulloblastoma: time-dose relationship based on a 30-year review." Int J Radiat Oncol Biol Phys **42**(1): 147-54.
- Di, C., S. Liao, et al. (2005). "Identification of OTX2 as a medulloblastoma oncogene whose product can be targeted by all-trans retinoic acid." Cancer Res **65**(3): 919-24.
- Douglas, J. G., J. L. Barker, et al. (2004). "Concurrent chemotherapy and reduced-dose cranial spinal irradiation followed by conformal posterior fossa tumor bed boost for average-risk medulloblastoma: efficacy and patterns of failure." Int J Radiat Oncol Biol Phys **58**(4): 1161-4.

- Douple, E. B., R. C. Richmond, et al. (1985). "Carboplatin as a potentiator of radiation therapy." Cancer Treat Rev **12 Suppl A**: 111-24.
- Doz, F., S. Neuenschwander, et al. (2002). "Carboplatin before and during radiation therapy for the treatment of malignant brain stem tumours: a study by the Societe Francaise d'Oncologie Pediatrique." Eur J Cancer **38**(6): 815-9.
- Doz, F. and R. Pinkerton (1994). "What is the place of carboplatin in paediatric oncology?" Eur J Cancer **30A**(2): 194-201.
- Duffner, P. K., M. E. Horowitz, et al. (1993). "Postoperative chemotherapy and delayed radiation in children less than three years of age with malignant brain tumors." N Engl J Med **328**(24): 1725-31.
- Eberhart, C. G., J. L. Kepner, et al. (2002). "Histopathologic grading of medulloblastomas: a Pediatric Oncology Group study." Cancer **94**(2): 552-60.
- Eberhart, C. G., J. Kratz, et al. (2004). "Histopathological and molecular prognostic markers in medulloblastoma: c-myc, N-myc, TrkC, and anaplasia." J Neuropathol Exp Neurol **63**(5): 441-9.
- Eberhart, C. G., J. E. Kratz, et al. (2002). "Comparative genomic hybridization detects an increased number of chromosomal alterations in large cell/anaplastic medulloblastomas." Brain Pathol **12**(1): 36-44.
- Eberhart, C. G., T. Tihan, et al. (2000). "Nuclear localization and mutation of beta-catenin in medulloblastomas." J Neuropathol Exp Neurol **59**(4): 333-7.
- Ellison, D. W., O. E. Onilude, et al. (2005). "{beta}-Catenin Status Predicts a Favorable Outcome in Childhood Medulloblastoma: The United Kingdom Children's Cancer Study Group Brain Tumour Committee." J Clin Oncol **23**(31): 7951-7.
- Evans, A. E., R. D. Jenkin, et al. (1990). "The treatment of medulloblastoma. Results of a prospective randomized trial of radiation therapy with and without CCNU, vincristine, and prednisone." J Neurosurg **72**(4): 572-82.
- Evans, D. G., P. A. Farndon, et al. (1991). "The incidence of Gorlin syndrome in 173 consecutive cases of medulloblastoma." Br J Cancer **64**(5): 959-61.
- Fan, X., I. Mikolaenko, et al. (2004). "Notch1 and notch2 have opposite effects on embryonal brain tumor growth." Cancer Res **64**(21): 7787-93.
- Fan, X., Y. Wang, et al. (2003). "hTERT gene amplification and increased mRNA expression in central nervous system embryonal tumors." Am J Pathol **162**(6): 1763-9.
- Fattet, S., C. Haberler, et al. (2009). "Beta-catenin status in paediatric medulloblastomas: correlation of immunohistochemical expression with mutational status, genetic profiles, and clinical characteristics." J Pathol **218**(1): 86-94.
- Fazekas, F., J. B. Chawluk, et al. (1987). "MR signal abnormalities at 1.5 T in Alzheimer's dementia and normal aging." AJR Am J Roentgenol **149**(2): 351-6.
- Feeny, D., W. Furlong, et al. (1995). "Multi-attribute health status classification systems. Health Utilities Index." Pharmacoeconomics **7**(6): 490-502.
- Fouladi, M., A. Gajjar, et al. (1999). "Comparison of CSF cytology and spinal magnetic resonance imaging in the detection of leptomeningeal disease in pediatric medulloblastoma or primitive neuroectodermal tumor." J Clin Oncol **17**(10): 3234-7.
- Frangé, P., C. Alapetite, et al. (2009). "From childhood to adulthood: long-term outcome of medulloblastoma patients. The Institut Curie experience (1980-2000)." Journal of Neuro-Oncology **95**(2): 271-279.
- Friedman, H. S., M. S. Mahaley, Jr., et al. (1986). "Efficacy of vincristine and cyclophosphamide in the therapy of recurrent medulloblastoma." Neurosurgery **18**(3): 335-40.
- Gajjar, A., M. Chintagumpala, et al. (2006). "Risk-adapted craniospinal radiotherapy followed by high-dose chemotherapy and stem-cell rescue in children with newly diagnosed medulloblastoma (St Jude Medulloblastoma-96): long-term results from a prospective, multicentre trial." Lancet Oncol **7**(10): 813-20.
- Gajjar, A., R. Hernan, et al. (2004). "Clinical, histopathologic, and molecular markers of prognosis: toward a new disease risk stratification system for medulloblastoma." J Clin Oncol **22**(6): 984-93.
- Garre, M. L., A. Cama, et al. (2009). "Medulloblastoma variants: age-dependent occurrence and relation to Gorlin syndrome--a new clinical perspective." Clin Cancer Res **15**(7): 2463-71.

- Gaynon, P. S., L. J. Ettinger, et al. (1990). "Carboplatin in childhood brain tumors. A Children's Cancer Study Group Phase II trial." Cancer **66**(12): 2465-9.
- Gentet, J. C., E. Bouffet, et al. (1995). "Preirradiation chemotherapy including "eight drugs in 1 day" regimen and high-dose methotrexate in childhood medulloblastoma: results of the M7 French Cooperative Study." J Neurosurg **82**(4): 608-14.
- Gentet, J. C., F. Doz, et al. (1994). "Carboplatin and VP 16 in medulloblastoma: a phase II Study of the French Society of Pediatric Oncology (SFOP)." Med Pediatr Oncol **23**(5): 422-7.
- Giangaspero, F., G. Perilongo, et al. (1999). "Medulloblastoma with extensive nodularity: a variant with favorable prognosis." J Neurosurg **91**(6): 971-7.
- Giangaspero, F., L. Rigobello, et al. (1992). "Large-cell medulloblastomas. A distinct variant with highly aggressive behavior." Am J Surg Pathol **16**(7): 687-93.
- Gilbertson, R., C. Wickramasinghe, et al. (2001). "Clinical and molecular stratification of disease risk in medulloblastoma." Br J Cancer **85**(5): 705-12.
- Gilbertson, R. J. (2004). "Medulloblastoma: signalling a change in treatment." Lancet Oncol **5**(4): 209-18.
- Gilbertson, R. J. and S. C. Clifford (2003). "PDGFRB is overexpressed in metastatic medulloblastoma." Nat Genet **35**(3): 197-8.
- Gilbertson, R. J. and D. W. Ellison (2008). "The origins of medulloblastoma subtypes." Annu Rev Pathol **3**: 341-65.
- Gilbertson, R. J., J. A. Langdon, et al. (2006). "Mutational analysis of PDGFR-RAS/MAPK pathway activation in childhood medulloblastoma." Eur J Cancer.
- Gioia, G. (2000). "Behavior rating inventory of executive function. Psychological Assessment Resources, Inc." from <http://www3.parinc.com/products/product.aspx?Productid=BRIEF>.
- Glaser, A., C. Kennedy, et al. (1999). "Standardized quantitative assessment of brain tumor survivors treated within clinical trials in childhood." Int J Cancer Suppl **12**: 77-82.
- Goodman, R. (1999). "The extended version of the Strengths and Difficulties Questionnaire as a guide to child psychiatric caseness and consequent burden." J Child Psychol Psychiatry **40**(5): 791-9.
- Goodrich, L. V., L. Milenkovic, et al. (1997). "Altered neural cell fates and medulloblastoma in mouse patched mutants." Science **277**(5329): 1109-13.
- Gottardo N.G., Hansford J.R., et al. (2014). "Medulloblastoma Down Under 2013: a report from the third annual meeting of the International Medulloblastoma Working Group." Acta Neuropathol **127**(2):189-201.
- Grill, J., V. K. Renaux, et al. (1999). "Long-term intellectual outcome in children with posterior fossa tumors according to radiation doses and volumes." Int J Radiat Oncol Biol Phys **45**(1): 137-45.
- Grill, J., C. Sainte-Rose, et al. (2005). "Treatment of medulloblastoma with postoperative chemotherapy alone: an SFOP prospective trial in young children." Lancet Oncol **6**(8): 573-80.
- Gringras, P., P. Santosh, et al. (2006). "Development of an Internet-based real-time system for monitoring pharmacological interventions in children with neurodevelopmental and neuropsychiatric disorders." Child Care Health Dev **32**(5): 591-600.
- Groen, H. J., S. Sleijfer, et al. (1995). "Carboplatin- and cisplatin-induced potentiation of moderate-dose radiation cytotoxicity in human lung cancer cell lines." Br J Cancer **72**(6): 1406-11.
- Grotzer, M. A., A. J. Janss, et al. (2000). "TrkC expression predicts good clinical outcome in primitive neuroectodermal brain tumors." J Clin Oncol **18**(5): 1027-35.
- Gudrunardottir, T., A. Sehested, et al. (2011). "Cerebellar mutism: review of the literature." Childs Nerv Syst **27**(3): 355-63.
- Gurney, J. G., N. S. Kadan-Lottick, et al. (2003). "Endocrine and cardiovascular late effects among adult survivors of childhood brain tumors: Childhood Cancer Survivor Study." Cancer **97**(3): 663-73.
- Hahn, H., C. Wicking, et al. (1996). "Mutations of the human homolog of Drosophila patched in the nevoid basal cell carcinoma syndrome." Cell **85**(6): 841-51.
- Hallahan, A. R., J. I. Pritchard, et al. (2004). "The SmoA1 mouse model reveals that notch signaling is critical for the growth and survival of sonic hedgehog-induced medulloblastomas." Cancer Res **64**(21): 7794-800.

- Hamilton, S. R., B. Liu, et al. (1995). "The molecular basis of Turcot's syndrome." N Engl J Med **332**(13): 839-47.
- Hernan, R., R. Fasheh, et al. (2003). "ERBB2 up-regulates S100A4 and several other prometastatic genes in medulloblastoma." Cancer Res **63**(1): 140-8.
- Hoppe-Hirsch, E., D. Renier, et al. (1990). "Medulloblastoma in childhood: progressive intellectual deterioration." Childs Nerv Syst **6**(2): 60-5.
- Hua, C., J. K. Bass, et al. (2008). "Hearing Loss After Radiotherapy for Pediatric Brain Tumors: Effect of Cochlear Dose." Int J Radiat Oncol Biol Phys.
- Huang, H., B. M. Mahler-Araujo, et al. (2000). "APC mutations in sporadic medulloblastomas." Am J Pathol **156**(2): 433-7.
- Jakacki, R., P. Burger, et al. (2012). "Outcome of children with metastatic medulloblastoma treated with carboplatin during craniospinal radiotherapy: A children's oncology group phase I/II study" Journal of Clinical Oncology **30**: 2648-2653.
- Johnson, R. L., A. L. Rothman, et al. (1996). "Human homolog of patched, a candidate gene for the basal cell nevus syndrome." Science **272**(5268): 1668-71.
- Kennedy, C. R. and K. Leyland (1999). "Comparison of screening instruments for disability and emotional/behavioral disorders with a generic measure of health-related quality of life in survivors of childhood brain tumors." Int J Cancer Suppl **12**: 106-11.
- Kirsch, D. G. and N. J. Tarbell (2004). "New technologies in radiation therapy for pediatric brain tumors: the rationale for proton radiation therapy." Pediatr Blood Cancer **42**(5): 461-4.
- Kleihues, P., B. Schauble, et al. (1997). "Tumors associated with p53 germline mutations: a synopsis of 91 families." Am J Pathol **150**(1): 1-13.
- Koch, A., A. Hrychyk, et al. (2007). "Mutations of the Wnt antagonist AXIN2 (Conductin) result in TCF-dependent transcription in medulloblastomas." Int J Cancer **121**(2): 284-91.
- Koch, A., A. Waha, et al. (2004). "No evidence for mutations or altered expression of the Suppressor of Fused gene (SUFU) in primitive neuroectodermal tumours." Neuropathol Appl Neurobiol **30**(5): 532-9.
- Koch, A., A. Waha, et al. (2001). "Somatic mutations of WNT/wingless signaling pathway components in primitive neuroectodermal tumors." Int J Cancer **93**(3): 445-9.
- Koch A, et al. (1999). "Childhood hepatoblastomas frequently carry a mutated degradation targeting box of the beta-catenin gene". Cancer Res **59**:269-73.
- Kool M, Korshunov A, et al. (2012). "Molecular subgroups of medulloblastoma: an international meta-analysis of transcriptome, genetic aberrations, and clinical data of WNT, SHH, Group 3, and Group 4 medulloblastomas." Acta Neuropathol **123**(4):473-84.
- Korshunov A, Remke M, et al. (2010). "Adult and pediatric medulloblastomas are genetically distinct and require different algorithms for molecular risk stratification." J Clin Oncol **28**(18):3054-60.
- Kortmann, R. D., J. Kuhl, et al. (2000). "Postoperative neoadjuvant chemotherapy before radiotherapy as compared to immediate radiotherapy followed by maintenance chemotherapy in the treatment of medulloblastoma in childhood: results of the German prospective randomized trial HIT '91." Int J Radiat Oncol Biol Phys **46**(2): 269-79.
- Kovnar, E. H., S. J. Kellie, et al. (1990). "Preirradiation cisplatin and etoposide in the treatment of high-risk medulloblastoma and other malignant embryonal tumors of the central nervous system: a phase II study." J Clin Oncol **8**(2): 330-6.
- Lamont, J. M., C. S. McManamy, et al. (2004). "Combined histopathological and molecular cytogenetic stratification of medulloblastoma patients." Clin Cancer Res **10**(16): 5482-93.
- Lannering, B., I. Marky, et al. (1990). "Long-term sequelae after pediatric brain tumors: their effect on disability and quality of life." Med Pediatr Oncol **18**(4): 304-10.
- Lannering B, S. Rutkowski et al. (2012). "Hyperfractionated versus conventional radiotherapy followed by chemotherapy in standard-risk medulloblastoma: results from the randomized multicenter HIT-SIOP PNET 4 trial." J Clin Oncol **30**(26):3187-93.
- Laughton, S. J., T. E. Merchant, et al. (2008). "Endocrine outcomes for children with embryonal brain tumors after risk-adapted craniospinal and conformal primary-site irradiation and

- high-dose chemotherapy with stem-cell rescue on the SJMB-96 trial." J Clin Oncol **26**(7): 1112-8.
- Le Gales, C., N. Costet, et al. (1999). "Cross-cultural adaptation of a health status classification system in children with cancer. First results of the French adaptation of the Health Utilities Index Marks 2 and 3." Int J Cancer Suppl **12**: 112-8.
- Lee, C. T., S. D. Bilton, et al. (2005). "Treatment planning with protons for pediatric retinoblastoma, medulloblastoma, and pelvic sarcoma: how do protons compare with other conformal techniques?" Int J Radiat Oncol Biol Phys **63**(2): 362-72.
- Lee, S. J., L. R. Schover, et al. (2006). "American Society of Clinical Oncology recommendations on fertility preservation in cancer patients." J Clin Oncol **24**(18): 2917-31.
- Lee, Y., R. Kawagoe, et al. (2007). "Loss of suppressor-of-fused function promotes tumorigenesis." Oncogene **26**(44): 6442-7.
- Lefkowitz, I. B., R. J. Packer, et al. (1990). "Results of treatment of children with recurrent medulloblastoma/primitive neuroectodermal tumors with lomustine, cisplatin, and vincristine." Cancer **65**(3): 412-7.
- Leung, C., M. Lingbeek, et al. (2004). "Bmi1 is essential for cerebellar development and is overexpressed in human medulloblastomas." Nature **428**(6980): 337-41.
- Livesey, E. A. and C. G. Brook (1988). "Gonadal dysfunction after treatment of intracranial tumours." Arch Dis Child **63**(5): 495-500.
- Livesey, E. A., P. C. Hindmarsh, et al. (1990). "Endocrine disorders following treatment of childhood brain tumours." Br J Cancer **61**(4): 622-5.
- Louis, D. N., H. Ohgaki, et al., Eds. (2007). WHO Classification of tumours of the central nervous system. Lyon, IARC.
- MacDonald, S. M., S. Safai, et al. (2008). "Proton radiotherapy for childhood ependymoma: initial clinical outcomes and dose comparisons." Int J Radiat Oncol Biol Phys **71**(4): 979-86.
- MacDonald, T. J., K. M. Brown, et al. (2001). "Expression profiling of medulloblastoma: PDGFRA and the RAS/MAPK pathway as therapeutic targets for metastatic disease." Nat Genet **29**(2): 143-52.
- Maddrey, A. M., J. A. Bergeron, et al. (2005). "Neuropsychological performance and quality of life of 10 year survivors of childhood medulloblastoma." J Neurooncol **72**(3): 245-53.
- Maddrey, A. M., J. A. Bergeron, et al. (2005). "Neuropsychological performance and quality of life of 10 year survivors of childhood medulloblastoma." Journal of Neuro-Oncology **72**(3): 245-253.
- Malkin, D., F. P. Li, et al. (1990). "Germ line p53 mutations in a familial syndrome of breast cancer, sarcomas, and other neoplasms." Science **250**(4985): 1233-8.
- Marino, S. (2005). "Medulloblastoma: developmental mechanisms out of control." Trends Mol Med **11**(1): 17-22.
- McCullagh, P. (1980). "Regression models for ordinal data (with discussion)." Journal of the Royal Statistical Society **42**: 109-142.
- McManamy, C. S., J. M. Lamont, et al. (2003). "Morphophenotypic variation predicts clinical behavior in childhood non-desmoplastic medulloblastomas." J Neuropathol Exp Neurol **62**(6): 627-32.
- McManamy, C. S., J. Pears, et al. (2007). "Nodule formation and desmoplasia in medulloblastomas-defining the nodular/desmoplastic variant and its biological behavior." Brain Pathol **17**(2): 151-64.
- Merchant, T. E., E. N. Kiehna, et al. (2006). "Modeling radiation dosimetry to predict cognitive outcomes in pediatric patients with CNS embryonal tumors including medulloblastoma." Int J Radiat Oncol Biol Phys **65**(1): 210-21.
- Merchant, T. E., L. E. Kun, et al. (2008). "Multi-institution prospective trial of reduced-dose craniospinal irradiation (23.4 Gy) followed by conformal posterior fossa (36 Gy) and primary site irradiation (55.8 Gy) and dose-intensive chemotherapy for average-risk medulloblastoma." Int J Radiat Oncol Biol Phys **70**(3): 782-7.
- Miralbell, R., S. Bieri, et al. (1999). "Prognostic value of cerebrospinal fluid cytology in pediatric medulloblastoma. Swiss Pediatric Oncology Group." Ann Oncol **10**(2): 239-41.

- Miralbell, R., A. Lomax, et al. (1997). "Potential role of proton therapy in the treatment of pediatric medulloblastoma/primitive neuro-ectodermal tumors: spinal theca irradiation." *Int J Radiat Oncol Biol Phys* **38**(4): 805-11.
- Morin, P. J. (1999). "beta-catenin signaling and cancer." *Bioessays* **21**(12): 1021-30.
- Mulhern, R. K., J. L. Kepner, et al. (1998). "Neuropsychologic functioning of survivors of childhood medulloblastoma randomized to receive conventional or reduced-dose craniospinal irradiation: a Pediatric Oncology Group study." *J Clin Oncol* **16**(5): 1723-8.
- Mulhern, R. K., S. L. Palmer, et al. (2005). "Neurocognitive consequences of risk-adapted therapy for childhood medulloblastoma." *J Clin Oncol* **23**(24): 5511-9.
- Nagel, B. J., D. C. Delis, et al. (2006). "Early patterns of verbal memory impairment in children treated for medulloblastoma." *Neuropsychology* **20**(1): 105-12.
- Nathwani, N. C., P. C. Hindmarsh, et al. (1998). "Gonadotrophin pulsatility in girls with the Turner syndrome: modulation by exogenous sex steroids." *Clin Endocrinol (Oxf)* **49**(1): 107-13.
- Nicholson, J. C., F. M. Ross, et al. (1999). "Comparative genomic hybridization and histological variation in primitive neuroectodermal tumours." *Br J Cancer* **80**(9): 1322-31.
- Ogilvy-Stuart, A. L., P. E. Clayton, et al. (1994). "Cranial irradiation and early puberty." *J Clin Endocrinol Metab* **78**(6): 1282-6.
- Ogilvy-Stuart, A. L., S. M. Shalet, et al. (1991). "Thyroid function after treatment of brain tumors in children." *J Pediatr* **119**(5): 733-7.
- Olshan, J. S., J. Gubernick, et al. (1992). "The effects of adjuvant chemotherapy on growth in children with medulloblastoma." *Cancer* **70**(7): 2013-7.
- Oyharcabal-Bourden, V., C. Kalifa, et al. (2005). "Standard-risk medulloblastoma treated by adjuvant chemotherapy followed by reduced-dose craniospinal radiation therapy: a French Society of Pediatric Oncology Study." *J Clin Oncol* **23**(21): 4726-34.
- Packer, R. J. (1999). "Childhood medulloblastoma: progress and future challenges." *Brain Dev* **21**(2): 75-81.
- Packer, R. J., A. Gajjar, et al. (2006). "Phase III study of craniospinal radiation therapy followed by adjuvant chemotherapy for newly diagnosed average-risk medulloblastoma." *J Clin Oncol* **24**(25): 4202-8.
- Packer, R. J., J. Goldwein, et al. (1999). "Treatment of children with medulloblastomas with reduced-dose craniospinal radiation therapy and adjuvant chemotherapy: A Children's Cancer Group Study." *J Clin Oncol* **17**(7): 2127-36.
- Packer, R. J., B. R. Rood, et al. (2003). "Medulloblastoma: present concepts of stratification into risk groups." *Pediatr Neurosurg* **39**(2): 60-7.
- Packer, R. J., L. N. Sutton, et al. (1994). "Outcome for children with medulloblastoma treated with radiation and cisplatin, CCNU, and vincristine chemotherapy." *J Neurosurg* **81**(5): 690-8.
- Packer, R. J., L. N. Sutton, et al. (1991). "Improved survival with the use of adjuvant chemotherapy in the treatment of medulloblastoma." *J Neurosurg* **74**(3): 433-40.
- Palmer, S. L., T. Hassall, et al. (2010). "Neurocognitive outcome 12 months following cerebellar mutism syndrome in pediatric patients with medulloblastoma." *Neuro Oncol* **12**(12): 1311-7.
- Paraf, F., S. Jothy, et al. (1997). "Brain tumor-polyposis syndrome: two genetic diseases?" *J Clin Oncol* **15**(7): 2744-58.
- Pasqualini, T., B. Diez, et al. (1987). "Long-term endocrine sequelae after surgery, radiotherapy, and chemotherapy in children with medulloblastoma." *Cancer* **59**(4): 801-6.
- Pezzotta, S., L. Cordero di Montezemolo, et al. (1996). "CNS-85 trial: a cooperative pediatric CNS tumor study--results of treatment of medulloblastoma patients." *Childs Nerv Syst* **12**(2): 87-96.
- Pfister, S., M. Remke, et al. (2009). "Outcome prediction in pediatric medulloblastoma based on DNA copy-number aberrations of chromosomes 6q and 17q and the MYC and MYCN loci." *J Clin Oncol* **27**(10): 1627-36.
- Pietsch, T., A. Waha, et al. (1997). "Medulloblastomas of the desmoplastic variant carry mutations of the human homologue of Drosophila patched." *Cancer Res* **57**(11): 2085-8.

- Pomeroy, S. L., P. Tamayo, et al. (2002). "Prediction of central nervous system embryonal tumour outcome based on gene expression." *Nature* **415**(6870): 436-42.
- Puget, S., N. Boddaert, et al. (2009). "Injuries to inferior vermis and dentate nuclei predict poor neurological and neuropsychological outcome in children with malignant posterior fossa tumors." *Cancer* **115**(6): 1338-47.
- Raffel, C., R. B. Jenkins, et al. (1997). "Sporadic medulloblastomas contain PTCH mutations." *Cancer Res* **57**(5): 842-5.
- Reardon, D. A., E. Michalkiewicz, et al. (1997). "Extensive genomic abnormalities in childhood medulloblastoma by comparative genomic hybridization." *Cancer Res* **57**(18): 4042-7.
- Reeves, C. B., S. L. Palmer, et al. (2006). "Attention and memory functioning among pediatric patients with medulloblastoma." *J Pediatr Psychol* **31**(3): 272-80.
- Reifenberger, J., M. Wolter, et al. (1998). "Missense mutations in SMOH in sporadic basal cell carcinomas of the skin and primitive neuroectodermal tumors of the central nervous system." *Cancer Res* **58**(9): 1798-803.
- Ribi, K., C. Relly, et al. (2005). "Outcome of medulloblastoma in children: Long-term complications and quality of life." *Neuropediatrics* **36**(6): 357-365.
- Ris, M. D., R. Packer, et al. (2001). "Intellectual outcome after reduced-dose radiation therapy plus adjuvant chemotherapy for medulloblastoma: a Children's Cancer Group study." *J Clin Oncol* **19**(15): 3470-6.
- Riva, D., C. Pantaleoni, et al. (1989). "Impairment of neuropsychological functions in children with medulloblastomas and astrocytomas in the posterior fossa." *Childs Nerv Syst* **5**(2): 107-10.
- Robertson, P. L., K. M. Muraszko, et al. (2006). "Incidence and severity of postoperative cerebellar mutism syndrome in children with medulloblastoma: a prospective study by the Children's Oncology Group." *J Neurosurg* **105**(6 Suppl): 444-51.
- Robinson G, Parker M, et al. (2012). "Novel mutations target distinct subgroups of medulloblastoma." *Nature* **2**;488(7409):43-8.
- Roncadin, C., M. Dennis, et al. (2008). "Adverse medical events associated with childhood cerebellar astrocytomas and medulloblastomas: natural history and relation to very long-term neurobehavioral outcome." *Childs Nervous System* **24**(9): 995-1002.
- Rutkowski, S., U. Bode, et al. (2005). "Treatment of early childhood medulloblastoma by postoperative chemotherapy alone." *N Engl J Med* **352**(10): 978-86.
- Rutkowski, S., A. von Bueren, et al. (2007). "Prognostic relevance of clinical and biological risk factors in childhood medulloblastoma: results of patients treated in the prospective multicenter trial HIT'91." *Clin Cancer Res* **13**(9): 2651-7.
- Rutkowski, S., K. von Hoff, et al. (2010). "Survival and prognostic factors of early childhood medulloblastoma: an international meta-analysis." *J Clin Oncol* **28**(33): 4961-8.
- Ryan SL et al. (2012). MYC family amplification and clinical risk-factors interact to predict an extremely poor prognosis in childhood medulloblastoma. *Acta Neuropathol.* **123**: 501-13.
- "Modification of the sample size and the schedule of interim analyses in survival trials based on data inspections." *statistics in medicine* **20**: 3741-3751.
- Schmahmann, J. D., R. Gardner, et al. (2009). "Development of a brief ataxia rating scale (BARS) based on a modified form of the ICARS." *Mov Disord* **24**(12): 1820-8.
- Schmiegelow, M., U. Feldt-Rasmussen, et al. (2003). "A population-based study of thyroid function after radiotherapy and chemotherapy for a childhood brain tumor." *J Clin Endocrinol Metab* **88**(1): 136-40.
- Schmiegelow, M., S. Lassen, et al. (2000). "Cranial radiotherapy of childhood brain tumours: growth hormone deficiency and its relation to the biological effective dose of irradiation in a large population based study." *Clin Endocrinol (Oxf)* **53**(2): 191-7.
- Schmiegelow, M., S. Lassen, et al. (1999). "Dosimetry and growth hormone deficiency following cranial irradiation of childhood brain tumors." *Med Pediatr Oncol* **33**(6): 564-71.
- Schofield, D., D. C. West, et al. (1995). "Correlation of loss of heterozygosity at chromosome 9q with histological subtype in medulloblastomas." *Am J Pathol* **146**(2): 472-80.
- Siddik, Z. H., M. Jones, et al. (1988). "Comparative distribution and excretion of carboplatin and cisplatin in mice." *Cancer Chemother Pharmacol* **21**(1): 19-24.

- Smets EM, B. Garssen, et al. (1995). The Multidimensional Fatigue Inventory (MFI) psychometric qualities of an instrument to assess fatigue. *J Psychosom Res.* Apr;39(3):315-25.
- Spoudeas, H. A., E. Charmandari, et al. (2003). "Hypothalamo-pituitary-adrenal axis integrity after cranial irradiation for childhood posterior fossa tumours." *Med Pediatr Oncol* **40**(4): 224-9.
- Spoudeas, H. A., P. C. Hindmarsh, et al. (1996). "Evolution of growth hormone neurosecretory disturbance after cranial irradiation for childhood brain tumours: a prospective study." *J Endocrinol* **150**(2): 329-42.
- Stearns, D., A. Chaudhry, et al. (2006). "c-myc overexpression causes anaplasia in medulloblastoma." *Cancer Res* **66**(2): 673-81.
- Stewart, J. and K. J. Turner (2005). "Inhibin B as a potential biomarker of testicular toxicity." *Cancer Biomark* **1**(1): 75-91.
- Stone, D. M., M. Murone, et al. (1999). "Characterization of the human suppressor of fused, a negative regulator of the zinc-finger transcription factor Gli." *J Cell Sci* **112** (Pt 23): 4437-48.
- Strauss, L. C., T. M. Killmond, et al. (1991). "Efficacy of postoperative chemotherapy using cisplatin plus etoposide in young children with brain tumors." *Med Pediatr Oncol* **19**(1): 16-21.
- Taipale, J. and P. A. Beachy (2001). "The Hedgehog and Wnt signalling pathways in cancer." *Nature* **411**(6835): 349-54.
- Tait, D. M., H. Thornton-Jones, et al. (1990). "Adjuvant chemotherapy for medulloblastoma: the first multi-centre control trial of the International Society of Paediatric Oncology (SIOP I)." *Eur J Cancer* **26**(4): 464-9.
- Taylor, M. D., L. Liu, et al. (2002). "Mutations in SUFU predispose to medulloblastoma." *Nat Genet* **31**(3): 306-10.
- Taylor MD, Northcott PA, et al. (2012). "Molecular subgroups of medulloblastoma: the current consensus." *Acta Neuropathol* **123**(4):465-72.
- Taylor, R. E., C. C. Bailey, et al. (2003). "Results of a randomized study of preradiation chemotherapy versus radiotherapy alone for nonmetastatic medulloblastoma: The International Society of Paediatric Oncology/United Kingdom Children's Cancer Study Group PNET-3 Study." *J Clin Oncol* **21**(8): 1581-91.
- Thompson, M. C., C. Fuller, et al. (2006). "Genomics identifies medulloblastoma subgroups that are enriched for specific genetic alterations." *J Clin Oncol* **24**(12): 1924-31.
- Thomson, A. B., H. O. Critchley, et al. (2002). "Late reproductive sequelae following treatment of childhood cancer and options for fertility preservation." *Best Pract Res Clin Endocrinol Metab* **16**(2): 311-34.
- Varni, J. W., T. M. Burwinkle, et al. (2002). "The PedsQL in pediatric cancer: reliability and validity of the Pediatric Quality of Life Inventory Generic Core Scales, Multidimensional Fatigue Scale, and Cancer Module." *Cancer* **94**(7): 2090-106.
- Varni, J. W., M. Seid, et al. (1999). "The PedsQL: measurement model for the pediatric quality of life inventory." *Med Care* **37**(2): 126-39.
- Verlooy, J., V. Mosseri, et al. (2006). "Treatment of high risk medulloblastomas in children above the age of 3 years: a SFOP study." *Eur J Cancer* **42**(17): 3004-14.
- von Hoff, K., W. Hartmann, et al. (2009). "Large cell/anaplastic medulloblastoma: outcome according to myc status, histopathological, and clinical risk factors." *Pediatr Blood Cancer* **54**(3): 369-76.
- von Hoff, K., B. Hinkes, et al. (2009). "Long-term outcome and clinical prognostic factors in children with medulloblastoma treated in the prospective randomised multicentre trial HIT'91." *Eur J Cancer* **45**(7): 1209-17.
- von Wolff, M., J. Donnez, et al. (2009). "Cryopreservation and autotransplantation of human ovarian tissue prior to cytotoxic therapy--a technique in its infancy but already successful in fertility preservation." *Eur J Cancer* **45**(9): 1547-53.
- Vorechovsky, I., O. Tingby, et al. (1997). "Somatic mutations in the human homologue of Drosophila patched in primitive neuroectodermal tumours." *Oncogene* **15**(3): 361-6.
- Wang, S. K. and A. A. Tsiatis (1987). "Approximately Optimal One-Parameter Boundaries for Group Sequential Trials." *Biometrics* **43**: 193-199.
- Wechsler-Reya, R. and M. P. Scott (2001). "The developmental biology of brain tumors." *Annu Rev Neurosci* **24**: 385-428.

- Wells, E. M., Z. P. Khademian, et al. (2010). "Postoperative cerebellar mutism syndrome following treatment of medulloblastoma: neuroradiographic features and origin." J Neurosurg Pediatr **5**(4): 329-34.
- Wetmore, C., D. E. Eberhart, et al. (2000). "The normal patched allele is expressed in medulloblastomas from mice with heterozygous germ-line mutation of patched." Cancer Res **60**(8): 2239-46.
- Wolden, S. L., I. J. Dunkel, et al. (2003). "Patterns of failure using a conformal radiation therapy tumor bed boost for medulloblastoma." J Clin Oncol **21**(16): 3079-83.
- Wolter, M., J. Reifenberger, et al. (1997). "Mutations in the human homologue of the Drosophila segment polarity gene patched (PTCH) in sporadic basal cell carcinomas of the skin and primitive neuroectodermal tumors of the central nervous system." Cancer Res **57**(13): 2581-5.
- Wolter, M., C. Scharwachter, et al. (2003). "Absence of detectable alterations in the putative tumor suppressor gene BTRC in cerebellar medulloblastomas and cutaneous basal cell carcinomas." Acta Neuropathol **106**(4): 287-90.
- Yuh, G. E., L. N. Lored, et al. (2004). "Reducing toxicity from craniospinal irradiation: using proton beams to treat medulloblastoma in young children." Cancer J **10**(6): 386-90.
- Zeltzer, P. M., J. M. Boyett, et al. (1999). "Metastasis stage, adjuvant treatment, and residual tumor are prognostic factors for medulloblastoma in children: conclusions from the Children's Cancer Group 921 randomized phase III study." J Clin Oncol **17**(3): 832-45.
- Zurawel, R. H., C. Allen, et al. (2000). "Analysis of PTCH/SMO/SHH pathway genes in medulloblastoma." Genes Chromosomes Cancer **27**(1): 44-51.
- Zurawel, R. H., S. A. Chiappa, et al. (1998). "Sporadic medulloblastomas contain oncogenic beta-catenin mutations." Cancer Res **58**(5): 896-9.

APPENDIX

A – National Documents (Country specific)

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Appendix B

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Appendix B

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C - Treatment Overview

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C.1. Treatment Overview PNET 5 MB - LR

Patient: _____ Surgery Date: _____

Radiotherapy:

Brain – 18.0 Gy in 10 daily fractions of 1.80 Gy
Spine - 18.0 Gy in 10 daily fractions of 1.80 Gy
Primary tumour boost – 36.0 Gy in 20 daily fractions of 1.80 Gy

Radiotherapy Start Date: _____

Radiotherapy End Date: _____

Chemotherapy:

Maintenance chemotherapy starts 6 weeks after the end of radiotherapy.

Regimen A

- **cisplatin:** 70 mg/m² intravenously (6 hour infusion) - day 1
- **CCNU (lomustine):** 75 mg/ m² orally - day 1
- **VCR (vincristine):** 1.5 mg/m² intravenously (max. dose 2 mg) - day 1, 8 and 15

Interval after cycle A: 6 weeks

Regimen B:

- **cyclophosphamide:** 1x 1000 mg/m²/d given as IV infusion, over one hour, days 1-2
- **VCR (vincristine):** 1.5 mg/m² intravenously (max. dose 2 mg) - day 1
- **MESNA:** 250mg/m² intravenously before first cyclophosphamide infusion. MESNA 750 mg/m²/24 hour, day 1-2 (according to local standard, please see chapter 11.3.9.)

Interval after cycle B: 3 weeks

Start Date 1. cycle A: _____

Start Date 1. cycle B: _____

Start Date 2. cycle A: _____

Date MRI: _____

Start Date 2. cycle B: _____

Start Date 3. cycle A: _____

Start Date 3. cycle B: _____

Date MRI: _____

Please regard necessary evaluations and dose modifications

C.2. Treatment Overview PNET 5 MB - SR

Patient: _____ Surgery Date: _____

Radiotherapy:

Brain – 23.40 Gy in 13 daily fractions of 1.80 Gy
Spine - 23.40 Gy in 13 daily fractions of 1.80 Gy
Primary tumour boost – 30.60 Gy in 17 daily fractions of 1.80 Gy

Randomisation: with or without concomitant Carboplatin: (please delete where inapplicable)

Carboplatin: 35 mg/m²/day intravenously over 15-60 minutes, 5 times a week
(Monday - Friday), 1-4 hours before radiation for 6 weeks (30 applications=total dose)

Radiotherapy Start Date: _____ End Date: _____

Chemotherapy:

Maintenance chemotherapy starts 6 weeks after the end of radiotherapy.

Regimen A

- **cisplatin:** 70 mg/m² intravenously (6 hour infusion) - day 1
- **CCNU (lomustine):** 75 mg/ m² orally - day 1
- **VCR (vincristine):** 1.5 mg/m² intravenously (bolus; max. dose 2 mg) - day 1, 8 and 15
Interval after cycle A: 6 weeks

Regimen B:

- **cyclophosphamide:** 1x 1000 mg/m²/d given as IV infusion, over one hour, days 1-2
- **vincristine:** 1.5 mg/m² intravenously (bolus; max. dose 2 mg) - day 1
- **MESNA:** 250mg/m² intravenously before first cyclophosphamide infusion. MESNA 750 mg/m²/24 hour, day 1-2 (according to local standard, please see chapter 11.3.9.)
Interval after cycle B: 3 weeks

Start Date 1. cycle A: _____

Start Date 1. cycle B: _____

Start Date 2. cycle A: _____

Date MRI: _____

Start Date 2. cycle B: _____

Start Date 3. cycle A: _____

Start Date 3. cycle B: _____

Date MRI: _____

Start Date 4. cycle A: _____

Start Date 4. cycle B: _____

Date MRI: _____

Please regard necessary evaluations and dose modifications

D- Standard Operating Procedures (SOP)

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D.1. Sample preparation, central pathology review and molecular diagnostics by National Reference Laboratories

Purpose and scope, applications

Sample Registration:

As part of the PNET 5 MB - LR and PNET 5 MB - SR clinical trials, it will be necessary for tissue samples from patients diagnosed with medulloblastoma to undergo central pathological review and have the biological status of prognostic molecular markers (MYC, MYCN, β -catenin IHC and mutation (mandatory) and chromosome 6 monosomy (optional)) established prior to starting adjuvant therapy, i.e. less than 22 days post-surgery. This will be achieved by treatment centres consenting patients for participation in the study using standard procedures and despatching frozen and paraffin tumour tissue to the national reference laboratory for preparation and assessment.

Pathology departments and national reference laboratories will play the central role in coordinating central pathology review and molecular diagnostics, to allow stratification of patients with medulloblastoma onto PNET 5 MB and its respective treatment arms.

The clinical trial requires mandatory submission of the following once a local diagnosis of medulloblastoma has been made:

Confirmation of consent for study

Notification of case to national reference centre and submission of:

1 x FFPE block - representative of the pathological process

1 aliquot of frozen tissue

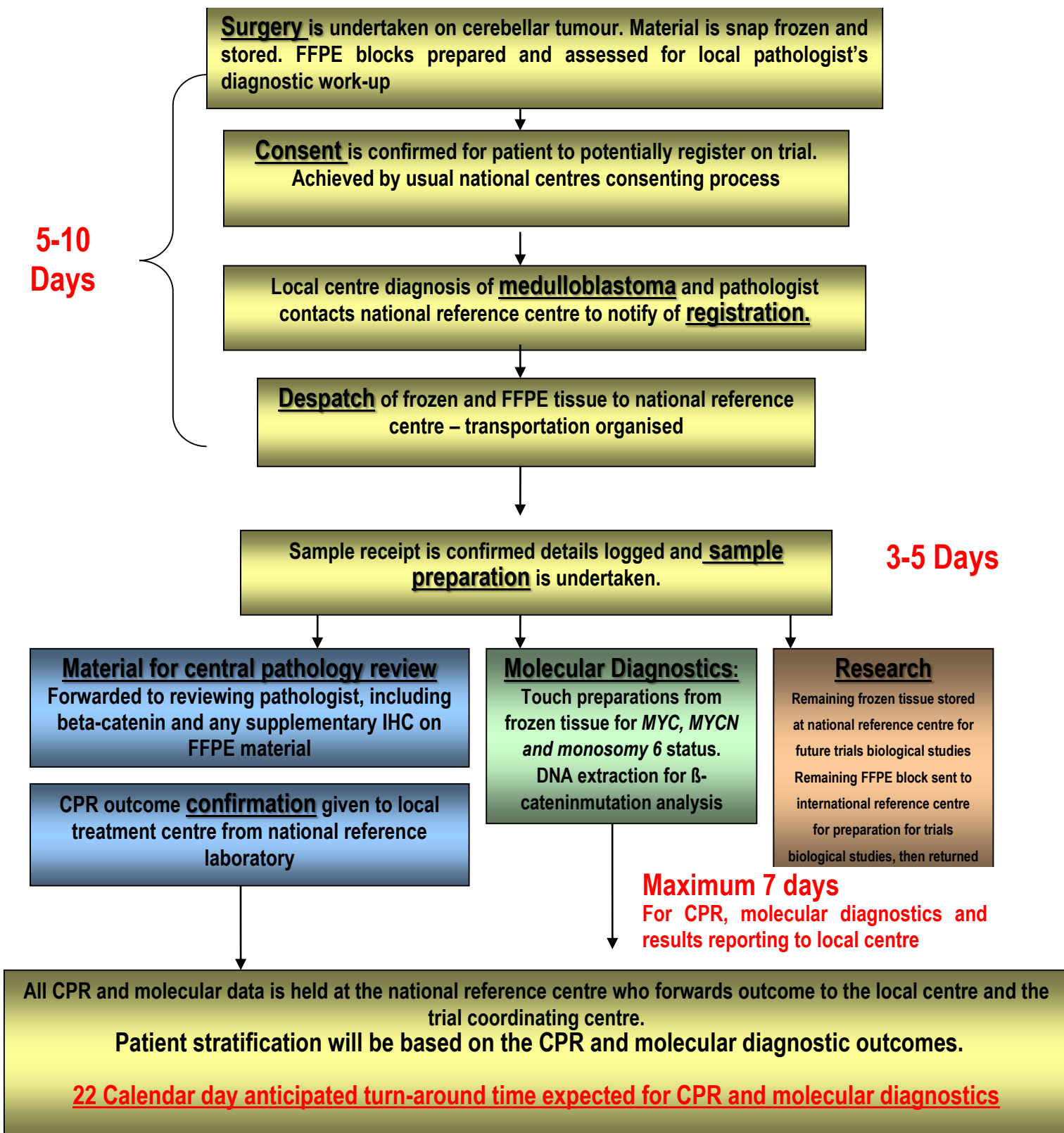
Copy of the local treatment centre pathology report

The national reference laboratory will be responsible for sample preparation to achieve central pathology review, molecular diagnostics and immunohistochemistry, including beta-catenin staining. **Submission of frozen tissue is mandatory.** At least 10mm³ is desirable; however any amount will be accepted to allow inclusion.

In addition to the technical procedures described here, administration and recording of the outcomes for every sample received will be logged into the central trial database (Marvin).

The flow diagram in Figure 1 (below) summarises the arrangements for biological study:

Central Pathology Review & Biological Studies of Paediatric Medulloblastoma in PNET 5 MB - LR and PNET 5 MB - SR



Sample Preparation by National Reference Laboratory

Central Pathology Review and Immunohistochemistry

Neuropathological central review is undertaken according to the World Health Organisation (WHO) Classification 2007. This will include examination of standard haematoxylin and eosin (H&E) and reticulin silver staining preparations as well as immunohistochemistry to look at factors such as expression of neuroepithelial protein markers for example, GFAP, synaptophysin and neurofilament protein, as well as proteins that may be expressed in other tumours of the posterior fossa, e.g. EMA (epithelial membrane antigen), cytokeratins, LIN28 and INI-1 in atypical teratoid/rhabdoid tumours to aid in confirming the local centre diagnosis.

As part of the molecular diagnostic component of the study, beta-catenin immunohistochemistry will also be assessed.

Once tissue is received by the national reference centre, it will be logged and receipt confirmed to the local study centre.

The detailed procedures of sample preparation involved are described later. The following will be prepared from the FFPE and frozen tissue at all national reference laboratories to aid up-front central pathology review and biological analysis as well as subsequent investigative biological studies:

FFPE - ***up to 20 x 5µm** sections for central pathology review purposes, including haematoxylin and eosin staining and immunohistochemistry panel – GFAP, synaptophysin, NFP, EMA, INI-1, vimentin, Ki-67 and reticulin special stain.

***2 x 5µm** sections for beta-catenin immunohistochemistry staining.

1 x 5 µm haematoxylin and eosin stained section for TMA mark-up by the reviewing pathologist.

Frozen - ***1 x 7µm** section for H&E – to be sent along with FFPE stained slides to reviewing pathologist for assessment of cell content.

***10 x (minimum of 4 x)** touch preparations for FISH *MYC* and *MYCN* (mandatory) and chromosome 6 (optional) status assessment.

*To be retained at the national reference centre for review and quality control purposes.

The remaining FFPE block will be forwarded to the international reference centre, for preparation of tissue microarray and processing for trials biological studies (*see separate international reference centre SOP*). The FFPE block will be returned to the local centre via the national reference centre.

The amount of tissue submitted may affect the amount of tissue processing that can be undertaken, however ***sufficient tissue will always be left in any FFPE blocks for further diagnostic use***, if requested by the local centre.

The remaining frozen material will be stored and made available for subsequent approved trials biological studies.

Fluorescence *in situ* hybridisation

Fluorescence *in situ* hybridisation will be undertaken to establish the *MYC* and *MYCN* gene amplification and monosomy 6 (optional) status of the sample.

Further methods for determination of *MYC* and *MYCN* gene amplification and monosomy 6 status

These may also be determined using validated array-based copy number analyses.

Central pathology review and molecular diagnostic outcomes will be notified to the local clinical study centre and the trial coordinating centre. If there are any discrepancies between the local and reviewing pathologist's diagnosis, they will liaise to reach a consensus.

PROCEDURES IN DETAIL

1. Local treatment centres

Collection

When surgery is planned for a paediatric cerebellar tumour, tissue must be sent fresh to the pathology department. The pathologist will prepare tissue for usual diagnosis, ensuring at least 1 aliquot is retained for frozen storage. Tissue should be snap-frozen in liquid nitrogen at the earliest opportunity from receipt into the lab. The frozen tissue is stored at -80°C or in liquid nitrogen, until ready for despatch to the national reference centre. The approximate time between surgical resection and tissue freezing should be recorded. FFPE blocks are prepared as per local centre SOPs, with one representative FFPE block used in the diagnostic process being submitted to the national reference centre once a diagnosis of medulloblastoma is confirmed locally, and the patient is consented for trial.

Consent

Once the pathologist has confirmed the tumour is medulloblastoma, it is the local centre's responsibility to undertake consent procedures as per their national SOPs to allow participation in the clinical trials, and to permit investigative biological studies to be undertaken on the material on a link-anonymised basis. These consents should include permission to use other materials (e.g. blood, cerebrospinal fluid samples; *see separate blood and CSF collection SOPs*) taken in investigative biological studies. Consent must be obtained early by the clinical team to allow rapid trial inclusion and submission of tissue to national reference centres.

Despatch

The local centre will contact the national reference laboratory immediately once consent and medulloblastoma diagnosis is confirmed. The local and national centres will liaise to arrange rapid submission to the national reference centre of the frozen and FFPE tissue, a copy of the local pathology report and relevant documentation for transport, **under national operating arrangements**. Receipt of material will be confirmed by the national laboratory, and FFPE blocks will be returned to local centres in a timely manner.

2. National reference centres

Receipt and received condition of tissue for study will be confirmed to the despatching local centre, logged and prepared as below by the national reference laboratory:

Frozen Tissue Preparation

Frozen tissue received will be stored at -80°C at all time. Frozen samples will be prepared for fluorescence *in situ* hybridisation and the production of a haematoxylin and eosin stained section to be sent for assessment alongside FFPE material for central pathology review.

Tissue received from centres will be checked to ensure the patient name/study number transcribed onto the tube matches the paperwork.

1. The frozen tissue will be prepared for sectioning on the cryostat.
2. 1 x 4-7µm section will be cut and transferred to a superfrost microscope slide, fixed in alcohol and haematoxylin and eosin stained as per local methodologies.
3. Touch preparations will be made by placing a superfrost slide on the surface of the tissue and taking two to three touch preps per sample on 4-8 separate superfrost slides.
4. Slides will be fixed in 3:1 methanol to acetic acid and submitted for *FISH* assessment
6. The remaining frozen tissue will be stored at -80°C in cryotubes for subsequent use in approved biological studies.

If multiple pieces of frozen tumour are received, each piece must be individually checked for composition by frozen section and H&E staining, with the help of an experienced pathologist / neuropathologist, to exclude necrotic or normal tissue contamination.

Optional: In countries where touch preparations are prepared for *iFISH* at the local treatment centre, these may be shipped to the national reference centre alongside the other required materials, using established national shipping arrangements. At least 10 slides, 5-6 touches each, preferably from two individual tumour pieces, are required. Tumour cell content should be assessed in all tumour pieces used.

FFPE Tissue Preparation

The paraffin block should at all times have sufficient tissue remaining to allow for further diagnosis if required by the local centre, therefore consideration for this should be given when preparing tissue required for the study.

To allow for central pathology review, up to 20 x 5µm sections are cut on the microtome and placed onto superfrost slides. Two slides will be stained with haematoxylin and eosin (H&E), another with a reticulin silver stain and the remaining spare sections will be available for immunohistochemistry required by the reviewing pathologist, which may include a basic panel of GFAP, synaptophysin, NFP, EMA, LIN28, INI-1 and Ki-67, leaving additional slides if required, to aid central pathology review.

Beta-catenin status will be assessed by immunohistochemistry.

The H&E stained frozen section, paraffin section, IHC panel and beta-catenin stained slides will be issued along with a copy of the local centre pathology report to the reviewing pathologist. These will be retained by the national reference centre for future review and quality control purposes.

If any additional immunohistochemistry to the standard panel is required, the reviewing pathologist will request this and it will be prepared urgently and despatched.

One of the FFPE H & E sections will be marked up by the review pathologist, to indicate regions of exclusive tumour cell content which are representative of the entire sample, in preparation for tissue micro-array construction by the international reference laboratory. A maximum of 5x 1mm diameter cores will be taken depending on tissue availability. (*See international reference laboratory SOP*)

Where tissue permits, all FFPE blocks will be submitted to the international reference laboratory to undergo tissue microarray construction and further processing for biological study. To allow this, the national reference laboratory will send the FFPE block and corresponding H&E slide with tumour areas marked up for coring (see 5) to the international reference laboratory. The FFPE blocks will be returned to the national laboratories within 6 weeks. The marked-up H&E will be retained by the international reference laboratory. (*See international reference laboratory SOP*)

The paraffin block will be returned to the local centre once all of the results have been confirmed and the block returned by the international reference laboratory.

Immunohistochemistry

Immunohistochemistry will be undertaken as per the national reference laboratory procedures. Beta-catenin antibody will be used for staining by the method of the individual national laboratories; however all will use the same primary antibody:

BD transduction laboratories beta-catenin (clone 14), catalogue number 610154

A sample is classified positive for beta-catenin when >10% of nuclei in the tissue section show nuclear positive accumulation.

In tumours with borderline positivity (i.e. 3% to 15% positive cells), repeat staining of the sample is strongly recommended.

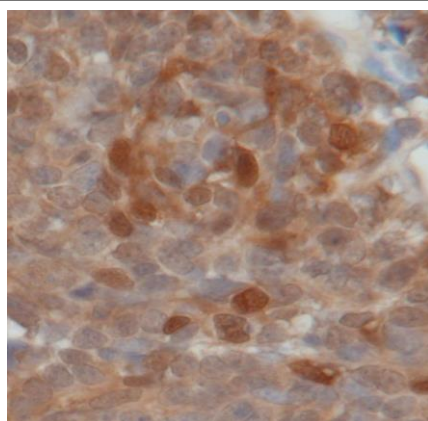


Figure 2: Immunohistochemistry using a beta-catenin primary antiserum allows the reaction to be visualised in tissue sections. If nuclear staining is present, the sample is positive for the WNT/WG pathway activation. The sample shown is positive in approximately 30% of cells.

(Image provided by David W Ellison, Neuropathology St Jude's Children's Hospital, Memphis, TN)

CTNNB1 sequencing

CTNNB1 (Gene ID: 1499; <http://www.ncbi.nlm.nih.gov/gene/1499>) sequencing is required for trial entry (see above):

DNA and RNA will be extracted from fresh-frozen samples according to standard procedures (*see separate DNA and RNA extraction SOP*). Up to 50ng DNA should be sufficient for PCR amplification, in order to preserve DNA for further analyses.

PCR products will span the mutation cluster region in exon 3 of *CTNNB1*, encompassing sequences encoding amino acids 30 to 45. The design of the primers must lead to amplicons that cover nucleotides 791-1070 of Genbank reference sequence X89579. Example oligonucleotide sequences for the amplification of this region may be found in:

Patricia Legoix et al. Beta-catenin mutations in hepatocellular carcinoma correlate with a low rate of loss of heterozygosity, *Oncogene*, 1999, 18:4044:4046. Koch A, et al., Childhood hepatoblastomas frequently carry a mutated degradation targeting box of the beta-catenin gene. *Cancer Res.* 1999; 59:269-73.

Mutations will be searched for using the Sanger direct DNA sequencing method on purified PCR-products. Sequence variants from the reference normal sequence ((Genbank reference sequence X89579)) must be confirmed independently on sequence analysis in both forward and reverse directions.

Variants (at the nucleotide and amino acid level) should be recorded and referenced according to the international nomenclature (<http://www.hgvs.org/mutnomen/>).

Positive results are those cases displaying confirmed non-synonymous missense mutations in the mutation cluster region.

Fluorescence *in situ* hybridisation

Interphase FISH will be carried out using commercial FISH probes to assess copy numbers of *MYC* and *MYCN* in relation to centromeric reference probes (both tests are mandatory), and to assess monosomy 6 status (optional test):

Probes recommended:

Abbott (Patho Vision) LSI *MYCN* SpectrumGreen DNA probe (Cat no. 32-191014)
Kreatech *MYCN* probe Cat. No. KBI-10106
(or other commercial locus-specific probe)

Abbott (Patho Vision) LSI *c-MYC* SpectrumOrange DNA probe (Cat no. 32-190006)
Kreatech *c-MYC* probe Cat. No. KBI-10706
(or other commercial locus-specific probe)

Chromosome 2 centromeric reference probe (*for use with MYC-N probe*)
e.g. Abbott (Patho Vision) CEP 2 SpectrumOrange DNA probe (Cat no. 32-191014)

Chromosome 8 centromeric reference probe (*for use with c-MYC probe*)
e.g. Abbott (Patho Vision) CEP SpectrumGreen DNA probe (32-132008)

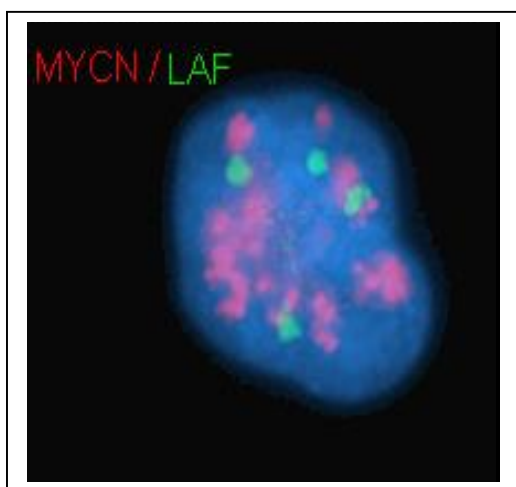
Metasystems Chromosome 6 XL 6q21 / 6q23 dual probe (Order no. D-5039-100-OG)
(or other commercial locus-specific probes. Note two loci must be assessed)

DAPI nuclear counterstain

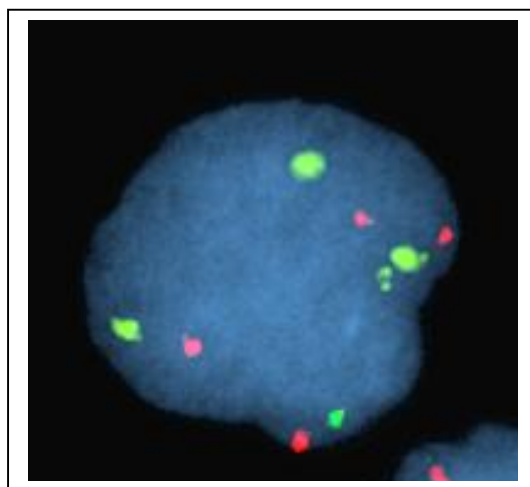
Analysis:

Following sample preparation and undertaking FISH of *MYC* and *MYCN* on medulloblastoma samples, they will be assessed to determine their amplification status.

The images below illustrate an example of a medulloblastoma sample nucleus with a *MYCN* amplification (a), in contrast to a non-amplified sample (b). (*Courtesy of Rachel Newby, NHS Northern Genetics Service, Newcastle*).



a. *MYCN* amplification



b. *MYCN* non-amplified

200 nuclei are counted and *MYC* or *MYCN* amplification-positive cases are determined as those with $\geq 5\%$ of nuclei showing evidence of gene amplification (signals consistent with double minute or homogenously staining region formation, and test probe copy number ≥ 4 times copy number of the reference signal) See Ryan SL et al. (2012) *MYC* family amplification and clinical risk-factors interact to predict an extremely poor prognosis in childhood medulloblastoma. Acta Neuropathol. 123: 501-13.

For monosomy 6 status, 200 nuclei are counted and positive cases are determined as those with $\geq 50\%$ of nuclei showing a single signal for both the p- and q-arm probes (i.e. 1:1).

Recording of FISH data

Representative images should be saved for all FISH investigations, sufficient to allow results to be reviewed by other national reference laboratories.

Determination of *MYC* and *MYCN* amplification and monosomy 6 status by array-based copy number analysis.

DNA and RNA will be extracted from fresh-frozen samples according to standard procedures (see separate DNA and RNA extraction SOP). Samples must contain at least 60% tumour cells.

Recommendations for array-based copy number analysis

Array-CGH, SNP array, and other array-based copy number platforms, are sensitive to contamination by DNA of normal cells. To use these techniques, a sample with a minimum of 60% of tumoural cells is required to obtain good results. In tumour samples containing less than 60% of tumour cells (samples from primary tumours with extensive necrotic, haemorrhagic and/or calcification areas), *MYC* or *MYCN* status must be assessed by FISH.

Copy number must be assessed using a validated commercial platform, or prepared slides, by an accredited genomics reference laboratory. The array should contain DNA sequences (BAC/PAC clones, oligonucleotide sequences, SNPs, etc.) distributed evenly along the whole genome. Chromosomal regions of special interest in medulloblastoma, such as chromosomes 6, 9q or 17q, may be covered more densely; *MYC* or *MYCN* loci should each be covered by at least several probes, and the close neighbouring regions (within 1Mb either side of the locus) should be covered densely.

The presence of replicate probes for specific DNA sequences, on the array, allow for calculation of a mean for a given locus, rendering results more robust.

For comparative hybridisation methods, tumour DNA is analysed with an equal amount of normal reference DNA, quality and quantity checked, digested, purified and labelled with distinct fluorochromes. Following precipitation and denaturing, the samples are hybridised to the slides. The slides are then washed and ready for acquisition of images. Images will be acquired using an appropriate software.

Definition of *MYC* and *MYCN* amplification

Based on published experience comparing copy number seen by FISH and log2ratios obtained by array-CGH [Bourdeaut et al (2013). *MYC* and *MYCN* amplification can be reliably assessed by aCGH in medulloblastoma. Cancer Genet. 206(4): 124-9], amplification is defined by a log2 ratio ≥ 1.5 in at least three probes within the *MYC* or *MYCN* loci. Any focal log2ratio peak suspected to reveal an amplicon at the *MYC* or *MYCN* locus, but with a <1.5 ratio, must be confirmed by FISH. Focal amplicons encompassing *MYC* or *MYCN* loci and not exceeding few megabases should be clearly distinguished from wider chromosome 2p or 8q gains.

Definition of monosomy 6

Monosomy 6 assessment by array-based copy number methods requires a tumour cell content at least equal to 60%. Monosomy is defined by a log2ratio ≤ -0.5 across the whole of chromosome 6, encompassing the p- and q-arms.

In order to appreciate the patterns of log2 ratios obtained for monosomic chromosomes in comparative hybridisation analyses, it is recommended to hybridise tumour from male patients with female control DNA. As an internal control, the minimal log2ratio for a complete monosomy in 100% of the cells is given by the X chromosome log2ratio when XY tumour DNA is matched with XX control DNA.

Data validation by a second national reference laboratory

Results from array-based copy number analysis should be confirmed by exchange and review of data with a second national reference laboratory for the trial.

D.2. Sample preparation by International Reference Laboratory

Purpose and scope, applications.

FFPE Block preparation

As part of the investigative biological studies associated with newly diagnosed medulloblastoma patients recruited to PNET 5 MB - LR and PNET 5 MB - SR clinical trials, the SIOP PNET Biology group international reference laboratory will be responsible for the construction of tissue microarray (TMA) composite blocks, and further processing of the FFPE material, to support biological studies within the trials.

This will be achieved through the submission of FFPE blocks from national reference laboratories of the participating countries, to the international reference centre, once they have completed central pathology review and molecular diagnostic testing of the samples.

Maintaining tissue integrity of the FFPE block for further diagnostic use by the local centre is of paramount importance. Therefore, TMA will only be undertaken if sample size is appropriate. The group recognises that undertaking TMA preparation at a international reference laboratory will optimise preparation of the FFPE resource for all contributing national laboratories and research groups.

Procedure:

1. National reference laboratories submit 1x FFPE block and corresponding haematoxylin and eosin (H&E) stained slide with tumour areas marked for coring (see separate national reference centre SOP), to the international reference laboratory at Newcastle University, UK:

Professor Steven C. Clifford
c/o Dr. Stephen Crosier
Cellular Pathology
Level 3, New Victoria Wing
Royal Victoria Infirmary
Queen Victoria Road
Newcastle upon-Tyne
NE1 4LP, United Kingdom.

Tel: +44 (191) 282 1303
Fax: +44 (191) 282 0656

e-mail: stephen.crosier@nuth.nhs.uk

2. Using the marked-up H&E for reference, up to 5 x 1.0mm tissue cores will be taken from the donor FFPE tissue blocks submitted and transferred to a recipient composite block. Tissue core positions will be robustly transcribed. The number of cores taken will depend upon the surface area and depth of the donor FFPE block.

3. If sufficient tissue remains in the block, the international reference laboratory will also cut the following to be available for subsequent investigative biological studies:
 - a. 2 x 20 micron FFPE curls for DNA extraction
 - b. 2 x 20 micron FFPE curls for preparation of nuclear extract slides
 - c. 2 x 20 micron curls for RNA extraction (deepest curls)

All curls will be stored at -80°C.

The German national reference centre will undertake this tissue processing for German and Swiss cases, using the same procedures as the international reference centre.

Material will subsequently be distributed to research groups for approved investigative biological studies conducted by the SIOP PNET Biology group.

All FFPE blocks will be returned to national reference laboratories within 6 weeks of receipt. The marked up H&E sections will be retained at the international reference laboratory.

D.3. Sampling guidelines for blood

Purpose

Blood of each patient included in PNET 5 MB should be stored to (i) extract constitutional DNA as control material for genomic analyses performed on the frozen tumour tissue and (ii) harvest the plasma for research of diagnostic markers.

Sample acquisition and preparation

A blood sample of 5-10 ml should be taken into an EDTA or citrate tube during a clinically needed blood sampling to avoid extra venopunctures:

The blood preparation should be performed ideally within 2 hours after taking of the blood sample.

1. Label 1.5 ml Eppendorf tubes with patient code and date.
2. Centrifuge the 10 ml EDTA or citrate blood vial at 4 °C: 1100 x g 10 min.
3. Take supernatant and aliquot into 1 ml aliquots, transfer aliquots into the labeled Eppendorf tubes and store at -80°C.
4. After having removed the supernatant fill up the tube to 10ml with a hypotonic buffer.
5. Incubate at 4°C for 30 min.
6. Centrifuge at 1500 rpm 5-10min.
7. Decanter supernatant.
8. Resuspend leukocytes with 1ml PBS.
9. Transfer (ideally 2 aliquots) them to a 1.5ml Eppendorf tube.
10. Spin down briefly and remove supernatant.
11. Snap-freeze the pellet(s) and store them at -80°.

If separation into leukocyte pellet and plasma is not possible, please store the whole blood sample at -80°C.

Retain tubes initially at the local treatment centre. Samples should periodically be shipped to the national reference centre, who will coordinate the shipment.

D.4. Sampling guidelines for cerebrospinal fluid (CSF) for research use

Purpose

CSF of each patient included in PNET 5 MB - LR and PNET 5 MB - SR trials should be stored for research use.

Sample acquisition and preparation

Lumbar puncture showing a CSF free of tumour cells is mandatory before randomisation. It is allowed (and recommended for keeping time lines for stratification and to start radiotherapy, if the clinical situation allows lumbar puncture) to perform lumbar puncture within the first 2 weeks after surgery. If a lumbar puncture is performed before 15 days after surgery and is negative for tumour cells, then this will be taken as evidence of non-metastatic disease. If, however, the CSF is positive for tumour cells on lumbar puncture taken before day 15, the lumbar puncture must be repeated at day 15 or later.

In case of equivocal CSF cytology, performance of a second lumbar puncture is also recommended. (see chapter 8.1. *Screening investigations*, page 58)

In addition to the CSF material routinely collected for clinical assessment, additional CSF should be harvested when possible, and the sample divided for preparation as follows.

Preparation of CSF (if procedures are possible at local treatment centre):

Label 1.5ml Eppendorf tubes with patient code, date and 'CSF supernatant'

Centrifuge CSF

After centrifugation save the supernatant

Aliquot supernatant into 0.5ml aliquots and transfer into labeled tubes

Store tubes at -80°C.

For preparation of any additional CSF collected (or if collection of CSF supernatant (above) is not possible):

Label 1.5ml Eppendorf tubes with patient code, date and 'whole CSF'

Aliquot CSF into 0.5ml aliquots and transfer into labeled tubes

Store tubes at -80°C.

Storage and shipment:

Retain tubes at local treatment centre.

Samples should periodically be shipped to the national reference centre, who will coordinate the shipment.

D.5. DNA and RNA extraction at national reference centres

Purpose

High molecular weight DNA and RNA will be extracted from frozen tumour biopsies at national reference centres. DNA will be used to conduct β -catenin (*CTNNB1*) mutation testing. Excess DNA and RNA will be made available to research centres to conduct approved biological studies, according to national regulation and consent of the patient.

Sample preparation

1. Frozen tumour biopsies provided by local treatment centres should be stored at -80°C at all times.
2. Frozen tumour biopsies with confirmed high tumour cell content (determined by H&E stain of a frozen section - see national reference centre sample preparation SOP) should be chipped into small aliquots under liquid nitrogen, and stored in cryovials.
3. High molecular weight DNA and RNA should be extracted from chipped tissue aliquots using the 'AllPrep DNA/RNA extraction mini kit' (Qiagen), according to the manufacturer's instructions.
4. Extracted high molecular weight DNA should be resuspended at a concentration >75ng/μl, in water or an appropriate buffer (e.g. TE), in 1.5ml eppendorf tubes. Concentration (ng/μl) and total yield (ng) should be assessed spectrophotometrically (absorbance at 260nm), and its 260/280nm ratio determined. These values should be recorded.
5. Extracted RNA should be resuspended in water at a concentration >75ng/μl, in 1.5ml eppendorf tubes. Concentration (ng/μl) and total yield (ng) should be assessed spectrophotometrically (absorbance at 260nm), and its 260/280nm ratio determined. These values should be recorded.
6. RNA quality should be assessed using a bioanalyser (Agilent), and the RIN value recorded.
7. Extracted nucleic acids should be stored at -80°C and retained at the national reference centre.

Aliquots of extracts should periodically be shipped on request to research centres, to undertake approved trials biological studies. Extract movements will be coordinated by the SIOP PNET Biology group.

D.6. National SOPs (Country specific)

E- Patient / Parent Information Forms and Informed Consent

Accepted national procedures for patient consent are to be used. Therefore these forms have to be designed separately by each participating national group.

The patient's and/or parent's written consent to participate in the study must be obtained after a full explanation has been given of the treatment options including the conventional and generally accepted methods of treatment and the manner of treatment allocation. For children and adolescents who are too young to give informed consent, depending on national definitions, informed consent will be obtained in written form from the parents. Patient assent may also be obtained, depending on national requirements. Additionally the child should receive an explanation as to his/her means of understanding and should give consent as well, if he/she is able to do so. Enough time and the opportunity to discuss participation before the decision for and start of treatment have to be given. The right of a patient to refuse to participate without giving reasons must be respected.

It is recommended to obtain a separate consent for the submission of tumour material, MRI, and patient data at the time when the pathological diagnosis of medulloblastoma has been made locally, as these need to be sent to the respective reference centres before eligibility for PNET 5 MB - LR and PNET 5 MB - SR can be checked.

Written informed consent should also be obtained for the biological studies, in accordance with national guidelines. These should include consent for the link-anonymised investigation of all biological materials collected (i.e. tumour biopsy, CSF, blood), including germline DNA.

E.1.	Information on Follow-up Evaluations - Template	2
E.2. and following	National Informed Consent Forms	4

E.1. - Information on Follow-Up Evaluations - Template

Information for adults and young adults about necessary follow-up evaluations

Dear Patient,

you have been treated for a medulloblastoma within the PNET 5 MB study. Although treatment has been completed, regular follow-up examinations through a specialized, multidisciplinary team is highly recommended.

Follow-up examinations are necessary to detect a possible relapse of the medulloblastoma, tumour and therapy-related late-effects, and a possible secondary neoplasm.

Relapses:

An intensive treatment as in PNET 5 MB is effective in long term disease control in the majority of patients, who have been diagnosed with a localised medulloblastoma of moderate or low biological risk group. However, some patients experience relapses after the end of therapy. While the risk of relapse is highest in the first 2 years after the end of treatment, in some cases relapses also occur several years after treatment.

Regular clinical and neurological examinations, and MRI evaluations of the brain will be performed for detection of relapse.

Late-effects:

Treatment can lead to side effects, which sometimes manifest after a prolonged interval after treatment. On the one hand the diagnosis of the late effects is necessary to arrange for the appropriate support, on the other hand evaluation and documentation of treatment related side effects is necessary for the assessment of the study treatment.

Examples for treatment related side effects, which can be detected even some time after the end of treatment may be:

- Hearing deficits:

Some patients experience clinically relevant hearing impairment after treatment for a medulloblastoma. Monitoring of hearing deficits has been performed while and after the therapy. However, even in the absence of a clinically relevant hearing deficit at the end of therapy, in very rare cases, this might be apparent at a later time point. Patients with documented hearing deficit at the end of therapy might experience worsening of hearing function over time. In case of apparent, clinical relevant hearing deficit, respective hearing aids can be prescribed.

- Deficits in hormone regulation:

Deficits in hormone regulations are commonly observed after treatment for medulloblastoma. These can be monitored using blood tests. Due to the age specific complex regulation of hormone excretion, deficits can also manifest sooner or later after the end of therapy, depending on the age of the patient. Hormonal dysfunctions can lead to:

- Problems with growth
- Problems with pubertal development
- Problems concerning fertility
- Thyroid problems

Therapy would include individual adapted hormone substitution.

- Renal function:

Very rarely, impairment of renal function can be experienced after treatment of medulloblastoma. Monitoring of deficits in renal function has been performed on treatment and may be required after completion of chemotherapy.

- Neurocognitive / neurologic impairments:

Some patients experience impairments in specific physical or mental abilities. This might lead to specific questions and problems in selecting appropriate educational or occupational options. In these cases, an experienced psychosocial team should be able to give necessary support.

Secondary neoplasm:

Rarely, another tumour within the brain, within the thyroid gland, or at another site can occur years after the end of the treatment. Surveillance within follow-up cannot encompass all possible manifestations, but a regular thyroid screening (yearly TSH level) is indicated.

The following evaluations should be performed for post treatment follow-up

	1. year after treatment	2. year after treatment	3-5. year after treatment	> 5 years after treatment
Clinical / Neurological evaluation	Every 4 month	At least every 6 month	At least every year	
Cranial MRI			At least every year	at suspicion
Audiometry	1 year, and 4 years after end of treatment*			individually
Auxiology/ Endocrinology	Refer to endocrinology by 2 years- then at least 6monthly			6monthly to 18years (adult) then 1-2yearly
Renal function	At least 1/year			individually
QoL	1 years and 4 years after end of treatment*, and at 18 years of age			None after 18years
additional individual evaluations, as due to clinical indication				

* i.e. 2 and 5 years after diagnosis

Documentation of late-effects for the PNET 5 MB study is necessary 2 and 5 years after treatment, and at age 18.

If due to your age you cannot be followed by your paediatric oncologic team anymore, please make shure, that the necessary documentation of late effects will be performed by the respective medical team in charge – or data will be transferred for documentation to the paediatric oncologic team.

If you are seeking medical help outside the specialized follow-up care, please inform your physician on the diagnosis and treatment of medulloblastoma.

F – Common Toxicity Criteria**COMMON TERMINOLOGY CRITERIA FOR ADVERSE EVENTS (CTCAE) Version 4.0 - EXCERPT**

ABBREVIATIONS: ADL = Activities of Daily Living
ULN = upper limit of normal
LLN = lower limit of normal

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CTCAE v4.0 Term	Grade 1	Grade 2	Grade 3	Grade 4	Grade 5	CTCAE v4.0 AE Term Definition
Anemia	Hemoglobin (Hgb) <LLN - 10.0 g/dL; <LLN - 6.2 mmol/L; <LLN - 100 g/L	Hgb <10.0 - 8.0 g/dL; <6.2 - 4.9 mmol/L; <100 - 80g/L	Hgb <8.0 g/dL; <4.9 mmol/L; <80 g/L; transfusion indicated	Life-threatening consequences; urgent intervention indicated	Death	A disorder characterized by a reduction in the amount of hemoglobin in 100 ml of blood. Signs and symptoms of anemia may include pallor of the skin and mucous membranes, shortness of breath, palpitations of the heart, soft systolic murmurs, lethargy, and fatigability.
Febrile neutropenia	-	-	ANC <1000/mm ³ with a single temp. of >38.3°C or a sustained temp. of ≥38°C for more than one hour	Life-threatening consequences; urgent intervention indicated	Death	A disorder characterized by an ANC <1000/mm ³ and a single temperature of >38.3 degrees C (101 degrees F) or a sustained temperature of ≥38 degrees C (100.4 degrees F) for more than one hour
Myocardial infarction	-	Asymptomatic and cardiac enzymes minimally abnormal and no evidence of ischemic ECG changes	Severe symptoms; cardiac enzymes abnormal; hemodynamically stable; ECG changes consistent with infarction	Life-threatening consequences; hemodynamically unstable	Death	A disorder characterized by gross necrosis of the myocardium; this is due to an interruption of blood supply to the area.
External ear inflammation	External otitis with erythema or dry desquamation	External otitis with moist desquamation, edema, enhanced cerumen or discharge; tympanic membrane perforation; tympanostomy	External otitis with mastoiditis; stenosis or osteomyelitis; necrosis of soft tissue or bone	Urgent operative intervention indicated	Death	A disorder characterized by inflammation, swelling and redness to the outer ear and ear canal.

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Cataract	Asymptomatic; clinical or diagnostic observations only; intervention not indicated	Symptomatic; moderate decrease in visual acuity (20/40 or better)	Symptomatic with marked decrease in visual acuity (worse than 20/40 but better than 20/200); operative intervention indicated (e.g., cataract surgery)	Blindness (20/200 or worse) in the affected eye	-	A disorder characterized by partial or complete opacity of the crystalline lens of one or both eyes. This results in a decrease in visual acuity and eventual blindness if untreated.
Dry eye	Asymptomatic; clinical or diagnostic observations only; mild symptoms relieved by lubricants	Symptomatic; multiple agents indicated; limiting instrumental ADL	Decrease in visual acuity (<20/40); limiting self care ADL	-	-	A disorder characterized by dryness of the cornea and conjunctiva.
Keratitis	-	Symptomatic; medical intervention indicated (e.g., topical agents); limiting instrumental ADL	Decline in vision (worse than 20/40 but better than 20/200); limiting self care ADL	Perforation or blindness (20/200 or worse) in the affected eye	-	A disorder characterized by inflammation to the cornea of the eye.
Constipation	Occasional or intermittent symptoms; occasional use of stool softeners, laxatives, dietary modification, or enema	Persistent symptoms with regular use of laxatives or enemas; limiting instrumental ADL	Obstipation with manual evacuation indicated; limiting self care ADL	Life-threatening consequences; urgent intervention indicated	Death	A disorder characterized by irregular and infrequent or difficult evacuation of the bowels.

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Diarrhea	Increase of <4 stools per day over baseline; mild increase in ostomy output compared to baseline	Increase of 4 - 6 stools per day over baseline; moderate increase in ostomy output compared to baseline	Increase of ≥ 7 stools per day over baseline; incontinence; hospitalization indicated; severe increase in ostomy output compared to baseline; limiting self care ADL	Life-threatening consequences; urgent intervention indicated	Death	A disorder characterized by frequent and watery bowel movements.
Dysphagia	Symptomatic, able to eat regular diet	Symptomatic and altered eating/swallowing	Severely altered eating/swallowing; tube feeding or TPN or hospitalization indicated	Life-threatening consequences; urgent intervention indicated	Death	A disorder characterized by difficulty in swallowing.
Mucositis oral	Asymptomatic or mild symptoms; intervention not indicated	Moderate pain; not interfering with oral intake; modified diet indicated	Severe pain; interfering with oral intake	Life-threatening consequences; urgent intervention indicated	Death	A disorder characterized by inflammation of the oral mucosal.
Nausea	Loss of appetite without alteration in eating habits	Oral intake decreased without significant weight loss, dehydration or malnutrition	Inadequate oral caloric or fluid intake; tube feeding, TPN, or hospitalization indicated	-	-	A disorder characterized by a queasy sensation and/or the urge to vomit.
Vomiting	1 - 2 episodes (separated by 5 minutes) in 24 hrs	3 - 5 episodes (separated by 5 minutes) in 24 hrs	≥ 6 episodes (separated by 5 minutes) in 24 hrs; tube feeding, TPN or hospitalization indicated	Life-threatening consequences; urgent intervention indicated	Death	A disorder characterized by the reflexive act of ejecting the contents of the stomach through the mouth.

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Fatigue	Fatigue relieved by rest	Fatigue not relieved by rest; limiting instrumental ADL	Fatigue not relieved by rest, limiting self care ADL	-	-	A disorder characterized by a state of generalized weakness with a pronounced inability to summon sufficient energy to accomplish daily activities.
Infusion related reaction	Mild transient reaction; infusion interruption not indicated; intervention not indicated	Therapy or infusion interruption indicated but responds promptly to symptomatic treatment (e.g., antihistamines, NSAIDS, narcotics, IV fluids); prophylactic medications indicated for ≤24 hrs	Prolonged (e.g., not rapidly responsive to symptomatic medication and/or brief interruption of infusion); recurrence of symptoms following initial improvement; hospitalization indicated for clinical sequelae	Life-threatening consequences; urgent intervention indicated	Death	A disorder characterized by adverse reaction to the infusion of pharmacological or biological substances.
Infusion site extravasation	-	Erythema with associated symptoms (e.g., edema, pain, induration, phlebitis)	Ulceration or necrosis; severe tissue damage; operative intervention indicated	Life-threatening consequences; urgent intervention indicated	Death	A disorder characterized by leakage of a pharmacologic or a biologic substance from the infusion site into the surrounding tissue. Signs and symptoms include induration, erythema, swelling, burning sensation and marked discomfort at the infusion site.
Multi-organ failure	-	-	Shock with azotemia and acid-base disturbances; significant coagulation abnormalities	Life-threatening consequences (e.g., vasopressor dependent and oliguric or anuric or ischemic colitis or lactic acidosis)	Death	A disorder characterized by progressive deterioration of the lungs, liver, kidney and clotting mechanisms.

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Allergic reaction	Transient flushing or rash, drug fever <38 degrees C (<100.4 degrees F); intervention not indicated	Intervention or infusion interruption indicated; responds promptly to symptomatic treatment (e.g., antihistamines, NSAIDs, narcotics); prophylactic medications indicated for <=24 hrs	Prolonged (e.g., not rapidly responsive to symptomatic medication and/or brief interruption of infusion); recurrence of symptoms following initial improvement; hospitalization indicated for clinical sequelae (e.g., renal impairment, pulmonary infiltrates)	Life-threatening consequences; urgent intervention indicated	Death	A disorder characterized by an adverse local or general response from exposure to an allergen.
Infection	-	Localized; local intervention indicated (e.g., topical antibiotic, antifungal, or antiviral)	IV antibiotic, antifungal, or antiviral intervention indicated; radiologic or operative intervention indicated	Life-threatening consequences; urgent intervention indicated	Death	A disorder characterized by an infectious process. A localisation must be given.
Dermatitis radiation	Faint erythema or dry desquamation	Moderate to brisk erythema; patchy moist desquamation, mostly confined to skin folds and creases; moderate edema	Moist desquamation in areas other than skin folds and creases; bleeding induced by minor trauma or abrasion	Life-threatening consequences; skin necrosis or ulceration of full thickness dermis; spontaneous bleeding from involved site; skin graft indicated	Death	A finding of cutaneous inflammatory reaction occurring as a result of exposure to biologically effective levels of ionizing radiation.

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Blood bilirubin increased	>ULN - 1.5 x ULN	>1.5 - 3.0 x ULN	>3.0 - 10.0 x ULN	>10.0 x ULN	-	A finding based on laboratory test results that indicate an abnormally high level of bilirubin in the blood. Excess bilirubin is associated with jaundice.
Creatinine increased	>1 - 1.5 x baseline; >ULN - 1.5 x ULN	>1.5 - 3.0 x baseline; >1.5 - 3.0 x ULN	>3.0 baseline; >3.0 - 6.0 x ULN	>6.0 x ULN	-	A finding based on laboratory test results that indicate increased levels of creatinine in a biological specimen.
Ejection fraction decreased	-	Resting ejection fraction (EF) 50 - 40%; 10 - 19% drop from baseline	Resting ejection fraction (EF) 39 - 20%; >20% drop from baseline	Resting ejection fraction (EF) <20%	-	The percentage computed when the amount of blood ejected during a ventricular contraction of the heart is compared to the amount that was present prior to the contraction.
Lymphocyte count decreased	<LLN - 800/mm ³ ; <LLN - 0.8 x 10 ⁹ /L	<800 - 500/mm ³ ; <0.8 - 0.5 x 10 ⁹ /L	<500 - 200/mm ³ ; <0.5 - 0.2 x 10 ⁹ /L	<200/mm ³ ; <0.2 x 10 ⁹ /L	-	A finding based on laboratory test results that indicate a decrease in number of lymphocytes in a blood specimen.
Neutrophil count decreased	<LLN - 1500/mm ³ ; <LLN - 1.5 x 10 ⁹ /L	<1500 - 1000/mm ³ ; <1.5 - 1.0 x 10 ⁹ /L	<1000 - 500/mm ³ ; <1.0 - 0.5 x 10 ⁹ /L	<500/mm ³ ; <0.5 x 10 ⁹ /L	-	A finding based on laboratory test results that indicate a decrease in number of neutrophils in a blood specimen.
Platelet count decreased	<LLN - 75,000/mm ³ ; <LLN - 75.0 x 10 ⁹ /L	<75,000 - 50,000/mm ³ ; <75.0 - 50.0 x 10 ⁹ /L	<50,000 - 25,000/mm ³ ; <50.0 - 25.0 x 10 ⁹ /L	<25,000/mm ³ ; <25.0 x 10 ⁹ /L	-	A finding based on laboratory test results that indicate a decrease in number of platelets in a blood specimen.
Weight loss	5 to <10% from baseline; intervention not indicated	10 - <20% from baseline; nutritional support indicated	>=20% from baseline; tube feeding or TPN indicated	-	-	A finding characterized by a decrease in overall body weight; for paediatrics, less than the baseline growth curve.
Paresthesia	Mild symptoms	Moderate symptoms; limiting instrumental ADL	Severe symptoms; limiting self care ADL	-	-	A disorder characterized by functional disturbances of sensory neurons resulting in abnormal cutaneous sensations of tingling, numbness, pressure, cold, and warmth that are experienced in the absence of a stimulus.
Peripheral neuropathy	Asymptomatic	Moderate symptoms; limiting instrumental ADL	Severe symptoms; limiting self care ADL	Life-threatening consequences; urgent intervention indicated	Death	A disorder characterized by inflammation or degeneration of the peripheral sensory or motor nerves (please indicate which).

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Seizure	Brief partial seizure; no loss of consciousness	Brief generalized seizure	Multiple seizures despite medical intervention	Life-threatening; prolonged repetitive seizures	Death	A disorder characterized by a sudden, involuntary skeletal muscular contractions of cerebral or brain stem origin.
Somnolence	Mild but more than usual drowsiness or sleepiness	Moderate sedation; limiting instrumental ADL	Obtundation or stupor	Life-threatening consequences; urgent intervention indicated	Death	A disorder characterized by characterized by excessive sleepiness and drowsiness.
Chronic kidney disease	eGFR (estimated Glomerular Filtration Rate) or CrCl (creatinine clearance) <LLN - 60 ml/min/1.73 m ² or proteinuria 2+ present; urine protein/creatinine >0.5	eGFR or CrCl 59 - 30 ml/min/1.73 m ²	eGFR or CrCl 29 - 15 ml/min/1.73 m ²	eGFR or CrCl <15 ml/min/1.73 m ² ; dialysis or renal transplant indicated	Death	A disorder characterized by gradual and usually permanent loss of kidney function resulting in renal failure.
Hematuria	Asymptomatic; clinical or diagnostic observations only; intervention not indicated	Symptomatic; urinary catheter or bladder irrigation indicated; limiting instrumental ADL	Gross hematuria; transfusion, IV medications or hospitalization indicated; elective endoscopic, radiologic or operative intervention indicated; limiting self care ADL	Life-threatening consequences; urgent radiologic or operative intervention indicated	Death	A disorder characterized by laboratory test results that indicate blood in the urine.

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Proteinuria	1+ proteinuria; urinary protein <1.0 g/24 hrs	Adults: 2+ proteinuria; urinary protein 1.0 - 3.4 g/24 hrs; Paediatric: urine P/C (Protein/Creatinine) ratio 0.5 - 1.9	Adults: urinary protein ≥3.5 g/24 hrs; Paediatric: urine P/C >1.9	-	-	A disorder characterized by laboratory test results that indicate the presence of excessive protein in the urine. It is predominantly albumin, but also globulin.
Dyspnea	Shortness of breath with moderate exertion	Shortness of breath with minimal exertion; limiting instrumental ADL	Shortness of breath at rest; limiting self care ADL	Life-threatening consequences; urgent intervention indicated	Death	A disorder characterized by an uncomfortable sensation of difficulty breathing.
Pulmonary edema	Radiologic findings only; minimal dyspnea on exertion	Moderate dyspnea on exertion; medical intervention indicated; limiting instrumental ADL	Severe dyspnea or dyspnea at rest; oxygen indicated; limiting self care ADL	Life-threatening respiratory compromise; urgent intervention or intubation with ventilatory support indicated	Death	A disorder characterized by accumulation of fluid in the lung tissues that causes a disturbance of the gas exchange that may lead to respiratory failure.
Erythroderma	-	Erythema covering >90% BSA without associated symptoms; limiting instrumental ADL	Erythema covering >90% BSA with associated symptoms (e.g., pruritus or tenderness); limiting self care ADL	Erythema covering >90% BSA with associated fluid or electrolyte abnormalities; ICU care or burn unit indicated	Death	A disorder characterized by generalized inflammatory erythema and exfoliation. The inflammatory process involves > 90% of the body surface area.
Rash	covering <10% BSA with or without symptoms (e.g., pruritus, burning, tightness)	covering 10 - 30% BSA with or without symptoms (e.g., pruritus, burning, tightness); limiting instrumental ADL	covering >30% BSA with or without associated symptoms; limiting self care ADL	associated with extensive superinfection with IV antibiotics indicated; life-threatening consequences	-	A disorder characterized by the presence of rash.

G – Ethical Considerations And Declaration Of Helsinki

Ethical Considerations

The trial protocol must be approved by the appropriate ethical committee in each country prior to patient entry. The patient's and/or parent's written consent to participate in the study must be obtained after a full explanation has been given concerning the treatment. As well as consent for the clinical trial, consent is required for participation in the biological studies and, depending on national law, consent is needed for data collection, storage, transfer and analysis. Additionally the child should receive an explanation as to his/her means of understanding and should give consent as well if he or she is able to do it.

The right of a patient to refuse to participate without giving reasons must be respected. The patient must remain free to withdraw at any time from protocol without prejudicing his/her further treatment. The study observes the rules for clinical research set out in the ICH/GCP and EC rules of good clinical practice.

World Medical Association Declaration of Helsinki: Ethical Principles for Medical Research Involving Human Subjects

Adopted by the 18th WMA General Assembly, Helsinki, Finland, June 1964, and amended by the:

29th WMA General Assembly, Tokyo, Japan, October 1975

35th WMA General Assembly, Venice, Italy, October 1983

41st WMA General Assembly, Hong Kong, September 1989

48th WMA General Assembly, Somerset West, Republic of South Africa, October 1996

52nd WMA General Assembly, Edinburgh, Scotland, October 2000

53rd WMA General Assembly, Washington, DC, USA, October 2002 (Note of Clarification added)

55th WMA General Assembly, Tokyo, Japan, October 2004 (Note of Clarification added)

59th WMA General Assembly, Seoul, Republic of Korea, October 2008

64th WMA General Assembly, Fortaleza, Brazil, October 2013

PREAMBLE

1. The World Medical Association (WMA) has developed the Declaration of Helsinki as a statement of ethical principles for medical research involving human subjects, including research on identifiable human material and data.

The Declaration is intended to be read as a whole and each of its constituent paragraphs should be applied with consideration of all other relevant paragraphs.

2. Consistent with the mandate of the WMA, the Declaration is addressed primarily to physicians. The WMA encourages others who are involved in medical research involving human subjects to adopt these principles.

GENERAL PRINCIPLES

3. The Declaration of Geneva of the WMA binds the physician with the words, "The health of my patient will be my first consideration," and the International Code of Medical Ethics declares that, "A physician shall act in the patient's best interest when providing medical care."

4. It is the duty of the physician to promote and safeguard the health, well-being and rights of patients, including those who are involved in medical research. The physician's knowledge and conscience are dedicated to the fulfilment of this duty.

5. Medical progress is based on research that ultimately must include studies involving human subjects.

6. The primary purpose of medical research involving human subjects is to understand the causes, development and effects of diseases and improve preventive, diagnostic and therapeutic interventions (methods, procedures and treatments). Even the best proven interventions must be evaluated continually through research for their safety, effectiveness, efficiency, accessibility and quality.

7. Medical research is subject to ethical standards that promote and ensure respect for all human subjects and protect their health and rights.

8. While the primary purpose of medical research is to generate new knowledge, this goal can never take precedence over the rights and interests of individual research subjects.

9. It is the duty of physicians who are involved in medical research to protect the life, health, dignity, integrity, right to self-determination, privacy, and confidentiality of personal information of research subjects. The responsibility for the protection of research subjects must always rest with the physician or other health care professionals and never with the research subjects, even though they have given consent.

10. Physicians must consider the ethical, legal and regulatory norms and standards for research involving human subjects in their own countries as well as applicable international norms and standards. No national or international ethical, legal or regulatory requirement should reduce or eliminate any of the protections for research subjects set forth in this Declaration.

11. Medical research should be conducted in a manner that minimises possible harm to the environment.

12. Medical research involving human subjects must be conducted only by individuals with the appropriate ethics and scientific education, training and qualifications. Research on patients or healthy volunteers requires the supervision of a competent and appropriately qualified physician or other health care professional.

13. Groups that are underrepresented in medical research should be provided appropriate access to participation in research.

14. Physicians who combine medical research with medical care should involve their patients in research only to the extent that this is justified by its potential preventive, diagnostic or therapeutic value and if the physician has good reason to believe that participation in the research study will not adversely affect the health of the patients who serve as research subjects.

15. Appropriate compensation and treatment for subjects who are harmed as a result of participating in research must be ensured.

RISKS, BURDENS AND BENEFITS

16. In medical practice and in medical research, most interventions involve risks and burdens.

Medical research involving human subjects may only be conducted if the importance of the objective outweighs the risks and burdens to the research subjects.

17. All medical research involving human subjects must be preceded by careful assessment of predictable risks and burdens to the individuals and groups involved in the research in comparison with foreseeable benefits to them and to other individuals or groups affected by the condition under investigation.

Measures to minimise the risks must be implemented. The risks must be continuously monitored, assessed and documented by the researcher.

18. Physicians may not be involved in a research study involving human subjects unless they are confident that the risks have been adequately assessed and can be satisfactorily managed.

When the risks are found to outweigh the potential benefits or when there is conclusive proof of definitive outcomes, physicians must assess whether to continue, modify or immediately stop the study.

VULNERABLE GROUPS AND INDIVIDUALS

19. Some groups and individuals are particularly vulnerable and may have an increased likelihood of being wronged or of incurring additional harm.

All vulnerable groups and individuals should receive specifically considered protection.

20. Medical research with a vulnerable group is only justified if the research is responsive to the health needs or priorities of this group and the research cannot be carried out in a non-vulnerable group. In addition, this group should stand to benefit from the knowledge, practices or interventions that result from the research.

SCIENTIFIC REQUIREMENTS AND RESEARCH PROTOCOLS

21. Medical research involving human subjects must conform to generally accepted scientific principles, be based on a thorough knowledge of the scientific literature, other relevant sources of information, and adequate laboratory and, as appropriate, animal experimentation. The welfare of animals used for research must be respected.

22. The design and performance of each research study involving human subjects must be clearly described and justified in a research protocol.

The protocol should contain a statement of the ethical considerations involved and should indicate how the principles in this Declaration have been addressed. The protocol should include information regarding funding, sponsors, institutional affiliations, potential conflicts of interest, incentives for subjects and information regarding provisions for treating and/or compensating subjects who are harmed as a consequence of participation in the research study.

In clinical trials, the protocol must also describe appropriate arrangements for post-trial provisions.

RESEARCH ETHICS COMMITTEES

23. The research protocol must be submitted for consideration, comment, guidance and approval to the concerned research ethics committee before the study begins. This committee must be transparent in its functioning, must be independent of the researcher, the sponsor and any other undue influence and must be duly qualified. It must take into consideration the laws and regulations of the country or countries in which the research is to be performed as well as applicable international norms and standards but these must not be allowed to reduce or eliminate any of the protections for research subjects set forth in this Declaration.

The committee must have the right to monitor ongoing studies. The researcher must provide monitoring information to the committee, especially information about any serious adverse events. No amendment to the protocol may be made without consideration and approval by the committee. After the end of the study, the researchers must submit a final report to the committee containing a summary of the study's findings and conclusions.

PRIVACY AND CONFIDENTIALITY

24. Every precaution must be taken to protect the privacy of research subjects and the confidentiality of their personal information

INFORMED CONSENT

25. Participation by individuals capable of giving informed consent as subjects in medical research must be voluntary. Although it may be appropriate to consult family members or community leaders, no individual capable of giving informed consent may be enrolled in a research study unless he or she freely agrees.

26. In medical research involving human subjects capable of giving informed consent, each potential subject must be adequately informed of the aims, methods, sources of funding, any possible conflicts of interest, institutional affiliations of the researcher, the anticipated benefits and potential risks of the study and the discomfort it may entail, post-study provisions and any other relevant aspects of the study. The potential subject must be informed of the right to refuse to participate in the study or to withdraw consent to participate at any time without reprisal. Special attention should be given to the specific information needs of individual potential subjects as well as to the methods used to deliver the information.

After ensuring that the potential subject has understood the information, the physician or another appropriately qualified individual must then seek the potential subject's freely-given informed consent, preferably in writing. If the consent cannot be expressed in writing, the non-written consent must be formally documented and witnessed.

All medical research subjects should be given the option of being informed about the general outcome and results of the study.

27. When seeking informed consent for participation in a research study the physician must be particularly cautious if the potential subject is in a dependent relationship with the physician or may consent under duress. In such situations the informed consent must be sought by an appropriately qualified individual who is completely independent of this relationship.

28. For a potential research subject who is incapable of giving informed consent, the physician must seek informed consent from the legally authorised representative. These individuals must not be included in a research study that has no likelihood of benefit for them unless it is intended to promote the health of the group represented by the potential subject, the research cannot instead be performed with persons capable of providing informed consent, and the research entails only minimal risk and minimal burden.

29. When a potential research subject who is deemed incapable of giving informed consent is able to give assent to decisions about participation in research, the physician must seek that assent in addition to the consent of the legally authorised representative. The potential subject's dissent should be respected.

30. Research involving subjects who are physically or mentally incapable of giving consent, for example, unconscious patients, may be done only if the physical or mental condition that prevents giving informed consent is a necessary characteristic of the research group. In such circumstances the physician must seek informed consent from the legally authorised representative. If no such representative is available and if the research cannot be delayed, the study may proceed without informed consent provided that the specific reasons for involving subjects with a condition that renders them unable to give informed consent have been stated in the research protocol and the study has been approved by a research ethics committee. Consent to remain in the research must be obtained as soon as possible from the subject or a legally authorised representative.

31. The physician must fully inform the patient which aspects of their care are related to the research. The refusal of a patient to participate in a study or the patient's decision to withdraw from the study must never adversely affect the patient-physician relationship.

32. For medical research using identifiable human material or data, such as research on material or data contained in biobanks or similar repositories, physicians must seek informed consent for its collection, storage and/or reuse. There may be exceptional situations where consent would be impossible or impracticable to obtain for such research. In such situations the research may be done only after consideration and approval of a research ethics committee.

USE OF PLACEBO

33. The benefits, risks, burdens and effectiveness of a new intervention must be tested against those of the best proven intervention(s), except in the following circumstances:

Where no proven intervention exists, the use of placebo, or no intervention, is acceptable; or

Where for compelling and scientifically sound methodological reasons the use of any intervention less effective than the best proven one, the use of placebo, or no intervention is necessary to determine the efficacy or safety of an intervention

and the patients who receive any intervention less effective than the best proven one, placebo, or no intervention will not be subject to additional risks of serious or irreversible harm as a result of not receiving the best proven intervention.

Extreme care must be taken to avoid abuse of this option.

POST-TRIAL PROVISIONS

34. In advance of a clinical trial, sponsors, researchers and host country governments should make provisions for post-trial access for all participants who still need an intervention identified as beneficial in the trial. This information must also be disclosed to participants during the informed consent process.

RESEARCH REGISTRATION AND PUBLICATION AND DISSEMINATION OF RESULTS

35. Every research study involving human subjects must be registered in a publicly accessible database before recruitment of the first subject.

36. Researchers, authors, sponsors, editors and publishers all have ethical obligations with regard to the publication and dissemination of the results of research. Researchers have a duty to make publicly available the results of their research on human subjects and are accountable for the completeness and accuracy of their reports. All parties should adhere to accepted guidelines for ethical reporting. Negative and inconclusive as well as positive results must be published or otherwise made publicly available. Sources of funding, institutional affiliations and conflicts of interest must be declared in the publication. Reports of research not in accordance with the principles of this Declaration should not be accepted for publication.

UNPROVEN INTERVENTIONS IN CLINICAL PRACTICE

37. In the treatment of an individual patient, where proven interventions do not exist or other known interventions have been ineffective, the physician, after seeking expert advice, with informed consent from the patient or a legally authorised representative, may use an unproven

intervention if in the physician's judgement it offers hope of saving life, re-establishing health or alleviating suffering. This intervention should subsequently be made the object of research, designed to evaluate its safety and efficacy. In all cases, new information must be recorded and, where appropriate, made publicly available.

H – National Certificates

H.1. National IRB Approval

H.2. Certificate of Insurance

H.3. DKG Gütesiegel

I – Case Record Forms (CRF)

J – Associated studies

K - Drug Information